

IRSTI 68.41.63

UDC 619:616.851.31

<https://doi.org/10.52269/RWEP25215>

PRODUCTION OF CAMPYLOBACTERIOSIS AGGLUTINATING MONOSPECIFIC SERUM

Bizhanov A.B. – Doctor of Veterinary Sciences, Professor, Chief Researcher, Department of bacteriology, Kazakh Scientific Research Veterinary Institute LLP, Almaty, Republic of Kazakhstan.*

Sembina F.Y. – Candidate of Veterinary Sciences, Leading Researcher, Department of bacteriology, Kazakh Scientific Research Veterinary Institute LLP, Almaty, Republic of Kazakhstan.

Baramova Sh.A. – Doctor of Biological Sciences, Professor, Chief Researcher, Department of bacteriology, Kazakh Scientific Research Veterinary Institute LLP, Almaty, Republic of Kazakhstan.

Kassenov M.M. – Candidate of Veterinary Sciences, Professor, Director General, Kazakh Scientific Research Veterinary Institute LLP, Almaty, Republic of Kazakhstan.

The article presents the results of experiments conducted to obtain campylobacteriosis agglutinating monospecific serum. Based on the conducted study, the Campylobacter fetus fetus B-0115 strain of KazSRVI was isolated from an aborted ovine fetus. This strain exhibits characteristic cultural, morphological, biochemical, and antigenic properties; previous research substantiated that this strain can be effectively used to produce monospecific agglutinating sera for the serological diagnosis of campylobacteriosis via agglutination reaction. High pathogenicity and virulence of this strain were established.

On the basis of the developed scheme of immunization and use of immunostimulant the antigenic load during immunization was significantly reduced and antibody titers of specific sera were increased due to increased antibody formation in the animals-producers. The activity of hyperimmune sera obtained using the immunostimulant was 1:1600-1:3200. The sera were strictly specific – they gave negative agglutination reaction with heterologous antigens. The effect of storage duration on the biological properties of campylobacteriosis antigens and monospecific agglutinating sera of types I, II, and III was investigated, and production testing of these preparations was conducted.

Based on the agglutination reaction results, the antigens retained their specificity and activity for up to 12 months post-manufacture under laboratory conditions. In all experiments, antigen self-agglutination controls yielded negative results.

On the basis of the Campylobacter fetus fetus B-0115 strain of KazSRVI and application of the developed immunization scheme, an active and highly specific campylobacteriosis monospecific agglutinating serum for the diagnosis of campylobacteriosis in the agglutination reaction was obtained.

Key words: agglutination, campylobacteriosis, immunization, monospecific serum.

КАМПИЛОБАКТЕРИЯЛЫҚ АГГЛЮТИНАЦИЯЛАЙТЫН МОНОСПЕЦИФИКАЛЫҚ САРЫСУ АЛУ

Бижанов А.Б. – ветеринария ғылымдарының докторы, профессор, бактериология бөлімінің бас ғылыми қызметкері, «Қазақ ғылыми-зерттеу ветеринария институты» ЖШС, Алматы қ., Қазақстан Республикасы.*

Сембина Ф.Е. – ветеринария ғылымдарының кандидаты, бактериология бөлімінің жетекші ғылыми қызметкері, «Қазақ ғылыми-зерттеу ветеринария институты» ЖШС, Алматы қ., Қазақстан Республикасы.

Барамова Ш.А. – биология ғылымдарының докторы, профессор, бактериология бөлімінің бас ғылыми қызметкері, «Қазақ ғылыми-зерттеу ветеринария институты» ЖШС, Алматы қ., Қазақстан Республикасы.

Касенов М.М. – ветеринария ғылымдарының кандидаты, профессор, Бас директор, «Қазақ ғылыми-зерттеу ветеринария институты» ЖШС, Алматы қ., Қазақстан Республикасы.

Мақалада Campylobacter агглютинациялайтын моноспецификалық сарысуды алу бойынша жүргізілген тәжірибелердің нәтижелері берілген. Жүргізілген зерттеулер негізінде түсік түсірілген қой ұрығынан Campylobacter fetus fetus B-0115 ҚазФЗВИ штаммы бөлініп алынды, оның әдеттегі культуралды-морфологиялық, биохимиялық, антигендік қасиеттері бар және бұл штаммды қолдану агглютинация реакциясында кампилобактериозды диагностикалау үшін кампилобактериоз агглютинациялайтын моноспецификалық сарысуды алуға мүмкіндік беретіні ғылыми дәлелденген. Бұл штаммның жоғары патогенділігі мен вируленттілігі анықталды.

Жасалған иммундау схемасы және иммуностимуляторды қолдану негізінде иммундау кезінде антигендік жүктеме айтарлықтай төмендеді және продуцент-жануарларда антидене түзілуінің артуы нәтижесінде спецификалық сарысулардың антидене титрлері жоғарылады.

Иммуностимуляторды қолдану арқылы алынған гипериммунды сарысулардың белсенділігі 1:1600-1:3200 болды. Сарысу қатаң спецификалық болып шықты – олар гетерологиялық антигендермен теріс агглютинация реакциясын берді. Сақтау мерзімінің кампилобактер антигендері мен I, II, III типті моноспецификалық агглютинациялаушы сарысулардың биологиялық қасиеттеріне әсері зерттеліп, олардың өндірістік сынақтары жүргізілді.

Агглютинация реакциясының нәтижелері бойынша зертханада өндірілгеннен кейін 12 айдан кейін антигендер ерекше және белсенді болып қалды. Барлық эксперименттерде антигендердің өздігінен агглютинациясын бақылау теріс нәтиже берді.

Campylobacter fetus fetus B-0115 ҚазҒЗВИ штаммы және әзірленген иммундау схемасын қолдану негізінде агглютинация реакциясында кампилобактериозды диагностикалау үшін белсенді және жоғары спецификалық кампилобактериоздың моноспецификалық агглютинациялаушы сарысуы алынды.

Түйінді сөздер: агглютинация, иммунизация, кампилобактериоз, моноспецификалық сарысу.

ПОЛУЧЕНИЕ КАМПИЛОБАКТЕРИОЗНОЙ АГГЛЮТИНИРУЮЩЕЙ МОНОСПЕЦИФИЧЕСКОЙ СЫВОРОТКИ

Бижанов А.Б.* – доктор ветеринарных наук, профессор, главный научный сотрудник отдела бактериологии, ТОО «Казахский научно-исследовательский ветеринарный институт», г. Алматы, Республика Казахстан.

Сембина Ф.Е. – кандидат ветеринарных наук, ведущий научный сотрудник отдела бактериологии, ТОО «Казахский научно-исследовательский ветеринарный институт», г. Алматы, Республика Казахстан.

Барамова Ш.А. – доктор биологических наук, профессор, главный научный сотрудник отдела бактериологии, ТОО «Казахский научно-исследовательский ветеринарный институт», г. Алматы, Республика Казахстан.

Касенов М.М. – кандидат ветеринарных наук, профессор, Генеральный директор, ТОО «Казахский научно-исследовательский ветеринарный институт», г. Алматы, Республика Казахстан.

В статье приведены результаты экспериментов, проведенных с целью получения кампилобактериозной агглютинирующей моноспецифической сыворотки. На основании проведенных исследований из абортрованного плода овцы выделен штамм *Campylobacter fetus fetus* B-0115 КазНИВИ, который обладает типичными культурально-морфологическими, биохимическими, антигенными свойствами, и научно доказано, что использование данного штамма позволит получить кампилобактериозные агглютинирующие моноспецифические сыворотки для диагностики кампилобактериоза в реакции агглютинации. Установлена высокая патогенность и вирулентность данного штамма.

На основе разработанной схемы иммунизации и использования иммуностимулятора значительно снижена антигенная нагрузка при иммунизации и повышены титры антител специфических сывороток за счет увеличения антителообразования у животных-продуцентов. Активность гипериммунных сывороток, полученных с использованием иммуностимулятора, составила 1:1600-1:3200. Сыворотки оказались строго специфичными – дали отрицательную реакцию агглютинации с гетерологичными антигенами. Изучено влияние сроков хранения на биологические свойства кампилобактериозных антигенов и моноспецифических агглютинирующих сывороток I, II, III типов и проведены их производственные испытания.

По результатам реакции агглютинации через 12 месяцев после изготовления в лабораторных условиях антигены оставались специфичными и активными. Контроль на самоагглютинацию антигенов во всех опытах дал отрицательный результат.

На основе штамма *Campylobacter fetus fetus* B-0115 КазНИВИ и применения разработанной схемы иммунизации получена активная и высоко специфичная кампилобактериозная моноспецифическая агглютинирующая сыворотка для диагностики кампилобактериоза в реакции агглютинации.

Ключевые слова: агглютинация, иммунизация, кампилобактериоз, моноспецифическая сыворотка.

Introduction. Campylobacteriosis, also known as vibriosis, is a venereal disease of cattle caused by the microorganism *Campylobacter fetus* subspecies *fetus* (formerly known as *Vibrio fetus* subsp. *venerealis*). Bovine genital campylobacteriosis (CGC) is a sexually transmitted disease (STD) recognized by the World Organization for Animal Health (OIE) as a major cause of early reproductive failure in naturally bred cattle under intensive housing, which places severe restrictions on international trade in animals and animal products. Characteristically, the disease causes infertility in females with increased services required for conception, and occasional late-term abortions are also observed [1, p. 327, 2, p. 1]. Most CGC cases or outbreaks occur following the recent introduction of an infected bull or cow into a susceptible breeding herd.

Often the disease goes undetected until late fall, when the cattle owner discovers that they have several females showing estrus [3, p. 650].

Infected bulls show no clinical signs and may become chronic carriers, whereas in cows, infection is usually self-limited and may cause embryonic death or early fetal loss.

Herds with CGC often experience decreased overall breeding performance, including lower than expected pregnancy rates, increased insemination attempts per conception, and prolonged calving intervals, resulting in significant economic losses in affected herds.

Beyond the reproductive tract, *Campylobacter* spp. are also of concern in public health. Since 2011, numerous outbreaks of campylobacteriosis in the European Union have been caused by the consumption of raw milk from infected dairy cattle [4, p. 1].

Cattle are frequent carriers of *Campylobacter* spp. and therefore these bacteria can be transmitted to humans through meat or milk [5, p. 3]. The entry of *Campylobacter* spp. into raw milk during milking is a most common route due to secondary fecal contamination; however, *Campylobacter* excretion from the udder may also be the cause of milk-borne infection.

A preliminary diagnosis of CGC can be made on the basis of herd history and is often confirmed by laboratory methods [6, p. 650].

C. fetus includes three subspecies. Two of them, *C. fetus* subsp. *fetus* (Cff) and *C. fetus* subsp. *venerealis* (Cfv), are highly relevant veterinary pathogens commonly associated with mammals. A third species, *C. fetus* subsp. *testudium* (Cft), is mainly associated with reptiles and shows clear genetic divergence with *C. fetus* subspecies associated with ruminants [7, p. 807, 8, p. 2945, 9, p. 2006].

Cfv can be found in the gastrointestinal tract of healthy ruminants and, by translocation through the intestinal mucosa, it can enter the bloodstream and colonize the fetus, causing abortions in sheep and sporadic abortions in cattle. Cases of sepsis and/or gastrointestinal disease caused by Cfv have also been reported in immunocompromised humans [10, p. 66, 11, p. 2, 12, p. 22, 13, p. 1].

In recent years, many advances have been made in understanding the immune response that occurs during infection and systemic immunization. Currently, in Kazakhstan, clinical and epizootological, serological and bacteriological methods are used to diagnose campylobacteriosis in animals. Of these, bacteriological method is the main standard, as only the isolation of the causative agent is the basis for the diagnosis of campylobacteriosis [14, p. 616, 15, p. 2, 16, p. 26].

Serologic method of diagnostics for campylobacteriosis includes agglutination reaction with vaginal mucus (RAVS) and fluorescent microscopy of smears. Serum agglutination reaction (RA) is also widely used, with RAVS being used only in the diagnosis of campylobacteriosis in cattle, and luminescent smear microscopy applicable to both cattle and sheep.

In this regard, obtaining campylobacteriosis agglutinating monospecific serum for the diagnosis of this infection is relevant.

The research purpose of this study is to develop campylobacteriosis agglutinating monospecific serum used further for agglutination reaction.

Research objectives:

1. To isolate and identify the *C. fetus fetus* strain.
2. To develop campylobacteriosis agglutinating monospecific serum from *C. fetus fetus* using the B-0115 KazSRVI (Kazakh Scientific Research Veterinary Institute) strain.

Materials and methods. The research of the production of campylobacteriosis agglutinating monospecific serum was conducted at the Kazakh Scientific Research Veterinary Institute (KazSRVI).

Bacterial strain. The *C. fetus fetus* strain B-0115 KazSRVI, deposited in the collection of KazSRVI for the study of the gene pool of microorganisms, was used as starting material. This strain was isolated from the aborted fetus of a sheep belonging to "Baiganinskiy" farm located in the Aktobe region.

The strain was identified by morphological, cultural and physiological-biochemical properties, according to Bergey's Manual of Systematic Bacteriology [17, p. 1147].

The strain selection, morphological features, culture properties, biochemical properties, antigenic structure, marker features, virulent and pathogenic properties, serological and immunochemical properties were performed according to generally accepted methods.

Serum production. Campylobacteriosis agglutinating monospecific serum was obtained by intravenous fourfold immunization of rabbits with antigen from the *C. fetus fetus* strain B-0115 KazSRVI at a concentration of 10 billion microbial bodies (bn m.b.) per dose, with an interval of 5 days in increasing doses from 0.5 cm³ to 2.0 cm³. Simultaneously, intramuscular injection of thymalin was administered at a dose of 5.0 mg, with further total exsanguination of rabbits on the 8th day after the last immunization, separation of serum and taking the target product in titers ranging from 1:1600 to 1:3200.

Ethical approval and experimental animals. All procedures involving animals were approved by the Ethical Commission of KazSRVI.

The experiments involved the following animal models: white laboratory mice (n = 20), weighing 18–20 g; guinea pigs (n = 15), weighing 0.7–0.8 kg; rabbits (n = 10), weighing ~2.0 kg; and female swine-sheep hybrids (n = 10), weighing 58–60 kg.

Results

Strain selection. Brain tissue of an aborted fetus was seeded with a Pasteur pipette into semi-liquid agar (SLA) with the addition of enzymatic hydrolysine. The tissue was then cultured under microaerophilic conditions (exicator) in the thermostat at 37°C for 36–48 hours. Following incubation, at the end of the specified period, characteristic growth appeared in the form of grayish-white disk under the agar surface and slight turbidity along the course of the medium column, indicating the presence of post-renal microflora. Further, purification was carried out using the Pasteur pipette method, designed to utilize the high mobility of *Campylobacter* spp., their accumulation and growth under the agar surface. For this purpose, SLA was introduced into Pasteur pipettes in an amount so that the column height was at least 10–12 cm.

Seeding of the test material was carried out by suction through a narrow part of a Pasteur pipette and after 36–48 hours of cultivation. Growth in the form of a thin disk was detected under the surface of the medium column, which was transferred to regular test tubes for further growth and to Petri dishes with meat-peptone liver agar to isolate individual colonies of *Campylobacter* transparent gray color in S-form. If the culture contained a large amount of mobile extraneous microflora, it was purified by intraperitoneal injection of a suspension of mixed two-day culture in the amount of 1.0–1.5 cm³ into guinea pigs, followed by their slaughter after 5–10 min and sowing of blood from the heart in SLA in 7–8 tubes with the addition of 10% enzymatic hydrolysine. After isolation of pure *Campylobacter* cultures, further study and identification were carried out.

As a result of these studies, an isolate designated *C. fetus fetus* B-0115 of KazSRVI was isolated and deposited in the collection of the laboratory for the study of the gene pool of microorganisms of KazSRVI.

Morphologic features. The strain *C. fetus fetus* B-0115 KazSRVI morphologically had the features of a curved bacillus in the form of a “flying gull”, 1–10 µm in length and 0.2–0.8 µm in width. *Campylobacter* was immobile, with monoracially arranged flagella, and spores and capsules were not formed.

Culture properties. They grew on semi-liquid agar under microaerophilic conditions, at an optimal growth temperature of 37°C and pH 7.0–7.2. Colonies appeared as a disk under the agar surface on SLA and as translucent, grayish, round, and convex colonies on MPLA.

Biochemical properties. *C. fetus fetus* B-0115 KazSRVI secreted catalase, did not form hydrogen sulfide, grew on media with the addition of 1% glycine, 8% glucose, 10% bile. But it did not grow on media with the addition of 3.5% chloride, did not secrete indole, ammonia, did not liquefy gelatin, did not curdle milk, convert nitrates into nitrites. The culture did not ferment carbohydrates (glucose, lactose, sucrose, maltose, mannitol), did not grow on SLA at 25°C.

Antigenic structure. *C. fetus fetus* B-0115 KazSRVI has two main antigenic complexes: O – antigen (somatic) is thermostable, H – antigen (flagellar) of protein nature and thermolabile.

Marker features. *C. fetus fetus* B-0115 KazSRVI, physiologically – chemoheterotroph, is a microaerophile. It grows on media with the addition of glycine and possesses the ability to dissociate from S- to R-form, acquisition of drug resistance.

Pathogenic properties. KazSRVI strain *C. fetus fetus* B-0115 had pathogenic properties for white mice weighing 16–18 g, causing the death of all 10 mice by subcutaneous injection of daily broth culture at a dose of 0.4 cm³.

By subcutaneous injection of 1–5 m.b./cm³, this strain caused abortions in all 10 bitter ewe lambs.

Virulent properties. The strain *C. fetus fetus* B-0115 KazSRVI possessed pronounced virulence for 10 white mice, weighing 16–18 g, causing mortality of 100% of animals at subcutaneous injection of 250,000 m.b./1 cm³ at a dose of 0.4 cm³ on 2–3 days.

Serologic properties. Serological typing of *Campylobacter* cultures was carried out in RA with biofactory antigen type 1 and antigens of types 2 and 3 produced in laboratory conditions and monospecific campylobacteriosis sera (serovars I, II, III).

Campylobacteriosis antigens of types I, II, III were obtained; in order to control the produced antigens for type specificity and activity, control (normal) and type-specific campylobacteriosis sera of three types were obtained by immunization of rabbits according to the generally accepted and developed immunization scheme with whole-cell antigens and immunostimulant. The immunization scheme is presented in Table 1.

Table 1 – Scheme of immunization of rabbits

№ injections	Interval between injection	Generally accepted		Proposed	
		Amount of antigen injected (ml)		Antigen (ml)	Immunocor-rection (mg in 1 ml)
		intra-venous	sub-dermally	intravenously	in/muscular
1	-	0,5	0,5	0,5	5
2	5	1,0	1,0	1,0	5
3	5	1,5	1,5	1,5	5
4	5	2,0	2,0	2,0	5

Table 1 shows that in our proposed immunization scheme the amount of administered antigen is reduced by half. The use of immunostimulant significantly reduced the antigenic load during immunization and increased antibody titers of specific sera due to increased antibody formation in the animals-producers.

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

According to the results of agglutination reaction, 12 months after production in laboratory conditions antigens remained specific and active. Control for self-agglutination of antigens in all experiments gave a negative result. The activity of hyperimmune sera obtained with the use of immunostimulant was 1:1600–1:3200. The sera were strictly specific – they gave a negative agglutination reaction with heterologous antigens.

In Akmola regional branch of RWL (Kokshetau) and in Karaganda regional branch of RWL (Karaganda), blood sera from aborted cows and sheep in the amount of 20 and 30 samples, respectively, were tested for campylobacteriosis in agglutination reaction (RA) using tested antigens of three types.

As a result, campylobacteriosis was not detected in the tested blood samples, the tested antigens were specific and active – they gave a positive reaction with homologous monospecific agglutinating sera against campylobacter in titer 1:800 and negative reaction with heterologous monospecific agglutinating sera against Campylobacter, normal rabbit serum; they did not self-agglutinate with 0.3% formalized 3% sodium chloride solution.

Immunochemical properties. The activity of hyperimmune sera obtained using the immunostimulant was 1:1600–1:3200.

Thus, on the basis of these studies, *C. fetus fetus* strain B-0115 KazSRVI was isolated, characteristic of subspecies *fetus*, its morphological features, culture properties, biochemical properties, antigenic structure, and marker features were studied, virulent and pathogenic properties, serological and immunochemical properties and scientifically proved that the use of this strain will allow to obtain campylobacteriosis agglutinating monospecific sera for diagnostics of campylobacteriosis in agglutination reaction.

Discussion. *Campylobacter jejuni* infection is one of the most common infectious diseases of the last century. Over the past decade, the incidence and prevalence of campylobacteriosis has increased in both developed and developing countries. The dramatic increase in incidence in North America, Europe and Australia is alarming, and data from parts of Africa, Asia and the Middle East indicate that campylobacteriosis is endemic in these regions, especially among children. In addition to *C. jejuni*, the clinical significance of new Campylobacter species, including *C. concisus* and *C. ureolyticus*, is increasingly recognized. Poultry is a major reservoir and source of transmission of campylobacteriosis to humans. Other risk factors include consumption of animal products and water, contact with animals and international travel. Strategic implementation of multilateral biocontrol measures to reduce transmission of this group of pathogens is of paramount public health importance. Overall, campylobacteriosis remains one of the most important infectious diseases that is likely to challenge global public health in the coming years.

C. fetus comprises three subspecies. Two of them, *C. fetus* subsp. *fetus* (Cff) and *C. fetus* subsp. *venerealis* (Cfv), are highly relevant veterinary pathogens commonly associated with mammals. A third species, *C. fetus* subsp. *testudium* (Cft), is mainly associated with reptiles and shows clear genetic divergence with *C. fetus* subspecies associated with ruminants [7, p. 807, 8, p. 2945, 9, p.2006].

The isolation of Campylobacter strains is often hindered by numerous challenges, as campylobacteriosis is a multifactorial disease. The virulence factors essential for the development of infection are typically poorly characterized, and some remain a subject of ongoing debate. The observed differences may be due to the range of host cell lines, reference strains, bacterial growth conditions or differences in experimental design and methodology applied.

Another reason is the lack of an adequate animal model that reproduces the pathologic symptoms of campylobacteriosis diarrhea in humans. A number of animal models have been used for this purpose, including the newborn pig, ferret, genetically modified mice and insects, but no ideal model exists. While the flagellar filaments FlaA and FlaB and the major adhesin CadF are considered reasonably well characterized, the exact role of many other virulence factors, such as CiaB and PEB, remains unclear. Further studies on this subject are needed to investigate the regulation and expression of virulence genes in vitro and in vivo, which will contribute to understanding the role of these factors in the pathogenesis of human and animal campylobacteriosis.

The *C. fetus fetus* B-0115 strain of KazSRVI isolated by us from the aborted fetus of a sheep belonging to the “Baiganinsky” farm of Aktobe region has typical cultural, morphological, biochemical and antigenic properties.

Campylobacteriosis agglutinating monospecific serum was obtained by intravenous fourfold immunization of rabbits with antigen from *C. fetus fetus* B-0115 strain KazSRVI at a concentration of 10 bn m.b., with an interval of 5 days in increasing doses from 0.5 cm³ to 2.0 cm³, with simultaneous intramuscular injection of thymalin in a dose of 5.0 mg, with further total exsanguination of rabbits on the 8th day after the last immunization, separation of serum and taking the target product in titer 1:1600–1:3200.

At the same time, in the immunization scheme proposed by us, the amount of administered antigen is reduced twofold. The use of immunostimulant significantly reduced the antigenic load during immunization and increased antibody titers of specific sera due to increased antibody formation in the animals-producers.

Further serological typing of *Campylobacter* cultures was carried out in RA with biofactory antigen type 1 and antigens of types 2 and 3 produced in laboratory conditions and monospecific campylobacteriosis sera (serovars I, II, III). According to the results of agglutination reaction, 12 months after in vitro manufacture, the antigens remained specific and active. Control for self-agglutination of antigens in all experiments gave a negative result. The activity of hyperimmune sera obtained with the use of immunostimulant was 1:1600–1:3200. The sera were strictly specific – they gave a negative agglutination reaction with heterologous antigens.

Thus, on the basis of the conducted studies, *C. fetus fetus* B-0115 strain KazSRVI, characteristic of subspecies *fetus*, was isolated, its morphological features, culture properties, biochemical properties, antigenic structure, marker features were studied, virulent and pathogenic properties, serological and immunochemical properties and scientifically proved that the use of this strain will allow to obtain campylobacteriosis agglutinating monospecific sera for diagnostics of campylobacteriosis in agglutination reaction.

Conclusion. Bovine campylobacteriosis is widespread in our country and poses a great danger to domestic cattle breeding [18, p.19].

We have isolated *C. fetus fetus* B-0115 strain of KazSRVI, which has typical cultural, morphological, biochemical, antigenic properties, from the aborted fetus of a sheep belonging to Production Company “Baiganinskiy” of Aktobe region. High pathogenicity and virulence of this strain was established.

On the basis of the immunization scheme developed by us and the use of immunostimulant it was possible to significantly reduce the antigenic load during immunization and increase the antibody titers of specific sera due to the increase of antibody formation in the animals-producers. The activity of hyperimmune sera obtained with the use of immunostimulant was 1:1600–1:3200. The sera were strictly specific – they gave negative agglutination reaction with heterologous antigens.

The influence of storage time on the biological properties of campylobacteriosis antigens and monospecific agglutinating sera of types I, II, III was studied 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.

On the basis of *C. fetus fetus* B-0115 strain of KazSRVI and application of the immunization scheme developed by us, an active and highly specific campylobacteriosis monospecific agglutinating serum for the diagnosis of campylobacteriosis in the agglutination reaction was obtained.

Acknowledgements.

The authors are grateful to the management of the Kazakh Scientific Veterinary Research Institute for the opportunity to conduct experiments.

REFERENCES:

1. Hoffer M.A. Bovine Campylobacteriosis: A Review. *The Canadian journal*, 1981, 22(12), pp. 327-330.
2. Pena-Fernández N., Cano-Terriza D., García-Bocanegra I. et al. Campylobacteriosis, associated risk, factors and spatial distribution in spanish beef cattle based on veterinary laboratory database records. *Frontiers in Veterinary Science*, 2021, 8, pp.1-12.
3. Anderson M.L. Infectious causes of bovine abortion during mid- to late-gestation. *Theriogenology*, 2007, 68, pp.474-486.
4. Knipper A.-D., Ghoreishi N., Crease T. Prevalence and concentration of *Campylobacter* in faeces of dairy cows: A systematic review and meta-analysis. *PLoS ONE*, 2022, 17(10), pp.1-20.
5. Kenyon J., Inns T., Aird H., Swift C., Astbury J., Forester E., Decraene V. *Campylobacter* outbreak associated with raw drinking milk, North West England, 2016. *Epidemiol. Infect.*, 2020, 148, pp.13-22.
6. World Organisation for Animal Health (OIE). Manual of Diagnostic Test and Vaccines for Terrestrial Animals: Bovine Genital Campylobacteriosis, 2021, 3.4.4, pp.1223.
7. Oporto B., Hurtado A. Emerging thermotolerant *Campylobacter* species in healthy ruminants and swine. *Foodborne Pathogens and Disease*, 2011, 8(7), pp. 807-813.
8. Fitzgerald C., Tu Z.C., Patrick M. et al. *Campylobacter fetus* subsp. *Testudinum* subsp. nov., isolated from humans and reptiles. *Int. J. Syst. Evol. Microbiol.*, 2014, pp. 2944-2948.
9. Gilbert M. J., Miller W. G., Yee E. et al. Comparative Genomics of *Campylobacter fetus* from Reptiles and Mammals Reveals Divergent Evolution in Host-Associated Lineages. *Genome Biol. Evol.*, 2016, 8(6), pp. 2006-2019.
10. Sprenger H., Zechner E. L., Gorkiewicz G. So close and yet so far-Molecular microbiology of *Campylobacter fetus* subspecies. *Eur. J. Microbiol. Immunol.*, 2012, 2, pp. 66-75.

11. Nadin-Davis S. A., Chmara J., Carrillo C.D. et al. A comparison of fourteen fully characterized mammalian-associated *Campylobacter fetus* isolates suggests that loss of defense mechanisms contribute to high genomic plasticity and subspecies evolution. *Peer J.*, 2021, 9, pp.1-16.
12. Sahin O., Yaeger M., Wu Z., Zhang Q. *Campylobacter* associated diseases in animals. *Annu. Rev. Anim. Biosci.*, 2017, 5, pp. 21-42.
13. Kang H., Thomas R.M. Bacteria and sepsis: microbiome to the rescue?. *J Clin Med.*, 2021, 10(16), pp. 1-9.
14. Özcan N., Bacalan F., Çakır F. et al. Culture and culture-independent diagnostic tests in *Campylobacter enteritis*. *J Infect Dev Ctries.*, 2022, 16(4), pp. 616-621.
15. Okada A., Tsuchida M., Aoyagi K., Yoshino A., Rahman, Md.M., Inoshima, Y. Research Note: Detection of *Campylobacter* spp. in chicken meat using culture methods and quantitative PCR with propidium monoazide. *Poultry Science*, 2023, 102(9), pp.1-4.
16. Kadiyala V., Kulkarni M., Kadiyala P., Yadav A.K. Clinico-bacteriological study and molecular detection of campylobacter in childhood diarrhoea: a cross-sectional study. *Journal of Clinical and Diagnostic Research.*, 2024, pp. 25-29.
17. Brenner D. J., Krieg N.R., Staley J.T., Garrity G.M. Volume two: the protobacteria, part c: the alpha-, beta-, delta- and epsilonbacteria. *Bergey's Manual of Systematic Bacteriology*, Michigan State University, USA, 2005, 2 (2), pp.1147-1160.
18. Zhanserkenova O.O., Luchko M.A. Specificeskaya profilaktika kampilobakterioza krupnogo rogatogo skota. Izuchenie immunogeny'h i antigeny'h svojstv opy'tny'h serij inaktivirovanny'h vacin na krolikah i korovah [Specific prevention of *Campylobacteriosis* in cattle. Research of immunogenic and antigenic properties of experimental series of inactivated vaccines on rabbits and cows]. *Byuleten'*, 1991, 6(75-76), pp. 19-22. (In Russian).

Information about the authors:

*Bizhanov Alim Baizhanovich** – Doctor of Veterinary Sciences, Professor, Chief Researcher, Department of bacteriology, Kazakh Scientific Research Veterinary Institute LLP, Republic of Kazakhstan, 050016, Almaty, 223 Raiymbek Ave., tel.: +7-777-370-12-40, e-mail: alimakyntai@mail.ru.

Sembina Fatima Yegimbayevna – Candidate of Veterinary Sciences, Leading Researcher, Department of bacteriology, Kazakh Scientific Research Veterinary Institute LLP, Republic of Kazakhstan, 050016, Almaty, 223 Raiymbek Ave., tel.: +7-702-787-93-57, e-mail: fatimsem@mail.ru.

Baramova Sholpan Auzarovna – Doctor of Biological Sciences, Professor, Chief Researcher, Department of bacteriology, Kazakh Scientific Research Veterinary Institute LLP, Republic of Kazakhstan, 050016, Almaty, 223 Raiymbek Ave., tel.: +7-771-403-44-59, e-mail: sholbar@mail.ru.

Kassenov Markhabat Melisbekovich – Candidate of Veterinary Sciences, Professor, General Director, Kazakh Scientific Research Veterinary Institute LLP, Republic of Kazakhstan, 050016, Almaty, 223 Raiymbek Ave., tel.: +7-701-585-05-58, e-mail: kassenov_mm@mail.ru.

*Бижанов Алим Байжанович** – ветеринария ғылымдарының докторы, профессор, бактериология бөлімінің бас ғылыми қызметкері, «Қазақ ғылыми-зерттеу ветеринария институты» ЖШС, Қазақстан Республикасы, 050016, Алматы қ., Райымбек даңғ., 223, тел.: +7-777-370-12-40, e-mail: alimakyntai@mail.ru.

Сембина Фатима Егimbaевна – ветеринария ғылымдарының кандидаты, бактериология бөлімінің жетекші ғылыми қызметкері, «Қазақ ғылыми-зерттеу ветеринария институты» ЖШС, Қазақстан Республикасы, 050016, Алматы қ., Райымбек даңғ., 223, тел.: +7-702-787-93-57, e-mail: fatimsem@mail.ru.

Барамова Шолпан Аузаровна – биология ғылымдарының докторы, профессор, бактериология бөлімінің бас ғылыми қызметкері, «Қазақ ғылыми-зерттеу ветеринария институты» ЖШС, Қазақстан Республикасы, 050016, Алматы қ., Райымбек даңғ., 223, тел.: +7-771-403-44-59, e-mail: sholbar@mail.ru.

Касенов Мархабат Мелисбекович – ветеринария ғылымдарының кандидаты, профессор, Бас директор, «Қазақ ғылыми-зерттеу ветеринария институты» ЖШС, Қазақстан Республикасы, 050016, Алматы қ., Райымбек даңғ., 223, тел.: +7-701-585-05-58, e-mail: kassenov_mm@mail.ru.

*Бижанов Алим Байжанович** – доктор ветеринарных наук, профессор, главный научный сотрудник отдела бактериологии, ТОО «Казахский научно-исследовательский ветеринарный институт», Республика Казахстан, 050016, г. Алматы, пр. Райымбека 223, тел.: +7-777-370-12-40, e-mail: alimakyntai@mail.ru.

Сембина Фатима Егimbaевна – кандидат ветеринарных наук, ведущий научный сотрудник отдела бактериологии, ТОО «Казахский научно-исследовательский ветеринарный институт», Республика Казахстан, 050016, г. Алматы, пр. Райымбека 223, тел.: +7-702-787-93-57, e-mail: fatimsem@mail.ru.

Барамова Шолпан Аузаровна – доктор биологических наук, профессор, главный научный сотрудник отдела бактериологии, ТОО «Казахский научно-исследовательский ветеринарный институт», Республика Казахстан, 050016, г. Алматы, пр. Райымбека 223, тел.: +7-771-403-44-59, e-mail: sholbar@mail.ru.

Касенов Мархабат Мелисбекович – кандидат ветеринарных наук, профессор, Генеральный директор, ТОО «Казахский научно-исследовательский ветеринарный институт», Республика Казахстан, 050016, г. Алматы, пр. Райымбека 223, тел.: +7-701-585-05-58, e-mail: kassenov_mm@mail.ru.

IRSTI 68.41.63

UDC 619:616.851.31

<https://doi.org/10.52269/RWEP252112>

PRODUCTION OF ANTIGEN FOR DIAGNOSIS OF BOVINE CAMPYLOBACTERIOSIS USING AGGLUTINATION REACTION

Sembina F.Y. – Candidate of Veterinary Sciences, Leading Researcher, Department of Bacteriology, Kazakh Scientific Research Veterinary Institute LLP, Almaty, Republic of Kazakhstan.

Bizhanov A.B.* – Doctor of Veterinary Sciences, Professor, Chief Researcher, Department of bacteriology, Kazakh Scientific Research Veterinary Institute LLP, Almaty, Republic of Kazakhstan.

Baramova Sh.A. – Doctor of Biological Sciences, Professor, Chief Researcher, Department of bacteriology, Kazakh Scientific Research Veterinary Institute LLP, Almaty, Republic of Kazakhstan.

Kassenov M.M. – Candidate of Veterinary Sciences, Professor, Director General, Kazakh Scientific Research Veterinary Institute LLP, Almaty, Republic of Kazakhstan.

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°C. 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

Сембина Ф.Е. – ветеринария ғылымдарының кандидаты, бактериология бөлімінің жетекші ғылыми қызметкері, «Қазақ ғылыми-зерттеу ветеринария институты» ЖШС, Алматы қ., Қазақстан Республикасы.

Бижанов А.Б.* – ветеринария ғылымдарының докторы, профессор, бактериология бөлімінің бас ғылыми қызметкері, «Қазақ ғылыми-зерттеу ветеринария институты» ЖШС, Алматы қ., Қазақстан Республикасы.

Барамова Ш.А. – биология ғылымдарының докторы, профессор, бактериология бөлімінің бас ғылыми қызметкері, «Қазақ ғылыми-зерттеу ветеринария институты» ЖШС, Алматы қ., Қазақстан Республикасы.

Касенов М.М. – ветеринария ғылымдарының кандидаты, профессор, Бас директор, «Қазақ ғылыми-зерттеу ветеринария институты» ЖШС, Алматы қ., Қазақстан Республикасы.

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.