Trakia Journal of Sciences, Vol. 3, No. 1, pp 30-36, 2005

Available online at:
http://www.uni-sz.bg

ISSN 1312-1723

*Original Contribution*

# POLYMORPHISM OF C3 COMPLEMENT COMPONENT IN SHEEP

**Lilian Sotirov[1]*, Ivan Dimitrov[2], Maria Djorbineva[2], Evgeni Gelyazkov[3]**

[1]Department of Animal Genetics; Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria
[2]Institute of Agricultural Science, 6000 Stara Zagora, Bulgaria
[3]Department of Animal Genetics; Agrarian Faculty, Trakia University, 6000 Stara Zagora, Bulgaria

**ABSTRACT**

This investigation aimed to determine whether the C3 complement component in sheep was of a polymorphic nature. The sheep included in this study were the milk type crossings as follows: 25 sheep and 6 rams - Stara Zagora × East-Friesian and (Stara Zagora × East-Friesian) × Blackhead Pleven breed; 10 male and 19 female lambs (their progeny) were also included in the study. The electrophoretic mobility and the phenotype expression of C3 complement component isovariants were expressed by one or two bands with differing electrophoretic mobility. The one that was moving slower was labelled with the letter S, whereas the faster one was labelled with the letter F. The homozygous phenotypes were marked with SS and FF, while the heterozygous with FS. Apart from these principal phenotypes, some others were observed, particularly in the heterozygous variant. They were the F5S, F7S and F10S phenotypes. They differed by the distance between the F and S bands. In the ordinary heterozygous phenotype, the F–S distance was 3 mm, whereas for the others they were 5, 7 and 10 mm, respectively. On the basis of familial analysis we concluded that the C3 bands are inherited in a co-dominant way.

**Key words:** C3; polymorphism; sheep; inheritance.

## 1. INTRODUCTION

Various investigators are studying the genetic determination of various polymorphic systems (proteins, erythrocyte and lymphocyte antigens) with regard to a possible interrelationship among them and some important production parameters and the resistance or susceptibility to some diseases. The theoretical background of such studies are the well-known genetic phenomena as gene linkage, the pleiotropic effect and the heterozygous combination of alleles in heterozygous polymorphic genotypes (1). For now, the results are promising. Yotsov et al. (2, 3, 4) reported that the observed resistance of chickens (broilers and layer hens) against Marek's disease was related to the alkaline phosphatase genotype AkpSS, the cholinesterase genotypes $E_{S4}AB$ and $E_{S5}AB$ and the heterozygous haemoglobin genotype $Hb^2AB$. Briles et al. (5), Bennejean et al. (6) and Iotova et al. (7) observed that hens from the $B^{21}B^{21}$ genotype were considerably more resistant to Marek's disease.

Other authors (8, 9,10, 11, 12, 13, 14) showed a relationship among various transferrin genotypes in swine, cattle, sheep and carps and resistance or susceptibility to leptospirosis in swine; mastitis, leukosis and trypanosomiasis in cattle; facial eczema in sheep and branchiomycosis in carps. Those facts are very interesting and could be a precondition for the initiation of a selection aimed at increasing the resistance of the mentioned animal species against the respective diseases.

Our present investigation aimed to determine whether the C3 complement component in sheep was of a polymorphic type.

## 2. MATERIALS AND METHOD

### 2.1. Animals

*Correspondence to: Assoc. Prof. Dr. L. Sotirov, Department of Animal Genetics, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria; E-mail: sotirov20@yahoo.com

The sheep included in this study were milk type crossings: 25 sheep and 6 rams - Stara Zagora × East-Friesian and (Stara Zagora × East-Friesian) × Blackhead Pleven breed; 10 male and 19 female lambs (their progeny) were also included in this study. Blood for analysis was drawn from the *v. jugularis* of the animals and collected in 10 ml tubes. The blood was allowed to clot for one hour at room temperature (25$^{o}$C) and subsequently centrifuged at 4000 rpm for 10 min.

### 2.2. Method

The polymorphism of C3 complement component was determined according to the method of Teisberg (15) and is briefly described here:

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.

The first buffer was used for preparation of agarose gel (1%) where serum samples were applied. After their absorption, the electrophoresis was carried out at 20 V/cm for about 2.5 h. It was stained for 1 min with Amido Black 10 B and destained for overnight.

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 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 for overnight. If necessary it could be filtered after a week.

### 3. RESULTS

The C3 polymorphism in sheep was determined by the method of Teisberg (15). It was found that the electrophoretic mobility and the phenotype expression of C3 complement component isovariants were manifested in a very characteristic way. Most commonly, one or two bands with varying electrophoretic mobility were seen. The one that moved slower was labelled with the letter S, whereas the faster one was labelled with the letter F.

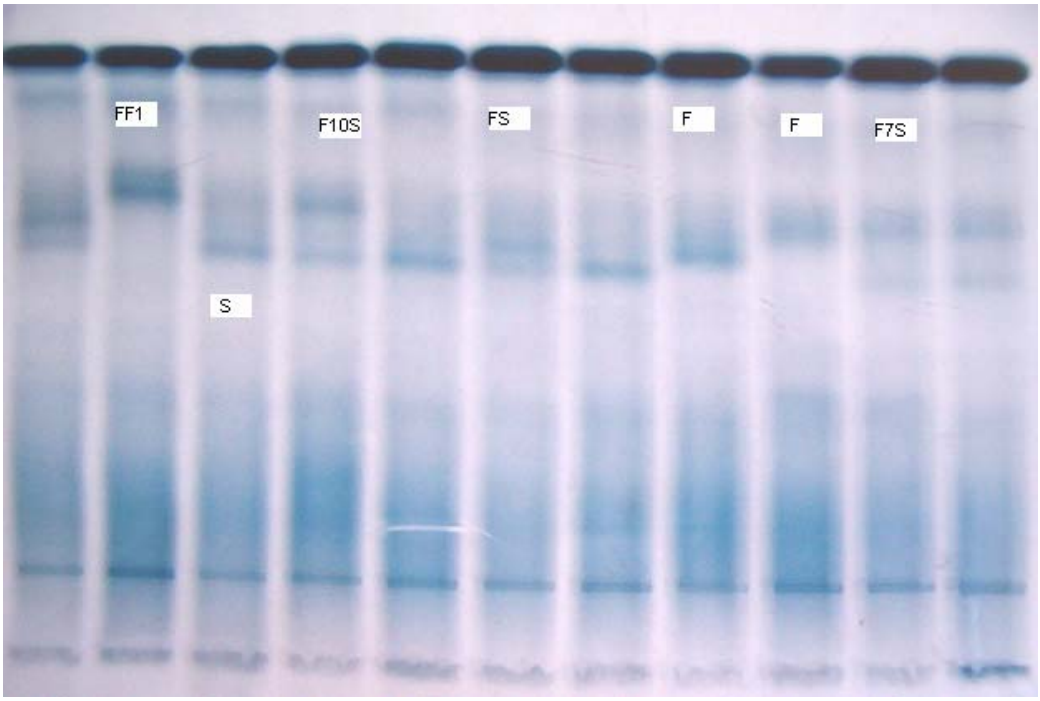


**Figure 1.** Different C3 phenotypes in sheep (FS, F5S, F7S, F10S, FF, SS).

The homozygous phenotypes were marked with SS and FF, while the heterozygous with FS. Apart from those principal phenotypes, some others could be observed particularly in the heterozygous variant. Such were the F5S, F7S and F10S phenotypes (**Figure 1**). They differed by the distance between F and S bands. In the ordinary heterozygous phenotype, the F–S distance was 3 mm, whereas for the others it was 5, 7 and 10 mm, respectively. This phenomenon is still not well understood, but the International Complement Society recognises them as separate phenotypes. Apart from the described phenotypes we found a very rare variant such as FF1 (**Figure 2**). It was however observed very infrequently and was not present in all studied sheep breeds.

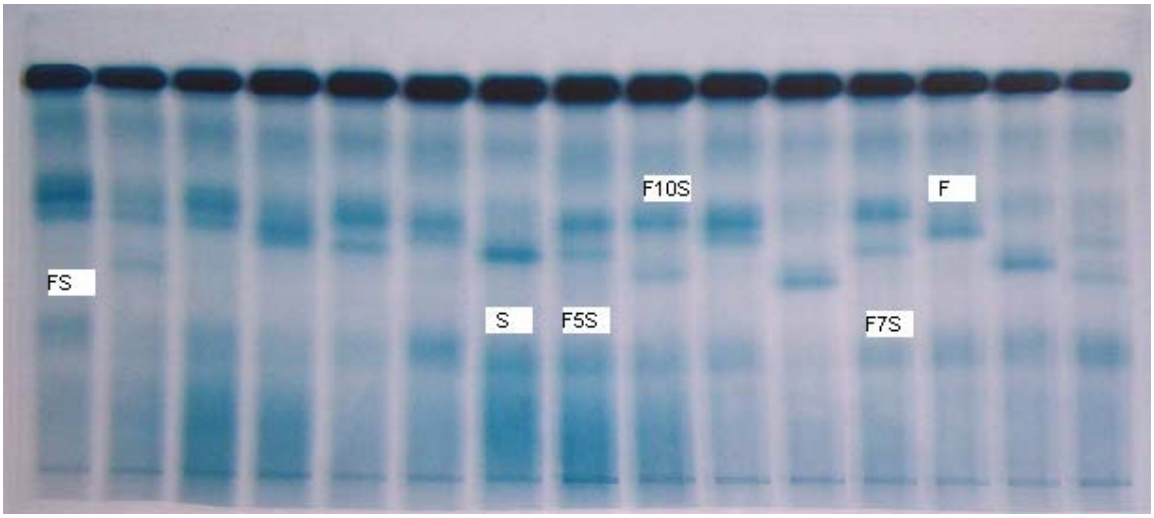


**Figure 2.** Rare C3 phenotype FF1.

A principle in the study of polymorphic proteins is the determination of the pattern of inheritance of bands. This is very important because it determines the method of calculation of genotype and gene frequencies.

This primary element of our investigation was elucidated using the method of family analysis. The schedule and the results of the analysis are presented on **Table 1**. It shows that in mothers, the phenotypic diversity was big – there was homozygous (SS and FF) as well as heterozygous (F7S) phenotypes. The rams were predominantly heterozygous – FS, F7S and F10S, but there was one homozygous animal (FF). The dissociation of signs in the progeny was very characteristic and supposed a co-dominant type of inheritance. This assumption was evidenced by all studied combinations, some of them being particularly distinctive (**Table 2**). Combination 1 shows that the mating of homozygous FF parents resulted in a homozygous progeny from the same phenotype. Combination 2 shows that the mating of homozygous parents from different phenotypes (SS × FF) resulted in heterozygous progeny (FS). The other combinations were an obvious example of various types of band dissociation in the progeny. The case Numbers 5, 6 and 7 were especially interesting. There were three lambs from two F7S parents, and the dissociation followed exactly the model of the co-dominant inheritance – one homozygous and two heterozygous phenotypes (F7S, SS and FF). It must be emphasised that in no case was there either a dominant or a recessive activity of genes determining the synthesis of bands. The inheritance could not be intermediate as well because there was an equivalent manifestation of two genes with independent expression. Therefore, the hypothesis about a co-dominant inheritance of bands was fully supported. Moreover, the phenotype manifestation of bands corresponded to the genotype of animals. Therefore, when we said "phenotype", we actually meant the genotype of sheep, so we shall consequently use only the term "genotype".

## 4. DISCUSSION

The C3 polymorphism is not peculiar to our studies. Wieme and Demeulenaere (16) were the first to deal with this problem. They studied the polymorphism of human serum proteins and were surprised that in some individuals, the $\beta_2$C globulin zone was presented by 2 bands. The electrophoresis was performed in 1% agar gel, but when the traditional starch gel was used, the results were identical. The performed family analysis

revealed that those bands were inherited as an autosomal sign, but it did not indicate how. An interesting observation was the fact that the person with two $\beta_2$C bands was a girl suffering from the Hodgkin's disease. This first observation motivated the work of many investigators thereafter.

**Table. 1:** *Family analysis of C3 genotypes in sheep.*

| Mother | Genotype | Father | Genotype | Progeny | Genotype |
|---|---|---|---|---|---|
| 0510 | SS | 98 | FF | 3424 | FS |
| 9603 | FS | A | F10S | 3538 | F7S |
| 0526 | SS | 9512 | F7S | 3520 | F7S |
| 8726 | FS | 0410 | F7S | 3565 | FS |
| 0514 | FS | A | F10S | 3535 | FS |
| 8634 | FF | A | F10S | 3414 | FF |
| 9617 | FF | A | F10S | 3514 | FS |
| 9660 | SS | A | F10S | 3406 | FS |
| 8711 | F7S | A | F10S | 3413 | F10S |
| 9664 | FF | 0409 | FS | 3419 | FF |
| 7701 | FS | 0409 | FS | 3512 | FF |
| 9643 | SS | 0410 | F7S | 3548 | F7S |
| 8611 | SS | 9512 | F7S | 3553 | F7S |
| 8622 | FF | A | F10S | 3405 | FS |
| 0501 | F7S | 9502 | F7S | 3521 | F7S |
| | | | | 3555 | SS |
| | | | | 3415 | FF |
| 7706 | SS | A | F10S | 3515 | FS |
| | | | | 3416 | F7S |
| 9621 | FS | A | F10S | 3503 | F10S |
| 9666 | F7S | 9512 | F7S | 3519 | FS |
| 0512 | SS | 0409 | FS | 3529 | SS |
| 0524 | FF | 9512 | F7S | 3523 | FS |
| 9608 | SS | A | F10S | 3567 | SS |
| 0529 | FF | 98 | FF | 3536 | FF |
| 9611 | SS | A | F10S | 3509 | FS |
| 7742 | SS | A | F10S | 3409 | F7S |
| 9658 | F7S | A | F10S | 3425 | F7S |

**Table. 2:** *The most typical combinations parents-progeny proving co-dominant way for inheritance of C3 bands in sheep.*

| № | Mother | Genotype | Father | Genotype | Progeny | Genotype |
|---|---|---|---|---|---|---|
| 1 | 0529 | FF | 98 | FF | 3536 | FF |
| 2 | 0510 | SS | 98 | FF | 3424 | FS |
| 3 | 8634 | FF | A | F10S | 3414 | FF |
| 4 | 8622 | FF | A | F10S | 3405 | FS |
| 5 | 0501 | F7S | 9502 | F7S | 3521 | F7S |
| 6 | | | | | 3555 | SS |
| 7 | | | | | 3415 | FF |
| 8 | 9660 | SS | A | F10S | 3406 | FS |
| 9 | 9608 | SS | A | F10S | 3567 | SS |
| 10 | 9611 | SS | A | F10S | 3509 | FS |
| 11 | 9617 | FF | A | F10S | 3514 | FS |
| 12 | 9664 | FF | 0409 | FS | 3419 | FF |

Only one year later, Alper and Propp (17), reported about 4 (probably 5) alleles, coding for C3 variants with various electrophoretic mobility in agarose gel with a better separation capability. They determined 15 various phenotypes. According to the authors, the $F_1$, F and S alleles were inherited autosomal genes and were co-dominant whereas the $S_1$ and $F_{0,8}$ alleles were via an unknown mechanism. Furthermore, the investigators have studied the influence of various C3 genotypes upon the blood serum C3 component concentration, the total complement activity and the adhesive properties of C3 molecules, but no significant differences were observed.

In the same year, Azen and Smithies (18), using starch gel electrophoresis, detected 4 co-dominantly inherited alleles that were determining 6 different genotypes within the same study. The allele frequency was as follows: $C3^1$ – 0.21; $C3^2$ – 0.77; $C3^3$ – 0.01 and $C3^4$ – 0.004.

The actual study on C3 polymorphism began however when Teisberg (15) published a really perfect method for C3 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. In 1975, Mauff et al. (19) reported about the existence of an occult C3 gene. During the investigation on a German family, they discovered that the mother's genotype was FF and the father's – FS. One of offspring however, was of the C3F0, 5S genotype. Seventeen additional studies have been performed in order to exclude any doubt about the parenthood. There was, therefore, one possible explanation: in the mother's genotype there was an "occult" or, "silent" gene. Alper et al. (20) were the first to report the existence of such genes.

The determination of amino acid sequences of those polymorphous 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 resistance) to some diseases.

The molecular mechanism of C3 complement component polymorphism in humans was made clear by Botto et al. (21). They found out that the difference between the genome organisation of C3S and C3F was a point mutation (cytosine towards guanine) at nucleotide 364 of 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 (restriction fragment length polymorphism) 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 with 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 (respectively the resistance) to some diseases.

All those facts suggest that the polymorphism of the C3 component of complement system was extensively studied in humans. In animals, this problem is almost left open. In our knowledge, there are only 4 publications on this subject. Gorman et al. (22) reported the existence of C3 polymorphism in dogs. It was established that the C3F and C3S genes were inherited by an autosome-co-dominant manner, but apart from those 2 principal alleles, others were not observed. The C3 locus was not linked and was neither inherited jointly with genes of the Major Histocompatibility Complex (DLA), nor with C6 and C7 complement component loci.

The studies of Kay et al. (23) in horses evidenced that in these 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 (24) 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 (25) discovered a C3-like protein in sheep blood plasma. Despite the earlier studies, they used an ordinary agarose gel electrophoresis followed by immunoblotting. Thus, they found out 4 principal alleles, three of them (F, I and M) being represented by one principal band and the fourth (S) – by 3 to 5 bands. The family analysis showed that the alleles were inherited in an autosome-co-dominant manner.

On the basis of our results, we suppose that the C3 complement component in sheep was a polymorphic system, built by 2 alleles, forming various sheep genotypes. The inheritance of C3 bands were by a co-dominant way.

## 5. REFERENCES


1. Makaveev, Tz. and Dobriyanov, D. Genetika I jivotnovodstvo, Zemizdat, Sofia, pp. 112-113, 1983.
2. Iotova, I. T., Ermenkova, L., Iotzov, S. Blood-group antigens in laying hens in relation to resistance to Marek's disease. *Animal Breeding*, 26:48-53, 1990.
3. Iotzov, S. and Iotova, I. On the correlation between the genetic polymorphism of haemoglobin in the blood of broiler chicks and their resistance to the virus causing the Marek's disease. *Animal Science*, 18, 8:94-98, 1981.
4. Iotzov, S., Iotova, I., Girginov, G., Chichibaba, V., Nikolova, M. Genetic polymorphism of cholin-esterase in the blood plasma of broiler chicks depending on their resistance to Marek's disease. *Animal Science*, 19, 4:63-71, 1982.
5. Briles, W. E., Stone, H. E., Cole, R. C. Marek's disease: effect of B-histocompatibility alleles in resistant chicken lines. *Science*, 195:193-195, 1977.
6. Bennjean G., Marat, P., Perramon, A., Guitted, M., Picualt, J. P. Blood groups and Marek's disease in fowl: resistance in a heterozygotic genotype. *Bulletin d'information Station Experimentale d'Aviculture de Ploufragan*, 16, 4:159-163, 1976.
7. Iotova, I. T., Ermenkova, L., Iotzov, S. Blood-group antigens in laying hens in relation to resistance to Marek's disease. *Animal Breeding*, 26:48-53, 1990.
8. Koromislov, G. F., Klimov, N. M., Yaremenko, I. I. Transferrin type in relation to the resistance of cattle to leucosis. *Trudy Vsesoyuznogo Instituta Experimentalnoi Veterinarii*, 48:61-64, 1978.
9. Loong, P. C., Wheeler, T. W., Ford, H. C., Campbell, A. G. Use of two-dimensional polyacrylamide gel electrophoresis to detect plasma protein variation between Romney sheep selected for facial eczema resistance or susceptibility. *New Zealand Journal of Agricultural Research*, 29, 3:449-456, 1986.
10. Nikitchenko, I. P., Dzumkov, V. A., Pleshkovich, I. S., Lemesh, V. M. Breeding Dairy cattle for resistance to leucosis. *Zhivotnovodstvo*, 6:35-36, 1984.
11. Przytulski, T. and Porzeczkowska, D. Polymorphism of blood serum amylase and transferrin and leptospirosis in Large White Polish pigs. *British Veterinary Journal*, 135, 1:103-107, 1979.
12. Queval, R. Transferrin polymorphism in trypanosensitive and trypanotolerant cattle of West Africa. Allele distribution and frequency. *Revue d'Elevage et de Medicine Veterinaire des Pays Tropicaux,* 35, 4:373-380, 1982.
13. Valenta, M., Stratil, A., Kalal, L. L., Smisek, J. The possibility of using carp polymorphic proteins for estimation of offspring viability and resistance to branchiomycosis. *Animal blood groups and biochemical polymorphism,* 11, suppl. 1:64, 1980.
14. Wijerante, W. V. S., Wilson, C. D., Hebert, C. N., Richards, S. R., Kingwill, R. G., Griffin, T. K., Spooner, R. L. Relationship of blood serum transferrin and amylase polymorphism with liability to mastitis in cattle. *Animal blood groups and biochemical polymorphism* , 8, suppl. 1:43, 1977.
15. Teisberg, P. High voltage agarose gel electrophoresis in the study of C3 polymorphism. *Vox Sang,* 19:47-56, 1970.
16. Wieme, R. J. and Demeulenaere, L. Genetically determined electrophoretic variant of the human complement component C3. *Nature*, 214, 3:82-83, 1967.
17. Alper, C.A. and Propp, R.P. Genetic Polymorphism of the Third Component of Human Complement (C3). *The Journal of Clinical Investigation*, 47:2181-2191, 1968.
18. Azen, E. A. and Smithies, O. Genetic polymorphism of C′3 ($\beta_{1c}$-Globulin) in human serum. *Science*, 162:905-907, 1968.
19. Mauff, G., Pulverer, G., Opperkuch, W., Hummel, K. Hidden C3-variants and diverse phenotypes of unconverted and converted C3. In: Protides of the Biological Fluids (ed. H. Peeters), vol. 22, Pergamon Press, Oxford, 1975.
20. Alper, C.A. and Johnson, A.M. Immunofixation Electrophoresis: A Technique for the Study of Protein Polymorphism. *Vox Sanguinis*, 17: 445-452, 1969.
21. Botto, M., Fong, K.Y., So, A.K., Koch, C., Walport, M.J. Molecular Basis of Polymorphisms of Human Complement Component C3. *J. Exp. Med*., The Rockefeller University Press, 172:1011-1017, 1990.
22. Gorman, N.T., Hobart, M.J., Lachmann, P.J. Polymorphism of the third component of canine complement. *Veterinary Immunology and Immunopathology*, 2:301-307, 1981.

23. Kay, P.H., Dawkins, R.L., Bowling, A.T., Bernoco, D. Electrophoretic polymorphism and molecular structure of equine C3. *Animal Genetics,* 17:209-215, 1986.
24. Bowling, A.T. and Dileanis, S. Population data and a fourth allele for equine complement component (C3). *Animal Genetics,* 21:83-86, 1990.
25. Gahne, B. and Amorena, B. Genetic polymorphism of a C3-like protein in sheep plasma. *Animal Genetics*, vol. 18, suppl. 1, p. 57, 1987.