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DIAGNOSIS OF CATTLE CAMPYLOBACTERIOSIS

Summary. Campylobacteriosis of cattle is one of the most economically important diseases of dairy cattle, leading to a decrease in the production of offspring, embryo death and abortion.

Data from literature sources showed that 55-80% of human campylobacteriosis cases are related to the consumption of products derived from poultry, as well as large and small cattle 20 - 40%.

Polymorphism of clinical manifestations in animals and humans does not allow to diagnose "campylobacteriosis" without laboratory confirmation. Microbiological diagnosis of campylobacteriosis is a time-consuming, multi-cost procedure, which is associated with the biological properties of the pathogen.

The lack of standards for the diagnosis of campylobacteriosis in the CIS has led to a distortion of the real picture of the prevalence of campylobacteriosis.

According to the OIE recommendations, along with bacteriological methods for the detection and differentiation of campylobacteria, molecular genetic methods, i.e. PCR, and modern modification of PCR with real-time detection can be used

This article reflects the improvement of the method for the isolation of DNA from clinical material with high analytical characteristics. As a result of researches Campylobacter fetus subspecies venerealis with the help of PCR in real time in the rate of cattle of the dairy direction are identified. In metagenomic studies found that the circulation of pathogenic strains of Campylobacter is associated with the metric in cows can also be important for the development of metritis in cows after calving.

The introduction of real-time PCR can improve the diagnosis of campylobacteriosis and improve the quality control of livestock products, which will have a favorable economic and social effect.

Abstract. As a result of epizootological monitoring, it was found that among the livestock of cattle campylobacteriosis is represented in the nosological profile of infectious diseases. The analysis of comparative diagnosis of campylobacteriosis of cattle microbiological and serological studies (ELISA) is presented. The real-time PCR study additionally revealed 5 heads of cattle positively reacting to campylobacteriosis, which had not previously been detected by culture isolation.

Keywords: campylobacteriosis, campylobacter fetus subspecies venerialis PCR mode rail time, cattle.

Introduction. Campylobacteriosis is an infectious disease of animals and humans caused by pathogens of the genus Campylobacter, characterized by varying degrees of severity and polymorphism of manifestations.

The genus Campylobacter includes several species that cause campylobacteriosis in animals, humans and birds, with predominant tropism to the gastrointestinal tract and reproductive system.

Campylobacteriosis is characterized by varying degrees of severity of the disease and polymorphism of clinical manifestations. Campylobacteria cause significant damage to agriculture, associated with a decrease in animal productivity, the cost of diagnostic studies and anti-epizootic measures.

The most pathogenic to ruminants are C. fetus subspecies fetus and C. fetus subspecies veneralis.

C. fetus subspecies veneralis is classified as a causative agent of genital campylobacteriosis (vibriosis - BVS) in cattle. In cows, it colonizes the epithelial cells of the vagina, cervical canal, uterus, uterine horns, and in bulls it is localized mainly in the prepucial cavity [1]. Clinical manifestation is characterized by inflammation of the reproductive system that causes abortions in 10% of cases, frequent iregular and infertility in 10% of cases [2, 3]. Private asymptomatic carriage and lifelong persistence are the main factors of transmission [4]. The disease is common in regions with extensive animal husbandry. There is a different level of infection of cattle, in some countries the infection reaches 29% [5].

C. fetus subspecies fetus is an obligate microflora of the gastrointestinal tract of ruminants, but can also cause sporadic abortions [6].

Campylobacteriosis of cattle belongs to list B, subject to registration of diseases of the international epizootic Bureau (OIE), consisting of diseases that have socio-economic significance (McMillen. L. et al., 2006).

Epidemiological studies have shown that 55-80% of human campylobacteriosis cases are associated with the consumption of products derived from poultry, as well as large and small cattle 20-40% [7, 8].

In carrying out activities related to the prevention and elimination of campylobacteriosis, much attention is paid to the diagnosis.

Currently, for the diagnosis of Campylobacter infection, there are methods - bacteriological, serological-enzyme immunoassay (ELISA) to detect antibodies, molecular genetic methods, i.e. PCR, and modern modification of PCR with real-time detection, DNA fingerprinting [9].

The classical bacteriological diagnosis of campylobacteriosis is difficult, since campylobacteria are microaerophiles, so their growth requires the creation of conditions with a high content of carbon dioxide, and, as a rule, requires the acquisition of expensive selective media (iron - etheric blood agar, Muller-Hinton medium, brucellosis agar, etc.).

Materials and methods. Research for the diagnosis of campylobacteriosis of cattle and identification of isolated cultures of Campylobacter was carried out in the laboratory of Green biotechnology and cellular engineering of the Kazakh-Japanese innovation center of the Kazakh national agrarian university.

For bacteriological, serological studies and highlight the culture of the causative agent of campylobacteriosis, samples were taken samples from cows – vaginal mucus, blood serum, aborted fruits (stomach, liver with gall bladder, lung, intestinal contents), pieces of the placenta – not later than the day after the abortion; the mucus from the cervix – the first time 3-4 days after abortion; from the bulls – prepucialna slime, a secret accessory genital glands and sperm.

For selective isolation of thermolerant campylobacters prepared agar Preston with a special modified additive (Preston, FD042, which contains-polymyxin sulfate, rifampicin, trimethoprim lactate, amphotericin B). Cultivation was carried out in microaerophilic conditions, on enriched media at 42°C for 2 to 3 hours, then at 37°C for 44 hours.

For transportation and storage of the selected crops, a semi-liquid medium (according to Wang) and a Thioglycolic medium with the addition of 5% defibrinated blood of cattle were used.

Identification of isolated cultures of campylobacteria was carried out by cultural, biochemical, serological and pathogenic properties of the pathogen.

Two collection strains were used as positive control - C. fetus subsp. venerealis No. 6829, Campylobacter jejuni subsp. jejuni № 70.2 t.

When setting ELISA used stages: the choice of antigen and its sensitizing dose, time and temperature of fixation of the antigen on the carrier, the choice of the pH of the buffer, the choice of the optimum conjugate dilutions, determination of optical limit for the determination of the titer and the establishment of a diagnostic titer. In the formulation of ELISA, an antigen destroyed by low-frequency ultrasound was used.

DNA isolation from Campylobacter cultures was carried out by phenol-chloroform extraction with modification, a set of "Cell and Tissue DNA Kit" with the help of an automatic isolation station KingFisher NK, a set of Purelink® Genomic DNA Mini Kit with modification, temperature change. Prepared solutions for phenol-chloroform method. 10% SDS-200 mGy SDS dissolved in 2 ml deionized water, 0.1 M Tris-1 ml 1M Tris diluted with 9 ml deionized water, phenol-chloroform p – 3 ml chloroform added 3 ml phenol.

The quality of the isolated DNA was assessed by quantitative determination of its concentration by spectrophotometric method using nanodrop 2000 spectrophotometer. The method is based on the existence of DNA maximum absorption at a wavelength of 260 nm. This means that in nucleic acid solutions the maximum photometric absorption is observed at 260 nm and directly correlates with the DNA concentration.

The quality of the isolated DNA was also analyzed by separating DNA fragments in agarose gel from 0.8-1.5%, depending on the length of the analyzed fragment, in the presence of an intercalating agent – ethidium bromide, which was used to visualize DNA in horizontal electrophoresis.

Real-time PCR setting. Optimization of the conditions for assessing the sensitivity and specificity of PCR protocols in real time was carried out using the device Real Time (StepOnePlus, Applied Biosystems, USA).

Selection and verification of specific primers were carried out using the Primer Select (DNASTAR) and BioEdit programs and the PrimerBlast web resource (NCBI). On primers were taken into account the main parameters: close annealing temperature of forward and reverse primer, primer length from 18 to 25 PN, a low probability of formation of secondary structures.

Based on the results of optimization, a Protocol of species identification of C. fetus, C. jejuni was developed by PCR in real time, which includes the optimal composition of the reaction mixture and the PCR amplification program. The optimal composition of the reaction mixture: primers of 10 pmol each, fluorescent probe 5 pmol, 10 mm Tris-HCl (pH 8.8 at 25 C), 50 mm KCl, 0.08% nonidet P40, 3 mm MgCl2, dntf at a concentration of 200 nm each, 5 nm Tetramethylammonium chloride, betaine – to a final concentration of 0.2 M, 1.5 units Taq DNA polymerase fermentas). PCR amplification program: long-term denaturation 95 seconds-5 minutes; 42 cycles 95 seconds-15 seconds, 61 seconds (C. fetus and C. jejuni)-15 seconds, 72 settings C-45 seconds. Accounting for the fluorescent signal at the stage of annealing primers.

Research results and discussion of the results. When a pure culture was isolated on selective Preston agar with a special modified additive Preston FD042, campylobacteria formed colonies on the 3rd day: smooth, colorless, diameter 1 mm.the Temperature optimum was 37-38^{0}C, when cultivated on this medium Campylobacter its color did not change. 9 cultures of the genus Campylobacter were isolated. The selected cultures of campylobacteria studied the enzymatic activity. Culture of C. fetus subsp. venerialis isolated catalase, did not form hydrogen sulfide, did not give rise to PJA with 1% glycine, 3,5% sodium chloride, 8% glucose, grow on PJA with 1% bile, do not ferment sugar and alcohol, do not dilute gelatin, do not develop on agar in aerobic conditions.

As a result, ELISA found that the FBI (pH 7.2) provides a stable adsorption of antigen on the surface of the microplate holes. The optimal time of antigen fixation is 18-20 hours, at 40C. In the reaction of ELISA seropositive were 16 heads of cattle, with a diagnostic titer of 1: 400.

In the framework of the studies, the comparative assessment of DNA isolation methods (table), the most effective method for gram-negative cultures has FHE. However, when days were isolated from gram-negative cultures, the greatest yield of days was achieved using the phenol-chloroform method of DNA isolation with modification.

Results of the quantitative evaluation of DNA

Name of samples	Density ng / µl	Absorption 260 nm	Absorption 280 nm	The ratio of 260/280 nm
FHE grammatricity 1	390,35	7,945	3,743	2,05
FHE grammatricity 2	146,10	3,062	1,338	2,07
FHE grammatricity 3	138,10	2,162	1,210	1,91
FHE grammatricity 4	69,15	2,672	1,342	2,31
FHE grammatricity 5	88,10	1,162	0,210	2,22
Modified FHE 1	338,10	5,472	2,242	2,39
Modified FHE 2	147,05	2,954	2,352	2,19
Modified FHE 3	189,85	3,797	0,373	1,02
Modified FHE 4	44,93	1,899	0,398	2,26
Modified FHE 5	99,05	2,707	1,207	2,02

Undoubtedly, the FHE method will give you a good result, with fewer uses in laboratory diagnostics, a hard-working method, and also a traction agent used by reagents. Cell and Tissue DNA Kit, effectively utilizing the DNA Kit, has come to terms with the ability to connect with the incubation of images with lysozyme and to penetrate the multiconductor phase in the new test tubes.

The high concentration of DNA from the prepaid mucus was found in the Purelink® Genomic DNA Mini Kit with modifications.

Taking shape, the effectiveness of the DNA sequence is up to 25% by comparing with the density of the sample. It is important to concentrate on the high density of DNA, as shown by the adsorption value of 260/280.

The spectrophotometric method used for electrophoretic analysis of the DNA results (figure 1).

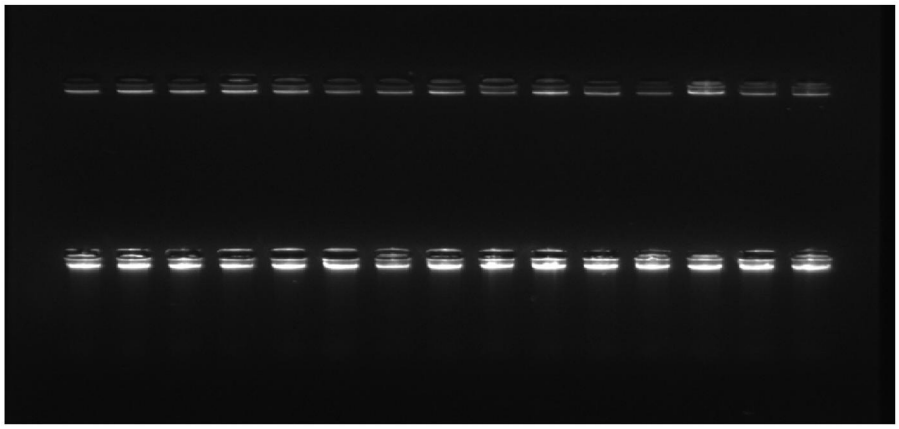

Figure 1 –Electrophoregram DNA Campylobacterium

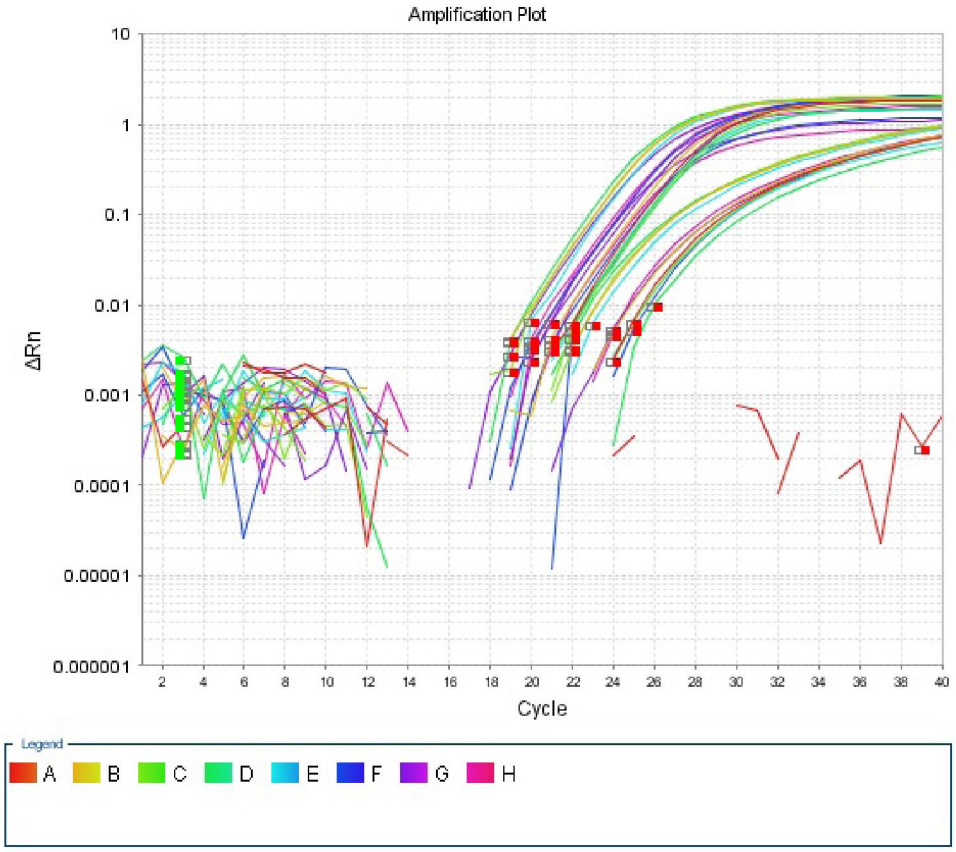

Figure 2 – Graphical image Result of Real - time PCR Diagnostic Campilobacteriosis Acne (FAM - DNA C fetus venerealis)

In consequence, metabolic research of vaginal mucous membranes with clinical triumphs of reproductive organs was established, as Helcococcus, Peptoniphilus, Peptostreptococcus, and Campylobacter bacteria should be able to mitigate the metritation of the meteorite (figure 3).

Metagenomed research has revealed that Campylobacter circulatory pathogenic strains are associated with metritis in the body, and can also be used for the development of metritin in the cockroach.

Figure 3 – Krona chart - nucleotide trace gene
16S bacterial vaginal microbioma of the rRNA of the coriander No. 19

Extraction. At this time, the diagnostics of campilobacteriosis and the detection of contamination of animal products using campilobacteria are using the "gold standard" - the identification and the identification of the pure culture. However, campilobacterium microorganisms, rusting on hardwood platelets, require the highest qualification of the staff. Diagnostics is a distinction in clinical development of campilobacteriosis. All this is why it is not easy to expose the film to the spread of carbohydrates in the infectious diseases of the animal and the human body.

As a result, the experimental laboratory experiments on campilobacteriosis revealed that microbiological methods and IFAs were unsatisfactory. Appropriate methods do not apply to all animal feeds.

The results of the IFA were found in the production of bullion-produsers. Bacteriological methods of production of pretzel slices were also produced by the producers of culture of campilobacterium.

The results of the experiments of serum serum test of 16 serum potassium immunoferential analysis were obtained by using bacteriological methods of 9 isolates of campilobacterium.

In addition, PCR detection of pathogens in clinical forms without all the culture allocation is practiced for diagnostics of labor (10, 11, 12, 13, 14).

Strengthening of PCR methods in diagnostic laboratories can help to maintain and efficacy of diagnostics of campilobacteriosis infections. Otherwise, IEC recommends that the MEB be developed with bacteriological methods for the discovery and differentiation of campilobacteria, which can be used by molecular genetic methods, PCR, and modern modification of PCR with detection in real time. The PCR method in diagnostic practice has been phased out as a highly sensitive and high-speci- fied method.

Replication of PCR can help to increase the effectiveness of the diagnostic campaigns and increase the control of quality of living products, which will have favorable economical and social effect.

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ІРІ ҚАРА МАЛДЫҢ КАМПИЛОБАКТЕРИОЗЫНБАЛАУ

Аннотация. Індеттанулық мониторинг нәтижесінде ірі қара мал басының арасында кампилобактериоз жұқпалы аурулардың нозологиялық профилінде көрсетілгені анықталды. Микробиологиялық және серологиялық зерттеулер (ИФТ) ІҚМ кампилобактериозының салыстырмалы балауы ұсынылған. ПТР әдісімен зерттеу кезінде нақты уақытта кампилобактериозға оң нәтиже берген 5 ірі қара мал басы анықталды,олардан бұрын-соңды өсінді бөліп алынбаған.

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ДИАГНОСТИКА КАМПИЛОБАКТЕРИОЗА КРУПНОГО РОГАТОГО СКОТА

Аннотация. В результате эпизоотологического мониторинга установлено, что среди поголовья крупного рогатого скота кампилобактериоз представлен в нозологическом профиле инфекционных болезней. Представлен анализ сравнительной диагностики кампилобактериоза КРС микробиологические и серологические исследования (ИФА). При исследовании методом ПЦР в реальном времени дополнительно были выявлены 5 голов КРС положительно реагирующих на кампилобактериоз, которые ранее не были обнаружены путем выделения культуры.

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REFERENCES

[1] Development and evaluation of an indirect enzyme-linked immunosorbent assay for the detection of antibodies against Campylobacter fetus in cattle / Hailing Zhao, Huifang Liu, Yanfen Du, Siguo Liu, Hongbo Ni, Yong Wang, Chunlai Wang, Wei SI, Jinguo Yang, Jingkai Ling // Research in Veterinary Science. 88 (2010). 446-451.

[2] Boom R. et al. *Rapid* and simple method for purification of nucleic acids // J. Clin. Microbiol. 1990. № 3. P. 495-503.

[3] Evaluation of two automated enzyme-immunoassays for detection of thermophilic campylobacters in faecal samples from cattle and swine / J. Hoorfar, E.M. Nielsen, H. Stryhn, S. Andersen // Journal of Microbiological Methods38 (1999) 101-106.

[4] Two outbreaks of campylobacteriosis associated with the consumption of rawcows' milk / Annet E. Heuvelink, Caroliene van Heerwaarden, Ans Zwartkruis-Nahuis, Jeroen J.H.C. Tilburg, M. Hanna Bosb, Frank G.C. Heilmann, Agnetha Hofhuis, Trynke Hoekstra, Enne de Boer // International Journal of Food Microbiology. 134 (2009) 70-74.

[5] A novel immunoproteomics method for identifying in vivo-induced Campylobacter jejuni antigens using pre-adsorbed sera from infected patients, Yuanqing Hu, Yuwei Shang, Jinlin Huang, Yan Wang, Fangzhe Ren, Yang Jiao, Zhiming Pan, Xinan Jiao, Biochimica et Biophysica Acta. 1830 (2013). 5229-5235.

[6] Campylobacter fetus subspecies: Comparative genomics and prediction of potential virulence targets, Amjad Ali, Siomar C. Soares, Anderson R. Santos, Luis C. Guimarães, Eudes Barbosa, Sintia S. Almeida, Vinícius A.C. Abreu, Adriana R. Carneiro, Rommel T.J. Ramos, Syeda M. Bakhtiar, Syed S. Hassan, David W. Ussery, Stephen On, Artur Silva, Maria P. Schneider, Andrey P. Lage, Anderson Miyoshi, Vasco Azevedo, Gene 508 (2012). 145-156.

[7] Keener K.M., Bashor M.P., Curtis P.A., Sheldon B.W., Kathariou S. Comprehensive review of Campylobacter and poultry processing // Compr. Rev. Food Sci. 2004. Vol. 3. P. 105-115.

[8] Rotariu O., Dallas J.F., Ogden I.D., MacRae M., Sheppard S.K., Maiden M.C., Gormley F.J., Forbes K.J., Strachan N.J. Spatiotemporal homogeneity of Campylobacter subtypes from cattle and sheep across northeastern and southwestern Scotland // Appl. Environ. Microbiol. 2009. Vol. 75. P. 6275-6281.

[9] The Campylobacterconundrum, RENDS in Microbiology Vol. 9, N 8. August 2001, Keith Jones Dept of Biological Sciences, IENS, Lancaster University, Lancaster, UK LA1 4YQ.

[10] Monke H.J., Love B.C., Wittum T.E., Monke D.R., Byrum B.A. 2002. Effect of transport enrichment medium, transport time, and growth medium on the detection of *Campylobacter fetus* subsp. *venerealis* // J. Vet. Diagn. Investig. 14: 35-39.

[11] Muller W., Hotzel H., Schulze F. 2003. Identification and differentiation of Campylobacter fetus subspecies by PCR // DtschTierarztlWochenschr. 110:55-59. (In German) .

[12] Nogva H.K., Bergh A., Holck A., Rudi K. 2000. Application of the 5'-nuclease PCR assay in evaluation and development of methods for quantitative detection of *Campylobacter jejuni* // Appl. Environ. Microbiol. 66: 4029-4036.

[13] On S.L.W., Harrington C.S. 2001. Evaluation of numerical analysis of PFGE-DNA profiles for differentiating *Campylobacter fetus* subspecies by comparison with phenotypic, PCR and 16S rDNA sequencing methods // J. Appl. Microbiol. 90: 285-293.

[14] Qi Y., Patra G., Liang X., Williams L.E., Rose S., Redkar R.J., DelVecchio V.G. 2001. Utilization of the *rpoB* gene as a specific chromosomal marker for real-time PCR detection of *Bacillus anthracis* // Appl. Environ. Microbiol. 67: 3720-3727.