

## The Role of Enterotoxins and Other Pathogenic Factors of Escherichia Coli in the Progression of Diarrhea in Calves

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### Abstract

*E. Coli* is a harmless inhabitant of the gastrointestinal tract of all mammals, including humans, as well as birds, reptiles and fish. However, under certain conditions, it may exhibit pronounced pathogenic properties that cause diarrhea and various extra-intestinal diseases.

According to the results of studies by a number of authors, diarrhea of newborn calves caused by *E. Coli* is the dominant unit in the nosology of diseases of these animals, is common in many developing countries, causing significant economic damage. The factors contributing to the disease of calves with diarrhea include their lack of colonization resistance, the duration of formation and the level of which directly depends on the health of the mother during childbirth and the sanitary condition of the premises where newborns get. In the case of domination in the intestinal tract in newborns conditionally micro flora of *enterobacteria*, *staphylococci*, *streptococci*, *enterococci*, clostridia and other bacteria over symbiotic microorganisms develops a dysbiotic state, which in its clinical and epizootological manifestation can be characterized as a mixed or associated intestinal infection. In this case, a special role is played by pathogenic diarrheal variants of coli, which differ in antigenic properties, a set of pathogenicity factors and the nature of clinical manifestations [13-15].

**The aim of the work** was to identify the main factors of pathogenicity and abilities of *E. Coli*.

**Keywords:** Escherichia Coli, Diarrhea, Enterobacteria, Pathogenicity.

### Materials and Methods of Research

The material of work was epizootic strains of *Escherichia Coli* in the amount of 95 isolates from the most frequently isolated from calves in the Krasnodar region for diarrheal diseases. Hemolytic activity (HA) of *Escherichia coli* was determined on Hottinger agar with the addition of 7% blood of sheep, without glucose. The adhesive activity of microorganisms was determined by the method proposed by Brilis [1] using native rabbit erythrocytes. The value of adhesive activity was estimated by the index of adhesion of microorganisms (IAM). Detection of General type fimbriae (type I, F1, MS pili) was carried out using a manose sensitive hem agglutination reaction with Guinea pig erythrocytes, for which equal volumes (one drop) of 0.85% NaCl solution, 5% erythrocyte suspension and 1 billion suspension of the tested enterobacteria cells were mixed on the slide. In parallel, the reaction with similar components was set, but instead of 0.85% NaCl solution, 1.5% d-mannose solution was used [2]. Anti-lysozyme activity (ALA) was detected in the solid nutrient medium with Bukharin [3]. The ability to produce exotoxins was detected in the mouse paw edema test using 48 h broth cultures, which were previously sterilized by centrifugation at 8000 rpm for

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60 min [3]. Biometric processing of the results was performed using Microsoft Excel 2010.

## Results and Discussion

As shown by our own research and reports of other authors [4-7], the share of *E. Coli* accounts for more than half of all cases of enterobacteria from the pathological material of calves in diarrhea.

As known, the pathogenesis of microbial diarrhea is characterized by complex relationships between the micro- and macroorganism, the implementation of which is carried out mainly due to the ability of the pathogen to adhesion, membrane-attack and cytolytic effect, as well as the ability to resist host protection factors [8,9].

Studies conducted by us with epizootic strains of *E. Coli* have shown the presence of the main factors of pathogenicity providing diarrheal properties in neonatal infants.

According to literature sources [2,8], adhesins promote binding to specific receptors of enterocytes for intestinal colonization and enterotoxins responsible for fluid secretion. According to the results of our studies (Table 1), 82.1% of the isolated *Escherichia coli* had the ability to attach to biological surfaces, but the activity of attachment on average did not exceed 4 bacterial cells on one erythrocyte (IAM was 3,980,31), which characterizes the *Escherichia coli* as a medium-adhesive [1].

It is known that the attachment of Enterobacteriaceae to host cells is provided by both fimbrial and non-fimbrial adhesion factors. Among fimbrial factors there are General MS fimbriae or pili and specific fimbriae or pili MR [1,10]. Despite the fact that the category of pathogenic *Escherichia* is accepted to include only those isolates that have specific saws, expressed in the form of fimbrial structures F4 (K88), F5 (K99), F6 (987P), F17 and F18, it is established that the variants possessing MS saws can also be pathogenic for animals [2]. The results of studies showed that MS saw possessed 32.6% of *Escherichia* isolates (Figure 1). This fact may indicate that in the process of attaching the studied enterobacteria to the eukaryotic cell, other adhesion factors, including those of non-fimbrial origin, may play a dominant role rather than MS pili.

Antilysozyme activity was in virtually all the tested strains of *Escherichia coli*, however most of them ALA was in the range of 3.38 µg/ml of inactivated lysozyme, which characterized them as intermediate level [3]. Despite the fact that lysozyme is active against gram-positive rather than gram-negative bacteria, however, the latter, apparently inactivating it, create a safe environment in the intestinal tract, organs and tissues for their frequent associates-enterococci and streptococci [6,11].

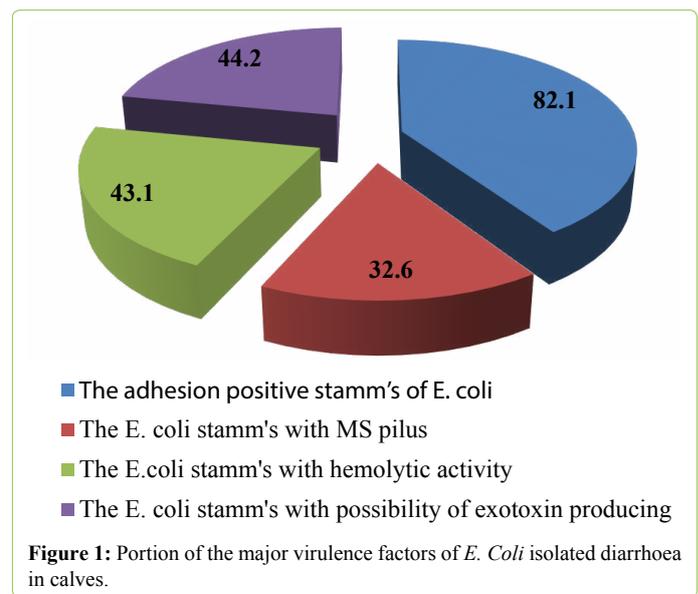
One of the pathogenetic elements of the development of enteric infections caused by enterobacteria is the production of hemolysin by them, rendering the membrane attack effect on blood cells. We found that *E. Coli* was mimicking hemolytic properties, at 43.1% of *Escherichia coli* strains was gemoliticski active.

If diareaha mechanisms of hemolysin enterobacteria are still not clear, varierende the action of exotoxins is not in doubt. Exotoxins Enterobacteriaceae by the nature of a specific action

on the cell structure of the intestine of the host is divided into cytotoxins and cytotoxins. Zerotonine do not change the cell structure, these include heat-stable (ST) and thermolabile (LT) toxins produced by esherihiami and other enterobacteria.

The main mechanism of action of these toxins is associated with the activation of intracellular adenylate and guanylate cyclase, which leads to the accumulation of cyclic adenyl monophosphate and guanyl monophosphate, which promote intensive secretion of epithelial cells of water and electrolytes. They colonize the villi of the small intestine without damaging them. Cytotoxins completely destroy enterocytes, this type of toxins includes cytotoxic necrotizing factor (CNF I, II) and shigatoxin (STX I, II) (Table 2) [8,11].

To indicate exotoxins in our studies, we used the classical method - the model of mouse paw edema, although initially it was focused on the detection of only ST [12]. However, according to research of Kotkova [13] in a comparative study of a number of biotests, it is shown that other exotoxins, in particular STX, can be detected in the test of edema of the mouse paw. In this connection, we considered this method as universal for revealing the ability to produce exotoxins without their identification [14,15].



**Table 1:** Pathogenic Factors of *E. Coli* responsible for adhesive properties of diarrhea in calves.

The pathogen	Indicators		
	The number of adhesion positive stamm's	The index of adhesion by microscopic organisms, bacteria/RBCs	The number of stamm's with MS pilus
<i>E. Coli</i> (n=95)	78	3,98±0,31	31

**Table 2:** Factors of pathogenicity of *Escherichia coli* responsible for antilysozyme, hemolytic activity and production of enterotoxins of diarrhea in calves.

The pathogen	Indicators		
	The anti-lysozyme activity mcg/ml	The number of stamm's with hemolytic activity	The number of stamm's with possibility of exotoxin producing
<i>E. Coli</i> (n=95)	3,38±0,06	41	42

The results showed that the amount of toxin producing strains among the tested *E. Coli* was large enough and was in the range of 44.2%.

Thus, the data obtained allow us to state the presence of the main factors of pathogenicity in *E. Coli* circulating on cattle farms in the Krasnodar region and causing diarrhea of newborn calves [16]. The results can be useful in deciphering the etiology and pathogenesis of diarrheal diseases in calves, as well as in the development of tools and methods for their prevention.

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