Immunobiological resistance and humoral protection factors in piglets

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Materials and methods

The quantitative and qualitative composition of the intestinal microflora was determined comprehensively using bacteriological, serological and coprological methods. The following media were used as growth media: Endo agar, yolk-salt agar, Wilson-Blair agar, Sabouraud agar, blood agar, culture medium for bifidobacteria (produced by the NPO «Culture Media»), Kvasnikov medium, author's environment for growing lactobacilli, medium for isolation of bacteroides with kanamycin, and medium for isolation of enterococci with potassium tellurite. Cultivation of anaerobic and microaerophilic microorganisms was carried out under conditions of anaerobiosis using gas-generating packages «Anaerogas» for 24-48 hours at 37 °C. Identification of isolated microorganisms was carried out using commercial MIKROLATEST test systems from Pliva Laechema Diagnostica (Erba Mannheim) – ENTERO-test 24, NEFERM-test 24, STAPHY-test 16, STREPTO-test 16, ENCOCCUS-test, ANAERO-test 23. The number of T-and B-lymphocytes was determined in the laboratory using the immunofluorescence method. Immunoglobulins of classes G and M were studied using a set of reagents for the enzyme immunoassay determination of the concentration of total immunoglobulin in blood serum (total-ELISA-BEST).
Materials and methods

A general blood test was performed on a hematological analyzer Abacus–3. The functional abilities of neutrophil granulocytes were evaluated by their phagocytic activity, phagocytic number, percentage of digestion, and mobilization coefficient, which were determined in the reactions of bacterial phagocytosis, spontaneous and stimulated NBT-test. To determine the degree of accumulation of antitoxic antibodies, newborn piglets were used, whose mothers were formed into groups and immunized twice at a dose of 5 cm3 intramuscularly according to the following scheme: I experimental group – broth culture of thermostable toxin (ST); II experimental group – broth culture of thermolabile toxin (LT); III experimental group – broth culture of shigell-like toxins (STX); IV experimental group – LT, ST, and STX – escherichia coli toxins; V – control group (0,85% sodium chloride solution). Toxigenic properties were deprived by adding formalin. Blood sampling from piglets for the study of blood serum for the presence of antitoxic antibodies was carried out on the 1st, 3rd, 5th and 7th day. An indirect hemagglutination reaction was used as a serological reaction.
Results and discussion

Studies have shown that piglets on the first day of their life, there is a uniform colonization of the anterior and posterior parts of the intestine with bacteria of the genus escherichia, streptococci, enterococci, staphylococci, lactococci and lactobacilli within the range of Ig 4-8 CFU/g of chymus. Colonization of the intestine with clostridium and bifidum bacteria has its own pattern – the first colonize more of the thick, and the second small intestine

On the second or third day, with a stable level of lactobacilli and an increase of 2-3 orders of magnitude in the number of lactococci in the small intestine, clostridia disappears almost completely, the level of hemolytic streptococci decreases, but hemolytic forms of escherichia appear.

By the fifth day of life, in addition to hemolytic and non-hemolytic streptococci, staphylococci, there was a decrease in the content of escherichia (up to Ig 2-3 CFU/g) and clostridia. Clostridia was found in isolated cases. The number of symbionts decreased, but their number still remained an order of magnitude higher than the conditionally pathogenic bacteria, in the range (lg 4-6 CFU/g).

By the age of ten days, the level of opportunistic and pathogenic bacteria (escherichia, streptococci, staphylococci, clostridia) continues to decrease in the anterior part of the intestine. Lactic acid bacteria and bifidum bacteria occur from several tens to several hundreds, and the number of lactic acid streptococci, on the contrary, increases to a concentration of Ig 7,7 CFU in 1 g of intestinal contents.

By the 15th day of life, the intestinal microbiocenosis and the blood picture do not change significantly, except for an increase in both the small and large intestine by 2-3 orders of magnitude in the number of lactic acid bacteria and a decrease in the presence of escherichia in the large intestine by Ig 2-3 CFU/g of chime. In the blood, the number of T-, B-lymphocytes and the concentration of immunoglobulins IgM (up to 0,81 mg/ml), IgG (up to 4,17 mg/ml) naturally increases.
Results and discussion

By 20-25 days of age in piglets in the small intestine, the content of escherichia grows and is lg 3,2 CFU/g, and their hemolytic variants are lg 1,51 CFU/g. The content of other conditionally pathogenic microflora also increases by an order of magnitude (staphylococci, enterococci, hemolytic streptococci and clostridia). Symbionts – lactic acid bacteria and bifidum bacteria, on the contrary, not only do not increase, but also decrease and occur from several hundred to several thousand in 1 g of chyme. In the large intestine, bifidum and lactic acid bacteria mainly prevail, their concentration is in the range of lg 6,6-10,0 CFU/g, the number of escherichia ranges from lg 6,90 CFU/g to lg 7,32 CFU/g, and their hemolytic variants are in the range of lg 3,2-4,2 CFU/g. The content of staphylococci and hemolytic streptococci increases to lg 3-6 CFU/g.

When studying the titer of antibodies in newborn piglets, it was found that the largest number of them was in the group of animals whose mothers were immunized with complex toxoid (3,4±0,4 log₂). In other groups, the level of immunoglobulins in piglets on the first day after birth was 1,2-1,4 times lower. It should be noted that in the control group, the animals also showed traces of antibodies, which reached values on the first day of 1,4±0,6 log₂, and in the following days, although it decreased, it remained at the level of 0,2±0,4 log₂.

On the third day, the titer of immunoglobulins in animals decreased by an average of 1,2-1,3 times for each group, while the maximum number of them was in piglets from the IV experimental group (2,8±0,4 log₂). On the fifth day, the dynamics of the decrease in antibodies in the blood serum of piglets increased, the minimum titer of immunoglobulins was in I and III groups – 1,2 log₂, and in II group – 1,4±0,4 log₂, in group IV, the maximum value was registered in this time period – 1,6±0,4 log₂. On the 7th day, the values of antibody titers were almost aligned with the control ones.