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CATTLE COLIBACILLOSIS IN PERM KRAI: PREVALENCE, SOURCES OF THE CAUSATIVE AGENT AND ITS BIOLOGICAL CHARACTERIZATION

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Abstract

Infectious diseases in agricultural enterprises of the Russian Federation are annually recorded in 50 % of the livestock, while the death of young calves during the first weeks of life ranges from 14 to 60 %. Colibacillosis remains the main infectious pathology in terms of morbidity and mortality, despite the widespread use of modern antibiotics and vaccines. To assess the prevalence of colibacillosis among cattle and identify the source of the infectious agent, for the first time an integrated approach was used covering a triad of sick animals—healthy animals—environment, which is of theoretical significance because it contributes to a better understanding the patterns of the epizootic process. An important practical aspect of the work was the analysis of data from long-term sanitary and zoo-hygienic records, which indicates that the control of the risk of *Escherichia coli* infections should be aimed not only at the natural reservoirs but also at the environment factors. The purpose of the study was to assess the prevalence of colibacillosis in cattle in various agricultural enterprises of the Perm Territory and to study the biological properties of the pathogen. To assess the *Escherichia coli* contamination of inventory, feeding and watering systems, data from sanitary and zoo-hygienic studies were analyzed. The incidence of the infections in cattle was summarized based on the reports from the Perm Veterinary Diagnostic Center, the livestock department of the Ministry of Agriculture of the Perm Krai for 2010–2020, statistical data from the Department of Veterinary Medicine of the Perm Krai and from laboratory records. The reports also covered bacteriological data for 22,480 samples from beef and dairy farms ($n = 146$). Sanitary and zoo-hygienic analyses of swabs from dairy equipment, inventory of slaughterhouses, feed of plant and animal origin, mixed feed were carried out for 29,207 samples from the same farms. The antigenic structure of the *E. coli* isolates was determined. The sensitivity of strains to antibacterial drugs was assayed by disk diffusion method (ampicillin 10 µg, cefoxitin 30 µg, ceftriaxone 30 µg, cefepime 30 µg, meropenem 10 µg, imipenem 10 µg, aztreonam 30 µg, amikacin 30 µg, gentamicin 10 µg, ciprofloxacin 5 µg, levofloxacin 5 µg, moxifloxacin 5 µg, tetracycline 30 µg, chloramphenicol 30 µg). In a prospective study (2020–2021), the prevalence of Shiga toxin-producing *E. coli* (STEC) strains in a population of healthy animals was assessed. Cultures ($n = 61$) were isolated from cattle feces. Genes encoding Shiga toxins 1 and 2 (*stx1* and *stx2*) were detected by polymerase chain reaction at the end point. The research results indicate that in recent years the prevalence of colibacillosis in the Perm Krai does not exceed 20 %. An increased number of dead animals and sick animals with diarrheal syndrome corresponded to a decreased immunization of pregnant cows. There were sporadic cases of the disease, but the risk of horizontal transmission of the pathogen, including through environmental objects, was high. *E. coli* was mostly isolated from swabs from dairy equipment and inventory of slaughterhouses, as well as from animal feed. Five most epizootically significant serogroups were identified, the *E. coli* O8, O15, O20, O101, and O115; in rare cases, *E. coli* O157 was isolated. The antibiotic resistance profiles of *E. coli* strains isolated from sick, dead and healthy animals did not differ significantly, except for tetracycline and chloramphenicol, resistance to which was significantly higher in *E. coli* strains of the first group. Of note is the higher proportion of cultures resistant to ampicillin and ciprofloxacin in the *E. coli* subpopulation from healthy cattle. In addition, the *stx1*

(2.0 %) and *stx2* (6.1 %) genes were found in *E. coli* from healthy cattle. That is, our data confirm that pathogens can persist in the gastrointestinal tract of both sick and healthy farm animals, which become the source of STEC. Additional sources of *E. coli* infection, including STEC strains, are drinking water, feed, and other abiotic components.

Keywords: cattle, colibacillosis, *Escherichia coli*, Shiga-like toxins, on-farm contamination

One of the priorities of the agro-industrial complex of the Russian Federation is to provide the population with environmentally friendly meat and dairy products [1, 2]. For this purpose, modern industrial technologies are actively used in livestock farms, which, however, have a number of disadvantages. Year-round stall keeping, high concentration of livestock in limited areas, lack of walking and insolation lead to an increase in the functional load on the animal's body and, as a result, to an increase in morbidity, especially of an infectious nature [3-5]. As a result, the number of stillborn and non-viable calves increases, which causes enormous economic damage, which is associated not only with the death of offspring, but also with a decrease in productivity, overspending of feed and significant treatment costs [6, 7]. Diseases of the gastrointestinal tract are the most significant in terms of mass and economic damage.

Infections of the gastrointestinal tract in livestock farms in Russia are annually recorded in 50% of the livestock, while the death of young animals in the first weeks of life ranges from 14 to 60% [8]. In newborn calves, diseases of the digestive system, accompanied by a diarrheal symptom complex, account for 60-70% of pathologies. Colibacillosis in terms of the number of cases and mortality, despite the widespread use of modern antibiotics and vaccines, remains the main infection in animals. This is an acute disease that occurs with diarrhea, signs of dehydration, intoxication, and dysfunction of the cardiovascular and central nervous system [9-11]. According to numerous studies, the prevalence of colibacillosis varies widely, from 5.4 to 90%, and the associated mortality of calves is estimated at approx. 20% [12]. Such variability in incidence rates is due to environmental problems, the conditions of keeping and raising animals, as well as the quality of sanitary and hygienic measures at agricultural enterprises in different countries [13]. The spread of colibacillosis in livestock complexes depends on the presence of a source of the infectious agent, the susceptibility of calves, and on the effectiveness of preventive measures [14].

In the etiology of colibacillosis in cattle (cattle), the most significant are enterotoxigenic (enterotoxigenic *Escherichia coli*, ETEC), enteropathogenic (enteropathogenic *E. coli*, EPEC) and enterohemorrhagic (enterohemorrhagic *E. coli*, EHEC) pathotypes of diarrheal *E. coli*, which cause enterocolitis in people, which makes this problem relevant not only for veterinary medicine, but also for medicine [12, 15, 16]. Representatives of enterotoxigenic serovars produce thermostable (STa or STb) and/or thermolabile (LT1 or LT2) enterotoxins that cause dehydration and are often found in calves in the first three days of life. Enterohemorrhagic and Shiga-toxin-producing (EHEC, STEC) strains of *E. coli* produce a toxin that, by damaging the intestinal microvilli, causes hemorrhagic diarrhea in calves at the age of 2-5 months [17, 18]. Infection of animals with less virulent cultures of extraintestinal *E. coli* leads to the occurrence of diseases of extraintestinal localization, in particular, polyarthritis, meningitis, less often uveitis and nephritis, which can become chronic.

It is known that representatives of diarrheagenic *E. coli* pathotypes are able to circulate for a long time in livestock enterprises among the livestock [19]. According to most researchers, the main source of the infectious agent is excreta (faeces) of apparently healthy animals, since *Escherichia* strains of enteropathogenic serogroups can persist in the intestines of adults [12, 18]. The udder of cows with a lack of bedding and irregular cleaning of manure can be contaminated with

bacteria, which leads to infection of calves through the digestive tract during sucking, as well as when drinking contaminated milk. Among calves, transmission of the pathogen occurs through direct contact (“nose-to-nose”) or through the respiratory tract [17]. Consequently, adult animals and young animals serve as a reservoir and/or source of the infectious agent, which allows the bacteria to persist in the herd, circulating among animals of all ages. In agricultural enterprises, colibacillosis pathogens can also be transmitted through drinking water and feed. The data of the microbiological study of feed on the territory of Russia for 2014-2018 indicate that representatives of enteropathogenic *E. coli* were most often isolated from feed of plant origin and compound feed [20].

Monitoring of pathogens of bacterial infections is an essential component of the system of epizootic surveillance of farm animals. Because of the increased cases of food poisoning when using animal products contaminated with Shiga toxin-producing *Escherichia*, control over the circulation of these pathogens in a population of healthy individuals is of particular importance.

This report presents for the first time data on the prevalence of colibacillosis and the biological properties of pathogens circulating in agricultural enterprises in the Perm region. For the first time, an integrated approach was applied to assessing the prevalence of colibacillosis among cattle (sick animals-healthy animals-environment), which is of theoretical importance, deepening the understanding of the patterns of the epizootic process in this disease. An important practical aspect was the analysis of data from long-term sanitary and zoohygienic studies, the results of which indicate that on-farm control of the risk of infection of animals and personnel of enterprises with diarrheal *Escherichia* should extend not only to the natural reservoir, but also to the environment.

The purpose of the work is to assess the prevalence of colibacillosis in cattle in various farms of the Perm Territory and to characterize the pathogen by biological properties. In order to control the contamination of equipment, feed and watering system with *Escherichia* in livestock farms, an analysis of the data of sanitary and zoohygienic studies was carried out.

Materials and methods. The dynamics of the incidence of bacterial infections in cattle was analyzed based on the reporting data of the Perm Veterinary Diagnostic Center, the Department of Animal Husbandry of the Ministry of Agriculture of the Perm Territory for 2010-2020, the statistical data of the Department of Veterinary Medicine of the Perm Territory (form No. 1-vet, 2-vet, form No. 3, 4), as well as laboratory journals for 2016-2020. The reporting included results for 146 beef and dairy enterprises of all categories, including 92 (63.0%) agricultural enterprises, 46 (31.5%) farms and 8 (5.5%) individual farms. The livestock for the entire observation period averaged 76754.2 ± 850.1 animals. Sanitary and zoohygienic studies were carried out at the same enterprises. Biological fluids, excreta and pathological material obtained from sick and dead animals were studied, i.e., feces, blood, urine, nasal mucus, vaginal mucus, preputial mucus, liver with gallbladder, a segment of the affected small intestine (22480 samples were analyzed in total). In addition, in dynamics, swabs from dairy equipment, inventory of slaughterhouses, feed of plant and animal origin, compound feeds were analyzed (29207 samples in total).

Bacteriological examination was carried out according to the “Guidelines for bacteriological diagnosis of colibacillosis (escherichiosis) in animals” (Moscow., 2000). The antigenic structure of the isolated *E. coli* strains was studied using the O-coli agglutinating sera kits (FKP Armavir Biofactory, Russia) and test sera for typing adhesive antigens of *Escherichia coli* F4 (K88), F5 (K99), F6 (987P), F41, A20 (Att25) (Vyshelsky Institute of Experimental Veterinary Medicine, Russia) according to the manufacturer’s instructions.

The sensitivity of strains to antibacterial drugs was determined according to MUK 4.2.1890-04. The strains were tested by disk-diffusion method using Muller-Hinton agar (SRC PMB, Russia) and disks (OOO NITsF, Russia) for sensitivity to penicillins (ampicillin, 10 µg), cephalosporins (cefoxitin, 30 µg; ceftriaxone, 30 µg; cefepime, 30 µg), carbapenems (meropenem, 10 µg; imipenem, 10 µg), monobactams (aztreonam, 30 µg), aminoglycosides (amikacin, 30 µg; gentamicin, 10 µg), fluoroquinolones (ciprofloxacin, 5 µg; levofloxacin, 5 µg; moxifloxacin, 5 µg), tetracyclines (tetracycline, 30 µg), and fenicol (chloramphenicol, 30 µg). Insensitivity of strains to at least one drug from three or more groups of antibiotics was considered multidrug resistance (MDR).

In a prospective study (2020-2021), the prevalence of Shiga toxin-producing *E. coli* strains was assessed in a population of healthy animals (calves and cows). Cultures ($n = 61$) were isolated from cattle feces at animal husbandry enterprises in the Perm Territory.

DNA was isolated by the following method. A separate colony of each strain was resuspended in 0.5 ml of ultrapure water in Eppendorf test tubes, incubated in a Termit solid-state thermostat (NPO DNA-Technology LLC, Russia) for 10 min at 98 °C, cooled and centrifuged for 5 min at 13000 rpm. The supernatant was used for genetic studies immediately or after storage at -18 °C. Genetic typing of cultures was carried out in a double control system by rep-PCR (repetitive element sequence-based PCR) with primers M13 (5'-GAGGGTGGC-GGTTCT-3') and ERIC1R/ERIC2 (5'-CACTTAGGGGTCCTCGAATGTA-3'/5'-AAGTAAGTGACTGGGGTGAGCG-3') using appropriate reaction modes [21, 22]. Detection of the *stx1* and *stx2* genes was performed using primers stx1-F/stx1-R (5'-ATAAATCGCCTATCGTTGACTAC-3'/5'-AGAACGCCCACT-GAGATCATC-3'; 180 bp fragment) and stx2-F/stx2-R (5'-GGCACTGTCTG-AAACTGCTCC-3'/5'-TCGCCAGTTATCTGACATTCTG-3'; 255 bp fragment). For both pairs, the general amplification regimen was used as recommended [23]: 3 min at 95 °C (initial denaturation); 1 min at 95 °C, 2 min at 60 °C, 1.5 min at 72 °C (25 cycles); 5 min at 72 °C (final elongation). Oligonucleotide primers were synthesized at OOO Sintol (Russia). DNA amplification was carried out using reagents manufactured by OOO Sintol (Russia) on a DNA Engine Dyad thermal cycler (Bio-Rad, USA) in 25 µl of the reaction mixture. PCR products were detected using a horizontal electrophoresis in 1.2% agarose gel at an electric field strength of 6 V/cm at room temperature. A buffer containing 0.25% bromophenol blue and 30% glycerol in ultrapure water was used to apply the samples. Agarose gels were stained with ethidium bromide solution (1-2 µg/ml) for 10-15 min. The bands were visualized and data were recorded using the Gel-Doc XR gel documentation system (Bio-Rad, USA).

Statistical data processing was carried out using Microsoft Office XP Excel 2013 and Statistica v.6.0 (StatSoft, Inc., USA). To compare qualitative features, the χ^2 test (with Yates correction) was used. At $p < 0.05$, the difference between the compared samples was considered statistically significant.

Results. According to statistical reports for the period from 2010 to 2020, 1361 cases of infectious diseases of bacterial etiology were registered among cattle at livestock enterprises in the Perm Territory. The share of colibacillosis in the infectious pathology of animals varied from 0.2 to 61.5% and averaged $14.4 \pm 11.9\%$ over 11 years (Fig. 1).

It should be noted the high incidence in 2011. The decrease in the proportion of colibacillosis among animal infections in 2012-2014 was apparently due to an increase in the prevalence of leptospirosis.

In 2020-2021, 39 cultures of diarrheal *E. coli* were isolated from sick and dead animals (calves and adult cattle). Among them, we identified 5 epizootically

most significant serogroups (O8, O15, O20, O101, O115), *E. coli* O157 was detected sporadically. The frequency of occurrence of A20 adhesin among pathogenic strains of *E. coli* was 6%. In most cases, *E. coli* cultures showed resistance to ampicillin and cefazolin (61.5% each). We found high resistance of cultures to other cephalosporin antibiotics, the ceftiofur (30.7%) and ceftriaxone (23.1%). Imipenem, meropenem, amikacin, tobramycin and moxifloxacin had the highest activity against *Escherichia*. The proportion of sensitive strains was 100, 97.4, 92.3, 100 and 92.3% respectively. A high frequency of resistant strains of *E. coli* has been reported to tetracycline (79.5%) and chloramphenicol (61.5%).

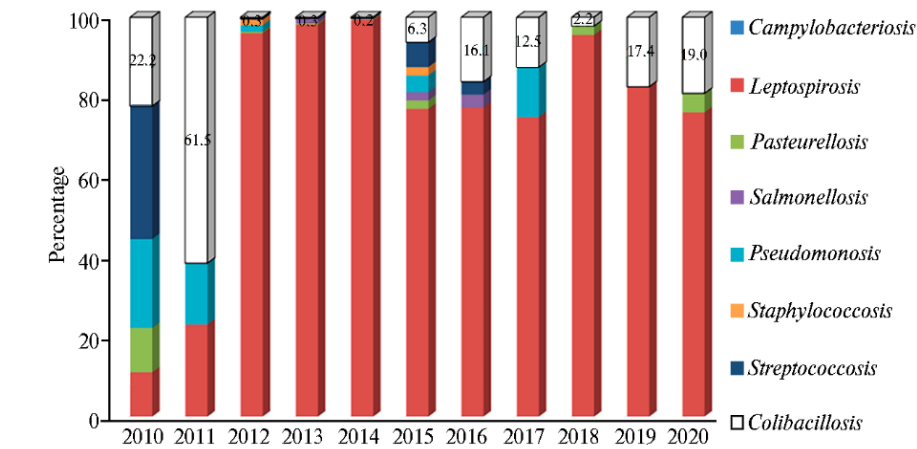


Fig. 1. Proportion of colibacillosis among cattle at livestock enterprises in the Perm Territory (statistical reporting, 22480 samples).

According to the results of ERIC typing of the primary collection of isolates ($n = 61$) isolated from the faeces of healthy calves and cows at industrial animal husbandry enterprises, we identified 49 representatives of individual genomogroups, which were used in further work. The *stx1* gene was detected in only one culture (2.0%), *stx2* was present in the genome of three (6.1%) cultures. That is, among these 49 *E. coli* strains, we found four STEC strains (8.1%).

1. The prevalence of antibiotic resistance among strains of *Escherichia coli* isolated from cattle at livestock enterprises in the Perm Territory (2020-2021)

Group of antibiotics	Antimicrobial agent	Resistant strains, %	
		sick/dead animals ($n = 39$)	healthy animals ($n = 49$)
Penicillins	Ampicillin	61.5	77,6
Cephalosporins	Cefoxitin (II)	30.7	20,4
	Ceftriaxone (III)	23.1	16,3
	Cefepime (IV)	23.1	14,3
	Aztreonam	25.6	16,3
Monobactams	Meropenem	2.6	0
Carbapenems	Imipenem	0	0
Aminoglycosides	Gentamicin (II)	7.7	2,0
	Amikacin (III)	7.7	0
Fluoroquinolone	Ciprofloxacin (II)	8.2	10,2
	Levofloxacin (III)	nd	6,1
	Moxifloxacin (IV)	7.7	nd
	Tetracycline	79.5	49,0* ($p = 0,0066$)
Tetracyclines	Chloramphenicol	61.5	20,4* ($p = 0,0002$)
Phenicols			

Н о т и е. In parentheses the generation of the antimicrobial agent is indicated, nd means no data.

* Differences from the group of sick/dead animals are statistically significant.

The formed collection of strains was tested for sensitivity to antimicrobial agents. Comparison of the antibiotic susceptibility profiles of *E. coli* strains isolated from sick and dead (group 1) and healthy (group 2) animals showed that for

most of the drugs the proportion of resistant strains was lower among cultures of the second group, but the difference was statistically significant only for tetracycline and chloramphenicol (Table 1). It should be noted a higher proportion of cultures resistant to ampicillin and ciprofloxacin in the subpopulation of *Escherichia* isolated from healthy cattle.

The results of microbiological studies of swabs from inventory and equipment, water samples from the animal watering system, feed and feed raw materials showed that the analyzed objects were contaminated to one degree or another by various microorganisms, but on average, the number of positive samples was a little more than 6%. Most often, the surfaces of equipment and inventory, as well as water sources, were contaminated. Representatives of *Salmonella* and *Escherichia*, *Proteus*, *Enterococcus*, *Staphylococcus*, *Streptococcus*, anaerobic bacteria and mold fungi were found in the studied samples. A total of 1806 bacterial cultures were isolated, of which almost 80% were *E. coli* isolates (Table 2).

2. The assessment of the sanitary and zoohygienic condition at livestock enterprises in the Perm Territory (2010-2020, statistical reporting)

Materials	<i>n</i>	Positive samples (%)	<i>Escherichia coli</i> (% of the number of positive samples)
Swabs:			
from dairy equipment ^a	6928	613 (8.8)	559 (91.2)
from the inventory of slaughterhouses ^b	7486	721 (9.6)	594 (82.4)
Feed:			
vegetable origin ^c	4414	71 (1.6)	50 (70.4)
compound feed ^d	3060	106 (3.5)	99 (93.4)
animal origin ^e	5445	25 (0.5)	7 (28.0)
Water for use ^f	1874	270 (14.4)	123 (45.5)
Total	29207	1806 (6.2)	1439 (79.7)

a, b, c, d, e, f Differences between the indicated material and materials marked with the corresponding Latin letters are statistically significant at $p < 0.01$.

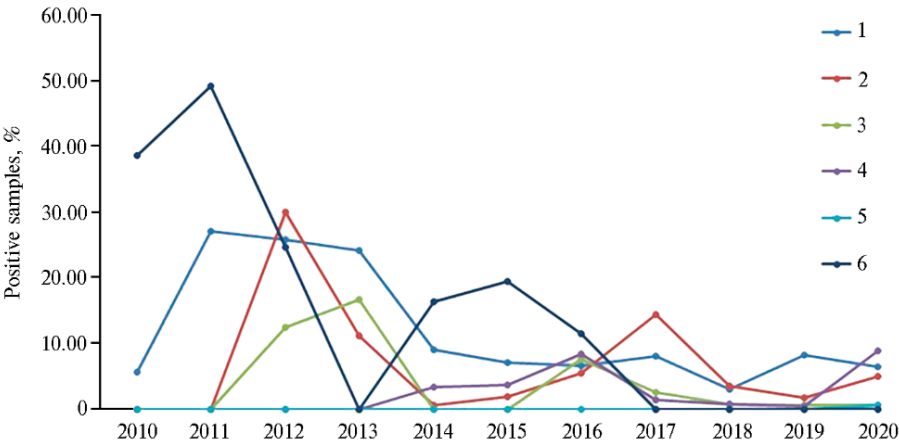


Fig. 2. Isolation of *Escherichia coli* from samples collected at livestock enterprises in the Perm Territory: 1 — swabs from dairy equipment, 2 — swabs from inventory of slaughterhouses, 3 — feed of plant origin, 4 — compound feed, 5 — feed of animal origin, 6 — water for use (statistical reporting, 29207 samples).

Most often, *Escherichia* was isolated from samples containing swabs from dairy equipment and inventory of slaughterhouses, as well as in animal feed. The proportion of samples containing *E. coli*, in most cases, did not exceed 20% of the number of all studies carried out in a year. In 2011, the highest percentage of

Escherichia-contaminated samples (49.1%) taken from drinking systems was revealed (Fig. 2). Apparently, these results indirectly reflect the situation of colibacillosis at the livestock enterprises of the region in that period (see Fig. 1).

It is known that gastrointestinal diseases of bacterial etiology occupy a special place among cattle infections, while enteropathogenic strains of *E. coli* play a leading role in the microbial profile of infectious pathology [6, 13, 24]. The high prevalence of colibacillosis of farm animals is recorded in the countries of South Asia and Africa. So, according to a number of studies, in animals with a diarrheal symptom complex EPEC occurs in 75% of cases in India, in 54% in Pakistan, in 86% in Iran, in 63.6-82.0% in Egypt. In European countries, this figure is significantly lower, for example, in Sweden, the frequency of isolation of *Escherichia* in intestinal diseases of cattle was 11.5%, in Germany 42.0%, in France 20.3%, in Spain 35.9% [12]. The variation in the prevalence of colibacillosis in different geographical regions may be associated with climate, the environmental situation, the form of keeping animals (free or stall), the practice of using antibiotics and carrying out sanitary and preventive measures.

In most regions of Russia, constant microbiological monitoring is carried out, and data on the incidence of colibacillosis differ significantly in different farms. An analysis of long-term (1996-2015) statistics of infections of farm animals in the Krasnodar Territory showed that the incidence of colibacillosis in calves remained high (33.5-53.6%) throughout the entire study period and averaged 40.5% of all registered infections [25]. Colibacillosis is widespread in the farms of the Amur region, the share in the infectious pathology of calves in some areas reached 60% [26]. The incidence of calves with escherichiosis on dairy farms of the GUSP MTS Tsentralnaya (2014-2016) in the Republic of Bashkortostan was more than 30% [9]. On the territory of the Irkutsk region in 2001-2010, the incidence of colibacillosis in cattle ranged from 0.2 to 20.5%, however, the authors state that the epizootological situation, both in general for gastrointestinal diseases, and for colibacillosis during the period under review was tense. This pathology remains one of the main problems in farms in almost all regions of the Irkutsk region, especially during the stall period of keeping animals [27]. Similar patterns in relation to colibacillosis were revealed by us in the Perm Territory: over an 11-year period, the proportion of the disease in cattle fluctuated widely, while it should be noted that the situation in this nosology in the region has been relatively stable in recent years.

E. coli bacteria are extremely heterogeneous in antigenic structure and toxigenic properties, which determine the pathogenetic features of their persistence in the macroorganism. More than 700 *Escherichia* serotypes are known, but only some of them have the ability to cause a diarrheal symptom complex in animals, therefore, simultaneously with the identification of bacteria, serotyping of cultures is carried out in an agglutination reaction with specific O-coli agglutinating sera. The main role in the development of diarrhea in newborn calves is played by enterotoxigenic *Escherichia* strains with adhesion antigens K88, K99, 987P, F41, F18, A20, which are more common among bacteria of serogroups O8, O9, O15, O41, O78, O86, O101, O115, O119, O137 [9, 12, 27]. Of particular importance is adhesin A20, which, according to the literature, is common among *Escherichia* that cause colibacillosis [9, 28]. STEC representatives do not usually cause disease in recovered or vaccinated animals with post-amnestic or post-vaccination immunity, but can cause mortality among young animals, hemorrhagic colitis and hemolytic uremic syndrome in humans [29]. Zoonotic strains of STEC include cultures of EHEC O157:H7, as well as representatives of other serogroups (non-O157 EHEC O26, O111, to a lesser extent O17, O56, O87, O108, and O109),

which have recently begun to circulate in agricultural enterprises with increasing frequency [19]. In the Perm Territory, the most significant bacteria in the etiology of colibacillosis in cows and calves turned out to be bacteria of the traditionally common serogroups O8, O15, O20, O101, and O115. It should be noted that strains of *E. coli* O157 were also recorded at agricultural enterprises, this serotype predominates in outbreaks of escherichiosis caused by STEC in Russia and other industrialized countries [30, 31].

Active use of antibiotics for the prevention and treatment of infectious animal diseases, as well as for fattening in many countries, leads to the emergence and spread of antibiotic resistance among commensal and pathogenic microbiota. Thus, studies of the antibiotic susceptibility of pathogenic isolates of *Escherichia* isolated from colibacillosis of calves at enterprises in the Altai Territory showed that most strains were resistant to seven to nine antibacterial drugs [32]. In Iran, of 63 *E. coli* isolates isolated from diarrheal calves, 76% were resistant to at least one of the drugs tested, and 62% of the cultures were multidrug resistant [33]. According to N.M. Sobhy et al. [34], 54.5% of isolates from calves with diarrheal syndrome in livestock farms in Egypt were multidrug resistant. The percent resistance to tetracycline, streptomycin, ampicillin, and trimethoprim/sulfamethoxazole (a combination known as co-trimoxazole) was 79.5, respectively; 67.0; 54.5 and 43.0%. Ceftazidime (14.8%), amoxicillin-clavulanate (13.6%), and aztreonam (11.3%) were the most effective, and none of the isolates was resistant to imipenem.

In our studies, the resistance of escherichiosis pathogens to beta-lactam antibiotics also varied widely, the proportion of those resistant to ampicillin was 61.5%, while all cultures were sensitive to imipenem. Interestingly, in *E. coli* strains isolated from sick/dead and healthy animals, the profiles of resistance to antibiotics did not differ significantly (with the exception of tetracycline and chloramphenicol, resistance to which was significantly higher in *Escherichia* of the first group). The MDR phenotype was observed in 14 (35.9%) and 16 (32.7%) cultures of the studied subpopulations, respectively, although, according to the literature, among the *E. coli* isolates circulating in livestock enterprises, more than half have multiple resistance [33, 35]. However, it should be noted that the percentage of beta-lactam-resistant *E. coli* strains exceeded those obtained by other researchers in relation to *Escherichia* isolated from both diseased and healthy animals [34, 36-38]. Significant differences in the prevalence of antibiotic-resistant microorganisms circulating in livestock farms in different countries may be due to the peculiarities of the conditions in which animals are kept and approaches to the use of antimicrobial drugs.

Cattle are the most important reservoir of zoonotic strains of STEC, which are transmitted to humans through dietary or faecal-contaminated water, and through direct contact with infected animals. The causative agents of infectious diseases can be isolated from the gastrointestinal tract not only of sick and dead, but also of healthy farm animals. Our studies confirm that the latter are the source of Shiga-like toxin-producing *E. coli* isolates, which occurred in 8.1% of cases. It should be noted that the published materials provide different data on the prevalence of *E. coli* producing Shiga toxin in herds of healthy animals, from 2.9 to 27.3% [39, 40]. At the same time, the frequency of detection of STEC in cattle with symptoms of intestinal infection, according to foreign studies, ranges from 10 to 40% [19, 34, 37]. The results of observations by V.I. Terekhova et al. [25] showed that 21.5% of *E. coli* strains isolated from calves with colibacillosis carried genes for Shiga-like toxins. Data on the prevalence of STEC are of great importance for the development of preventive measures in livestock enterprises and

the assessment of food safety, so monitoring the spread of STEC among livestock, including among healthy individuals, is one of the elements of infection control. The identification of factors that influence the carriage and shedding of STEC in cattle and lead to the development of the disease in humans is of both veterinary and medical importance [41].

On farms, the main sources of infection of cattle with *Escherichia*, including STEC strains, are drinking water, feed, and other abiotic components of the ecological system [19]. To a large extent, the health of farm animals, their reproductive functions and the biological value of final food products depend on the sanitary quality of feed. An analysis of data from microbiological monitoring of feed in the Russian Federation for 2014-2018, conducted in 2020, showed that pathogenic *E. coli* serotypes were most often isolated from feed of plant origin and compound feed [20]. In our studies, similar data were obtained: samples contaminated with *Escherichia* decreased in the series feed > feed of plant origin > feed of animal origin (see Table 2).

Vaccination of livestock with drugs containing various *Escherichia* antigens of adhesion factors and enterotoxins is extremely important for the prevention of colibacillosis in livestock enterprises. Currently in Russia, including on the Perm Territory, commercial preparations used for active immunization against colibacillosis of farm animals are OKZ (*E. coli* O9: K99, *E. coli* O138: K88) (OOO Agrovet, Russia), Kombovak-K (*E. coli* O9, O78, O115; capsular polysaccharides K80, K30; adhesive antigens K99, F41) (OOO Vetbiokhim, Russia), Rotagal which contains *E. coli* EC/17 with adhesive antigen F5 (K99) (OOO Vetbiokhim, Russia), Skaugard 4KS (ScourGuard 4KS, enterotoxigenic strains of *E. coli* with adhesion factor K99) (Zoetis, Inc., USA). At the same time, it can be assumed that for the prevention of colibacillosis in different regions, it is more appropriate to use local strains of microorganisms for the preparation of vaccines and hyperimmune sera [26]. In this regard, interesting studies were carried out at the Vyshel'sky Belarusian Research Institute of Experimental Veterinary Medicine in 2002, the purpose of which was to determine the correspondence between the O-serogroup affiliation of isolated epizootic strains and vaccine cultures [28]. The authors showed that O-serotypes of the applied *E. coli* vaccine strains are found in field isolates with a frequency of 30.3 to 55.2%, while in diseased and dead calves there was an increase in the number of isolates (up to 20%) carrying antigens that are absent in commercial vaccines.

In the Perm Territory, *E. coli* of non-vaccine serogroups were also encountered as etiological agents of colibacillosis in cows and calves, which confirms the need for specific prevention of acute intestinal diseases in calves, taking into account the spectrum of circulating enteropathogenic strains. Its basis is the immunization of pregnant cows, which allows you to protect young animals in the first days after birth. This approach is supported by data on the increase in the number of diseases of the gastrointestinal tract, recorded against the background of a decrease in the proportion of pregnant cows immunized against colibacillosis. Thus, according to the reports of the livestock department of the regional Ministry of Agriculture, in 2016, 57.6% of cows were vaccinated in the region, in 2020 - no more than 40%, while the number of sick calves increased from 21.9 to 24.8%, and the number fallen/forcibly killed, from 2.3 to 2.4% (data not fully presented). Nevertheless, it should be noted that over the past 5 years, the total number of cattle in the Perm Territory has remained stable in terms of numbers with an upward trend (172,029 heads in 2016, 173,944 heads in 2020).

The development of the epizootic process is mainly due to the peculiarities of the relationship of the infectious agent with the populations of the obligate and potential hosts, which, in turn, is determined by the biological properties of the

etiopathogen, the immune status of the macroorganism, as well as natural and economic factors [42]. Our comprehensive study, which analyzed the proportion of colibacillosis in the infectious pathology of animals, the prevalence of *Escherichia* in the on-farm environment, and also described some of the biological properties of the pathogen, allows us to better understand the epizootic process in colibacillosis and optimize preventive measures.

Thus, our sanitary and zoohygienic analysis indicates the need for more thorough bacteriological control of feed, dairy equipment, water and water supply sources, and inventory of slaughterhouses. In addition, on-farm control of the risk of infection of employees with diarrheal *Escherichia* is mandatory, which should extend not only to the natural reservoir of infection, but also to the environment. Vaccination, currently used as the main method of preventing colibacillosis, is not always effective enough. To reduce the colonization of the intestines of cattle by pathogenic *E. coli*, it is necessary to use probiotics, bacteriophages, as well as modification of the nutrition of young and adult animals.

So, the epizootic situation for colibacillosis in cattle in the Perm region in 2010-2020 was quite favorable: its share among all bacterial infections did not exceed 20%. The epizootic process was characterized by sporadic cases of the disease, while there was a high risk of transmission of the infectious agent in a horizontal way, including through environmental objects. *Escherichia* was mainly isolated from swabs from dairy equipment and inventory of slaughterhouses, as well as from animal feed. Among the isolates, five most epizootically significant serogroups were identified, the O8, O15, O20, O101, O115, in several cases *Escherichia coli* O157 were isolated. Antibiotic resistance profiles in *E. coli* isolates from diseased/deceased and healthy animals did not differ significantly (except for tetracycline and chloramphenicol, resistance to which was significantly higher in *Escherichia* of the first group). It should be noted a higher proportion of cultures resistant to ampicillin and ciprofloxacin in *Escherichia* from healthy cattle. In addition, we detected the *stx1* (2.0%) and *stx2* (6.1%) genes in the latter, i.e., our findings have confirmed that pathogens of infectious diseases can be present in the gastrointestinal tract of not only sick, but also healthy farm animals, which become a source of Shiga toxin-producing *E. coli*.

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