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ON POPULATION IMMUNOLOGY OF ESTONIAN RED BREED CATTLE

Population immunology is a new branch of science; it stimulates the development of biology, medicine, veterinary medicine, and phytopathology. Population immunology investigates the immunity of populations (herds) both in space and in time (see Pavel, 1987; Pavel, Fedotovski, 1987). One of the main tasks of veterinary medicine is to raise the viability of cattle herds (longevity, performance ability and resistance). However, a satisfactory solution of these problems involves veterinary laboratories.

The immune system of a vertebrate animal has not only an antimicrobial function, but it also takes part in the development of the organism; the reproductive potentials, and a good state of this system is also an important premise in establishing a high milk production (see Бабаева, Зотиков, 1987; Pavel, 1977).

Presently, one of the important tasks facing veterinary immunology is elaborating simple express methods for determining the state of cow's nonspecific as well as specific defense reactions. If it succeeds (and in chicken the decision has been almost reached to this effect) the next task will be an immunological characterization of the population of cattle herds in a region, i. e. determining the frequencies of immunological phenoclasses in various populations of cattle. The preliminary results are promising (see Павличенко et al., 1985; Меркурьева, Ксейби, 1987), though the authors have not determined the immunological phenoclasses, which could have made the results much more effective.

In parallel, it is necessary to establish the state of the virulence of the potential pathogenic microbes circulating in cattle-sheds (see Pavel, Peterson, 1989). So the population immunological method for the characterization of population immunity comprises the research on both components of the system — parasite-host. The population immunological monitoring allows to follow up immunological processes taking place in cattle-sheds.

The present paper is devoted to the population immunological monitoring of the cows of a cattle-shed in Estonia, i. e. to the determination of the frequencies of immunological phenoclasses in this particular cattle herd and to elucidate the connection between the phenoclasses and some zootechnical and veterinary traits.

Material and methods

The object of this study was the Estonian Red Breed cows of Lepiku cattle-shed of the Ülenurme Teaching-Experimental Farm of the Estonian Agricultural Academy. There were formed 4 comparison groups consisting of 30 cows of the same reproductive phase.

Two immunological traits (the antibacterial activity of blood serum to *Escherichia coli* O86 and to *Micrococcus lysodeikticus*, abbreviated accordingly Bae and Bam), and two clinical traits (the total blood serum protein, Prn, and the content of hemoglobin of the blood, Hb) were determined.

Antibacterial activity of the blood serum was determined by the method of O. V. Smirnova and T. S. Kuzmina (Смирнова, Кузьмина, 1966) modified by us. It was performed as follows. 3.5 ml of 1% peptone water (containing 0.5% NaCl), 1 ml (Bae) or 0.5 ml (Bam) blood serum was poured into two test-tubes (accordingly Bae and Bam) and into control test-tubes. Into the control ones was taken respectively 1 ml and 0.5 ml peptone water. To all test-tubes the bacterial culture (of the density of $1 \cdot 10^9$ bacterial cells in 1 ml) was added à 0.5 ml (respectively *E. coli* or *M. lysodeikticus*). After that the optical density (OD instantly) was determined using the photoelectrocolorimeter-nephelometer ФЭК-56М (green filter, the diameter of the cuvette 3 mm). The test-tubes were incubated in the thermostate for 3 hours at +37 °C. Thereafter the optical density (OD after 3 hours) and the antibacterial activity (in percentage) were calculated as follows:

$$100 - \frac{(\text{OD trial after 3 h} - \text{OD trial instantly})}{(\text{OD control after 3 h} - \text{OD control instantly})} \times 100.$$

The concentration of hemoglobin in blood was determined by the method of N. I. Romanenko and M. J. Ustselemov (Романенко, Усцелемов, 1988). We used an anticoagulant 0.5 ml of 2.9% Na-citrate solution to 5 ml of blood. 4 ml of 0.1 N HCl solution and 0.02 ml of blood was poured into the test-tube. The mixture was cautiously mixed. After that the concentration of hemoglobin was determined by electrophotocolorimeter (ФЭК-56М, violet filter, the diameter of the cuvette 5 mm). The resulting index was multiplied by 200, which gave us the blood hemoglobin concentration in g/l.

The total protein of blood serum was determined refractometrically (refractometer ИФР-454Б).

The immunophysiological phenoclasses (Bae Bam Hb Prn) and clinical phenoclasses (Hb Prn) were determined as follows. The cows were differentiated according to four immunophysiological traits into weak (−) and strong (+). By doing it we got the corresponding phenotypes (for example $Bae^- Bam^+ Hb^+ Prn^-$, $Bae^- Bam^- Hb^+ Prn^-$ etc.); the phenotypes were assembled into phenoclasses (see Pavel, Fedotovski, 1987). In the case of four traits (Bae Bam Hb Prn) one gets 5 phenoclasses (0 — all traits are weak — $Bae^- Bam^- Hb^- Prn^-$; 1 — one trait is strong — $Bae^+ Bam^- Hb^- Prn^-$ + $Bae^- Bam^+ Hb^- Prn^-$ + $Bae^- Bam^- Hb^+ Prn^-$ + $Bae^- Bam^- Hb^- Prn^+$; 2 — two traits are strong; 3 — three traits are strong; 4 — all four traits are strong). Such a simple way makes it possible to compare the animals studied in different seasons, and to compare different herds.

We also differentiated the cows according to two immunological (Bae Bam) and two clinical traits (Hb Prn). In both cases we got three phenoclasses: 0, 1 and 2.

As to zootechnical and veterinary characteristics, we determined milk production (during 305 days, in kg), the production of milk fat (kg), calf mortality (%), inseminations per animal served, and the stillborn (%) by lactations.

Results

In Tables only the data of four lactations are presented. It became evident that milk production, calf mortality and inseminations per cow served are not correlated with cow's antibacterial activity of blood serum or with such clinical indicators as the concentration of blood hemoglobin and the amount of total protein in blood serum.

Table 1

Phenoclass and the amount of milk fat, kg

Phenoclass	Bae Bam				Hb Prn			
	Lactation				Lactation			
	I	II	III	IV	I	II	III	IV
0	148.3* (22)	176.3* (15)	195.3 (7)	172.5 (4)	162.1* (29)	196.1** (17)	180.5 (6)	191.0 (5)
1	161.8 (54)	192.0 (38)	184.3 (21)	175.8 (12)	158.8 (42)	194.6 (32)	205.7 (16)	200.6 (9)
2	159.3* (34)	194.9* (30)	197.9 (17)	191.6 (13)	154.6* (37)	182.4** (32)	187.7 (21)	170.4 (14)

The number of cows is given in brackets. The comparison of the opposite phenoclasses is carried out (i. e. phenoclass 0 and 2).
* $P<0.05$.
** $P<0.01$.

Table 2

Stillborn calves, %

Phenoclass	Hb Prn			
	Lactation			
	I	II	III	IV
0	10.3* (29)	0 (29)	0 (18)	0 (6)
1	7.14 (42)	0 (42)	0 (27)	7.7 (13)
2	2.7* (37)	0 (36)	0 (28)	5.5 (18)

The number of cows is given in brackets. The comparison of the opposite phenoclasses is carried out (i. e. phenoclass 0 and 2).
* $P<0.05$.

Table 3

Phenoclassic structure of Lepiku herd

Phenoclass	Bae Bam Hb Prn [1]	Bae Bam [2]	Hb Prn [3]
0	7(5.6%)	22(20.0%)*	29(26.9%)*
1	29(23.2%)**	54(49.1%)	42(38.9%)
2	34(27.2%)	34(30.9%)*	37(34.2%)*
3	46(36.8%)**		
4	9(7.2%)		
Total: 5	125(100%)	110(100%)	108(100%)

[1] — The comparison of the opposite phenoclasses is carried out (i. e. phenoclass 1 and 3).
[2, 3] — The comparison of the opposite phenoclasses is carried out (i. e. phenoclass 0 and 2).
* $P<0.05$.
$P<0.01$.

But there were two tendencies (see Tables 1 and 2). Namely, the amount of milk fat was connected with the antibacterial activity of blood serum (positive correlation). So the higher was the cow's immunological phenoclass, the higher was the amount of produced milk fat during four lactations. But the produced milk fat was negatively correlated with the clinical indicators of the blood (Hb Prn), i. e. the lower was the cow's phenoclass, the higher was the milk fat production (Table 1).

In Table 2 the correlation between stillborn and clinical phenoclasses of cows is demonstrated. Contrary to our hopes, no correlation was observed between antibacterial activity in the mother's blood serum and the vitality of the embryo. But the stillborn frequency was correlated with the mother's clinical traits (Hb Prn) of the blood. So the higher was the clinical phenoclass of the mothers, the lower was the percentage of the stillborn, i. e. the higher was the vitality of the embryo.

Thus, the vitality as well as the calf resistance in postnatal period cannot be assigned to the cow's nonspecific resistance (as Bae Bam Hb Prn, as Bae Bam).

The immunological and physiological (clinical) structure of Estonian Red Breed cows in the cattle-shed studied (which is expressed by the frequencies of different phenoclasses), is presented in Table 3. It can be seen that in the case of four traits (Bae Bam Hb Prn) as well as two traits — Bae Bam and Hb Prn, the frequencies of strong animals as compared to the frequencies of weak animals were about 15% higher. The comparison with opposite phenoclasses (in the case of four traits; 0 and 4, and 1 and 3, or in the case of two traits: 0 and 2) the stronger phenoclasses are more frequently represented in the population. So, one can confirm that the natural selection and the artificial selection in this farm both prefer immunophysiologically strong cows (Bae Bam Hb Prn).

Discussion

For the purpose of eliminating seasonal fluctuations in the values of immunological traits (see Малинина, 1982; Зборовский et al., 1983; Белкина, Шаталов, 1985) and to get comparable data, we have proposed to measure the values of immunophysiological traits in the so-called comparison groups. Further, we recommend to use phenoclasses instead of phenotypes (Pavel, Fedotovski, 1987).

In this paper we have used two parallel ways — the four-trait system and the two-trait system. In the case of four traits there were notably 5 phenoclasses; in the case of two characters there were correspondingly 3 phenoclasses. This way enabled us to study the "specific weight" of the traits in determining herd immunity potentials. By using phenoclasses it is possible to compare cows in different seasons and in different herds.

If in the present work only the nonspecific resistance factors were used for characterizing the defense potential of cows, then in the next stage of research one should also measure the specific resistance factors (i. e. the immune response of the animals). One should also pay attention to the local defense factors (see Bourne, Newby, 1981). At the same time one should keep in mind that in different genotypes the activities of resistance factors (both nonspecific as well as specific) are different. One can even say that in different genotypes the different genes are "at work".

In addition to the above-mentioned we dare say that it might be reasonable to increase the amount of a comparison group from 30 animals to 60.

Among the defense mechanisms of animal organism the T-lymphocytes play an important role. Indirectly it has been confirmed by A. V. Gerassimtshuk (Герасимчук, 1986) whose findings assure that the so-called "general" immunological reactivity which is characterized by the skin reaction to foreign antiserum, is more effective resistance indicator as compared to the bactericidal activity of blood serum. The same is valid concerning the capability of antibody synthesis.

One should also pay attention to the circumstance that in some lines of chicken artificial selection is directed against natural selection (Pavel, 1989, unpublished data).

We think that in the nearest future one should check up the idea of using the V. I. Joffe's (Иоффе, 1944) skin test to measure the delayed hypersensitivity in cows. With the same purpose one can use histamine (Goto et al., 1978; Белкина, Шаталов, 1984). The determination of the state of "general" reactivity with the purpose of getting information about the "general" resistance, which is so widely used by selectionists (see Crittenden, 1983; Gavora, Spencer, 1978), does not seem very reasonable.

One should take into consideration the fact that many bacterial strains are resistant to blood serum (Jessop, Lambert, 1986; Кузник et al., 1989). Also, it is worth remembering that the sensitivity of *E. coli* and *Salmonella* to antibodies is dependent on the presence of the complement (Schiller, Joiner, 1986; Vreede et al., 1986).

According to Z. P. Ljubimova and N. N. Smirnova (Любимова, Смирнова, 1985), immunologically weak bulls are characterized by low fertilization of their daughters and high percentage of stillborn.

Conclusions

As the immunophysiological traits used in our work do not characterize the defense capability of the cow to potential bacteria to a desired extent, in further research we shall have to measure the status of T-lymphocytes and the antibacterial activity of milk or saliva as well. The presented data enable us to conclude that the population immunology will add an appreciable contribution to veterinary medicine. Also, it is reasonable to ascertain the part of natural and artificial selection in different genotypes. The frequency of opposite phenoclasses (4 traits — Bae Bam Hb Prn) in the herd in the cattle-shed studied refers to the circumstance that in this population operates directional selection.

REFERENCES

Bourne, J., Newby, T. Mucosal immunity // In Practice, 1981, **3**, N 5, 5—11.

Crittenden, L. B. Recent advances in the genetics of disease resistance // Avian Pathology, 1983, **12**, N 1, 1—8.

Gavora, J. S., Spencer, J. L. Breeding for genetic resistance to disease; specific or general? // World's Poultry Sci. J., 1978, N 3, 137—148.

Goto, N., Kodama, H., Okada, K. Fudjimoto, Y. Suppression of phytohemagglutinin skin response in thymectomized chickens // Poultry Sci., 1978, **57**, N 1, 246—250.

Jessop, H. L., Lambert, P. S. The role of surface polysaccharide in determining the resistance of *Serratia marcescens* to serum killing // J. Gener. Microbiol., 1986, **132**, N 9, 2505—2414.

Pavel, Ü. Veterinaargeneetika. Tallinn, 1977.

Pavel, Ü. Põllumajandusloomade resistentsus ja selle selektsioon. Populatsiooniimmunoloogia alused. Tallinn, 1987.

Pavel, Ü., Fedotovski, A. Population immunology and veterinary medicine // Proc. Acad. Sci. ESSR. Biol., 1987, **36**, N 4, 327—330.

Pavel, Ü., Peterson, K. On veterinary population immunology // Proc. Acad. Sci. ESSR. Biol., 1989, **38**, N 4, 266—273.

Schiller, N. L., Joiner, K. A. Interaction of complement with serum — sensitive and serum-resistant strains of *Pseudomonas aeruginosa* // Infection and Immunity, 1986, 54, N 3, 689—694.
Vreede, R. W., Marcelis, J. H., Verhoef, J. Antibodies raised against rough mutants of Escherichia coli and Salmonella strains are opsonic only in the presence of complement // Infection and Immunity, 1986, 52, N 3, 892—896.
Бабаева А. Г., Зотиков Е. А. Иммунология процессов адаптивного роста, пролиферации и их нарушений. М., 1987.
Белкина Н. Н., Шаталов С. В. Оценка общей иммунологической реактивности крупного рогатого скота // Повышение молочн. и мясн. продукт. крупн. рогатого скота. Персиановка, 1984, 95—99.
Герасимчук А. В. Связь признаков естественной резистентности с молочной продуктивностью, долголетием и воспроизводительными качествами коров // Повышение генетического потенциала молочного скота. М., 1986, 183—188.
Зборовский Л. В., Лебенгарц Я. З., Нестеров В. М., Семенюта А. Т., Колесников М. К. Иммунологическая реактивность телок различного генотипа в онтогенезе // Вестн. с.-н. науки, 1983, № 2, 98—101.
Иоффе В. И. Об очередной задаче в иммунологическом анализе инфекционных болезней // Клиническая медицина, 1944, № 9, 3—10.
Кузник Б. И., Васильев Н. В., Цыбиков Н. Н. Иммуногенез, гемостаз и неспецифическая резистентность организма. М., 1989.
Любимова З. П., Смирнова Н. Н. Иммунологическая реактивность крупного рогатого скота в связи с генетической принадлежностью животных. Генетические методы оценки сельскохозяйственных животных. М., 1985, 76—83.
Малинина Г. М. Влияние возраста, сезона года и генотипа на активность лизоцима, бетализинов и комплемента сыворотки крови норок // Автореф. дис. канд. биол. наук. Л., Ленингр. ветерин. ин-т, 1982.
Меркурьева Е. К., Бассам Ксейби. Влияние коров-матерей на уровень факторов естественной резистентности дочерей в ранний постэмбриональный период // Доклады ВАСХНИЛ, 1987, № 9, 31—33.
Павличенко В. П., Косякова Г. П., Быковченко Ю. Г., Милованов О. В. Уровень резистентности и жизнестойкости скота при разных методах разведения // С.-х. биология, 1985, № 5, 80—84.
Романенко Н. И., Усцелемов М. Е. Нормативы биохимических показателей крови холмогорских коров. Методические указания. М., 1988.
Смирнова О. В., Кузьмина Т. А. Определение бактерицидной активности крови методом фотонефелометрии // Ж. микробиол., эпидемиол. и иммунобиологии, 1966, № 4, 8—11.

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EESTI PUNAST TÕUGU KARJA POPULATSIOONIIMMUNOLOOGIAST

On toodud andmeid Lepiku lauda eesti punast tõugu karja populatsiooniimmunoloogilise inventeerimise tulemustest ning näidatud, et karjas toimib suunav valik.

Хели НООРСАЛУ, Юло ПАВЕЛ, Карл ПЕТЕРСОН, Юхан СИМОВАРТ

О ПОПУЛЯЦИОННОЙ ИММУНОЛОГИИ СТАДА ЭСТОНСКОЙ КРАСНОЙ ПОРОДЫ

Приводятся данные о популяционно-иммунологической инвентаризации коров эстонской красной породы Лепикуской фермы. Показано, что в стаде действует направленный отбор.