

# Alternative pathway of complement activation (APCA) in different sheep breeds

L. SOTIROV<sup>1\*</sup>, I. DIMITROV<sup>2</sup>, M. DJORBINEVA<sup>2</sup> and S. TANCHEV<sup>3</sup>

<sup>1</sup> Department of Genetics, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria

<sup>2</sup> Institute of Agricultural Science, 6000 Stara Zagora, Bulgaria

<sup>3</sup> Department of Genetics, Agrarian Faculty, Trakia University, 6000 Stara Zagora, Bulgaria

\* Corresponding author : [sotirov20@yahoo.com](mailto:sotirov20@yahoo.com)

## SUMMARY

Sera from 486 sheep belonging to four breeds (Milk type crosses, Trakia merino, Charollais, Ile de France, and lambs from dairy crossings) have been tested for determining intensity of alternative pathway of complement activation (APCA). The highest average values of APCA were obtained in Ile de France sheep and Milk type rams. Breed related differences for this parameter was evidenced but variations dependent on the gender were contradictory. Besides, APCA concentrations in lambs were partially influenced by APCA status of parents, suggesting that selection of breeders based on this parameter would improve innate immune resistance in progeny.

**Keywords :** natural immunity - complement - alternative pathway - sheep - breeds - lambs.

## RÉSUMÉ

**Intensité de l'activation du complément par la voie alterne dans différentes races ovines. Par L. SOTIROV, I. DIMITROV, M. DJORBINEVA et S. TANCHEV.**

Le degré d'activation du complément par la voie alterne a été mesuré dans 486 sérums de moutons appartenant à 4 races (croisements laitiers, Mérinos de Thrace, Charollais, Ile de France et chez des agneaux laitiers). Les valeurs moyennes maximales ont été observées chez les brebis Ile de France et chez les béliers de type laitier. Des différences dues à la race ont été mises en évidence, mais l'influence du sexe sur le degré d'activation du complément n'a pas été univoque. De surcroît, l'intensité de l'activation du complément par la voie alterne chez les agneaux est partiellement reliée aux statuts parentaux ce qui suggère qu'une sélection des reproducteurs sur ce paramètre pourrait améliorer la résistance immunitaire innée des nouveau-nés.

**Mots-clés :** immunité naturelle - complément - voie alterne - ovins - races - agneaux.

## Introduction

The alternative pathway of complement activation (APCA) is a primary humoral factor of innate immunity. It is active against Gram-negative bacteria, viruses, virus-infected cells, neoplastic cells, agarose, lipopolysaccharides, contrast media used in radiology etc. [5, 11]. GREWAL and BABIUK [9] have studied the cytotoxic effect of neutrophils against herpes virus infected cells and reported that it was dependent not only on the classical pathway of complement activation, but on APCA as well. The antiviral activity of APCA was confirmed by the experiment of OHTA *et al.* [12]. They have treated cell cultures of chicken embryos infected by the fowlpox virus with normal chicken serum and observed the lack of cytopathogenic effect, viral growth and plaque formation.

Other investigators reported that the APCA was active against some blood parasites too. In sheep, infected with *Trypanosoma brucei*, the complement activity disappeared completely by post infection day 5 and persisted until the lethal issue, if not treated. If an adequate treatment was however performed and recovery occurred, the activity of this important factor was completely regained [3].

The cited facts evidence the considerable significance of APCA for systemic defense against various pathogens. The objective of the present study was to determine the occurrence of APCA differences among various sheep breeds, in

adults and in lambs, before employing this parameter as a marker of immune defenses during infectious diseases.

## Material 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 breed;

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 ewes and 6 rams from the Charollais breed;

D. Furthermore, 45 lambs (21 male and 24 females - dairy crossings) were included in the study.

For family analysis, rams and ewes (milk type) were selected according their APCA activities. They presented high and middle APCA activities, and we could not find out animals with low APCA activity (i.e. below 80 CH50). After mating of breeders, APCA activities were determined on each progeny.

At the beginning of the experiment, the sheep and the rams

were 2-3-year-old. They were housed in separate premises. Blood for analysis was sampled in 10 mL tubes from *v. jugularis*. The blood was allowed to clot for one hour at room temperature (25°C) and the samples were centrifuged at 2000 g for 10 min.

#### APCA DETERMINATION :

The alternative pathway of complement activation (APCA) was studied by the method of SOTIROV [16]. Each serum sample was first diluted by mixing 100 mL serum with 350 mL veronal - veronal Na buffer (in final concentrations: 146 mM NaCl, 1.8 mM 5,5-diethylbarbituric acid sodium salt, 3.2 mM 5,5-diethylbarbituric acid, 1 mM EGTA and 0.8 mM MgCl<sub>2</sub>). In U bottomed plates (Flow Laboratories, UK), 7 other dilutions from each diluted serum were again prepared in veronal-veronal Na buffer : 80 mL diluted serum + 20 mL buffer, 70 mL diluted serum + 30 mL buffer, 60 mL diluted serum + 40 mL buffer, 50 mL diluted serum + 50 mL buffer, 40 mL diluted serum + 60 mL buffer, 30 mL diluted serum + 70 mL buffer and 20 mL diluted serum + 80 mL buffer. The final serum dilutions were respectively 8/45, 7/45, 6/45, 5/45, 4/45, 3/45 and 2/45. Then, 50 mL buffer and 100 mL of 1% rabbit erythrocyte suspension were added to each well. After incubation for 1 hour at 37°C, samples were centrifuged at 150 g for 3 minutes at room temperature (23°C). Thereafter, 150 mL of each supernatants were removed and placed in flat bottomed plates for measurement of optic density at 540 nm by "Sumal-PE2" ELISA reader (Karl Zeiss, Germany). The final APCA activity was calculated using special computer programmes developed in the Trakia University, and expressed as CH50 units (CH50 units correspond to 50% of complement-induced haemolysis of applied erythrocytes).

#### STATISTICAL ANALYSIS :

Data were analysed using the fixed effect MANOVA model (Program Statistica, Statsoft, Inc., USA) and evaluated through the following formula:

$Y_{ij} = m + a_i + e_{ij}$  where  $y_{ij}$  is the observation value of the investigated trait,  $m$  the population mean,  $a_i$  the breed effect and  $e_{ij}$  the random errors. Differences were considered as significant when  $p$  values were less than 0.05.

## Results and discussion

Four sheep breeds from different productivity type were used in the present study. The results are presented in Table I. The Ile de France sheep presented the highest APCA activity, whereas the lowest activity was observed for Charollais breed, and particularly for rams (the difference between Ile de France and Charollais breeds was statistically significant :  $p < 0.001$ ). The rams from dairy type presented similar complement activity as the Ile de France sheep and the differences with Charollais rams and ewes were also significant ( $p < 0.05$  and  $p < 0.01$ , respectively). Intermediate activities were found for Merino breed and milk type sheep.

Similar results were communicated by AUDRAN *et al.*

[2]. They studied 5 sheep breeds : Préalpe, Ile de France, Limousine, Mérinos d'Arles and Bouchara and reported the highest complement activities in Ile de France sheep (171.4 CH50) followed by Préalpe (168.8 CH50) and Mérinos d'Arles sheep (167.7 CH50), but the differences among these breeds were not significant. The average complement activities in Limousine (159.6 CH50) and Bouchara (138.1 CH50) sheep were however statistically lower. The breed-related differences showed that the APCA activities were under a genetic control. Dissimilarities related to the breed were observed in other animal species too. FOX *et al.* [7] have studied the cytotoxic effect of rabbit complement on sensibilised murine lymphocytes. The source of complement was stemmed from various rabbit lines. It was shown that out of the pure lines, the highest lymphocyte activity was that of rabbits from the IIIC/J and IIIVO/J lines whereas the lowest ( from the IIIVO/vptj line. The highest cytotoxic effect was obtained after the crossing over of the IIIC/j and IIIVO/J lines, probably due to the heterosis effect.

ABE *et al.* [1] have performed a very detailed analysis of classical pathway of complement activation (CPCA) in rabbits. For this purpose they experimented with two lines from the New Zealand White rabbit breed (NZB-Chiba and NZW-Ikaken), 2 lines from the Japanese White rabbit breed (JW-Nagano and JW-Tokyo) and 1 line from the Angora rabbit breed (A-Nagano). The highest activity was observed in the JW-Nagano breed and the lowest ( in Angora rabbits, where half of studied samples did not exhibit any haemolysis. For explanation of this phenomenon, the authors analyzed all components of the complement system and observed the lowest values for C6. After the addition of purified C6, the haemolytic activity of deficient sera was restored. Therefore, in such animals there was a C6 immune deficiency and thus the determination of the total complement titers could serve as a first sign for the incidence of immuno-deficiencies. The latter are scarcely identified in animals whereas in humans, they are well recognized.

TEDESCO and LACHMAN [17] reported that the normal levels of C6 in rabbits were 35 g/L. ABE *et al.* [1] were the first to detect a complete C6 deficiency in Angora rabbits, i.e. to prove that they were homozygous by respect to the C6<sup>o</sup> gene.

Breeds	n	Mean ± SE	VC (%)
Milk type sheep	118	133.66 ± 2.04 <sup>bc</sup>	16.50
Milk type rams	14	149.71 ± 6.93 <sup>ab</sup>	16.68
Trakia Merino sheep	80	147.98 ± 2.73 <sup>abc</sup>	16.42
Trakia Merino rams	9	131.25 ± 5.62 <sup>bc</sup>	12.11
Ile de France sheep	107	153.05 ± 2.00 <sup>a</sup>	13.48
Charollais sheep	107	130.57 ± 1.98 <sup>c</sup>	15.62
Charollais rams	6	125.4 ± 8.11 <sup>c</sup>	14.47
Male lambs	21	125.04 ± 4.44 <sup>c</sup>	10.66
Female lambs	24	123.04 ± 2.79 <sup>c</sup>	9.64

TABLE I. — APCA activities in different sheep breeds (expressed as CH50). Results are expressed as means ± standard error (SE). VC(%) : Coefficients of variations. <sup>a,b,c</sup> Mean values with different superscripts within a column differ significantly ( $p < 0.05$ ).

Rams		Dams		Lambs	
N°	APCA	number	APCA	number	APCA
98	120.23	5	120.28 ± 10.85	8	142.67 ± 7.15 <sup>a</sup>
0409	123.71	5	162.28 ± 20.16	8	117.51 ± 1.67 <sup>b</sup>
9512	143.61	5	122.27 ± 9.83	7	118.71 ± 3.34 <sup>b</sup>
0410	181.05	5	155.70 ± 40.11	8	123.87 ± 12.58 <sup>ab</sup>
A	181.05	12	127.25 ± 4.96	14	124.76 ± 3.76 <sup>ab</sup>

TABLE II. — Average APCA activities (expressed as CH50) in milk type rams, dams and their progenies used for family analysis. Results are expressed as means ± standard errors. <sup>a,b</sup>. Mean values with different superscripts within a column differ significantly ( $p < 0.05$ ).

HINZOVA *et al.* [10] thought that the genes controlling the levels of complement in mice, were related to the H-2 complex. This suggestion was further confirmed by DEMANT *et al.* [6] and CAPKOVA and DEMANT [4], who have proved that the genes determining the synthesis of the Ss-S1p protein were in the H-2K locus. Later it was pointed out that the Ss-S1p component was identical with the C4 component [13 - 15]. GOLDMAN and GOLDMAN [8] found out that the H-2 complex controlled the expression of the early components of complement.

WAMBURA *et al.* [18] compared 3 zebu breeds (Meru, Mbulu, and Iringa Red) and their crosses with Friesian cattle by respect to their resistance against ticks. It was observed that purebred zebu were more resistant to parasites compared to crossbreds. Also, the activity of complement in purebred zebu was higher than that in crossbreds. It was assumed that the highest complement activity was important for the higher resistance of the three zebu breeds. This suggestion was supported by the statistically significant negative correlation between the degree of infection with parasites and the complement activities.

Despite the observed breed-related differences, there are probably variations dependent on the gender, but with this regard, our results were contradictory. Trakia Merino and Charollais rams had a lower APCA activity than females, but differences were not significant. By contrast, rams from the dairy type showed a similar APCA activity as Ile de France sheep and the difference with Charollais sheep was significant ( $p < 0.05$ ). But again, in this milk type, the difference between males and females was not significant. In both male and female dairy lambs, the activities were almost identical (males : 125.04 ± 4.44 CH50 ; females : 123.04 ± 2.79 CH50). All those facts did not support the hypothesis for gender-related differences in complement activities.

The aforementioned facts emphasized the importance of APCA for the defense of animals against pathogenic agents and the possibility for a serious genetic control on this important humoral factor. A family analysis was performed in order to elucidate the effects of parents upon the inheritance of the factor by the progeny. There was a definite phenotypical diversity of APCA in the various types of animals: in mothers - between 92.23-184.06 CH50 ; in fathers - between 120.68-181.05 CH50 and in offsprings - between 102.68-143.82 CH50. Generally, the complement system is a conservative and relatively stable parameter. When we studied how the APCA activity was inherited in the progeny, we

noticed that offsprings tend to exhibit relatively high APCA values when their parents had already high values (Table II). For example, 14 lambs from ram "A" (which presented the highest complement activity) showed values equal or above the average value of the total progeny (124.76 ± 3.76 CH50). However, the highest APCA values (142.67 ± 7.15 CH50) were observed for ram 98's progeny, although this male exhibited the lowest APCA concentration among rams (Table II). The differences between ram 98's progeny and other lambs stemmed from other rams were significant ( $p < 0.05$ ). Besides, no positive significant correlation between APCA concentrations of lambs and fathers was found. It was probable that some other factors, such as mother influence, would interfere in the determination of APCA activity in lambs. But again, even if contribution of dams would be out of importance in APCA status, it was not sufficient for explaining the great APCA variations encountered in lambs. Indeed, APCA activities in progenies did not significantly correlate with APCA activities of mothers, and APCA activities have greatly differed among lambs belonging to a same litter.

As conclusion, our results demonstrated that, firstly, the APCA in the studied sheep breeds were different, and secondly APCA activities in lambs would partially be related to APCA status of parents. Consequently, by selection of breeders, values of this parameter would be increased in progeny for achieving a higher innate immune resistance in lambs.

## References

1. — ABE T., KOMATSU M., OISHI T., YAMAMOTO K. : Development and genetic differences of complement activity in rabbits. *Anim. Blood Grps biochem. Genet.* 1979, **10**, 19-26.
2. — AUDRAN R., MOULLEC J., MILLOT P. : Influence of breed of sheep on the titre of haemolytic complement. *C. R. Acad. Sci., Paris*, 1962, **254**, 2874-2876.
3. — BOUTEILLE B., DARBE M. L., MONTEIL J., PESTRE-ALEXANDRE M. : Complement, an indicator of *Trypanosoma brucei* infection in sheep used as an experimental model. Complement levels following treatment. *Bull. Soc. Pathol. Exo. Fil.*, 1988, **81**, 522-529.
4. — CAPKOVA J., DEMANT P. : Genetic studies of the H-2 associated complement gene. *Folia Biol. (Prague)*. 1974, **20**, 101-115.
5. — CORBEIL L.B. : Role of the complement system in immunity and immunopathology. *Vet. Clin. North Am.*, 1978, **8**, 585-611.
6. — DEMANT P., CAPKOVA J., HINZOVA E., VORACOVA B. : The role of the histocompatibility-2-linked SS-S1p region in the control of mouse complement. *Proc. Nat. Acad. Sci.* 1973, **70**, 863-864.
7. — FOX R. R., CHERRY M., SHULTZ K. L. : Effect of rabbit strain on activity levels and cytotoxicity of serum complement. *J. Heredity*. 1978, **69**, 107-112.

8. — GOLDMAN M. B., GOLDMAN J. N. : Relationship of levels of early components of complement to the H-2 complex of mice. *Fed. Proc. Fed. Amer. Soc. Exp. Biol.*, 1975, **34**, (abstract 4309).
9. — GREWAL A.S., BABIUK L. A. : Complement dependent, polymorphonuclear neutrophil-mediated cytotoxicity of herpes virus-infected cells : possible mechanism(s) of cytotoxicity. *Immunol.*, 1980, **40**, 151-161.
10. — HINZOVA E., DEMANT P., IVANYI P. : Genetic control of haemolytic complement in mice : association with H-2. *Folia Biol. (Prague)*, 1972, **20**, 237-243.
11. — KULBERG A. Ya. : *Molekularnaya immunologija*. (Eds R. V. Petrov, N. E. Kucherenko, Moskow Medical Institute and A. A. Bogdanov, Moskow Government University), Moskow, 1985, 175-178.
12. — OHTA H., KAI C., YOSHIKAWA Y., YAMANUCHI K. : Activation of chicken alternative complement pathway by fowlpox virus-infected cells". *Inf. Imm.*, 1983, **42**, 721-727.
13. — SHREFFLER D.C. : The S region of the mouse major histocompatibility complex (H-2) : genetic variation and functional role in the complement system. *Transplant. Rev.* 1976, **32**, 140-167.
14. — SHREFFLER D.C. : *Frontiers in Immunogenetics*. (ed. W. Hildemann). Amsterdam. 1981, 107-120.
15. — SHREFFLER D.C., ATKINSON J. P., BROWN J. L., PARKER K. L., ROOS M. H. : Genetic structure and function of murine S region gene products. *In* : *Immunology of the Major Histocompatibility Complex, Seventh International Convocation on Immunology*, (Eds. M. Zaleski, C. J. Abeyounis and K. Kano), Niagara Falls, N. Y. 1980, S. Karger A. G., Basel., 1981, **17**, 78-88.
16. — SOTIROV L. K. : Phenotype characteristic and inheritance of lysozyme and complement activity in swine. Thesis, Trakia University, Stara Zagora, 1991.
17. — TEDESKO F., LACHMANN P. J. : The quantitation of C6 in rabbit and human sera. *Clin. Exp. Immunol.* 1971, **9**, 359-370.
18. — WAMBURA P. N., GWAKISA P. S., SILAYO R. S., RUGAIMUKAMU E. A. : Breed-associated resistance to tick infestation in *Bos Indicus* and their crosses with *Bos taurus*. *Vet. Parasitol.*, 1998, **77**, 63-70.