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PRODUCTION OF ANTIGEN FOR DIAGNOSIS OF BOVINE CAMPYLOBACTERIOSIS USING AGGLUTINATION REACTION

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The article presents the results of experiments conducted to get a sensitive antigen for the diagnosis of campylobacteriosis. The conducted studies established the correspondence of the Campylobacter fetus fetus B-0115 KazNIVI strain to the subspecies Campylobacter fetus subsp. fetus (Cff), it has been proven that when typing with diagnostic monospecific agglutinating sera of types I, II, III, it has a positive reaction with type II serum and a negative reaction with type I and III sera.

A method has been developed for getting somatic antigen from this strain, based on boiling the bacterial mass obtained by the original method for 60 min at 100^0C. When performing a test tube agglutination reaction using the antigen we obtained, agglutination of somatic O-antigens is observed in lower titers compared to whole-cell OH-antigens, which indicates the specificity of somatic antigens for each subspecies of campylobacteria and confirms that the cultures belong to one or another type.

Based on the studies, Campylobacter fetus fetus B-0115 KazNIVI strain, characteristic of the fetus subspecies, was isolated, and a technology for producing a sensitive somatic antigen from it was developed. It was scientifically proven that the use of this antigen enables the diagnosis of campylobacteriosis in cattle using a test-tube agglutination reaction.

Key words: *agglutination, antigen, diagnosis, campylobacteriosis, serum.*

ІРІ ҚАРА МАЛДЫҢ КАМПИЛОБАКТЕРИОЗЫН АГГЛЮТИНАЦИЯЛЫҚ РЕАКЦИЯ АРҚЫЛЫ ДИАГНОСТИКАЛАУ ҮШІН АНТИГЕН АЛУ

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Мақалада ірі қара малдың кампилобактериозын пробиркалық агглютинация реакциясы арқылы диагностикалауға арналған сезімтал антиген алу мақсатында жүргізілген тәжірибелердің нәтижелері келтірілген. Жүргізілген зерттеулер Campylobacter fetus fetus B-0115 KazNIVI штамының Campylobacter fetus subsp. fetus (Cff) кіші түріне сәйкестігін анықтады, диагностикалық моноспецификалық агглютинациялаушы I, II, III типті сарысулармен типизациялау кезінде II типті сарысумен оң және I және III типті сарысумен теріс реакция болатыны дәлелденді.

Оригиналды әдіспен алынған кампилобактер бактериялық массасын 100^{0}С температурада 60 минут қайнату негізіндегі Campylobacter fetus fetus B-0115 KazNIVI штаммынан соматикалық антиген алу әдісі құрастырылды.

Біз алған антигенді пайдаланып, пробирканың агглютинация реакциясын жүргізген кезде соматикалық О-антигендердің агглютинациясы толық жасушалы ОН-антигендермен салыстырғанда төменгі титрлерде байқалады, бұл соматикалық антигендердің кампилобактериялардың әрбір кіші түрі үшін ерекшелігін көрсетеді және өсінділердің бір немесе басқа түрге жататынын растайды.

Жүргізілген зерттеулер негізінде ұрық түршелеріне тән Campylobacter fetus fetus B-0115 KazNIVI штаммы бөлініп алынып, одан сезімтал антиген алу технологиясы жасалды және осы соматикалық антигенді қолдану ацетилдік бактериялардың кемпилобактериоз реакциясында диагностика жасауға мүмкіндік беретіні ғылыми дәлелденді.

Түйінді сөздер: *агглютинация, антиген, диагностика, кампилобактериоз, сарысу.*

ПОЛУЧЕНИЕ АНТИГЕНА ДЛЯ ДИАГНОСТИКИ КАМПИЛОБАКТЕРИОЗА КРУПНОГО РОГАТОГО СКОТА В РЕАКЦИИ АГГЛЮТИНАЦИИ

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В статье приведены результаты экспериментов, проведенных с целью получения чувствительного антигена для диагностики кампилобактериоза крупного рогатого скота в пробирочной реакции агглютинации. Проведенными исследованиям установлено соответствие штамма Campylobacter fetus fetus B-0115 КазНИВИ к подвиду Campylobacter fetus subsp. fetus (Cff), доказано, что при типизации с диагностическими моноспецифическими агглютинирующими сыворотками I, II, III типов имеет положительную реакцию с сывороткой II типа и отрицательную реакцию с сыворотками I и III типов.

Разработан способ получения соматического антигена из штамма Campylobacter fetus fetus B-0115 КазНИВИ, основанный на кипячении, полученной оригинальным методом бактериальной массы кампилобактерий, в течение 60 мин при 100^{0}С.

При постановке пробирочной реакции агглютинации с использованием полученного нами антигена, агглютинация соматических О-антигенов отмечается в более низких титрах по сравнению с цельноклеточными ОН-антигенами, что свидетельствует о специфичности соматических антигенов для каждого подвида кампилобактерий и подтверждает принадлежность культур к тому или иному типу.

На основании проведенных исследований выделен штамм Campylobacter fetus fetus B-0115 КазНИВИ, характерный для подвида fetus, разработана технология изготовления из него чувствительного антигена и научно доказано, что использование данного соматического антигена позволит осуществит диагностику кампилобактериоза крупного рогатого скота в реакции пробирочной агглютинации.

Ключевые слова: *агглютинация, антиген, диагностика, кампилобактериоз, сыворотка.*

Introduction. Campylobacter spp. are among the leading causative agents of acute intestinal infections (AII) in developed countries, exceeding the incidence of salmonellosis and escherichiosis in some regions. In one third of cases, they are the cause of “traveler's diarrhea” in residents of economically developed countries visiting regions with a high degree of circulation of Campylobacter spp. in the population, animals, and the environment [1, p. 677]. According to the latest WHO estimates, campylobacteriosis (CB) is one of the most common infectious diseases transmitted via food. CB is reported in all age groups, more often among children aged 1 to 3-5 years; a relative increase in cases is noted in older children and young adults (compared to other age groups). The thermophilic C. jejuni and C. coli [2, p. 38], which are characterized by a variety of genetic determinants that determine the pathogenetic and clinical features of the disease, are of the greatest importance in human infectious pathology.

In contrast to other bacterial pathogens of AII, thermophilic Campylobacter spp. are among the most difficult microorganisms to culture, as they require special conditions and equipment. In laboratory diagnosis

of CB, the isolation of pure culture of the pathogen from feces samples is a particularly difficult task due to their massive associated microbial contamination. In this regard, information on the incidence of this infection is fragmentary and does not give a complete picture of the true extent of the disease [3, p. 687, 4, p. 87]. In recent years, the use of molecular methods of investigation is considered not as an alternative, but as a mandatory addition to the regulated schemes of AII diagnostics, which allows rapid and effective detection of AII pathogens, including thermophilic Campylobacter spp. At the same time, it does not imply species identification and determination of sensitivity to antimicrobials.

It is known that most Campylobacter spp. are resistant to the action of bile [5, p. 88] and have the ability to colonize all parts of the intestine with the development of inflammatory changes, swelling, hyperplasia of the mucous membrane at the site of introduction and the appearance of erosions [6, p. 1654]. The pathogenic properties of campylobacteria are largely determined by their mobility, ability to adhesion, invasion and production of toxins. The flagella of Campylobacter are responsible for their motility and movement along the epithelium [7, p. 2, 8, p. 1734]. Adhesion and penetration of enterocytes contributes to the destruction of the intestinal mucosa, a pronounced inflammatory response and the development of hemorrhagic colitis [9, p. 2726]. Severe forms of CB are associated with the production of thermostable and/or thermolabile enterotoxins and/or endotoxin (cell wall lipopolysaccharide), which affect the absorption of fluid and electrolytes, determining the development of diarrhea [10, p. 170].

Campylobacter fetus comprises three subspecies. Two of them, Campylobacter fetus subsp. fetus (Cff) and Campylobacter fetus subsp. venerealis (Cfv), are highly relevant veterinary pathogens commonly associated with mammals. A third species, Campylobacter fetus subsp. testudium (Cft), is mainly associated with reptiles and shows clear genetic divergence with C. fetus subspecies associated with ruminants.

Campylobacter fetus subsp. venerealis (Cfv) is the causative agent of bovine genital campylobacteriosis (BGC), also known as bovine venereal campylobacteriosis, which is an internationally traded disease listed by the World Organization for Animal Health (WOAH).

Often clinically silent bovines are a reservoir for the spread of infection. Cows become infected during natural service or after artificial insemination with infected semen. Bulls can become infected by servicing an infected cow, and transmission can occur between bulls during mating. Vertical transmission has not been reported. Infections (BGC) in cows usually clear up on their own, and most cows regain fertility within 5 months after the infection is removed from the uterus. Bulls may be lifelong carriers of the pathogen.

In contrast to other causative agents of acute intestinal infections (AII) of bacterial nature, thermophilic Campylobacter spp. are among the most difficult microorganisms to culture, as they require special conditions and equipment. In the laboratory diagnosis of campylobacteriosis is a particularly difficult task is the isolation of pure culture of the pathogen from samples of feces and other objects due to their massive associated microbial contamination.

In this regard, obtaining antigen from Campylobacter fetus fetus for diagnosis of bovine campylobacteriosis in agglutination reaction, used for diagnosis of this disease, is of great scientific and important practical importance.

The research purpose is to obtain a sensitive antigen for the diagnosis of bovine campylobacteriosis in vitro agglutination reaction.

Objectives:

1.To obtain antigen for diagnosis of bovine campylobacteriosis in agglutination reaction.

2. Staging of in vitro agglutination reaction using the somatic antigen obtained.

Materials and methods. Scientific research on obtaining campylobacteriosis antigen was carried out in the Kazakh Research Veterinary Institute (KazNIVI).

The strain Campylobacter fetus fetus B-0115 of KazNIVI, which is deposited in the collection for the study of the gene pool of microorganisms of KazNIVI, was used as starting material.

To identify the strain, we used traditional routine tests based on the determination of key phenotypic features: cell morphology and Gram staining, cytochrome oxidase and catalase production, hydrolysis of sodium hippurate and indoxyl acetate, as well as MALDI-TOF mass spectrometry (“Bruker Daltonik MALDI Biotyper”).

The nutrient medium for bacteriologic mass production during antigen production to produce campylobacteriosis agglutinating monospecific serum was 0.15% semi-liquid liver agar (SLA) with the addition of 10% enzymatic hydrolysine. To prepare the nutrient medium, 1 part of meat water, 1 part of liver broth, 2 parts of distilled water were used, 1% peptone, 0.5% sodium chloride and 0.15% agar were added, then 10% enzymatic hydrolysine was added, the concentration of hydrogen ions should be 7.0-7.2. Autoclaved at 1 atm for 30 min.

The agglutination reaction was performed according to the generally accepted method using factory-made monospecific agglutinating campylobacteriosis sera of types I, II and III and somatic O and -OH-antigens (somatic and whole-cell campylobacteriosis antigens) in triplicate [11, p. 81].

Results.

Identification of the strain. The conducted studies have established the conformity of Campylobacter fetus fetus strain B-0115 KazNIVI to Campylobacter fetus subsp. fetus (Cff) subspecies, proved that when

typing with diagnostic monospecific agglutinating sera of I, II, III types has a positive reaction with serum type II and negative reaction with sera types I and III.

Preparation of a sensitive antigen for diagnosis of bovine campylobacteriosis in vitro agglutination reaction and determination of its sensitivity. Somatic antigen was obtained by using bacterial culture of Campylobacter fetus fetus strain B-0115 KazNIVI, which was grown in test tubes on 0.15% semi-liquid meat-peptone liver agar (SLMPLA) with the addition of 10% enzymatic hydrolysine, in the thermostat at 370C in exicators under microaerophilic conditions for 2 days, then in order to accumulate bacterial mass the grown culture was transferred into vials with 0.15% semi-liquid meat-peptone liver agar (SLMPLA) with addition of 10% enzymatic hydrolysine, after two days of cultivation were checked for pure growth and dispersed into Tartakovsky flasks with 2.5-3.0% meat-peptone liver agar, after 2-3 days of cultivation the pure growth of the culture was washed off with sterile physiological solution, then the bacterial mass was boiled for 60 min at 100^0C and washed twice on centrifuge at 3000 rpm, suspended in 0.9% sterile physiological solution to 1 billion concentration according to the optical turbidity standard and used for agglutination reaction. Campylobacteriosis antigens of types I, II, III were obtained, agglutination reaction using the above antigens was performed according to the generally accepted methodology in tubes in the volume of 1.0 cm^3.

6 rows (2 rows of each serum) of serial dilutions of factory-made monospecific agglutinating campylobacteriosis sera of I, II, III types in 3% sodium chloride solution were prepared: 1:50; 1:100; 1:200; 1:400; 1:800; 1:1600; 1:3200; 1:6400. Then 0.5 cm^3 of somatic O-antigens under study were added to the first three rows of sera, and the common whole-cell OH-antigen was added to the remaining tubes for comparison and placed in the thermostat for 16-18 hours; 3-4 hours after removal from the thermostat the reaction results were taken into account. Normal rabbit serum with factory whole-cell antigen type 1 served as reaction controls, in addition, control for self-agglutination of antigens was carried out.

In this case, the results of the reaction were recognized as specific, if there was no agglutination in the control tubes. The serum dilution, where agglutination was observed not lower than two crosses, was considered as the limit titer.

The results of the whole-cell and somatic antigen assays are presented in Table 1.

Table 1 – Agglutination reaction results

№	S/Ag	Fetus WC	Fetus S	Venerealis (control) WC
1	I	-	-	3200
2	II	3200	1600	-
3	III	-	-	-
4	Normal rabbit	-	-	-

Note: S-serum, Ag-antigen, whole-cell, S-somatic.

Table 1 shows that agglutination of somatic O-antigens is observed in lower titers compared to whole-cell OH-antigens, which indicates the specificity of somatic antigens for each subspecies of Campylobacter and confirms the belonging of cultures to one or another type.

The influence of storage time on biological properties of campylobacteriosis antigens and monospecific agglutinating sera of types I, II, III at 2-5 °C was studied in laboratory conditions and their production tests were carried out.

According to the results of agglutination reaction, 12 months after manufacture in laboratory conditions antigens remained specific and active. Control for self-agglutination of antigens in all experiments gave a negative result.

Thus, on the basis of the conducted researches the strain Campylobacter fetus fetus B-0115 of KazNIVI, characteristic for subspecies fetus, the technology of production of sensitive antigen from it is developed and it is scientifically proved that the use of this somatic antigen will allow to carry out diagnostics of campylobacteriosis of cattle in test tube agglutination reaction.

Discussion. The problem of campylobacteriosis (CB) has been widely covered in domestic and foreign literature in the last 10-20 years. The interest in this topic is probably due to a number of reasons. From the microbiological point of view, the causative agent of CB was discovered relatively recently and is an actively studied microorganism. At the same time, the responsibility of the microbe for the development of diseases in domestic animals and birds puts it in the category of problems of veterinary pathology. In medical terms, CB, taking into account modern epidemiologic studies, is classified in the group of the most common bacterial intestinal infections in children of different age groups, as it causes from 5 to 44.9% of all cases of disease.

Unfortunately, despite the wide geographic distribution and intensive circulation of CB among humans and animals, practicing physicians do not often diagnose this disease. This is due to the fact that laboratory diagnosis of this infection requires special modified media and conditions for cultivation; the instability of the

pathogen in the environment makes it difficult to perform even such simple methods as bacterioscopy of native smears. However, in the classical course of the disease, generalized forms are extremely rare. This may be one of the reasons for misdiagnosis, due to which the patient does not receive appropriate etiotropic therapy.

Campylobacter fetus includes three subspecies. Two of them, Campylobacter fetus subsp. fetus (Cff) and Campylobacter fetus subsp. venerealis (Cfv), are highly relevant veterinary pathogens usually associated with mammals. A third species, Campylobacter fetus subsp. testudium (Cft), is mainly associated with reptiles and shows clear genetic divergence with C. other causative agents of acute intestinal infections (AII) of bacterial nature, thermophilic Campylobacter spp. are among the most difficult microorganisms to culture, as they require special conditions and equipment. In the laboratory diagnosis of campylobacteriosis is a particularly difficult task is the isolation of pure culture of the pathogen from samples of feces and other objects due to their massive associated microbial contamination. In this regard, the isolation of field isolate, study of all its properties, identification and obtaining a sensitive somatic antigen for use of in vitro agglutination reaction is an urgent task.

By the conducted researches it was established that the Campylobacter fetus fetus strain B-0115 of KazNIVI, isolated by us from the aborted fetus of a sheep belonging to “Baiganinsky” farm of Aktobe region, corresponds to Campylobacter fetus subsp. fetus (Cff), it was proved that at typing with diagnostic monospecific agglutinating agglutinating sera of types I, II, III it has positive reaction with serum of type II and negative reaction with sera of types I and III.

Earlier, a method for obtaining whole-cell antigen for agglutination reaction in the diagnosis of animal campylobacteriosis was developed, consisting of growing campylobacteria on semi-liquid nutrient medium, washing with 0.3% formalized physiological solution, washing and suspending campylobacteria in 3% formalized physiological solution [12, p. 17]. The disadvantage of this method was the presence of a multitude of thermolabile antigens in Campylobacter and, consequently, the impossibility of intertype clear differentiation using whole-cell typing.

Taking into account this fact, we have developed a method of obtaining somatic antigen from Campylobacter fetus fetus strain B-0115 of KazNIVI, based on boiling of Campylobacter bacterial mass obtained by the original method for 60 min at 100^0C.

In vitro agglutination reaction using the antigen obtained by us, agglutination of somatic O-antigens is observed in lower titers compared to whole-cell OH-antigens, which indicates the specificity of somatic antigens for each subspecies of Campylobacter and confirms the belonging of cultures to one or another type.

The influence of storage time on the biological properties of Campylobacteriosis antigens and monospecific agglutinating sera of types I, II, III was studied in laboratory conditions and their production tests were carried out. According to the results of the agglutination reaction, the antigens remained specific and active 12 months after manufacture under laboratory conditions. The control for self-agglutination of antigens in all experiments gave a negative result.

Thus, on the basis of conducted researches the strain Campylobacter fetus fetus B-0115 of KazNIVI, characteristic for subspecies fetus, the technology of manufacturing of sensitive antigen from it is developed and it is scientifically proved that the use of this somatic antigen will allow to carry out diagnostics of campylobacteriosis of cattle in test tube agglutination reaction.

Conclusion. Despite the fact that a large number of scientific works are devoted to campylobacteriosis pathology, there is still no domestic test for rapid and reliable diagnosis of campylobacteriosis of farm animals. By the conducted researches it has been established that Campylobacter fetus fetus strain B-0115 of KazNIVI, isolated from aborted fetus of sheep belonging to PC “Baiganinsky” of Aktobe region, corresponds to Campylobacter fetus subsp. fetus (Cff), it was proved that when typing with diagnostic monospecific agglutinating sera of types I, II, III it has a positive reaction with serum of type II and negative reaction with sera of types I and III. The method of obtaining somatic antigen from Campylobacter fetus fetus strain B-0115 KazNIVI was developed, based on boiling of Campylobacter bacterial mass obtained by the original method for 60 min at 100^0C.

In vitro agglutination reaction using the antigen obtained by us, agglutination of somatic O-antigens is observed in lower titers compared to whole-cell OH-antigens, which indicates the specificity of somatic antigens for each subspecies of Campylobacter and confirms the belonging of cultures to one or another type.

On the basis of the conducted researches the strain Campylobacter fetus fetus B-0115 of KazNIVI, characteristic for subspecies fetus, is allocated, the technology of manufacturing of sensitive antigen from it is developed and it is scientifically proved that the use of the given somatic antigen will allow to carry out diagnostics of campylobacteriosis of cattle in test tube agglutination reaction.

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АНАЛИЗ РИСКОВ ЗАГРЯЗНЕННОСТИ ПРОДУКТОВ ЖИВОТНОГО ПРОИСХОЖДЕНИЯ ЭНТЕРОПАТОГЕННЫМИ МИКРООРГАНИЗМАМИ В КОСТАНАЙСКОЙ ОБЛАСТИ

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Обеспечение безопасности пищевых продуктов животного происхождения является одной из приоритетных задач пищевой промышленности и здравоохранения, особенно в Костанайской области, где активно развивается сельское хозяйство и переработка продуктов животноводства. Загрязнение мясных и молочных продуктов энтеропатогенными микроорганизмами, такими как Salmonella enterica, Staphylococcus aureus и Escherichia coli, представляет собой серьезную опасность для здоровья населения.

Целью исследований является выявление и анализ степени загрязнения мясной и молочной продукции энтеропатогенными бактериями, определение факторов, способствующих их распространению.

В ходе исследований использовались бактериологические и биохимические методы анализа.

Материалом исследований были отобранные в розничных торговых точках 413 образца продуктов животного происхождения.

Основные результаты исследований показали, что в 413 пробах животноводческой продукции выявлено 108 (26%) бактериальных изолятов: E. coli – 69 (63,8%), S. aureus – 36 (33,3%), S. enterica – 3 (2,77%). Наибольшее загрязнение выявлено в мясе птицы (27%), сыром молоке (21,2%), полуфабрикатах (19,4%) и молочных продуктах (14,8%).

Полученные результаты исследований указывают на необходимость соблюдения санитарно-гигиенических норм, улучшения условий хранения и переработки продукции, внедрения дополнительных мер контроля на всех этапах пищевой цепи.

Ключевые слова: *пищевая токсикоинфекция, энтеропатогенные микроорганизмы, продукты животного происхождения, пищевая безопасность, S. enterica, S. aureus, E. coli.*