

STUDIES ON SOME IMMUNE PARAMETERS IN VARIOUS PHYSIOLOGICAL STATES AND AFTER INFECTION WITH RABIES VIRUS IN SHEEP *

The Veterinary Research Institute, Puławy
Department of General Epizootiology
Head: Prof. Dr. St. Krauss

EWA SOMMER

In consequence of intensification of sheep breeding, an important question, both theoretically and practically, has become the problem of natural immunity of these animals to infectious diseases. Therefore, it seems purposeful to investigate various aspects of the defence system of

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organism, i.e. its immune reactivity in different physiological states and under the influence of external stimuli.

One of the methods used for determination of the immunity state is *in vitro* examination of the immune indices to which, among other things, belong: complement, bactericidal activity of serum, phagocytosis, and antibodies. As yet, the studies dealing with natural immunity of sheep are relatively rare. No investigations on the bactericidal properties of sheep serum and influence of viral infection on the activity of defence systems of these animals, were found.

The purpose of the presented investigations was to study the development of some immune parameters in sheep from birth to the 6th month of life, and the influence of pregnancy, season of the year, and infection with rabies virus on the activity of these parameters.

Materials and Methods

Twelve ewes and ten offsprings were used in the study. The ewes were examined periodically before mate, during pregnancy, parturition, and lactation, up to the period before the next breeding season. The lambs were examined just after birth, before colostrum feeding, 12 and 24 hours after ingestion of colostrum, on the 3rd and 7th days of age, afterwards, every week up to the 8th week of age, and then every month.

The following immune parameters were examined: 1. Haemolytic activity of complement according to the method of Osler given by Truszczyński (11), using rabbit red blood cells sensitized with amboceptor prepared in sheep; 2. Bactericidal activity of serum against *E. coli* serotypes: O8:K87,K88, O24:K? and O78:K80, using the plate method of Dvořák (2); 3. Opsonocytophagic index by the Huddleson method (5), calculating according to Harris (4); 4. Gammaglobulins by paper electrophoresis; and 5. Titre of antibodies against O antigen of *E. coli* serotypes according to Sojka (10). Gammaglobulins and anti-O antibodies were determined also in colostrum whey and milk.

Haemolytic activity of complement and bactericidal properties of serum were estimated in 35 sheep used for the production of rabies vaccine. The parameters were determined before infection with fixed rabies virus, on the 2nd and 4th days after infection, and during agony. Control group consisted of 3 sheep injected intracerebrally with a sterile saline solution.

Results and Discussion

The influence of pregnancy and season of the year

It was demonstrated that pregnancy influenced all the investigated immune indices. The complement activity, after an initial increase in the middle of pregnancy, decreased and was nearly by 50% (the mean titre: 6.4 CH₅₀ units/ml) lower than that before mate (the mean titre: 10 units/ml). Similar decrease of complement was observed in women during parturition (6).

A pregnancy elicited also an effect on the bactericidal activity of serum against all the investigated *E. coli* serotypes. The activity declined during pregnancy and its lowest values: 0.063 for serotype O8K87, K88, 0.062 for O78:K80, and 0.157 for O24:K?, were observed during parturition. Before mate these values were: 0.017, 0.020, and 0.083, respectively.

No significant effect of pregnancy on phagocytic activity of sheep blood was found, however, a slight decrease of the activity was noted during parturition. The lowest level (17%) of gammaglobulins in pregnant

ewes was demonstrated during parturition. Before mate this level was on an average 25%. Similar changes were noted in the titre of antibodies against O antigen of *E. coli* serotypes.

Since it was accepted that passive immunity is transferred to offsprings *via* colostrum, gammaglobulins and antibodies in colostrum whey and milk of sheep were examined. The highest concentration of gammaglobulins (about 50%) was demonstrated in the colostrum whey at parturition. Within a few days' time after parturition, gammaglobulins declined rapidly, and after 2 weeks of lactation reached 14% remaining on this level up to the end of lactation. The same kinetics was seen in the anti-O antibody response measured in the colostrum whey and milk.

Evaluation of season influence on immunity was based on the examination of bactericidal activity of sheep serum against serotype O8:K87, K88. Unfertilized ewes were examined on an average every two months during the year. The lowest bactericidal activity was found in winter, and the highest one in summer. Similar relationship between bactericidal activity and season was observed by Borkowska-Opacka (1) in pigs.

Development of immune indices in lambs

An examination of the activity of immune indices in lambs from birth to the 6th month of age demonstrated that newborn lambs showed the haemolytic complement activity, bactericidal activity of serum, and phagocytic activity of blood before ingestion of colostrum. At this time, no serum gammaglobulins and anti-O agglutinins specific for the investigated *E. coli* serotypes, were found. The complement activity in serum of newborn lambs was 5-fold weaker than that observed in adult animals, and was on an average 2CH_{50} units/ml. During the first days of life, a progressive increase of titre was observed, and this titre was 8CH_{50} units/ml at one month of age. These findings seem to indicate that the complement could be formed during foetal life (3, 8, 9).

It was demonstrated that serum of pre-colostral lambs showed bactericidal activity against smooth *E. coli* serotype O8:K87,K88 and its bactericidal titre was 0.352 ml. After colostrum feeding, the bactericidal activity increased rapidly and was 7-fold higher as early as on the 3rd day of life. A slight decline of the activity was observed at the 4th month of life and then it reached the values of adult animals at the age of 6 months. The phagocytic activity to all of the investigated *E. coli* serotypes was found also in the blood of newborn lambs.

Gammaglobulins were detected in lamb serum 12 hours after colostrum feeding and their concentration was about 34%. Afterwards, they showed a rapid decrease, and their level was only 19% at 2 weeks of age. The lowest level of gammaglobulins (14%) was found during weaning, i.e. at 3 months of age. Presumably, the decrease of gammaglobulins observed at this time resulted from stress caused by weaning and replacement milk and vegetable feed with vegetable one (7). After this period, the concentration of gammaglobulins increased and reached the values characteristic for adult animals (about 25%) at 5 months of life.

The antibodies against O antigen of *E. coli* serotypes were demonstrated in lamb serum 12 hours after colostrum feeding, and their titre

was by one dilution lower than that found in maternal serum. Then, the titre declined rapidly and was undetectable at 4 weeks of age. The antibodies were detected again at the 3rd month of life.

The influence of infection with rabies virus

The influence of infection with rabies virus on haemolytic complement activity and bactericidal activity of serum against *E. coli* serotypes O8:K87,K88 and O24:K? was the last problem undertaken in the present study. The investigation may help to explain the pathogenesis of some diseases and role of immune indices in the defence system of organism. Similar studies were performed on pigs infected with hog cholera virus (1, 2). Borkowska-Opacka (1) suggests that the decrease of bactericidal activity of pig serum observed on the 6th day after infection with hog cholera virus might be the cause of frequent bacterial complications occurring in this disease.

In the present investigation, statistically significant increase of haemolytic complement activity and bactericidal activity of serum on days 2 and 4 after infection with rabies virus, and then a significant decrease during agony, were demonstrated. Similar increase of the examined immune indices was found in the control animals injected intracerebrally with a sterile solution, however, this increase remained unchanged for a longer time.

Conclusions

1. Pregnancy causes a decrease of the activity of all the investigated immune indices in ewes. The highest decrease is observed during parturition.
2. The investigation shows the influence of season on the bactericidal activity of sheep serum.
3. Pre-colostral newborn lambs show haemolytic complement activity, bactericidal activity against smooth *E. coli* strain, and phagocytic activity of blood to all the investigated strains.
4. No gammaglobulins and antibodies can be detected by used methods in the serum of pre-colostral lambs. Large amounts of gammaglobulins and antibodies can be demonstrated 12 hours after the ingestion of colostrum.
5. There is a transitory fall of gammaglobulins, complement activity, and antibodies in weaned lambs at 3 months of life.
6. The increase of the activity of some immune parameters in sheep infected with rabies virus is not due to a specific action of the virus alone.

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