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ON VETERINARY POPULATION IMMUNOLOGY

Veterinary medicine has to solve two important problems: the prophylaxis and elimination of still occurring infectious diseases and the prophylactic medical examination of the herds of animals (especially of cattle). According to V. M. Shishkov and co-workers (Шишков et al., 1986) the prophylactic examination should take into account the main traits of general nonspecific resistance and also the immunological status of animals. They emphasize that the majority of diseases of animals are caused by the weakening of nonspecific resistance and immunological status of animals. We are of the opinion that it is necessary to determine the viability and endurance of cattle as well (Pavel, 1987). This can be realized by the population immunological method. This method enables us to study the nonspecific (chemical) barrier of herd defense as well as the specific (immunological) one. In medicine the main attention has been called to the specific defense barrier of organism (the establishment of the immunological status; see Тотоляш et al., 1987) since the 1980s. In veterinary medicine, on the contrary, the main object is the determination of the nonspecific defense barriers.

Population immunology (PI) is a new branch of population biology. It determines changes in the phenotypic and phenoclassic structure of microbial, plant and animal populations in space and time. Therefore, the main object of PI is the variability of the pathogens' virulence (also that of the potentially pathogenic microbes!). PI can elucidate the mechanisms of physiological and genetic adaptations in plants and animals, without using the time-consuming genetic analysis methods. In PI the immunological, immunochemical, biochemical and biophysical methods are mainly used. The integral research method may be called "the population immunologic method". This method enables us to study the expression of traits, and also the possibility of assessing the endurance (and viability) of phenotypes.

Veterinary population immunology (VPI) connects the population biology with physico-chemical biology. Population genetics of pathogens, immunogenetics, epizootology and population phenetics (called also ecophenetics; see Ростовцев, 1986) are closely connected with VPI. Immunogenetics deals with the system host—microorganism while VPI as well as PI deals with the interaction of the p o p u l a t i o n s of the pathogens and host (plant and animal). The relations of these populations are investigated on phenotypical level. The natural selection acts on phenotypes, not on genotypes. VPI analyzes the interactions of corresponding traits of macro- and microorganisms on the population level, while epizootology limits itself mostly to the registration of disease outbreaks, i. e. epizooties. Furthermore, VPI searches also the potentially pathogenic microbes. It must be added that VPI also determines the reactions and adaptation of animal organisms to the low molecular mass chemicals on the population level. VPI and phenetics differ mainly in the genetic specification of traits — VPI deals with polygenic traits, such as immunity and resistance, while phenetics deals with monogenic characters — and also in the study methods used (Яблоков, 1987).

Our population immunological method is quick and simple and it does not require genetic analysis (Павел et al., 1981). This method does not replace the genetic methods, it is a complementary factor (Павел et al., 1986; Pavel, Fedotovski, 1987; Павел et al., 1987).

In this paper we shall only mention the plant ecological systems. It is to be emphasized that the concept of gene-for-gene action has been developed namely for plant systems (Flor, 1956), which explains the co-evolution of the plant populations and pathogens on the basis of the interaction between the plant's resistance gene and pathogen's virulence gene (see Vanderplank, 1978). The phytopathologists have explained the ecological and genetic interaction mechanisms (bases) of the system macroorganism—microorganism.

Concerning the role of pathogens in the evolution of ecological systems, one has to mention that up to the present time the live-stock breeding has ignored the pathogenic factor. Therefore the application of mathematical methods does not give precise information, since the host resistance mechanisms alone do not provide a complete survey of the system explored! So, if the biological basis is incorrect, the mathematical apparatus does not give the results desired. Phytopathology and medical pathology differ from veterinary medicine, as the latter calls little attention to the population structure of pathogens. We think that the population biology of pathogens must be the main task of veterinary medicine shared also by Ya. Tsilinski and D. Lvov (Цилинский, Львов, 1977). In their prominent monograph the authors stress that the viral gene pool of a species is quite heterogeneous. The viral populations are divided into subpopulations, which change constantly. In the process of viral transmission a genetic drift takes place, which leads to noticeable changes in the genetic structure of the population. Virus is regarded as the evolutionary factor to macroorganism, while the host is, in its turn, the evolutionary factor to viruses. The selection of new mutants should be taken into account. The main factors of the changeability of viral populations are mutations, recombinations and intragression. Viral mutants, especially the defective ones, will persist by wild strains.

The fact of the formation of more virulent strains is of practical value. The smaller is the viral population, the more noticeable is the role of occasional factors, e. g. genetic drift.

The subject is thoroughly studied by W. Brown (Браун, 1968). In this book he concentrates on the population variability of bacteria *in vivo*. It is particularly important to refer to the results of the investigations devoted to the changeability of virulence. The main selection factor is the host's defense mechanism, which causes the changes in the virulence and the antigenic structure of pathogens. The changes concern the selection of new mutants and their fixation in the population, especially of those having an advantage in the fitness. These changes depend on the host's resistance factors and the biological nature of bacterial population as well. The pathogens of low degree of virulence may persist in the herds for a long time. W. Brown points out that the fitness of pathogenic bacteria depends both on its virulence and the host's resistance (the metabolic peculiarities of the host).

In Table 1 we present the results of our earlier investigation on the virulence factors of *Escherichia coli*. In case of diarrhoea one is able to isolate various strains of great variety of virulence (Pavel, 1958).

From the data presented in Table 1 one can conclude that the pathogenicity of *E. coli* does not always correlate with its toxicity. Also these two traits do not always correlate with the extensity of induced skin reaction of the rabbit. However, the different reactivity of two rabbits depends on the catalase activity of the blood of the animal.

Table 1

Pathogenicity of the strains of *Escherichia coli* (after Pavel, 1958)

Strain	Pathogenicity in mice	Toxicity in mice	Skin changes in two rabbits		Trypaflavin agglutination
1	3	4	4	2	—
2	5	3	1	1	—
3	5	0	4	2	+
4	2	0	5	2	±
5	2	3	4	2	—
6	5	5	4	2	—
7	5	5	4	2	+
8	4	3	4	2	—
9	5	3	0	1	—
10	5	2	0	2	—
11	5	5	1	0	—
12	5	4	1	1	—
13	4	0	0	0	—
14	5	2	1	0	—
15	5	5	3	3	+
16	5	0	1	0	—
17	4	5	1	1	—
18	5	3	1	4	—
19	4	3	4	1	—
20	5	2	4	1	—
21	2	1	0	0	±
22	0	0	0	0	—
23	0	0	0	0	—
24	5	0	1	0	—
25	0	0	0	0	+
26	2	0	1	0	—
27	2	0	0	0	±
28	2	0	1	0	—
29	0	0	0	0	—
30	2	0	0	0	—

Skin changes: 0 — absent, 1 — oedema, 2 — haemorrhagic imbibition, 3 — large haemorrhagic infiltrate, 4 — necrosis up to 4 mm in diameter, 5 — necrosis more than 4 mm in diameter. **Pathogenicity and toxicity**: 5 — both mice died within 48 hours, 4 — one mouse died within 48 hours, another in 6 days, 3 — both mice died in 6 days, 2 — one mouse died in 48 hours, another survived, 1 — one mouse died in 6 days, another survived, 0 — both mice survived.

Biologists have only recently begun to study the genetic structure of natural bacterial populations. It has been found that due to the restricted recombination, the natural populations consist in the individual clones (Selander, 1985). R. K. Selander concludes that the serotype of bacteria has only limited value in signalizing the degree of its virulence.

Table 2

Antigenic structure of *Salmonella* isolates

Group	Number of strains		O-antigen					H-antigen	
	abs.	%	1	4	5	9	12	Phase 1 *i*	Phase 2 1,2
I	38	48.7	+	—	—	+	+	—	—
II	33	42.3	—	—	—	+	+	—	—
III	3	3.9	+	—	—	+	—	—	—
IV	4	5.1	+	+	+	—	+	+	+

In Table 2 the data about the frequency of *Salmonella* serotypes isolated from the sick chickens and hens are presented. As can be seen there exist different *Salmonella gallinarum* strains in the hen flocks.

The changeability of virulence factors in *Staphylococcus aureus* are presented in Table 3. We can conclude that in cow's milk there exist many different strains of *S. aureus*. Also it can be seen in the observed phagotypes of *S. aureus* (Table 4).

Table 3

The variability of virulence factors in *Staphylococcus aureus*

Strain		Year							
		1985		1986		1987		Total	
		abs.	%	abs.	%	abs.	%	abs.	%
Type of haemolysin	A (αδ or α)	32	8.0	41	7.9	20	5.2	33	7.1
	B (δβ or αβ)	124	31.0	94	18.0	93	24.0	311	23.8
	C (β)	135	33.7	175	33.6	111	28.7	421	32.2
	D (δ)	67	16.8	150	28.8	115	29.7	332	25.4
	Nonhaemolytic	42	10.5	61	11.7	48	12.4	151	11.5
Coagulation of blood plasma	Rabbit pos.	400	100.0	521	100.0	387	100.0	1308	100.0
	neg.	0	0	0	0	0	0	0	0
	Human pos.	354	88.5	478	91.7	379	97.9	1211	92.6
	neg.	46	11.5	43	8.3	8	2.1	97	7.4
	Cattle pos.	50	12.5	55	10.6	60	15.5	165	12.6
	neg.	350	87.5	466	83.4	327	84.5	1143	87.4
Mannite fermentation	pos.	317	73.3	487	93.5	374	96.6	1178	90.1
	neg.	83	20.7	34	6.5	13	3.4	130	9.9

Table 4

The phagotypes of *Staphylococcus aureus*

Strain		Year					
		1985		1986		1987	
		abs.	%	abs.	%	abs.	%
I	29, 52A	4	1.5	2	0.8	—	—
II	3A, 116	72	27.6	96	39.5	84	42.2
III	6, 42E, 53, 75, 84	34	13.0	20	8.2	27	13.6
IV	42D, 102, 107, 117	148	56.7	120	49.4	86	43.2
V	78, 118, 119	3	1.2	5	2.1	2	1.0
Total		261	100.0	243	100.0	199	100.0

Besides we have found that in a cattle-shed there circulate many strains of staphylococci (Плакк et al., 1986). Yu. Yezepchuk (Езепчук, 1985) confirms that the pathogenicity of microorganisms is the function of biomolecules. An excellent method for the determination of the degree of bacteria's virulence has been described by I. L. Reed and H. Muench (1938) and the methodology of the assessment of microbial pathogenicity by H. Smith (1987). The host resistance factors are as follows (Pavel, 1971):

A. Immunologic reactivity (recognition of the antigen and the reaction produced).

1. **Nonspecific reactivity** (determines the nonspecific response of organism). It depends on the individuality (structure and function) of: (a) nonspecific factors of resistance (barriers, humoral factors, etc.); (b) systemic factors (endocrine and nervous systems); (c) absence of a certain metabolite.

2. **Specific reactivity** (determines the immune response). It depends on the individuality of lymphoid apparatus and of the presence of antibody-like substances.

B. Resistance, the function of immunologic reactivity; it does not depend on the strength (+ or −) of argument; it can be subdivided: (a) nonspecific resistance (depends on the nonspecific reactivity), (b) specific resistance — immunity (depends on both forms of immunological reactivity), (c) resistance based on hyporeactivity of the individual. The nonspecific (1) and specific (2) immunological processes may appear in the following combinations: (1) and (2) have the same direction, and (1) and (2) have different directions.

The changeability of resistance factors in 3 hen lines reflects the genetic diversity of these lines:

	A	B	C
$Bae^{-}Bas^{-}Lam^{-}Ins^{-}$	5.4%	8.3%	14.3%
$Bae^{-}Bas^{-}Lam^{-}Ins^{+}$	7.3%	8.3%	12.5%
$Bae^{+}Bas^{+}Lam^{-}Ins^{+}$	5.4%	10.4%	0%
$Bae^{\pm}Bas^{+}Lam^{-}Ins^{+}$	5.4%	6.2%	3.6%
$Bae^{-}Bas^{-}Lam^{\pm}Ins^{-}$	3.6%	2.1%	5.4%
$Bae^{\pm}Bas^{-}Lam^{-}Ins^{-}$	0%	4.2%	7.1%
$Bae^{-}Bas^{\pm}Lam^{-}Ins^{+}$	1.8%	6.2%	1.8%
$Bae^{-}Bas^{-}Lam^{-}Ins^{\pm}$	3.6%	0%	3.5%

These more frequent phenotypes (Bae and Bas — bactericidal activity of blood serum to *E. coli* and *S. aureus*, Lam — lysozyme activity of blood serum, and Ins — the interferon titer of the blood serum; + strong; ± mediocre and — weak) are differently presented in these hen lines (Вальдман et al., 1984). The same situation has been established, when determining the phagocytic activity of pseudoeosinophilaes 'Pps) (Pavel, 1987):

	A	B	C
$Bae^{+}Bas^{+}Lam^{-}Pps^{-}$	15.6%	17.2%	3.1%
$Bae^{\pm}Bas^{+}Lam^{-}Pps^{-}$	7.8%	6.2%	0
$Bae^{-}Bas^{-}Lam^{-}Pps^{-}$	6.2%	4.7%	1.6%
$Bae^{+}Bas^{+}Lam^{-}Pps^{+}$	6.2%	0%	3.1%
$Bae^{\pm}Bas^{-}Lam^{-}Pps^{-}$	0%	6.2%	3.1%
$Bae^{-}Bas^{-}Lam^{-}Pps^{\pm}$	1.6%	0%	7.8%
$Bae^{+}Bas^{-}Lam^{-}Pps^{-}$	1.6%	0%	7.8%
$Bae^{+}Bas^{\pm}Lam^{+}Pps^{-}$	3.1%	1.6%	3.1%
$Bae^{\pm}Bas^{\pm}Lam^{\pm}Pps^{-}$	1.6%	6.2%	0%

Having in mind the needs of practical veterinary medicine we can say that:

1) the diseases complicate seriously the accomplishment of artificial selection of farm animals and pond fishes;

2) in the conditions of intensive animal breeding the veterinary science and service have to subject the elite herds of animals to the prophylatic veterinary examination (mainly using the population immunological method) and to prognosticate the health and productivity of cows in breeding herds;

3) in the process of biotechnological evolution of population the degree of homozygosity of herds will considerably enhance, and therefore the danger arises that one mutant microorganism will destroy the results of animal breeders;

4) it is necessary to increase the resistance of animal populations while the vaccination method does not solve the problem;

5) it is necessary to carry out the comparative investigation of the resistance and virulence genes in different populations of animals and microorganisms.

We are accustomed to the opinion that heterozygosity is always beneficial to its carrier. This circumstance is not universal, e. g. the mother's heterozygosity in mucin-globulins is more noxious to the successor when compared with homozygotes (Pavel, Peterson, 1969).

As we presumed (Павел, 1976) there exists no universal resistance mechanism in animals. We have succeeded in demonstrating that various groups of related animals react differently, i. e. the different defense factors "work" at varying intensity (Павел et al., 1985). Out of the three investigated lines of hens only one, the line B, has the correlation between the immunological phenoclass and the egg productivity per basic hen (Pavel, Fedotovski, 1987; Павел et al., 1987). On the contrary — the line A is observable without any correlation, whereas in the line C, the reaction norm of individuals was different during two years. Population immunological method enables to prognosticate to a certain degree the viability and endurance of animals basing on the immunological phenoclass. The data suggest that the differential ability of the immunological phenoclasses (based on the bactericidal activity of blood serum to *Escherichia coli* — Bae, the lysozyme activity of blood serum to *Micrococcus lysodeikticus* — Lam, the haemoglobin content of blood — Hb and the content of whole protein of blood serum — Prn) are in the three lines essentially different (Table 5). So in the line B the differential values of opposite immunological phenoclasses (i. e. 4 and 0) fluctuated between 30—50 eggs per

Table 5

Correlation between the phenoclasses of nonspecific resistance and egg productivity (per basic hen)

Phenoclass (Bae, Lam, Hb, Prn)	Year	Line		
		A	B	C
4	1982	0	252.25 (3)	0
3		0	223.26 (23)	205.36 (13)
2		214.72 (13)*	224.63 (55)	200.31 (43)
1		217.11 (56)	215.43 (31)	198.26 (54)
0		215.88 (52)	200.10 (10)	210.96 (15)
4	1983	166.50 (2)	255.66 (3)	0
3		240.11 (9)	222.95 (23)	212.70 (17)
2		220.16 (37)	228.94 (54)	197.66 (48)
1		211.16 (59)	214.32 (31)	198.81 (48)
0		220.00 (14)	200.10 (10)	211.50 (12)
4	1984	143.50 (4)	230.57 (7)	251.00 (2)
3		186.04 (22)	210.69 (13)	230.07 (27)
2		203.63 (41)	203.90 (32)	226.75 (40)
1		203.97 (45)	199.41 (21)	223.48 (41)
0		205.41 (12)	199.60 (5)	207.36 (11)

* The number of hens is given in brackets.

basic hen, while in the line C there was a remarkable correlation only in 1984. In 1982 (Павел et al., 1986, 1983; Pavel, Fedotovski, 1987) there was a slight difference (only reaching 5 eggs) in case of eliminating the extreme phenoclasses (4 and 0). Concerning the line A the difference was observed only in 1983, when the extreme phenoclasses were not included. So the genetically different animal groups react differently and their reaction norm is different. This may be explained only by the fact that in different genotypes different genes "work". The elimination of extreme phenoclasses of immunological characters from the selection is recommended by L. B. Crittenden (1983) and G. Biozzi (1979; cit. van der Zijpp, 1983). By using the immunological phenoclasses it is possible to prognosticate the endurance in young animals and the viability of hens (expressed in terms of egg production per basic hen) (Table 5).

From the preceding data it is obvious that the main task of veterinary population immunology is to determine defense factors, the activity of which reflects the viability of animals in the corresponding groups of related animals. So the selection for viability and endurance in cattle herds and in hen flocks proceeds in three stages. The first stage consists in establishing the main immunological traits of nonspecific resistance which indicate the viability of the animal. The second stage lies in the selection of the corresponding animals and the third in carrying out "the veterinary selection", i. e. in speeding up the selection process by prognosticating the viability and endurance in young animals.

In conclusion we can say that the determination of the activity of nonspecific defense factors gives sufficient information on the immunological status of animals. Namely in the last case it would be better to prove not the amount of B- or T- lymphocytes but the amount of lymphocytes activated with polyclonal inductors. Secondly we infer that it is more appropriate to use the term poly- or multiresistance instead of the general resistance.

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VETERINAARSEST POPULATSIOONIIMMUNOLOOGIAST

Artiklis on arutatud veterinaarse populatsiooniimmunoloogia probleeme ja toonitatud immunoloogiliste tunnuste esitamisel fenoklasside kasutamist. Andmed näitavad, et kaks kana liini uuritud kolmest on oma eluvõime poolest prognoositavad juba noores eas.

Юло ПАВЕЛ, Карл ПЕТЕРСОН

О ВЕТЕРИНАРНОЙ ПОПУЛЯЦИОННОЙ ИММУНОЛОГИИ

В целях характеристики иммунологических признаков целесообразно использовать феноклассы. Полученные данные показывают, что из трех изученных линий кур две являются прогнозируемыми в отношении жизнеспособности уже в молодом возрасте.