Immunological reactivity of calves with associated respiratory infections

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***Abstract*— Associated respiratory infections are a special type of infection. They have a combined effect on the organism. We conducted this study in order to determine impairment of immunological reactivity in calves aged from 5 days to 4 months for respiratory diseases caused by pathogen associations. Determination of etiological agents was carried out by a complex of diagnostic studies: clinical examination of calves, serological screening of paired blood sera from diseased calves; ELISA, PCR, microbiological, scatological and hematological studies. Among the examined animals, 19% had no clinical signs of the disease and there were no positive laboratory tests for the presence of pathogens. The etiological role in the development of respiratory diseases in calves belongs to the causative agent PI-3. Associating pathogens in the virus group are: rotavirus B bovine and Bovine herpesvirus 1. In the virus-bacteria group: E. coli 99. In the virus-bacteria and invasive group: rotavirus B bovine, E. coli 99 and Eimeria bovis. An index analysis of hemogram indices gives an idea of the complex changes in the immune system of calves with mixed respiratory infections. Mono-infections cause a moderate compensatory response of the organism. Endogenous intoxication with the "virus-bacteria" and "virus-bacteria and invasive" infections is expressed due to the poly-antigenic load on the body.**

***Keywords*— immunological reactivity, acute respiratory viral infections, bovine virus 1, bovine rotavirus B, E. coli 99, Eimeria bovis, calves, associated infections**

I. Introduction

Numerous studies by Russian and foreign authors have shown that the high mortality rate of respiratory viral infections in young cattle is due to the addition of cofactor infections [3, 5, 8, 10, 11, 15]. Combined antigenic load on the calf organism leads to the development of a complex of pathological changes in the immune system, which in turn initiates structural and functional damage to organs and systems [2, 4]. In the initial phase of the infectious process, the accumulation of toxic products occurs in the tissues of the primary source of infection. Neutrophilic leukocytes, macrophages, platelets, reticulocytes and others are specific "target cells" for the endotoxins. Under the action of endotoxins, the structural and functional state of the cellular and subcellular membranes is modified, which causes a second wave of intoxication. In the bloodstream and in the tissues of the primary source of infection, there is an intense accumulation of toxins in concentrations exceeding the functionality of the body's natural detoxification systems [2, 6, 14, 16]. The process of endogenous intoxication development becomes more alike a cascade. One of the first clinical symptoms are signs of endogenous intoxication of the body: anemia of the mucous membranes, dehydration, tachycardia, rapid breathing, hyperthermia. The severity of endogenous intoxication may serve as an indirect criterion for evaluating the condition of the diseased animal [13, 14].

Scales and systems, based on the assessment of hematological blood parameters, are used for early and fairly accurate diagnosis of endogenous intoxication in clinical medicine [14]. A number of indices were proposed, in which leukocyte indicators were used, taking into account other hematological parameters, which makes it possible to assess the state of various immunity units both in the prenosological period and in the early stages of the disease, without using special immunological methods. In veterinary laboratory practice, the determination of immunological markers is an expensive and long-lasting procedure requiring special equipment. Rapid assessment of the state of immunity in calves with diseases of the respiratory tract can serve as the basis for the use of effective and rational treatment regimens, allowing to save livestock and minimize economic losses [1, 7, 9, 12].

Our objective is to determine the impaired immunological reactivity in calves with respiratory tract diseases caused by associated pathogens.

II. Methods

The studies were performed in the "Ural Federal Agrarian Scientific Research Center, Ural Branch of the Russian Academy of Sciences".

The object of study is young cattle aged from 5-10 days to 4 months ($n = 265$); the "Diseased" group contained animals with clinical symptoms of respiratory tract diseases ($n = 214$); the "Comparison" group – animals without clinical symptoms of diseases of the respiratory and gastrointestinal tracts ($n = 51$). Calves were kept in farms of the Sverdlovsk region.

To determine the etiological causative agent of the disease, these complex diagnostic studies were conducted:

1) serological screening of paired sera from diseased calves;

2) detection of antigens by ELISA in blood and feces samples.

The studies were performed using commercial test systems: "Erythrocyte diagnostic kit for serodiagnosis of infectious bovine rhinotracheitis in indirect hemagglutination test"; "Erythrocyte diagnostic set for serodiagnosis of bovine viral diarrhea in indirect hemagglutination test"; "Kit for the diagnosis of bovine parainfluenza-3 in hemagglutination inhibition test" produced by Agrovet, Russia; "IDEXX Rota-Corona-K99", "Bovine Viral Diarrhoea Virus (BVDV) Antigen Test Kit / Serum Plus"; "IDEXX Neospora Ab (Neospora caninum)"; "IDEXX Chlamydia Total Ab"; "IDEXX IBR gE Ab Test"; "IDEXX IBR gB X3 Ab Test" manufactured by IDEXX Laboratories, Inc., USA. Recording of ELISA results was carried out on a SUNRISE reader (Tecan, Austria). The results were interpreted using the original x Chek Assay Management System software (IDEXX Laboratories Inc., USA);

3) detection of the pathogen genome by PCR, using commercial test systems, according to the instructions;

4) microbiological and scatological studies of feces are performed according to generally accepted methods;

5) hematological studies were performed on an Abacus Junior Vet analyzer (Diatron, Austria). Blood leukocyte count was determined by the standard method on an MC 50 microscope (MICROS, Austria).

The following indicators were used as indices characterizing the severity of the condition and impaired immunoreactivity in diseased calves:

Nuclear index (NI) – the indicator of the severity of a diseased animal.

$$NI=\frac{(\text{Myelocytes}+\text{Metamyelocytes}+\text{Band neutrophils})}{\text{Segmented neutrophils}}$$

Blood leukocyte shift index (BLSI) – the indicator of impaired immunological reactivity and severity of the inflammatory process.

$$BLSI=\frac{(\text{Eosinophils}+\text{Basophils}+\text{Myelocytes}+\text{Metamyelocytes}+\text{Band neutrophils}+\text{Segmented neutrophils}}{\text{Monocytes}+\text{Lymphocytes}}$$

Leukocyte index (LI) – the indicator of the ratio of humoral and cellular immunity.

$$\text{Leukocyte index} = \frac{\text{leukocytes}}{\text{neutrophils}}$$

Neutrophil/lymphocyte ratio (NLR) – the indicator of the ratio of specific and nonspecific protection.

$$NLR = \frac{(\text{Myelocytes}+\text{Metamyelocytes}+\text{Band neutrophils}+\text{Segmented neutrophils})}{\text{Lymphocytes}}$$

III. Research Results

In 76.5% of the cases, associations of infectious pathogens were identified in the population of the examined young cattle with diseases of the respiratory tract: "virus-virus", "virus-bacteria", "virus-bacteria and invasive" (Tab. 1).

TABLE I. ASSOCIATIONS OF INFECTIOUS PATHOGENS IN YOUNG CATTLE (N = 265)

Associated pathogens group	The "Diseased" group (n=214)	The "Comparison" group (n=51)
Mono-infections		
"viral" (group 1)	27	No infectious agents detected
"bacterial" (group 2)	23	
Mixed infections		
"virus-virus" (group 3)	49	No infectious agents detected
«virus-bacteria» (group 4)	85	
«virus-bacteria and invasive» (group 4)	30	

As part of all associations of infectious pathogens, bovine parainfluenza virus type 3 (BPIV3) was determined, indicating its etiological role in the development of respiratory disease in calves. In the "virus-virus" associations rotavirus B bovine (RVB) was diagnosed in 14.9% of the cases; Bovine herpesvirus 1 (BoHV-1) – in 7.9% of the cases. In case of "virus-bacteria" infections, E. coli 99 was detected in 39.4% of cases. Combined "virus-bacterial and invasive" infections were diagnosed in 14% of the examined calves with diseases of the respiratory tract. The antigenic landscape was introduced by BPIV3, rotavirus B bovine, E. coli 99 and Eimeria bovis.

Clinical manifestations of the respiratory tract disease were absent in calves of the "Comparison" group. A comprehensive diagnostic study confirmed the absence of pathogens of the respiratory viral infections in these animals: rotavirus B bovine, E. coli 99 and Eimeria bovis.

In 12.6% of the cases, the etiological agent of the disease was bovine parainfluenza virus type 3. In 10.9% of the cases, calves were diagnosed with bacterial mono-infection (Tab. 2).

TABLE II. ANTIGENIC LANDSCAPE OF ASSOCIATIONS OF PATHOGENS OF INFECTIOUS DISEASES (N = 265)

Etiological agents	Detected cases (n=214)
Bacteria	23
BPIV3	27
BPIV3 + BoHV-1	17
BPIV3+ RVB	32
BPIV3+ *E. coli 99*	67
BPIV3+ *E. coli 99* + yeast fungi Candida	18
BPIV3+ RVB + *E. coli 99* + *Eimeria bovis*	30

Condition of diseased animals during the survey was defined as moderately severe (Nuclear index 0.62-0.94) in 77.6% of the cases; severe (Nuclear index 1.21-1.6) – in 22.4%. In the calves of the "Comparison" group, the Nuclear index was 0.28 ± 0.09. It should be noted that "crucial condition" was recorded only in calves with "virus-bacterial" and "virus-bacterial and invasive" infections (Tab. 3).

TABLE III. THE RESULTS OF THE INDEX ANALYSIS OF HEMOGRAM INDICATORS OF CALVES IN THE ACUTE PERIOD OF THE DISEASE

Animal group	**Index**	
	NI	***BLSI***
"Comparison" group	0.15±0.37	0.47±1.01
Group 1	0.69±0.07	1.94±0.13
Group 2	0.74±0.12	1.85±0.18
Group 3	0.72±0.1	2.07±0.21
Group 4	0.84±0.17	1.97±0.25
Group 5	0.82±0.12 in 60% 1.38±0.22 in 40%	2.21±0.19
Animal group	**Index**	
	LI	***NLR***
"Comparison" group	0.96±1.68	0.57±0.99
Group 1	0.62±0.07	1.32±0.13
Group 2	0.58±0.1	1.37±0.16
Group 3	0.65±0.09	1.27±0.11
Group 4	0.54±0.08	1.39±0.19
Group 5	0.57±0.1	1.42±0.23

The Blood leukocyte shift index in all examined calves was increased (from 1.85 ± 0.18 to 2.21 ± 0.19), which indicated an active inflammatory process. Its increase in diseased animals is associated with an increase in the number of stab and segmented neutrophils. In the calves of the "Comparison" group, the Blood leukocyte shift index was 0.67 ± 0.07. An analysis of the relationship between the humoral and cellular components of the immune system showed that the cellular components functionally prevailed in the diseased calves – the Leukocyte index was determined in the range from 0.54 ± 0.08 to 0.65 ± 0.09; Leukocyte index in calves of the "Comparison" group – 1.27 ± 0.11. The increase in the neutrophil/lymphocyte ratio by 1.6-2 times compared with the "Comparison" group (0.72 ± 0.1) also testified to the prevalence of non-specific protection in diseased animals.

Significant differences in the immunoreactivity indices in calves with respiratory tract diseases were noted on the 14th day of observation (Tab. 4).

TABLE IV. THE RESULTS OF THE INDEX ANALYSIS OF HEMOGRAM INDICATORS OF CALVES ON THE 14TH DAY OF OBSERVATION

Animal group	**Index**	
	NI	***BLSI***
"Comparison" group	0.15±0.37	0.47±1.01
Group 1	0.60±0.05 in 26%	1.64±0.14 in 26%
Group 2	0.41±0.06 in 74%	1.23±0.18 in 74%
Group 3	0.61±0.01	1.63±0.21
Group 4	0.62±0.11 in 24% 0.79±0.01 in 76%	1.28±0.19 in 24% 1.58±0.21 in 76%
Group 5	0.63±0.07 in 17 % 1.01±0.15 in 83%	1.43±0.18 in 17 % 1.97±0.14 in 83%
Animal group	**Index**	
	LI	***NLR***
"Comparison" group	0.96±1.68	0.57±0.99
Group 1	0.61±0.09 in 26%	1.11±0.19 in 26%
Group 2	0.98±0.1 in 74%	0.89±0.17 in 74%
Group 3	0.74±0.09	1.08±0.14
Group 4	0.61±0.07 in 24% 0.57±0.09 in 76%	1.14±0.17 in 24% 1.44±0.19 in 76%
Group 5	0.67±0.1 in 17 % 0.59±0.09 in 83%	1.03±0.19 in 17 % 1.38±0.17 in 83%

The condition of 37 calves with mono-infections was interpreted as "satisfactory", which was confirmed by a decrease in the Nuclear index to 0.41 ± 0.06; the Blood leukocyte shift index went down to 1.23 ± 0.18. The balance of the humoral and cellular components of the immune system (Leukocyte index 0.98 ± 0.1) and the ratio of specific and nonspecific defence of the body (neutrophil/lymphocyte ratio 0.89 ± 0.07) were stabilized. A similar situation was observed in 49 animals with mixed "virus-virus" infections; however, these changes were less pronounced: the Nuclear index – 0.61 ± 0.08; the Blood leukocyte shift index – 1.63 ± 0.21; the Leukocyte index – 0.74 ± 0.09; the neutrophil/lymphocyte ratio – 1.08 ± 0.14. The results testified to the positive dynamics during the course of the disease on the one hand, and also to the persisting tension in the nonspecific defence of the organism on the other hand.

In animals (n = 85) with a mixed "virus-bacteria" infection a positive trend in the immunoreactivity indices in ¼ of the calves was observed on the 14th day of observation. In most cases, integral indices showed inflammatory processes in the organism of diseased animals and disturbances in immunological reactivity: the Nuclear index – 0.79 ± 0.09; the Blood leukocyte shift index – 1.58 ± 0.21; the Leukocyte index – 0.57 ± 0.09; the neutrophil/lymphocyte ratio – 1.44 ± 0.19. In calves (n = 30) with mixed "virus-bacteria and invasive" infections, the number of individuals with positive dynamics in terms of immunoreactivity was 5. The remaining animals showed no significant changes in following indicators: the Nuclear index – 1.01 ± 0.15; the Blood leukocyte shift index – 1.97 ± 0.14; the Leukocyte index – 0.59 ± 0.09; the neutrophil/lymphocyte ratio – 1.38 ± 0.17.

IV. CONCLUSION

The studies have shown that the main etiological agent in the development of respiratory diseases in calves aged from 5 days to 4 months is bovine parainfluenza virus type 3. This pathogen was detected in 100% of the cases. Animals had mono-infections and mixed infections. Mono-infections were viral or bacterial. Mixed infections were caused by viruses, bacteria, fungi and protozoa in different ratios. The obtained data on the distribution of respiratory diseases in young cattle caused by associated pathogens are consistent with the data of the authors Basqueira, Natalia Sobreira, Martin, Camila Cecilia, dos Reis Costa, Julia Franca, Brealey JC, Sly PD, Young PR, Chappell KJ, Gaeta, Natalia C .; Ribeiro, Bruno L.M .; Aleman, Mario A.R., Johansson, Kalin M, Hedlund J., Wani S.A., Bhat M.A .; Qureshi S. In various regions, the main etiological agent is most often a virus from the respiratory viral infections group. These can be the Bovine viral diarrhea virus, Bovine herpesvirus 1, Rotavirus B bovine, Bovine parainfluenza virus type 3, etc. We identified associations with Bovine parainfluenza virus type 3, Rotavirus B bovine, Bovine herpesvirus 1. Besides the virus associations, the bacterial coinfections are being developed (Brealey JC, Sly PD, Young PR, Chappell KJ, Johansson, Kalin M, Hedlund J., Wani SA, Bhat MA; Qureshi S.,). E. coli 99 was detected in 30% of the examined animals.

Other participants in the associations are identified: yeast fungi Candida and Eimeria bovis. The spread and negative impact of Eimeria bovis on the growth and development of the organism is of widespread significance. The distribution of Eimeria bovis in the cattle population is confirmed by Kim, Hyeon-Cheol, Choe, Changyoung, Kim, SuHee et al.

The development of new diagnostic methods allows us to identify many pathogens of respiratory diseases. The clinical manifestation of associated infections is not evaluated enough. It is important for the clinical practice to timely assess the state of the immune system and to plan treatment regimens and prognosis of the disease. Clinically associated infections manifest signs only of a bacterial infection, which causes a wrong diagnosis. The use of evaluation indices makes it possible to timely assess the condition of the patient and develop treatment tactics. Index assessment of the organism’s potential has spread in medical practice (Çağlayan Serin, Pullukçu H., Ciçek C., Sipahi O.R., Taşbakan S., Atalay S., Volkova V.V., Bel'ski V.V., Khovrina M.P.). It is also important to conduct screening in this direction in veterinary practice, for effective therapeutic and preventive measures and diagnosis (Donnik, I.M., Shkuratova, I.A., Gaeta, Natalia C.; Ribeiro, Bruno L.M.; Aleman, Mario A.R.).

The hemogram indices used by us make it possible to reveal complex changes of the immune system in calves with the respiratory tract diseases. It was shown that the severity of endogenous intoxication and inflammatory reactions had a moderate intensity in the animals with mono-infections.

Changes in the immunoreactivity of calves corresponded to an adequate response to the introduction of an antigen. Under the conditions of specific and symptomatic treatment, positive dynamics – “recovery” with stabilization of the humoral and cellular components of the immune system and the ratio of specific and nonspecific body defences were recorded in 87% of the cases. In calves with mixed “virus-bacteria” and “virus-bacteria and invasive” infections, endogenous intoxication was most noticeable, which was due to the poly-antigenic load on the body. Intensity of non-specific protection persisted for a long time. Positive change dynamics of the immunoreactivity was recorded only in 21.7% of all calves.

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