болса, кем дегенде инфекциялық белсенділігі 7,62 $\log_{10}$ЭИД$_{50}$/см^3 болатын жоғары вирусты материалды алуға болады, ол ұсақ мал бруцеллезіне қарсы векторлық вакцинаны дайындауға әбден лайықты.

Кілт сөздер: Тұмаулық вирустық векторлар, бруцеллез, антиген, рекомбинанттық штамдар, тауық эмбриондары, ұсақ малдар,өсіру.

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OPTIMIZATION IN CHICKEN EMBRYOS CULTIVATION CONDITIONS OF INFLUENZA VIRAL VECTORS EXPRESSING BRUCELLA IMMUNODOMINANT OMP19 OR CU-ZN-SOD PROTEINS

Abstract

To develop a vaccine against ruminants brucellosis the influenza viral vectors (IVV) expressing Brucella immunodominant Omp19 or Cu-Zn-Sod proteins were constructed. In this paper, we have established the optimal parameters for the cultivation of these IVV in chicken embryos (CE): the age of the CE is 10 days, infection dose 100-1000 EID50, the temperature and duration of incubation are 32-34±0,5ºC and 48 h, respectively. Culturing under specified parameters can stably obtain a highly virus-containing material with potency of at least 7.0 $\log_{10}$ EID$_{50}$/ml, which is suitable for the preparation of a vector vaccine against brucellosis.

Key words: Influenza viral vectors, brucellosis, antigen, recombinant strains, chicken embryos, cultivation.

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IDENTIFICATION OF LISTERIA IN TURKEY MEAT

Annotation

L. monocytogenes is food pathogen, it is often found in meat raw materials and semi-finished products, since they are resistant to low temperatures and are able to multiply at ambient temperatures and household refrigerators.

In this research, samples of turkey meat, feed and water were tested for the presence of Listeria by using classical method, biochemical tests and biotest. Results from microscopy showed bacteria with characteristics of Listeria. Biochemical tests and results from mice infection proved the presence of Listeria in all samples.

Keywords: *L. monocytogenes*, turkey meat, feed, water, mouse.

Introduction

Listeriosis is a relatively rare but serious disease which occurs in various animals, and there were reported many cases of human infection. The causative agent of this disease is bacteria of Listeria species [1]. Infection, caused by Listeria species, can pose major risks for certain populations, pregnant women, new born babies, older adults, and individuals with weakened immune systems (AIDS patients) [2].

Listeria is genus of Gram positive, rod-shaped with rounded ends, non-sporulative, facultative anaerobic bacteria [2]. The organism is psychotropic and grows at temperature 0-40^{0}C. Optimal growth temperature for the members of the genus Listeria is 30-37^{0}C, but also can

grow at 4-10^{0}C. All species of Listeria are motile at 20-28^{0}C by means of one of the five peritrichous flagella [3].

Listeria monocytogenes (L. monocytogenes) is widespread in the environment and commonly has been isolated from soil, vegetation, silage, faecal material, sewage and water. *L. monocytogenes* is capable of surviving under different extreme environmental conditions for long periods [4]. Listeria spp. show unusual tolerance to refrigeration temperature, high salt concentration and can grow on dry and moist surfaces. *L. monocytogenes* is a normal inhabitant of the intestinal tract in humans, that why antibodies to Listeria spp. are common in healthy people [5].

L. monocytogenes relates to food pathogens, which are found in meat raw materials and semi-finished products, but often they are found in poultry products. Poultry meat refers to dietary products, but it also serves as a favorable environment for microbial grouth, which reduces the level of its safety for consumers. Contamination of poultry meat occurs during the life through feed and water, and when processing on a separate technological operations. In primary processing of poultry contamination of carcasses by pathogenic microorganisms can be enhanced by cross-contamination when removing the feathers, gutting and chilling [6].

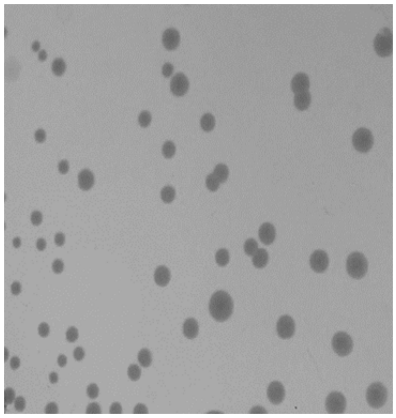
Materials and methods

Research work on the identification of Listeria was carried out at the Kazakh-Japan Innovation Center in the laboratory of "Microbiological Safety" in September 2017. 12 samples of turkey meat, 7 samples of feed, 2 samples of water (from slaughterhouse and storage tank) from Orenburg poultry farm of the Russian Federation served as a material for bacteriological analysis.

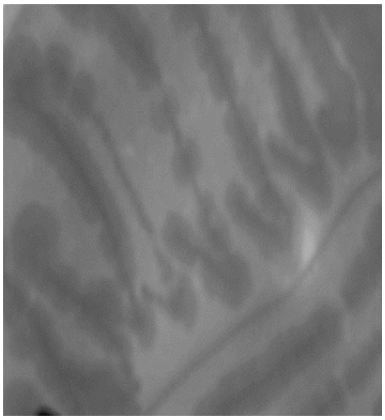
Microbiological analysis was carried out according to GOST 32031-2012 and ST RK GOST R 51921-2010. Crops suspension of meat of physiological solution in the ratio 1:9 was done on a nutrient medium MPB (meat peptone broth), broth Fraser. Broth samples were incubated in a thermostat at 37°C. Grown broth culture seeded with bacteriological loop on MPA (meat peptone agar) and on selective medium Palkam and blood agar to obtain the growth over the entire surface of the agar, grown at a temperature of 37^{0}C 24-48 hours. From the grown colonies prepared smears and stained by gram. There was conducted biochemical studies, as well as infection of laboratory white mice, were injected with a suspension of 1:5 subcutaneously in physiological solution. Laboratory mice were observed for 10 days.

Results and discussion

After 24 h cultivation of crops in the thermostat at 37°C MPB and broth Fraser was observed a slight uniform clouding and the formation of surface biofilm. For MPA was observed a gentle growth of shiny, small and convex colonies. (Picture 1).



Picture 1.Listeria growth on MPA



Picture 2.Listeria growth on blood agar

After 24 hours incubation on the selective medium Palkam noted abundant growth of small, grayish-green or green colonies with a diameter of 0.5–1.0 mm. In blood agar of the selected crops were found the zone of hemolysis of varying intensity (Picture 2).

When inoculating selected crops on a carbohydrate medium the following results were obtained.

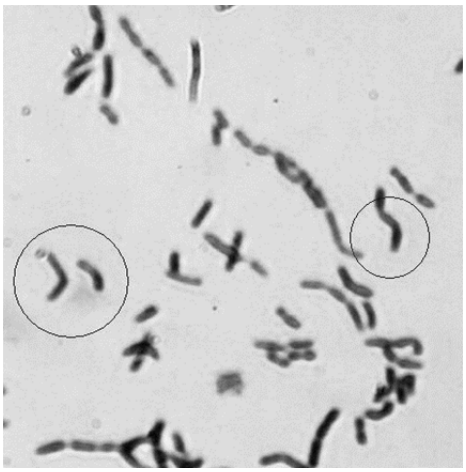
As can be seen from table 1, the reaction of β-hemolysis is positive. Culture of Listeria has charolaise properties. Listeria fermented rhamnose, but not fermented mannitol and xylose. It has a catalase activity, oxidase negative.

Table 1. Biochemical properties of selected cultures of Listeria

№	Source of sample	The reaction to β -hemolysis	Fermentation of carbohydrates				
			mannitol	rhamnose	xylose	oxidase	catalase
1	Liver	+	-	-	+	-	+
2	Heart	+	-	-	+	-	+
3	Spleen	+	-	-	+	-	+
4	Lungs	+	-	-	+	-	+
5	Intestine	+	-	-	+	-	+

From infected mice were observed the death of one mouse during the first day, and then the death of 2 mice on the third day. Performed the autopsy and made the seeding of the internal organs (liver, heart, spleen, lungs and intestines) on the MPB, MPA and was making preparations for study with the microscope.

In gram-stained preparations of bacteria of the genus Listeria are installed in the form of short rods, arranged single and in pairs. The causative agent of listeriosis is a gram-positive with rounded ends of the sticks, which can be polymorphic. A characteristic feature of Listeria is that some bacteria are arranged relative to each other in the form of a Roman numeral V or in parallel. The daily cultures of *L. monocytogenes* isolated from turkey meat are presented in (Picture 3).



Picture 3. Culture of Listeria monocytogenes in a smear stained by the gram stain

Conclusion

Isolated cultures of Listeria have characteristic morphological, cultural, biochemical and pathogenic properties characteristic of pathogenic strains of Listeria and may be causing the illness and deaths of birds. In further studies disinfectants and antibiotics need to be analyzed against to Listeria that was found in samples of turkey meat, water and feed.

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КҮРКЕТАУЫҚ ЕТІНЕН ЛИСТЕРИЯНЫ АНЫҚТАУ

Аңдатпа

L. monocytogenes шикі етте және жартылай фабрикаттарда кездесетін төмен температураға төзімді тағам патогені болып табылады. Бұл зерттеу жұмысында күркетауық еті, азығы және су классикалық әдіс, биохимиялық тест және биосынама әдісі арқылы зерттелді. Алынған нәтижелер барлық үлгілерде листерия бар екендігін көрсетті.

Кілт сөздер: *L. monocytogenes,* күркетауық еті, азық, су, тышқан.

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ИДЕНТИФИКАЦИЯ ЛИСТЕРИИ В МЯСЕ ИНДЕЙКИ

Аннотация

L. monocytogenes является пищевым патогеном, который часто встречается в мясном сырье и полуфабрикатах, поскольку они устойчивы к низким температурам. В этом исследовании мясо индейки, корма и вода были протестированы на присутствие листерии с использованием классического метода, биохимических тестов и методом биопробы. Результаты показали наличие листерии во всех образцах.

Ключевые слова: *L. monocytogenes*, мясо индейки, корм, вода, мышь.