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Annotation

Results of the conducted researches on definition of the immunizing dose and term of offensive of immunity of the inactivated bivalent vaccine against RRSS of the American and European genotypes are presented in this article. The most optimum volume of the bivalent vaccine entered to subepidemic parotitis is the dose of 4,0 ml the term of offensive of immunity of 14 days.

Key words: immunizing dose, term of offensive, divalent vaccine, PRRS.

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ANTIMICROBIAL SUSCEPTIBILITY AND BIOFILM FORMATION ABILITY OF *LISTERIA MONOCYTOGENES* ISOLATED FROM POULTRY PLANTS

Abstract

In this study we have isolated 15 *listeria* strains from 72 samples from poultry plants. The isolates were identified as *listaeria* by their cultural and biochemical properties. Their antimicrobial susceptibility and an ability to form biofilms was assessed. This work in ongoing in the frames of the project "Development of the monitoring system and eradication methods of biofilm-forming strains of listeria in poultry plants based on microbiological and molecular genetic methods".

Key words: listeria monocytogenes, biofilms, antimicrobial susceptibility.

Introduction

Poultry farming is a dynamically developing branch of the agro-industrial complex, which provides the population with biologically complete healthy food. At the same time, in the poultry processing industry close attention is paid to food-borne diseases, including listeriosis. Pollution of food products by*L. monocytogenes* can occur at all stages of the food chain. Listeria can accumulate while storing products in refrigerators, when many other bacteria die or do not multiply, thus, do not compete with listeria for space and nutrients.

According to the Berjeesystematics of the microorganisms, the genus *Listeria* consists of 7 species, which manifest themselves differently in the pathogenic process. 2 species are pathogenic*: L. monocytogenes* and *L. ivanovii*. The genus *Listeria*also includes following non-pathogenic species: *L. innocua, L. 'welshimeri, L. seeligeri, L. murrayi* and *L. grayi*. The resistance of the pathogen to various environmental factors is high: in soil, manure, water, on plants, they remain viable until 600 days, on the contaminated surfaces of agricultural premises in summer (9 ... 22 ° C) listeria remain viable up to 25 days and in winter (-2 ... -23 ° C) up to 130 days. Lakes contaminated with *Listeria*pose a danger in epizootic and epidemiological terms. In the ice*listeria* can survive from 5.5 months up to 2.5 years.

*Listeria*haveincreasedviability to the influence of various factors and techniques used in the technology of meat and meat products' production. According to many researchers, meat cooling up to 17 days (shelf-life of chilled meat) reduces the viability of the causative agent of listeriosis 4 fold in comparison with their original content, but during this period there is no

complete dying out of the *listeria*. So complete eradication of the pathogen does not occur. This feature explains the common name of *listeria*- a "refrigerator microbe".

Listeria has a high thermal stability within the temperature ranges of pasteurization and sausages cooking. The heat resistance of *listeria* decreases with an increase in the content of connective tissue in the meat. Fat has a protective effect on the heat resistance of *listeria*. The cooking of the "tea sausage" (the temperature of the heating medium is 75-80 ° C) with a diameter of 35-50 mm inactivates the *listeria*in 75 minutes and at the diameter of 65 mm in 90 minutes. When cooking 8-10 cm thick lamb pieces weighing 1-2.5 kg, the causative agent of listeriosis perishes within 1 hour [1].

The emergence of resistant strains significantly hampers effective control of the pathogen. In addition, the persistence of *listeria* in the body and on theenvironmental objects of, especially on technological equipment, is associated with its ability to form biofilms, which in turn increases the resistance of cells to disinfectants, heating and prevents physical removal [2, 3].

The acquired resistance of *listeria*was noted for a number of antibiotics. Among them, resistance to tetracyclines, macrolides, trimethoprim. Also*L.monocytogenes* has an innate resistance to phosphomycins, producing phosphomycin-resistant protein FosX, which catalyzes the hydration of antiphaticphosphomycin, (1R, 2S) -epoxypropylphosphonic acid. The mechanisms of *listeria* resistance described in the literature are given in Table 1 [4, 5].

Table 1. Genes of resistance to antibiotics identified in listeria.

Gene	Resistance mechanism	Antibiotic type, to which resistance is acquired
erm(B)	methylation of one adenine in 23S rRNA, which is a component of 50S rRNA	cross resistance to macrolides, (except erythromycin) to lincosamides and streptogramin B
dfrD	Modification of the goal of trimethoprim - dehydrofolic acid	trimethoprim
tet(M) and *tet(S)*	Protectionofthe ribosomes	tetracycline

The problem is also compounded by the ability of bacteria to form biofilms on different surfaces used in the food industry, which allows it to persist and survive for a long time, significantly reducing the product shelf - life and posing a threat to public health. In addition, biofilms can "shelter" so-called persister cells, which maintain a pool of resistance genes to certain antibacterial agents, and transfer them to other bacteria. Interspecies transmissions of resistance genes in biofilms were also reported [6, 7, 8]. This exacerbates the worldwide problem of combating the resistance of microorganisms to antimicrobials.

The purpose of our research was to identify *listeria* from poultry products and to determine their ability to form biofilms and test their antimicrobial sensitivity. This researchwas carried outin the framework of the research project: "Development of the monitoring system and eradication methods of biofilm-forming strains of listeria in poultry plants based on microbiological and molecular genetic methods".

Materials and methods

Work on isolation of *listeria*strains have been carried out since 2015. The subjects of the study were samples of poultry meat and bird carcasses delivered from poultry farms, samples of meat purchased in the markets of Almaty.

GOST RK 51921-2002 "Food products. Methods for the isolation and detection of bacteria *Listeriamonocytogenes* », EN ISO 11290-1 ((ISO 2005) standards were used during the work.

Pre-selective enrichment was carried out by applying 5 g of poultry meat into 225 cm^3 of a selected liquid medium (Fraser broth). The contents were shaken in a circular motion. Cultures were incubated at 30 ° C for 24 hours. The study of the biological properties of *Listeria* continued using the cultures that induced blackening of the medium after 48 hours, which is usually observed in the presence of *listeria*.

Further, culturingwas carried out in the MPA; PALCAM agar and blood agar.
Cultures from poultry meat in physiological saline in a ratio of 1: 5 were made on MPB (meat-peptone broth), MPA (meat-peptone agar). Cultures were incubated in a thermostate at 25 ° C.
The 24 - hour broth cultures, grown at 25 ° C, were spread with a bacteriological loop on 2 test tubes of MPA and grown at the room temperature for 24 to 30 hours.

After 24 hours, with the appearance of a continuous growth of the colonies, colonies were taken to selective diagnostic mediaPalkam and blood agar. From the zones of maximal medium blackening with colonies were taken to 2-3 Petri dishes with a selective differential diagnostic medium for obtaining isolated colonies.

TSA (Tryptic soy agar) was used for the biochemical test. Cultures were incubated at 30 ° C for 24 hours. Colonies with a typical for *listeria* growth were taken from Petri dishes to prepare smears for a microscopy and stained by Gram. A catalase test (with 3% H_2O_2) was performed and the mobility was determined.

To study the stability of *listeria* at various factors (change ofpH of the medium, various concentrations of NaCI), cultures were grown on MPB with a pH in the range of 7.2; 6.5; 5.5 and on BCH with the content of NaCl6%, 10%, 14%, 24%. The plates were incubated for 24 hours at 25 ° C, then kept at 4 ... 6 ° C for the entire observation period (30 days). Every 3 days, re-culturingwas carried out from each test tube to the Frazer broth. When the medium was blackened, it was believed that *listeria* retained its viability.

The ability to form a biofilm was evaluated by staining with the crystalviolet. Cultures were grown in a liquid media MPB in Petri dishes at 30 ° C for 48-hours. The broth was then drained, and the dishes were rinsed twice with distilled water. The dishes were then dried and dyed with 0.1% crystal violet and rinsed twice with distilled water. The biofilm formation was assessed visually (picture 3).

Antibiotic susceptibility testing was performed by disk diffusion in agar plates, using 2% MPA. The list of tested antibiotics and the results of a sensitivity study are displayed in Table 3.

Results

A total of 72 samples of meat were analyzed during this period, of which 15 *listeria* cultures were isolated in 9 samples. The isolated cultures of listeria had a typical morphology: in smears, they had the appearance of small, often polymorphic, gram-positive rods located singly at an angle in the form of a Roman numeral V, or arranged parallel to each other in the form of a palisade.

On MPA colonies of *listeria* grew in the form of small, round, transparent colonies clearly visible under a transmitted light, after a few days the colonies becameturbid. On the MPB*listeria* caused a uniform turbidity of the medium, wave – like movement was observed when shaking. After 8-10 day a precipitate that rises upwards in the form of a pigtail while shaking was observed (Picture 1). In young cultures (6-24 hours), *listeria* are motile; their motility is better visible after cultivation at room temperature. The study of biochemical properties showed that the isolated cultures fermented salicin, glucose, lactose and glycerin by formation of an acid

without gas; They did not ferment mannitol, dulcite; Did not liquefy gelatin; Did not change milk; reduced methylene blue. The assay for catalase was positive: when 1 ml of 10% hydrogen peroxide was added to the test tube with the 12-24-hour cultures on the MPB the liquid frothed.

The results of a study of the stability of *listeria* at the changing pH and NaCl concentrations are shown in Table 2.

Picture 1- Listeria grown in MPB, Raised in a pigtail pattern while shaken

Picture 2- Listeria growth on MPA

Picture 3- Growth of Listeria on PALCAM media visualised under microscope at 8x

Table 2 Stability of Listeria at pH Changes and Different Concentrations of NaCI

Listeria cultures	Media pH range			NaCI concentration			
	7.2	6.5	5.5	6%	10%	14%	24%
L. monocytogenes 2/1	30	30	30	30	27	21	9
L. monocytogenes 2/2	30	30	30	30	24	18	9
L. monocytogenes 2/5	30	30	30	30	24	24	18
L. monocytogenes 2/6	30	30	30	30	27	21	15
L. monocytogenes 4/2	30	30	30	30	27	24	15
L. monocytogenes 4/4	30	30	30	30	27	24	15
L. monocytogenes 5/1	30	30	30	30	27	21	12
L. monocytogenes 5/3	30	30	30	30	27	24	15
L. monocytogenes 8/2	30	30	30	30	21	21	12
L. monocytogenes 8/4	30	30	30	30	27	21	15
L. monocytogenes 9/2	30	30	30	30	27	24	18
L. monocytogenes 9/3	30	30	30	30	24	21	12
L. monocytogenes 10/2	30	30	30	30	21	18	12
L. monocytogenes 10/5	30	30	30	30	24	15	9
L. monocytogenes 11/1	30	30	30	30	27	21	-
L. monocytogenes 11/2	30	30	30	30	27	24	9
L. monocytogenes 12/1	30	30	30	30	30	30	21
L. monocytogenes 12/2	30	30	30	30	24	21	9
L. monocytogenes 12/5	30	30	30	30	24	18	15
L. monocytogenes 13/4	30	30	30	30	27	24	18
L. monocytogenes 14/2	30	30	30	30	30	27	21
L. monocytogenes 14/4	30	30	30	30	21	15	9
L. monocytogenes 15/1	30	30	30	30	27	21	-
L. monocytogenes 15/4	30	30	30	30	21	15	-
L. monocytogenes 1/1	30	30	30	30	24	24	12
L. monocytogenes ½	30	30	30	30	24	15	-
L. monocytogenes 1/3	30	30	30	30	30	24	9

Studies have shown that the viability of *listeria* are not affected by changes in pH of the medium, since all the cultures studied showed a different growth intensity when they were transferred to the Fraser broth. The growth of *listeria* was observed on the 2-5th day.

The growth of *listeria* on media with NaCl content was inversely proportional to the increase in NaCl concentration in the MPB. Thus, *listeria* retained viability in meat-peptone broth (MBP) with a content of 6% NaCl for the entire observation period (30 days), with the concentration of salt 10% - from 21 days to 1 month, and with 24% NaCI - up to 21 days.

Antibiotics / Cultures	Levomycetin Disk	Streptomycin Disk	Ampicillin	Tetracycline Disc	Interspektin-L	Amoxicillin-150	Doximac-0	Acvatil	Enrokoli-10%	Thiemikol	Gentoquinol	Tyrmicosin	Zinaprim	Tylosin Tartrate
L. monocytogenes 2/1		S		S			R	I	S	I				I
L. monocytogenes 2/2				R		I	S		S	S			R	
L. monocytogenes 2/5	I	I	R	S		I	S	R	S	S	S	S	R	I
L. monocytogenes 2/6		I	R	S		I	S					I	R	
L. monocytogenes 4/2	R		R	S		S		S	R		R		R	S
L. monocytogenes 4/4	S		S	S						S			R	S
L. monocytogenes 5/1	R	S		R			R	I	R	S	S			S
L. monocytogenes 5/3	S			S			S	S	S		S		R	S
L. monocytogenes 8/2				S			S	S			S			S
L. monocytogenes 8/4	I		S				S	S	S	S	S			
L. monocytogenes 9/2	S	S	I	I	S	I	S	I		S	S	S		
L. monocytogenes /3				S			S		S		S			
L. monocytogenes 10/2				I	S	I	S				S	S	R	I
L. monocytogenes 10/5	I	S	R	S			S	I		S	S			
L.	I	I		I		I	S	S		S			R	

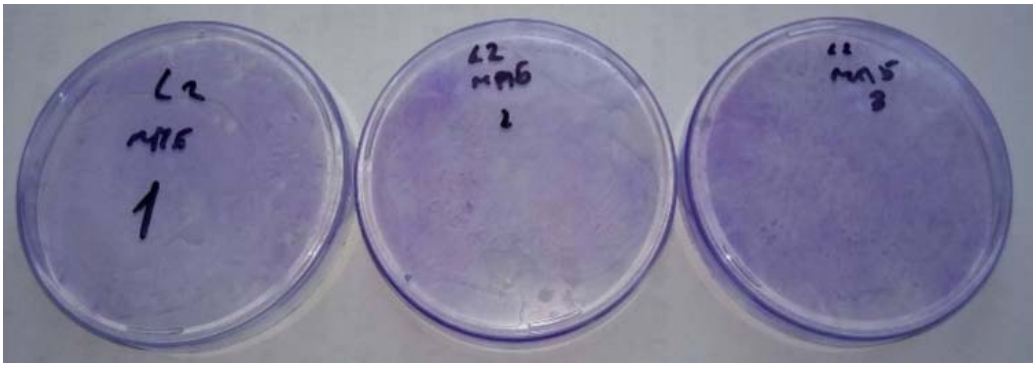
monocytogenes 11/1														
L. monocytogenes 11/2	R	I		S	S	I	S	I	S		R	I	R	
L. monocytogenes 12/1	R	I		R		S	S	I	R		R	I	R	
L. monocytogenes 12/2	R	I		R		S	S	I	R		R	I	R	
L. monocytogenes 12/5	I	I		S		S	S			S			R	
L. monocytogenes 13/4	I	I	R		S	S	S							I
L. monocytogenes 14/2	R	I		R		S	S	I	R		R	I	R	
L. monocytogenes 14/4		S	S	I			S			S				
L. monocytogenes 15/1	S		S				S			S				
L. monocytogenes 15/4		I	I				S			S				
L. monocytogenes 1/2	R	I		R		S	S	I	R		R	I	R	
L. monocytogenes 1/3	R	S		R			R	I	R	S	S			S

Note: S - sensitivity is high (up to 5 mm); I - sensitivity is average (up to 10 mm); R - sensitivity is low (above 10 mm, bold - above 20 mm)

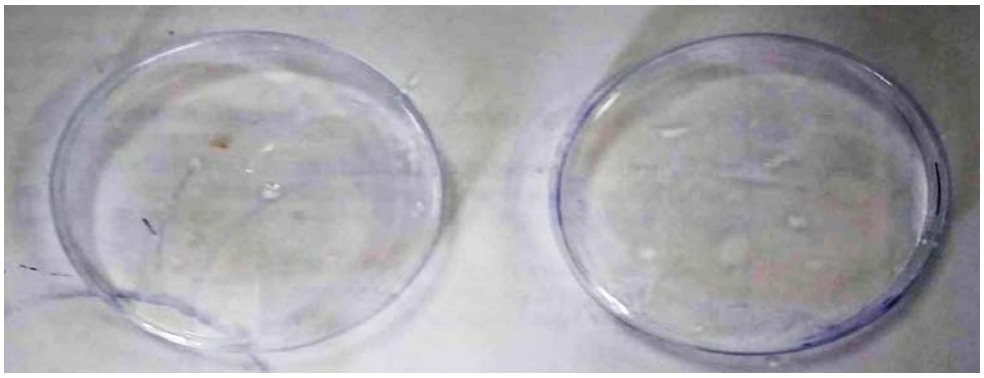
As can be seen from the table, the most resistant*listeria* cultures were *L. monocytogenes 1/3, L. monocytogenes 4/2. L.monocytogenes 12/2 L.monocytogenes 2/5*. The resistance can be associated either with the ability to form a biofilm or with the presence of genes that cause resistance to a particular drug. Most often, the studied strains of *listeria* showed resistance to following antibiotics: zinaprim, levomycetin, enrokoli and tetracycline.

All tested strains had the ability to form biofilms. That complies with the results reported by other researchers in the World [9, 10, 11]. The ability of bacteria, including *listeria* to form biofilms, is described as evolutionary adaptability to the influence of stress factors, such as exposure to antibiotics and disinfectants, lack of nutrients and water, the presence of shear force (in pipes, vessels), and other factors inducing the mechanisms of chemical communication of bacteria (Quorum sensing).

Presence of extracellular matrix and genes controlling biofilm formation, a complex architectural structure are distinctive features of biofilms in comparison with their planktonic counterparts. *Listeria* has the *LuxS*-like gene *lmo1288* (which initiates and produces AI-2) and the *agrD* gene, which produces AI peptides and regulates functions such as pathogenicity, motility and biofilm formation [12, 13].



Picture 4- Biofilms *L. Monocytogenes 2/1*, visualized by crystal violet staining



Picture 5- Controls that do not contain bacteria, treated and stained in the same way

Discussion

Excessive use of antibiotics in poultry plants creates a medium that is oversaturated with antimicrobial agents, which in turn leads to the selection of resistant strains of *listeria.* Our study proves the importance of limiting the inappropriate use of antibiotics. It is necessary to conduct regular monitoring of the circulation of resistant strains of *listeria* for more effective use of suitable antibiotics. When using antimicrobial agents, it is necessary to take into account the biofilm formation of bacteria that can interfere with the effective penetration of antibiotics through the extracellular matrix, and also allows to resist chemical and mechanical removal by bacteria.

Bibliography

1. Методические рекомендации: «Лабораторная диагностика листериоза животных и людей», М. 1987.

2. Fagerlund A., Moretro T., Heir E., Briandet R., Langsrud S. Cleaning and disinfection of biofilms composed of listeria monocytogenes and background microbiota from meat processing surfaces. 2017. Appl. Environ. Microb. Doi: 10.1128/AEM.01046-17.

3. Gunther F., Scherrer M., Kaiser SJ., DeRosa A., Mutters NT. Comparative testing of disinfectant efficacy on planktonic bacteria and bacterial biofilms using a new assay based upon kinetic analysis of etabolic activity. 2016. Doi: 10.1111/jam.13358.

4. Morvan A., Moubareck C., Leclercq A., Herve – Bazin M., Bremont S., Lecuit M., Courvalin P., Le Monnier A. Antimicrobial resistance of Listeria monocytogenes strains isolated from humans in France. 2010. Antimicrobial agents and chemotherapy. Vol. 54. No. 6. P. 2728 – 2731

5. Conter M., Paludi D., Zanardi E., Ghidini S., Vergara A., Ianieri A. Characterization of antimicrobial resistance of foodborne Listeria monocytogenes. 2009. International journ. Of food microb. 128. P. 497 -500.

6. Gutierrez D., Rodriguez-Rubio L., Martinez B., Rodriguez A., Garcia P. Bacteriophages as weapon against bacterial biofilms in the food industry. 2016. Front. Microbial. 7:825.

7. Du L., Liu F., Zhao P., Zhao T., Do MP. Characterization of *Enterococcus durans*152 bacteriocins and their inhibition of *Listeria monocytogenes* in ham. 2017. Food microbiology.

8. Akbas M.Y. Bacterial biofilms and their new control strategies in food industry. 2015. The Battle Against Microbial Pathogens: Basic Science, Technological Advances and Educational Programs. P. 383-394.

9. Purkrtova S., Turonova H., Pilchova T., Demnero K., Pazlarova J., Resistance of Listeria monocytogenes biofilms to desinfectants. 2010. Czech J. of Food Sci. Vol. 28. No. 4. P. 326 – 332.

10. Pan Y., Breidt F. Jr., Kathariou S. Resistance of Listeria monocytogenes biofilms to sanitizing agents in a simulated food-processing environment. 2006. Appl. And environ. Microbial. P. 7711 – 7717.

11. Oloketuyi SF., Khan F. Inhibition strategies of Listeria monocytogenes biofilms – current knowledge and future outlooks. 2017. Journ. Of Basic Microbiol. 9999. P. 1 – 16.

12. Piercey J., Hingston PA., Hansen LT. 2016. Genes involved in *listeria monocytogenes* biofilm formation at a stimulated food processing plant temperature of 15C. Int. jour. Of food microbial. doi: 10.1016/j.ijfoodmicro.2016.02.009

13. Sela S., Frank S., Belausov E., Pinto R. 2006. A mutation in the *luxS*gene influences *listeria monocytogenes*biofilm formation. Appl. AndEnv. Microbial. Vol. 72. No.8. p. 5653 – 5658.

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ҚҰС ӨНІМДЕРІН ӨНДІРУ КӘСІПОРЫНДАРЫНАН БӨЛІНІП ШЫҒАРЛҒАН LISTERIA MONOCYTOGENES – ТІҢ АНТИБИОТИКСЕЗІМТАЛДЫҒЫ ЖӘНЕ БИОҚАБЫРШАҚ ҚҰРАУ ҚАБІЛЕТІ

Аңдатпа

Осы зерттеулерде, біз құс өнімдерін өндіру кәсіпорындарынан алынған 72 пробадан 15 листерия штаммдарын бөліп шығардық. Листерия изоляттары олардың мәдени және биохимиялық қасиеттері негізінде анықталды. Олардың антибиотиксезімталдығы және биокабыршақ дамыту мүмкіндігін бағалады. Бұл жұмыс «Микробиологиялық және молекулярлық-генетикалық әдістер негізінде құс фабрикаларында листерияның биоқабыршақты қалыптастыратын штамдарын жоюдың мониторинг жүйесімен әдістерін әзірлеу» жобасы аясында жүзеге асырылады.

Кілт сөздер: listeria monocytogenes, биоқабыршақ, антибиотиксезімталдық.

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АНТИБИОТИКОЧУВСТВИТЕЛЬНОСТЬ И СПОСОБНОСТЬ К ОБРАЗОВАНИЮ БИОПЛЕНКИ LISTERIA MONOCYTOGENES ВЫДЕЛЕННЫХ С ПТИЦЕВОДЧЕСКИХ ПРЕДПРИЯТИЙ

Аннотация

В этом исследовании мы выделили 15 штаммов листерий из 72 образцов из птицеводческих предприятий. Изоляты были идентифицированы как листерия по их культуральным и биохимическим свойствам. Была оценена их антибиотико-чувствительность и способность образовывать биопленки. Эта работа проводится в рамках проекта «Разработка системы мониторинга и методов эрадикации биопленкообразующих штаммов листерий в птицеводческих предприятиях на основе микробиологических и молекулярно-генетических методов».

Ключевые слова: listeria monocytogenes, биопленки, антибиотикочувствительность.

УДК 639.3.09

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ІЛЕ-БАЛҚАШ СУАЛАБЫНДАҒЫ ШЫҒЫС ТАБАНЫНЫҢ (*ABRAMIS BRAMA ORIENTALIS*) ПАРАЗИТОФАУНАСЫ

Аңдатпа

Мақалада Іле-Балқаш суалабындағы өнеркәсіптік балық аулау жұмыстары жүргізілетін суаттарға интродукцияланған шығыс табанының (*Abramis brama orientalis*) паразитофаунасы туралы мәліметтер келтірілген.

Кілт сөздер: шығыс табаны, интродукция, паразитофауна, протозооздар, гельминтоздар.

Кіріспе

Көптеген паразиттер балықтардың өсуін бәсеңдетеді және қоңдылығын төмендетеді, бұл жағдай өз кезегінде балық өнімдерінің тауарлық сапасын нашарлатып, аквакультураның экономикалық тиімділігіне талдау жүргізілгенде есепке алынбайтын қосымша шығынға алып келеді. Осыған байланысты, Қазақстанның балық шаруашылығының дамуы мен балық өнімінің көбеюіне балықтардың инвазиялық ауруларымен күресу жоспарлы және басты іс-шаралардың бірі болуы тиіс.

Іле-Балқаш суалабы толығымен Полеоарктикалық ихтиогеографиялық ауданның Таулы-Азия бөлімшесіне кіреді, осыған байланысты аталмыш суалап жүйесіне кіретін суаттарда тіршілік ететін балықтар мен басқа да гидробионттардың түрлік құрамы ұқсас. Гидрофаунаның түрлік құрамының бір келкі болуына байланысты паразитоценоз суалап бойынша бірегей болуы тиіс. Алайда, XX ғасырда жүргізілген көптеген ихтиоинтродукциялық жұмыстар нәтижесінде Іле-Балқаш суалабының паразитофаунасының түрлік құрамы түбегейлі өзгерген.

Шығыс табаны Қазақстанның оңтүстік-шығыс бөлімінің суаттары үшін аборигенді балығы емес, мақсатты интродуцент болып табылады. Бұл оңтүстік-шығыс Қазақстан суаттарының қоректік базасын толық қанды пайдалану мақсатында жерсіндірілген.

Балқаш көлі. Қазіргі таңда көлдегі балықтарды аулауға шекті рұқсат етілген ауланым 5520 тоннаны құрайды, оның 60%-ы шығыс табанына тиесілі. Көлдегі табанның қоректік