The pathogenetic aspects of acute intestinal infections of suckling piglets

Alexey G. Shakhov¹, Larisa Yu. Sashnina¹, Julia Y. Vladimirova¹, Maria Y. Jeines¹, and Anatoly Y. Samuilenko²

¹ All-Russian Scientific Research Veterinary Institute of Pathology, Pharmacology and Therapy, 394087 Voronezh, Russia
² Federal Research and Technology Institute of Biological Industry, 141142 Moscow, Russia

Abstract. According to the studies piglets with intestinal infections compared to the clinically healthy animals, have lower the content of total protein, gamma globulins and general immunoglobulins, higher levels of malondialdehyde and endogenous intoxication, low molecular weight circulating immune complexes, lysozyme and complementary blood serum activity, phagocytic activity of neutrophils, their absorption and metabolic ability with a low functional reserve of phagocytes. The revealed changes in the biochemical, antioxidant, and immune status should be considered one of the pathogenetic mechanisms of the complex process of inflammation, as well as their diagnostic value in case of intestinal pathology caused by bacteria.

1 Introduction

The concentration and specialization of pig breeding, its transfer to an industrial basis, make possible to widely use the scientific discoveries and advanced experience as well as receive significantly more products at lower costs.

At the same time, due to the intensive industry management, qualitatively new keeping and operating methods, characterized by the constant presence of animals in enclosed spaces, there is a high concentration in limited production areas, the exposure of the body to numerous stress factors which adversely affect the physiological state of their natural resistance. This leads to the emergence of many diseases.

Against the background of the indicated predisposing factors, various viruses, bacteria, fungi, protozoa, etc., both individually and most often in various associations, take part in their etiology.

Intestinal infections caused by pathogenic bacteria are widespread in industrial pig farms. Colibacteriosis and diseases caused by the association of potentially pathogenic microorganisms occupy stay one of the most popular [1, 2].

The most important of the measures aimed at reducing the incidence of intestinal infections, are the improvement of specific prevention and rational therapy, as well as monitoring the recovery completeness of animals.

Despite the large number of works devoted to the pathogenesis and treatment of intestinal infections [3–6], an important area of scientific research is still known the decoding of the pathogenetic aspects of intoxication syndrome.

According to the researches the trigger mechanism for the development of intoxication syndrome with intestinal infections become endotoxins of gram-negative bacteria - lipopolysaccharides (LPS) [7].

Excessive formation of active oxygen in the body, lipid peroxidation products (lipid peroxidation), leading to suppression of non-specific immunological resistance factors occurs as a result of segmented neutrophils activation by lipopolysaccharides [8].

Endotoxemia, recorded in intestinal infections, also leads to an increase in the level of low molecular weight immune complexes, medium molecular peptides, affecting the quantitative and qualitative characteristics of immunocompetent cells, lowering the immune status [9].

Thus, the pathogenesis of intestinal infections is a complex and insufficiently resolved problem, considering that it is caused by several factors, the result of the interaction of which leads to the infectious process development. On the one hand, these factors, are associated with the pathogen (pathogenicity and antigenic properties), and on the other hand, macroorganism (its immune, metabolic and antioxidant status).

The purpose of the research is to study the state of natural resistance, free radical processes, and endogenous intoxication syndrome during the development of acute intestinal infections in piglets.

2 Material and methods

The studies were conducted in the industrial pig-breeding farm of LLC Vishnevskoye in the Verkhne-Khavsky District of the Voronezh Region on sick (n = 6) and clinically healthy (n = 6) piglets aged 7 days.
Blood tests from animals were carried out at the laboratories of the Federal Research Veterinary Institute of Pathology, Pharmacology and Therapy at the Russian Academy of Agricultural Sciences. The blood was determined by the content of malondialdehyde (MDA), the activity of catalase and glutathione peroxidase (GPO) (indicators of the enzymatic link of antioxidant protection (AOP), the level of nitric oxide metabolites (NOx), indicators of endogenous intoxication – average molecular peptides (AMP) and index endogenous intoxication (IEI) in accordance with the “Methodological Aids” [10] and using techniques [11–13].

Indices of natural resistance are bactericidal (BASK), lysozyme (LASK) and complementary (CASK) blood serum activity, cellular immunity indicators (phagocytic neutrophil activity (FNA), phagocytic index (PHI) and phagocytic the number (PHN), metabolic (functional) activity of neutrophils (NBT test spontaneous (SP) and stimulated (ST), reserve index (RI)) was determined in accordance with the “Methodological recommendations” [14].

The content of large (3 %), medium (3.5 %) and small (4 %) circulating immune complexes (CIC), total protein, proteinogram [15] pathogenicity coefficient of CIC (C4 / C3 ratio) [16] and total immunoglobulins (Ig) [17].

The etiology of intestinal infections was established based on the results of generally accepted bacteriological and molecular genetic (PCR) studies.

Statistical processing of the obtained data was carried out using the Statistica v.6.1 program, and reliability was assessed using Student’s criterion.

3 Results and discussion

Piglets with intestinal infections had diarrhea, fever at the onset of the disease, lethargy, depression, refusal to suck a sow, rapid exhaustion. Postmortem examination revealed catarrhal - hemorrhagic inflammation of the mucous membrane of the small intestine, covered with mucus, an increase and hyperemia of the mesenteric lymph nodes, a slight increase in the spleen, anemia of the liver and kidneys.

During bacteriological examination of pathological material (blood from the heart, liver, spleen, kidneys, mesenteric lymph nodes, small intestine) from enteric pathogens from 10 dead from gastrointestinal diseases piglets aged 4, 5, and 7 days out of 9 samples were isolated coli (serovarants O35 and O137), while sample generalized monoinfection was established in one, the rest had generalized infection caused by enteropathogenic Escherichia in association with Str. suis (in 7 samples) and Ent. faecium in 1 sample. From the pathological material of one piglet at the age of 5 days, Str. suis (generalized infection).

PCR studies in pathological material did not reveal the genomes of pathogens of transmissible gastroenteritis and epizootic diarrhea of pigs, rotavirus infection, campylobacteriosis and cryptosporidiosis.

Biochemical studies have found significant changes in protein metabolism in sick piglets. They had a lower total protein content of 12.6 %, which is associated with a decrease in synthetic processes in the liver due to the high antigenic load and the intensive consumption of gamma globulins, providing humoral protection of the body against infection.

They also recorded a slight excess (by 3.7 %) of the amount of albumin, the most important factor in plasma detoxification, binding and removal of toxins, 16.9 % alpha globulins, transporting lipids, forming lipoprotein complexes with them, and those participating in the functioning of the blood coagulation system and complement system, as well as β-globulins by 7.2 %, which are mainly based on low-density lipoproteins, complement components (participating in immunity reactions) and part of immunoglobulins, due to the development of acute inflammation casting process (Table 1).

Table 1. Biochemical parameters of piglets

| Indicators             | Clinically healthy | Sick          |
|-----------------------|-------------------|---------------|
| Protein g/l           | 74.1±1.21         | 64.8±2.99*    |
| Albumen, %            | 36.9±0.25         | 38.25±0.86    |
| Globulins: α, %       | 12.8±0.22         | 15.4±0.98*    |
| β, %                  | 23.6±0.34         | 25.0±0.91     |
| γ, %                  | 26.7±0.43         | 21.4±0.74*    |
| A / G ratio           | 0.58:1            | 0.62:1        |

Note: * P <0.05; ** P <0.0001 relative to clinically healthy piglets.

The presence in sick animals of a lower level of gamma globulins (19.9 % less than in healthy piglets), containing mainly anti-bodies, is apparently due to a decrease in the absorption capacity of intestinal epithelial cells and their increased consumption body protection against infection.

The albumin / globulin ratio (A / G ratio), which was higher (0.62:1) than clinically healthy animals (0.58:1), also indicates a change in the proteinogram in patients.

When studying the antioxidant status as a combination of pro- and antioxidant processes, its significant changes in sick piglets were established (Table 2).

Table 2. Antioxidant status of piglets

| Indicators         | Clinically healthy | Sick            |
|--------------------|--------------------|-----------------|
| MDA, µM / L        | 1.41±0.18          | 3.13±0.42**     |
| Catalase, µH2O2/I/ min | 55.7±2.47          | 69.4±2.19**     |
| GPO, µMGSH/I/ min  | 4.71±0.17          | 11.3±0.74**     |
| NOx, µM/l          | 36.7±2.19          | 81.1±11.45**    |
| AMP, cond.         | 0.73±0.01          | 0.81±0.03*      |
| IEI                | 25.8±0.68          | 33.0±1.82**     |

Note: * P <0.05; ** P <0.001; *** P <0.0001 relative to clinically healthy piglets.

In them, the development of diarrheal syndrome is accompanied by activation of the processes of lipid peroxidation and the accumulation of its toxic products in the blood. This is evidenced by an excess of 2.2 times the level of MDA in the blood.
It was established that the initiator of free radical lipid oxidation in acute intestinal infections is the active forms of oxygen generated by phagocytes. In this case, the main role in enhancing lipid peroxidation processes belongs to endotoxin, which, in addition to neutrophil activation, damages epithelial cells and causes neutrophil-independent oxidative stress [7].

The damaging effect of reactive oxygen species and lipid peroxidation products is opposed by the antioxidant defense system, which includes enzymatic and non-enzymatic units and is of exceptional importance in the body's implementation of protective and adaptive reactions [10].

In response to the intensification of free radical oxidation in sick animals, the activity of antioxidant protection enzymes was higher – 24.6 % of catalase, which neutralizes hydrogen peroxide, a significant amount of which is formed during the activation of phagocytosis in the infection process, and glutathione peroxidase in 2.4 times involved in the neutralization of both organic and inorganic hydroperoxides, the amount and value of which increases due to the activation of lipids, as well as the restoration of hydroperoxide of fatty acids, perki and protein and nucleic prois.

In patients with piglets, the level of stable metabolites of nitric oxide was significantly higher by 2.2 times, which takes part in the regulation of cellular and tissue metabolism in various pathological conditions in oxidative stress reactions and antioxidant defense mechanisms.

Since the nitric oxide system includes a mechanism to protect the body from excessive stress, limiting the intensification of free radical oxidation associated with its ability to increase antioxidant enzyme activity and expression coding genes an increase of nitric oxide production is recorded within moderate or short-term stress loads [8].

When exposed to exogenous or endogenous factors, NO* is involved in providing its protective responses.

Activation of lipid peroxidation processes, accompanied by increased formation of reactive oxygen species of sick piglets, enhances the oxidative modification of proteins, increases their accessibility to proteases and, as a result, increases the average molecular peptides and the index of en- of toxic intoxication by 11.0 and 27.9 %.

Moreover, medium-molecular peptides, accumulating in high concentrations, act as secondary endotoxins, causing a disorder of various processes, and their individual components enhance the processes of lipid peroxidation [13].

Endogenous intoxication is the leading pathogenetic syndrome of gastrointestinal diseases. Its universal marker shows the average molecular peptides represented by intermediate and final products of normal and impaired protein and lipid metabolism, the degree of its manifestation, the index of endogenous intoxication. It stimulates the generation of reactive oxygen species in the area of inflammation, and promotes the development of oxidative stress, leading to overproduction of free radicals and membrane destruction with impaired antioxidant defense function.

When studying the humoral link of nonspecific resistance in sick animals compared with clinically healthy piglets, the content of lysozyme produced by phagocytes and being an antibacterial enzyme was significantly higher (by 91.0 %). This is associated with an adaptive reaction directed to increase the stability of the body, and the need for inactivation of bacterial pathogens circulating in piglets.

The activity of complement was 27.3 % higher in patients with pigs (P <0.05). The activity of complement is an important part of inflammation and the development of the body's resistance to infectious agents. The complement system is important in enhancing phagocytosis, since direct or indirect (through anti-bodies) binding of complement components to bacteria is a necessary condition for phagocytosis (Table 3).

### Table 3. Indicators of natural resistance in piglets

| Indicators                                                                 | Clinically healthy | Sick |
|---------------------------------------------------------------------------|-------------------|------|
| Bactericidal (BASK),%                                                    | 78.8±2.02         | 76.1±3.26 |
| lysozyme (LASK), µg/ml                                                   | 1.56±0.14         | 2.98±0.45* |
| complementary (CASK), % heme                                              | 8.8±0.37          | 11.2±0.95* |
| Total Ig mg/ml                                                           | 16.7±0.16         | 12.4±0.009* |
| circulating immune complexes (CIC), 3.5 µg/ml                           | 0.54±0.03         | 1.06±0.13** |
| CIC, 3.0 µg/ml                                                           | 0.48±0.02         | 0.8±0.12* |
| CIC, 4.0 µg/ml                                                           | 0.29±0.03         | 1.02±0.12*** |
| CIC C4/C3                                                                | 0.59±0.047        | 1.2±0.31* |
| (phagocytic neutrophils activity) PHNA, %                                | 89.0±1.13         | 90.6±0.23 |
| PHI                                                                      | 6.8±0.19          | 7.3±0.09* |
| Phagocytic number (PHN)                                                  | 6.2±0.20          | 6.8±0.10** |
| SP, %                                                                    | 36.0±0.73         | 70.5±2.50** |
| ST, %                                                                    | 59.0±1.26         | 82.0±1.41** |
| RI                                                                       | 1.6±0.05          | 1.2±0.01*** |

Note: P <0.05; ** P <0.001; *** P <0.0001 relative to clinically healthy indicators

The integral indicator of the humoral link of nonspecific resistance – the bactericidal activity of blood serum is insignificant (by 3.4 %), and the number of total immunoglobulins is significantly (by 34.7 %) lower in sick animals compared with clinical healthy ones.

The decrease in the bactericidal activity of blood serum and total immunoglobulins is due to excessive accumulation in the body of toxic products resulting from the breakdown of hydroperoxides. They have a depressing effect on the molecular structure of humoral immune factors circulating in the body that have a protein nature.

When determining the level of circulating immune complexes, which are the product of the reaction of antigen, antibodies and complement and which play a large role in maintaining the homeostasis of the organism, a significant excess of large (3 %), medium (3.5 %) in sick animals was found and small (4 %) CICs by 66.7; 96.3 % and 3.5 times, respectively, compared with that of clinically healthy piglets.
At the same time, the pathogenicity coefficient (C4/C3 ratio) in patients was 2 times higher mainly due to the increase in low molecular weight complexes, which worse than complement large complexes activate complement, as a result of which they circulate for a long time blood, are deposited in organs and tissues, causing inflammation or suppression of the immune system [16].

A significant increase in the level of small and medium circulating immune complexes is observed in acute processes and correlates with the severity of the disease [16].

When studying the cell link of nonspecific resistance (Table 3), the phagocytic activity of neutrophils in patients with piglets, compared with healthy animals, was not significantly higher (1.9 %). The absorption activity was 7.4 %, the phagocytic index and phagocytic number by 9.7 %.

A slight increase in the phagocytic activity of neutrophils and a higher level of their absorption capacity in sick animals are apparently associated with the activation of phagocytes by complement and exo-endotoxins accumulating in their bodies, which play a major role in stimulating phagocytosis and activating the polynuclear neutrophil system.

There are significant changes occurred in sick piglets and in the metabolic (functional) activity of neutrophils. This is evidenced by the reduction reaction of nitro-blue tetrazolium, which is a very informative method for assessing the functional state of leukocytes in various pathologies of bacterial and toxic nature.

Thus, the spontaneous NBT test, which allows assessing the degree of antigenic irritation of inactivated blood granulocytes and characterizing the degree of activation of intracellular antibacterial systems, in animals with the development of diarrhea syndrome compared with clinically healthy pigs was 95.8 %, while the stimulated NBT test, considered as a criterion for the readiness of neutrophils for complete phagocytosis, is 39.0 %.

Nevertheless, the functional reserve of cells (PR), revealing the metabolic potential of phagocytes and characterized by their digestible ability, was lower by 33.3 %, reflecting the low functional reserve of oxygen-dependent mechanism biocide-ness of phagocytes and the depletion of the cellular component of nonspecific defense in patients with piglets [18].

4 Conclusion

The experiment on patients with intestinal infections, piglets showed changes in the biochemical status (low level of total protein, gammaglobulins), lipid peroxidation-AOP system (high content of malondialdehyde, accompanied by an increase in endogenous intoxication), humoral (high lysozyme and complementary activity blood serum content, the content of small and medium circulating immune complexes and a low level of total immunoglobulins) and cellular (a slight excess of the phagocytic activity of neutrophils, an absorber and metabolic ability, with a decrease in the functional reserve of phagocytes) links of non-specific resistance.

The revealed changes should be considered as one of the pathogenetic mechanisms of the complex process of inflammation, as well as their diagnostic value in case of intestinal infections in piglets.

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