Bovine coronavirus: virus isolation, laboratory diagnostics and specific prevention

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Abstract. Diseases of young cattle of infectious etiology are the most important problem of cattle breeding. Among these diseases, the greatest economic damage is caused by diseases in calves of the digestive system, collectively called "neonatal diarrhea". Numerous studies carried out in recent years have irrefutably proven that the main role in the occurrence of diarrhea in calves is played by rota-, coronaviruses, less often viral diarrhea-mucosal disease, as well as bacteria from the genus Escherichia (separately or in associations). These infectious agents cause a similar clinical picture of the disease; therefore only laboratory methods (detection of antigen in feces, organs, and tissues of sick or dead animals, as well as antibodies in the blood) are the basis for the diagnosis. Besides, with the help of diagnostic kits, it is possible to determine the presence of immunity to a particular disease and the need for vaccination. The article provides an overview of certified kits and vaccines for the diagnosis and prevention of coronavirus infection in cattle and discusses the benefits of a comprehensive regimen of their use.

1. Background
The successful implementation of the State Program for the Development of Agriculture, as a result of which the country will acquire the necessary level of food independence, is largely due to the formation of highly productive livestock breeding. The solution to this problem is restrained by the spread of massive infectious animal diseases, which cause huge economic losses. Diseases of the gastrointestinal tract of newborn calves are a scourge for livestock farms, no matter what form of ownership they belong to: be it a large livestock enterprise or a small farm or subsidiary farm. The infection incidence of animals can be 50-80%, mortality - up to 30-50% [1-2].

The literature data of recent years indicate that the main causes of mass enteritis in newborn calves are coronaviruses, rotaviruses, and bacteria E. coli. Besides, the coronavirus and the bovine viral diarrhea virus play one of the main roles in winter dysentery of cattle and respiratory pathology of young animals [3-4].

The first information about the coronavirus and its role in the pathology of the digestive system in calves dates back to 1972 when this virus was discovered by a research group at the University of Nebraska, Lincoln, USA [5]. In our country, the first reports on the detection of coronavirus in calf feces by electron microscopy were written by O.V. Bogatyrenko and others in 1976 [6].

The causative agent of the disease – bovine coronavirus belongs to the family Coronaviridae. The source of the virus in natural conditions is sick or recovered virus-carrying animals. Coronavirus enterocolitis affects calves from one day to 3 months old. The incubation period for oral infection in the
experiment is 20 hours. Under natural conditions, its duration depends on a load of viral infection and the resistance of the animal's organism. The most widespread and acute disease occurs in the winter-spring period, especially during mass calving. The mortality rate of calves at this time can reach 30%, and the total losses, taking into account the culling of ill young animals due to the impossibility of restoring normal development and the planned gain in live weight, -40%. Studies on the isolation of coronavirus were first carried out in 1981 by V.S. Avilov, N.L. Sokolova, etc [7]-[8]. A difficult epizootic situation for acute gastrointestinal diseases of calves in our farms developed in the 70s. due to the spread of corona and rotavirus infections [9]. There are three different forms of coronavirus infection: diarrhea in newborn calves, winter dysentery with the hemorrhagic syndrome in cows, and transport fever. The latter is a complex of respiratory diseases in cattle of different ages [10].

Coronavirus infection in newborn calves with symptoms of enterocolitis is diagnosed mainly in the first three weeks [11]. The main symptoms: diarrhea with the discharge of yellow or greenish liquid feces with a lot of mucus, sometimes mixed with blood. When ulcers form on the oral mucosa, there is profuse salivation. The duration of the disease is 5-6 days. In case of complications by secondary bacterial microflora, the lethal outcome is recorded on days 2-3. Autopsy reveals hemorrhagic diathesis in the intestine, ulceration of the mucous membrane and thinning of the wall, hypertrophy of the lymph nodes of the abdominal cavity [12]. All known bovine coronavirus isolates, regardless of the clinical manifestation of the disease, belong to the same serotype. But at the same time, from 2 to 3 subtypes of the virus are distinguished, which differentiate in reaction with monoclonal antibodies. Several phenotype and genotype indicators also make it possible to distinguish between respiratory and diarrheal virus isolates [13].

2. Virus isolation

When selecting a sensitive cell culture for the isolation and accumulation of coronavirus, both continuous (BK, BEL, MDBK, HPT-18) and primary (BEK, CK) cell cultures were used. The continuous cell lines MDBK, BEL, and HPT-18 were found to be sensitive to the bovine coronavirus and suitable for its cultivation. Four field strains of the coronavirus were isolated from the feces and intestinal homogenates of sick and dead calves with signs of diarrhea in continuous cell cultures HPT-18 and MDBK: CL-2, KB-6, KM1-14, and KR-10. A method for cultivating the causative agent of coronavirus enteritis in calves in continuous culture of MDBK cells using domestically available culture media based on FHM-d with commercial cattle serum has been worked out. The strain KL-2 of the coronavirus reproduces in the culture of MDBK cells, inducing a persistently expressed CPE, the first signs of which were observed one day after infection. After 4 days, the monolayer of infected cells looked like a network formed from groups of cells and cytoplasmic cords. Electron and immunoelectron microscopy were used to identify isolated strains of coronavirus.

3. Laboratory diagnostics

To solve the most important economic problem of animal husbandry, the diagnosis and specific prevention of gastrointestinal diseases of viral and bacterial etiology of young farm animals are of paramount importance. The diagnosis of coronavirus enteritis is based on the analysis of clinical and epizootic data, pathological changes, and laboratory results with confirmation of serological studies (hemagglutination assay – HA, hemagglutination inhibition reaction - HI, immunofluorescent antibodies test - IFAT, enzyme-linked immunosorbent assay - ELISA, etc.). To detect the coronavirus antigen in the feces and intestines of sick and dead calves and specific antibodies in the blood serum, colostrum, and milk of cows, the optimal conditions for setting the HA and HI have been worked out. A kit for the diagnosis of coronavirus enteritis of cattle by the hemagglutination method has been developed, consisting of lyophilized specific antigen, specific and control (negative) sera. The kit was developed in the country for the first time in the late 80s, protected by copyright certificate No. 1628294 dated 17.10.1990. For the manufacture and control of industrial series, regulatory and technical documentation (RTD) was developed (TU 10.07-28-90), registered in the State Standard of the Russian Federation for No. 005/023405 dated 29.11.90. An order was issued by the Ministry of Agriculture on the introduction
of the kit into veterinary practice No. 107 of 12.12.90. With the help of a diagnostic kit, a wide circulation of causative agents of coronavirus enteritis was established in the calves with dysfunction for gastrointestinal diseases. For the detection of coronavirus antigen in pathological samples from sick and dead calves during mass examinations of cattle to diagnose coronavirus enteritis, for the first time in the country, a kit has been developed for diagnosing coronavirus enteritis in cattle by enzyme immunoassay. High sensitivity, specificity, ease of staging the reaction, the ability to automate research processes, allow in a short time to carry out mass examinations of animals to establish the spread of the disease in farms unfavorable for gastrointestinal infections. For the differential diagnosis of rota-, coronavirus enteritis and viral diarrhea in cattle by the method of ELISA, a kit "RODIKOR-TEST VIEV" has been developed. Developed and approved by RTD for the production of sets TU 9384-025-00496165-2006. Regulatory documents are registered in the Russian Federation: registration certificate No.PVR-1-1.6 / 01778 dated December 29, 2006. The kit is a new type of product that has no domestic analogs; it allows the simultaneous diagnosis of three viral diseases, which significantly reduces the time of diagnosis, and therefore, proceeds with the timely implementation of medical and preventive measures. Colostral antibodies are essential for gastrointestinal infections in calves. The effectiveness of colostral immunity is primarily due to three factors: the timely receipt of colostrum by newborn calves, the number of antibodies contained in colostrum, and their specificity to epizootically significant strains of pathogens. To detect antibodies to rota-, coronaviruses, and bovine diarrhea virus by enzyme-linked immunosorbent assay, a kit “ANTITELA-RODIKOR-ELISA VIEV” was created. The kit is a new type of product. Normative documents for the production of the kit are registered in the Russian Federation, Certificate of Conformity No. ROSS RU FV01.C24175, Declaration of Conformity No. ROSS RU FV01.D07404. It is possible to increase the protective function of passive immunity by immunizing mothers during the dry period.

4. Specific prevention
Analysis carried out to study the antigenic properties of inactivated coronavirus in laboratory animals and cattle gave rise to the design and testing of a series of an inactivated vaccine against coronavirus enteritis of calves. The inactivated aluminum hydroxide vaccine against bovine coronavirus enteritis has been developed for the first time and is protected by the copyright certificate. Documentation for batch production of the drug was developed and approved. The vaccine is designed to immunize pregnant cows before calving, induces the formation of high levels of antibodies in colostrum and milk in cows, which provides 85-90% protection of calves from coronavirus enteritis. The production and control of a pilot-industrial batch of vaccines were carried out based on the experimental-production laboratory of VIEV under the normative and technical documentation approved by the General Directorate of Veterinary Medicine in Russia in the prescribed manner (TU 10.07.237-91). The vaccine was prepared from a formalin-inactivated strain of the coronavirus “KL-2”, cultivated in continuous culture of MDBK cells, for the cultivation of which domestic nutrient media were used. Each batch of vaccine was tested for sterility, harmlessness, and antigenic activity in laboratory animals. The pilot vaccine series has passed production tests on several farms. Epizootological assessment of the effectiveness of the vaccine was determined by reducing the incidence and mortality of calves. The vaccine proved to be harmless for pregnant cows and prevented morbidity and mortality in young animals. The developed method for the manufacture of inactivated aluminum hydroxide vaccine against coronavirus enteritis in calves, methods of its control, and technology of pilot production ensure the serial production of the drug with stable quality indicators. The inactivated vaccine against coronavirus enteritis of calves has a pronounced immunogenic activity in pregnant cows for the induction of antibodies in colostrum. Feeding colostrum from vaccinated cows to calves reduced the infection incidence of young calves and prevented their death. The vaccine retained its antigenic activity for 12 months if stored at a temperature of 8-12º C.

Mass diarrhea of calves is polyetiological, caused by the association of pathogenic agents, most often corona-, rotaviruses, and Escherichia coli, which have a complex effect on the host organism. In this case, the most effective means of prevention are multi-component vaccines, including both viral and
bacterial components. A trivalent virus-bacterial vaccine has been developed against rota-, coronavirus infection and E. coli enteritis. It was found that the immunization of pregnant cows for 40 and their re-immunization 20 days before calving with the associated vaccine against rota-, coronavirus enteritis and E. coli enteritis does not hurt the physiological parameters of the body and significantly affects the accumulation of antibodies in the blood and colostrum of animals. An increase in the immunogenic activity of a trivalent virus-bacterial vaccine against rota-, coronavirus infection and colienteritis in calves was obtained when used together with the immunomodulator “Splenivit”. For the first time in the country, an inactivated vaccine associated with rotavirus, bovine coronavirus enteritis, and viral diarrhea “RODÍKOR-VAK VIEV” was developed and approved under the established procedure by RTD for batch production of the drug (TU 9384-025-00496165-2006). The vaccine is registered in the Russian Federation under the number PVR-1 1.6 / 01779.

It is intended for the immunization of pregnant cows to obtain immune colostrum and feed it to newborn calves in farms that are unfavorable for gastrointestinal diseases. The peculiarity of the vaccine is that it protects calves from three diseases at once - rotavirus, coronavirus enteritis, and viral diarrhea.

5. Conclusion
Given the widespread of gastrointestinal diseases in the farms of the Russian Federation and the need to vaccinate a large number of animals, the economic effect of the use of the developed means and methods of diagnostics and specific prevention can be significant since it will reduce the infection incidence and mortality of calves, and facilitate the course of the disease.

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