Research Article

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ETIOLOGY AND CLINICO-MORPHOLOGICAL MANIFESTATION OF ANAEROBIC ENTEROTOXAEMIA OF YOUNG CATTLE

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ABSTRACT

In the article the questions of etiology and clinical-morphological manifestations of anaerobic enterotoxaemia in young cattle are investigated. The problem of anaerobic enterotoxaemia is stationary in nature and is characterized by high mortality, which leads to significant economical loss for the farming. During research we used the following methods, such as clinical, pathological, bacteriological and serotyping. We carried out our researches of anaerobic enterotoxaemia at various farms from 10 constituents of the Russian Federation. Cases of anaerobic enterotoxaemia were found for 5 cows, which were characterized by diarrhea, malnutrition, anemia mucosal, infiltration in the subcutaneous tissue and abortion. At cow autopsy in the abdominal and thoracic cavities seroplastic exudat, hemorrhagic inflammation of the small intestine, softening of the kidneys, and petechial hemorrhages on the surfaces of parenchymal organs were observed. Bacteriological studies determined pathogens - *Clostridium perfringens*, which were pathogenic for laboratory animals. In pure culture we extracted 18 isolates and *Clostridium perfringens*, 5 of them had high pathogenicity. *Clostridium perfringens* isolates of "C" type was allocated in 55.6 % of cases, "D" type – in 11.1% and "A" type in 33.3 % of cases. "A" type of *Clostridium perfringens* dominated in neonatal calves (83%) in anaerobic enterotoxaemia etiology and "C" type of *Clostridium perfringens* were typical for older cattle (75%). The analysis of our own studies results and literature data showed that the anaerobic enterotoxaemia pathogen of young cattle belongs to *Clostridium perfringens* of "C", "A", "D" serotypes. The development of specific prophylaxis means against anaerobic enterotoxaemia in cattle farming should be based on the inclusion of the above-noted serotypes into biologic antigens composition.

Keywords: anaerobic enterotoxaemia; "A", "C", "D" serotypes of *Clostridium perfringens*, calves, cattle.

INTRODUCTION

Today the pathology of the gastrointestinal tract of young cattle occupies one of the leading places in the structure of morbidity and death of animals. Among the young animal diseases, accompanied by the digestive system affection, anaerobic enterotoxaemia from the group of clostridium is widespread on the territory of our country and abroad. In spite the fact that the disease manifests in sporadic cases, they have a clear consistency. The infection is characterized by a steady character and a high mortality rate that results in significant economical losses for the cattle farms.

Nowadays more than 100 species of Clostridia are known already. New, understudied, modified, and rare types of this pathology¹ are coming into existence.

The causative agent of anaerobic enterotoxaemia are *Clostridium perfringens* – large, fixed, anaerobic, spore-forming, gram-positive rods, which are divided into six types that differ from each other in producing of toxins and antigenic structure².

According to various published data anaerobic enterotoxaemia of the cattle is caused by *Clostridium perfringens* of "A", "C",

"D" serotypes. As for "B" serotype, it has not been extracted²⁻⁴ among the sick animals. At most the diseases are registered in the farms where zoohygienic technology and animal health requirements are not observed, and there are violations in the feeding and management of animals⁵.

According to the researches of Spiridonov A.G., Kapustin A.V., Kurlovich D.V., Moskalyova N.V. today the specific preventive measures against anaerobic enterotoxaemia are inadequate and insufficient to prevent morbidity and mortality of calves^{3,6,7}.

The problem of antigenic composition for the construction of specific prophylaxis means against anaerobic enterotoxaemia in calves is still understudied. Thereby, monitoring researches of manifestations and anaerobic enterotoxaemia etiology in cattle in various farms of the Russian Federation are of vital importance.

Purpose and Objectives of research

The purpose of our research is anaerobic enterotoxaemia monitoring, of *Clostridium perfringens* serotypes identification, etiopathogenic for calves.

To achieve the purpose of the work the following objectives were formulated:

1. To conduct a study of cases of anaerobic enterotoxaemia at the farms of the Russian Federation.
2. To select mortem samples of pathologicoanatomic material from fallen or slaughtered animals with symptoms of anaerobic enterotoxaemia and to conduct bacteriological tests for the differentiation of clostridia.
3. To study the antigenic composition and biological properties of pathogens anaerobic enterotoxaemia in calves.
4. To identify etiologically significant *Clostridium perfringens* at anaerobic enterotoxaemia.

MATERIAL AND METHODS

Within the research framework fallen or slaughtered young cattle with the anaerobic enterotoxaemia symptoms were subjected to autopsy. In total 18 autopsies of 6 calves under 10 days of age, 4 animals aged from 10 to 30-days and 3 calves aged 1-12 months. Also 5 cow corpses with the typical symptoms of anaerobic enterotoxaemia were subjected to autopsy and bacteriological examination. For bacteriological examination the pathologicoanatomic material from heart (blood, myocardium pieces), muscle tissue, as well as the small intestine sections with the contents and pieces of parenchymal organs (liver, kidney and spleen) was taken. Forcedly slaughtered animals autopsy was done by the method of isolated extraction of internal organs (R. Virchow) and by the method of complete extraction of organs (H. Shore)². The digest medium: MPA, MPB, Kitt-Tarozzi, MPPA with the addition of 10% sheep blood and 0.5% glucose were used. The «RapID ANA II» kit, designed to determine the species identity of anaerobic organisms by enzymatic properties was used. To identify the *Clostridium perfringens* type the neutralization reaction was used.

To create anaerobic conditions anaerobic culture apparatus with gas-generator package was used. Tinctorial properties and morphology of the isolates were studied in the smear microscopy of cultures being Gram-stained.

To determine the extracted isolates pathogenicity the experimental infection of the laboratory animals was performed. Rabbits of chinchilla breed, weighing $2,5 \pm 0,54$ kg, guinea pigs of self breed, weighing $250,0 \pm 4,32$ g and white scrub mice weighing $17,0 \pm 2,64$ g were used.

The extracted clostridia antibiotic resistance was investigated by agar diffusion method using standard disks.

RESULTS

The study of the cases of anaerobic enterotoxaemia of cattle was carried out in the Kirov region, the Saratov region, the Samara region, the Moscow region, the Tver' region, the Belgorod region, the Chelyabinsk region, the Kursk region, the Nizhny Novgorod region and the Republic of Mordovia. Altogether we researched pathologicoanatomic material of 18 animals.

The disease in neonatal calves was registered in 6 cases. The disease is characterized by fever up to $42,5 \pm 0,16$ °C, increased heart rate $157 \pm 2,3$ hb / min and breathing $61,5 \pm 1,4$ dd / min, refusal of feed, lethargy, dystaxia, pareses and paralyses. Diarrhea mixed with blood was also marked. Initially feces were yellow-green in color, containing gas bubbles, and then it became fulvous-brown. 4 calves bled from natural orifices. In the submandibular space, neck, dewlap, abdomen, back, extremities the infiltrates in the subcutaneous tissue were marked. 5 calves out of 6 cases died, 1 was forcedly slaughtered.

At autopsy of the fallen or forcedly slaughtered calves the most characteristic pathologicoanatomic symptoms were recorded: anemic subcutaneous tissue and bloody fluid accumulation in the abdominal cavity. The mucous membrane of the small intestine is inflamed catarrhal, sometimes hemorrhages were detected. Bleeding was also seen from the natural orifices (mouth, nose and anus). Hemorrhages in the subcutaneous tissue were observed in 4 cases.

At the beginning of the disease calves of the age of 10-30 days had liquid feces with gray-yellow slime, unpleasant smell, gas bubbles and blood admixture. Later it became profuse diarrhea, defecation was involuntary. Incompletely closed anus was marked. Organism dehydration symptoms were developing: dry mucous membranes, decreased skin elasticity, hair-coat matte. Body temperature was increased to $42,1 \pm 0,6$ °C. Pulse was $129 \pm 0,7$ hb / min, arrhythmic. Breathing was heavy, frequent: $73,3 \pm 0,6$ dd / min. Calves had symptoms of severe intoxication: depression - sopor, in 3 cases - convulsions. Animals were constantly lying up stretching neck or throwing back head sidewise, slowly responding to surrounding. The death of 4 animals was recorded. The fallen calves autopsy discovered accumulation of serofibrinous exudate in the chest and abdominal cavities, petechial hemorrhages on the epicardium, pulmonary edema. Mesenteric lymph nodes were edematous, enlarged, with dripping turbid liquid on a section. Liver was filled with blood, increased, of flaccid texture. Kidneys were enlarged, softened; the borderline between cortical and medullary areas was absent. In the brain 2 calves had focal purulent meningitis with the lateral sides of the cerebellum base.

Anaerobic enterotoxemia of cattle aged from 1 to 12 months was observed with diarrhea as well as of younger age cattle. The disease is characterized by increased heart rate to 119 bpm $\pm 2,1$ hb/min and breath to $57,6 \pm 1,4$ dd / minute and body temperature increase up to $40,6 \pm 0,4$ °C.

Animals were sedentary, sluggish and they refused from feed. In 4 cases, twitching and paralyses were observed. In the dewlap, neck, abdomen, back, limbs of sick animals the soft crepitant, with increased local temperature infiltrations in the subcutaneous tissue were detected. 3 cases of disease in young animals of this age group were studied, 2 of them died and 1 was forcedly slaughtered.

Typical pathomorphological changes were recorded. Rigor mortis was short during 4-6 hours. Corpses were inflated, quickly biodegraded. Muddy foam mixed with blood exuded from the nasal and oral cavities. At autopsy of fallen animals and animals forcedly slaughtered manifested hemorrhagic inflammation of the mucosa of the small intestine with ulcerations, thinned intestinal walls. Also softening of kidneys, liver granular dystrophy was recorded.

Material for bacteriological examination was also obtained from 5 cows, as they manifested diarrhea, emaciation, mucous membranes anemia, subcutaneous infiltrations in the area of back, abdomen, extremities, which is characteristic of *Clostridium* infection. Temperature, pulse, breathing was within the physiological norms. One cow was marked by an abortion; aborted fetus was subjected to bacteriological examination. *Clostridium perfringens* were not found.

At cow autopsy serofibrinous exudate was found in abdominal and thoracic cavities. Hemorrhagic inflammations of the small intestine, kidney softening were detected. On the surfaces of parenchymal organs petechial hemorrhages were observed.

For bacteriological examinations pathologicoanatomic material from heart, muscle tissue, as well as portions of the small intestine with the contents and parenchymal organs pieces (liver, kidney and spleen) were taken.

Altogether we have selected 108 samples, which were seeded on MPA, MPB under anaerobic conditions. Media Kitt-Tarozzi, MPPA with additions of 10% sheep blood and 0.5% glucose were used to differentiate microorganisms. The Petri dishes were placed in anaerobic culture apparatus. They were incubated at the temperature of 37°C during 24-48 hours. The typical growth for *Clostridium perfringens* on digest media in the form of abundant gas formation, turbidity in its initial growth stage, followed by the medium bleaching, the formation of sediment at the tube bottom on medium Kitt-Tarozzi, large, smooth, convex colonies with smooth edges surrounded by β -hemolysis zones on MPPA with sheep blood and 0.5% glucose in the absence of growth on MPA and MPB, allowed to select cultures for further research.

In parallel, pathological material was examined in impression smears being Gram-stained, microscopically. At impression smears microscopy large, fixed, with spores, Gram-positive rods, measuring 4...8×0,6...1,5 μ m were observed.

Characteristic for *Clostridium perfringens* colonies were seeded into 2 tubes on slanted blood agar, one of which was cultivated in an incubator (control), and the other was cultivated under anaerobic conditions. After 24-48 hours at the presence of the microflora growth on agar cultured in anaerobic culture apparatus, and the absence of growth in the control, the impression smears from culture were prepared and Gram-stained. Rods with morphology typical for *Clostridium perfringens* uncontaminated by extraneous microflora were detected. All in all, 18 isolates obtained from the bacteriological study were extracted, 5 of which were certificated.

For microorganism's identification the enzymatic method with the «RapID ANA II» kit usage was applied. The bacterial suspension was prepared using standard turbidity, which corresponds to an amount of 1 billion of microbic cells. The prepared suspension was distributed in the wells uniformly and cultivated in the incubator at 37 °C for 4-6 hours. Further, the appropriate reagents were put in the wells. The appearance of different colors in the wells was compared with the table appended to the «RapID ANA II» kit. The results were entered the computer program to obtain confirmation of *Clostridium perfringens* identification from pathologicoanatomic material.

To determine the *Clostridium perfringens* serotypes the neutralization reaction with serum of antitoxic *Clostridium perfringens* of types A, C, D (produced by FSUE "Kursk biofabrika") was used. The obtained results are given in Table 1.

Table 1: Seroprevalence *Clostridium perfringens*, extracted from cattle

The test animals	Type A	Type C	Type D
calves under age of 10-days	5	1	-
calves of age from 10 to 30 days	-	3	1
youngsters from 1 to 12 months	-	3	-
Cows	1	3	1

Table 1 demonstrates that serotype C is the most frequent, it was identified in 10 cases, serotype A - in 6 cases, and type D - only in 2 cases. *Clostridium perfringens* serotype B was not extracted.

Research of antibiotic resistance was carried out among 13 extracted isolates. The obtained data are given in Table 2.

Table 2: Antibiotic resistance of *Clostridium perfringens*

Drug	N	B	C	H	Y
Azithromycin	10	0	0	1	9
Amoxicillin	13	8	5	0	0
Ampicillin	13	2	11	0	0
Gentamicin	12	0	0	3	9
Doxycycline	11	0	2	6	3
Chloramphenicol	10	0	0	8	2
Neomycin	11	0	0	10	1
Tetracycline	10	0	1	7	2
Cefazolin	13	1	11	1	0
Cephalexin	13	3	8	2	0
Ciprofloxacin	10	6	4	0	0
Enrofloxacin	11	2	7	2	0
Erythromycin	9	0	0	1	8

Designations: N - number of examined isolates; B - highly sensitive; C - medium sensitive; H - low sensitive, Y - resistant to antibiotic.

Data presented in Table 2 show multiple resistance of *Clostridium perfringens* to antibiotics. The most active antibiotics are: ciprofloxacin, amoxicillin; advantageously antibiotics with an average activity are: cefazolin, ampicillin, cephalexin, enrofloxacin.

DISCUSSION

To monitor *Clostridium perfringens* we studied pathologicoanatomic material from forcedly slaughtered and fallen 18 heads of cattle from the cattle farms of 10 constituents of the Russian Federation. The animals demonstrated pathologicoanatomic symptoms typical for anaerobic enterotoxaemia such as hemorrhagic inflammation of the mucosa of the small intestine with ulcerations, kidney softening, granular degeneration of the liver, bleeding from the natural orifices, infiltrations in the subcutaneous tissue. At bacteriological examinations we identified *Clostridium perfringens* which are pathogenic for laboratory animals. Moreover, in pure culture 18 isolates were identified, 5 of which had high pathogenicity. *Clostridium perfringens* isolates of type C were identified in 55.6% of cases, type D - in 11.1% and type A - in 33.3% of cases. It should be noted that type A of *Clostridium perfringens* (83%) dominated in the newborn calves' etiology of anaerobic enterotoxaemia, while type C (75%) was in older cattle etiology.

The data that we have obtained are not exactly coherent with the research of Spiridonov G.N. and co-authors, conducted in the Republic of Tatarstan in 2010-2013, who extracted from calves *Clostridium perfringens* of type D in 7 cases and type C - only in one case. But the data about the predominant serotype - A (15 cases) are exactly coherent with the results of our studies^{4,6}. Unfortunately, in the article of Spiridonov G.N. and co-authors the exact age of the calves is not mentioned, and probably our results will contribute to the G.N. Spiridonov's data and actualize the problem of anaerobic enterotoxaemia of calves caused by *Clostridium perfringens* of three serotypes: A, D, C.

N.V. Moskaleva in her research conducted in the farms of the Republic of Belarus in 1989-1998 underlines that the most frequent type of *Clostridium perfringens* is type A (40.5%), type D was isolated in 35.1% of cases, the share of type C is 24,3% of isolates⁷.

According to the data of Kapustin A.V. and co-authors, based on the studies of postmortem material from 30 heads of cattle, being obtained from livestock farms of six administrative regions of the Russian Federation, *Clostridium perfringens* of type A were extracted in the 3 cases, of type D in 3 cases, of type C in 2 cases².

Thus, on the whole, our results complete the available data about three main types of *Clostridium perfringens*, which are etiopathogenetic for young cattle and about the absence of epizootic role of serotype B.

Based on the materials of our research and the published data it should be noted that the anaerobic enterotoxaemia agent of young cattle is referred to *Clostridium perfringens* mostly of C, A, D serotypes.

Therefore, the creation of active immunization means against calves anaerobic enterotoxaemia requires the inclusion of these *Clostridium perfringens* serotypes antigens in their composition.

CONCLUSION

Observations of anaerobic enterotoxaemia in 10 farms of the Russian Federation showed that young cattle disease was accompanied with fever, acceleration of breathing and heart rate. Feces were liquid and of gray-yellow color, with blood admixture and gas bubbles. Subcutaneous infiltrates in the dewlap, neck, abdomen, back and extremities were observed. Autopsy of the fallen and forcedly slaughtered animals Postmortem of the fallen and slaughtered animals displayed hemorrhagic inflammation of the small intestine, kidney softening, and bloody fluid accumulation in the abdominal and chest cavities, exudation of the muddy foam with blood admixture from the natural orifices. From 108 samples of pathologicoanatomic material 18 cultures of *Clostridium perfringens* were extracted with characteristic morphological, tinctorial, antigenic properties. Pure culture was extracted from them in 18 cases. 5 strains of *Clostridium perfringens* which are the most highly pathogenic for laboratory animals were certified. In the etiology of cattle anaerobic enterotoxaemia the most significant *Clostridium perfringens* serotypes are types A, C, D, which must be considered in the vaccine prophylaxis tools design.

Studies carried out in accordance with international principles of ethics and the humane treatment of animals.

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