UDC 597-12 : 578.233.44 : 639.3

RESULTS OF SURVEILLANCE STUDIES OF INFECTIOUS FISH DISEASES IN FRESHWATER AQUACULTURE OF UKRAINE

N. M. Matvienko, A. V. Vashchenko, I. O. Tsiganok, L. P. Buchatsky

Institute of Fisheries, NAAS
135, Obukhivska Str, Kyiv, Ukraine, 03164

e-mail: mnarine73@mail.ru

Received on March 24, 2015

Aim. To investigate the epizootic state of fisheries in Ukraine; to study the biological specificities of viral and bacterial isolates of fish in freshwater aquaculture. **Methods**. The epizootic state of fisheries was defined according to the surveillance plan for fisheries, virological (biosampling of sensitive fish species, virus isolation on sensitive passaged cell cultures), serological (enzyme immunoassay, virus neutralization test using sensitive passaged cell cultures) and molecular-biological (reverse transcriptase polymerase chain reaction − RTPCR) methods of investigation were used. The pathogenicity of the isolated bacteria was studied in the biosample. The identification was performed using Bergey's Manual. The express-identification of bacteria was performed using the standardized test-system API 20E Bio Merieux (France). **Results.** The IPNV isolates of rainbow trout were first isolated in the fisheries of different forms of ownership in the western regions of Ukraine (Volyn, L'viv, Transcarpathian, Chernivtsi regions). It was demonstrated that different age groups of carp are infested with the virus in the fisheries of L'viv, Donetsk, Chernihiv, Kyiv, and Odesa regions which testifies to a wide spread of the virus in Ukraine. Out of fish infectious diseases the red spot-like disease and the swim bladder inflammation of carp, the diseases of young trout and sturgeon were detected in the investigated fisheries of Ukraine. **Conclusions**. The epizootic data were used to estimate the condition of the fisheries in Ukraine in terms of fish infectious diseases. An infectious pancreatic necrosis virus, new for Ukraine, was revealed. It was found to affect rainbow trout (*Oncorhynchus mykiss* , Walbaum, 1792) and the spread of SVCV in carp fisheries was demonstrated. As for bacterial fish diseases, the decrease in the epizootic situation was described along with considerable extension of the range of species of bacterial pathogens of fish. Annual systematic monitoring and measures of preventing the introduction of the agents of infectious diseases are the guarantee of protection of the specialized fisheries of Ukraine.

Key words: fish, monitoring, bacteria and viruses of fish.

DOI: 10.15407/agrisp2.02.032

INTRODUCTION

Surface waters of Ukraine, an integral part of natural resources of the country, are of high economic relevance. The processes of fish farming are impossible without the introduction of new working methods, including the application of polyculture, stocking condensation, introduction and acclimatization of new aquaculture objects, the use of highly nutritious feeds and immunostimulators. Specific attention should also be paid to non-controlled transfer of fish from one fishery to others, located in different geographic and climatic zones with corresponding ecosystems, which can lead to the occurrence of new fish diseases, often not prominent for a region or a specific fishery for a long period of time.

Viral and bacterial infectious diseases of fish constitute a significant problem for aquaculture, which often results in mass fish mortality and considerable economic losses. A considerable amount of bacteria live in aquatic medium, but only 70 species cause fish dis-

eases. The microflora composition of different water bodies varies to a great degree. However, fish ponds are characterized by a relatively stable spectrum of microorganisms with dominating saprophyte microflora, bacteria of genera *Aeromonas*, *Pseudomonas* predominantly [1]. Due to massive transfer of fish and other hydrobionts from different geographic zones (including the ones from abroad) there have appeared some diseases, which were previously not registered in the territory of Ukraine. Along with the intensification of the processes of cultivating different species of fish there appear new infectious diseases, which cause conside-rable damage to the aquaculture [2, 3]. Over 250 viruses were revealed for fish, the main part of them being in the countries with highly developed aquaculture (USA, France, Germany, Great Britain, Italy, Norway, Japan, *etc*.). However, the degree of their spreading varies for different countries. It is known that the greatest damage to the fisheries is done by the diseases, caused by RNA-containing viruses, in particular, spring viraemia virus of carp (SVCV), infectious haematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV) and viral hemorrhagic septicemia (VHS) [4–6].

Due to limited possibilities of treating the mentioned fish diseases, general prevention is an obligatory method, including relevant nutrition and prevention of stress factors, which can reduce natural immunity in fish. The prevention of infectious diseases should be viewed in the context of fish farming factors. It is extremely important to maintain proper sanitary living conditions as well as physical and chemical parameters of water. There is a real danger of further spread of infectious fish diseases along the territory of Ukraine which is a hazard of considerable economic losses in future, when domestic fisheries will work in the full scope.

The investigations were aimed at the study of the epizootic condition of fisheries in Ukraine and exploration of viral and bacterial isolates of fresh water aquaculture fish.

MATERIALS AND METHODS

The epizootic investigation was conducted twice a year: in early spring and late autumn, at water temperature of 9−17 °C, and throughout the year in case of clinical symptoms of fish diseases, the cases of its death, and in accordance with the surveillance plan for fisheries [7]. The material for virological investigations was sampled in the fisheries and surface waters in different regions of Ukraine. Most frequently the viral isolates were isolated from carp, juvenile and adult rainbow trout with the symptoms of viral infection.

The objects of the research were apparently healthy and sick fish of fresh water ponds of different age groups as well as spring viraemia virus of carp, family *Rhabdoviridae,* genus *Vesiculovirus*, hemorrhagic septicemia virus of trout, family *Rhabdoviridae*, genus *Novirhabdovirus*, infectious pancreatic necrosis virus, family *Birnaviridae,* genus *Aquabirnavirus*, as well as bacteria of different etiological groups, infesting hydrobionts.

The epizootic, virological (biosampling of sensitive fish species, virus isolation on sensitive passaged cell cultures), serological (enzyme immunoassay, virus neutralization test using sensitive passaged cell cultures) and molecular-biological (reverse transcriptase polymerase chain reaction − RTPCR) methods of investigation were used [7–9]. The pathogenicity of the isolated bacteria was studied in the biosample. The identification was made according to the common procedure using Bergey's manual to define the cultural, tinctorial and biochemical properties of each microorganism isolate [10, 11]. The express-identification of bacteria was performed using the standardized test-system API 20E Bio Merieux (France), based on 21 biochemical tests. The cultures were incubated for 18–24 h. The results were visually estimated, comparing the reaction color against the color identification table.

The isolation and identification of viruses were performed using classic virological methods [12, 13].

The continuous cell lines of fish were used in the work: epidermal neoplasms of pox-infested carp (EPC) [14]; tail stem of blue gill sunfish (BF-2) [15]; tail stem of black fathead minnow (FHM) [16], rainbow trout gonad (RTG) [17].

The sampling of pathological material, the isolation and accumulation of virus in cell cultures, the experimental restoration of the diseases and virus titration in cell cultures were conducted in accordance with the international regulatory documents [7]. Virus isolates were purified and concentrated in polyethylene glycol (PEG) by Mahi and Kangro method [18].

The sets "Ribosol-A" and "Revertase-L" (AmpliSens™, RF) were used for PCR-analysis, including the isolation of RNA-viruses, cDNA synthesis, amplification and detection of the results. The reaction was conducted according to the manufacturer's protocol. The results were detected by electrophoresis method [19].

RESULTS AND DISCUSSION

The investigation of the epizootic situation involved the study of fisheries of different forms of ownership, specialized in breeding rainbow trout and carp in 2003–2014. Natural surface waters, providing water for these fisheries, were also studied. In 2003–2014 about 400 isolates of microorganisms were isolated from fish of different fisheries of Ukraine in total.

Different age groups of rainbow trout were studied for the presence of viral infections. The integrated diagnosis was made with the consideration of the type of a fishery, the epizootic data, the clinical symptoms, postmortem examinations and the results of laboratory research [20].

A considerable part of trout-breeding fisheries are full system institutional establishments . Most fisheries are located in western regions of Ukraine (Transcarpathian, L'viv, Chernivtsi, Volyn, Rivne) and the AR of Crimea. Natural climatic conditions in these regions are favorable for trout breeding, which is rather a labor-intensive process. Normal functions of rainbow trout requires specific conditions, in particular, maintaining low water temperature, high flowage and oxygen saturation of water.

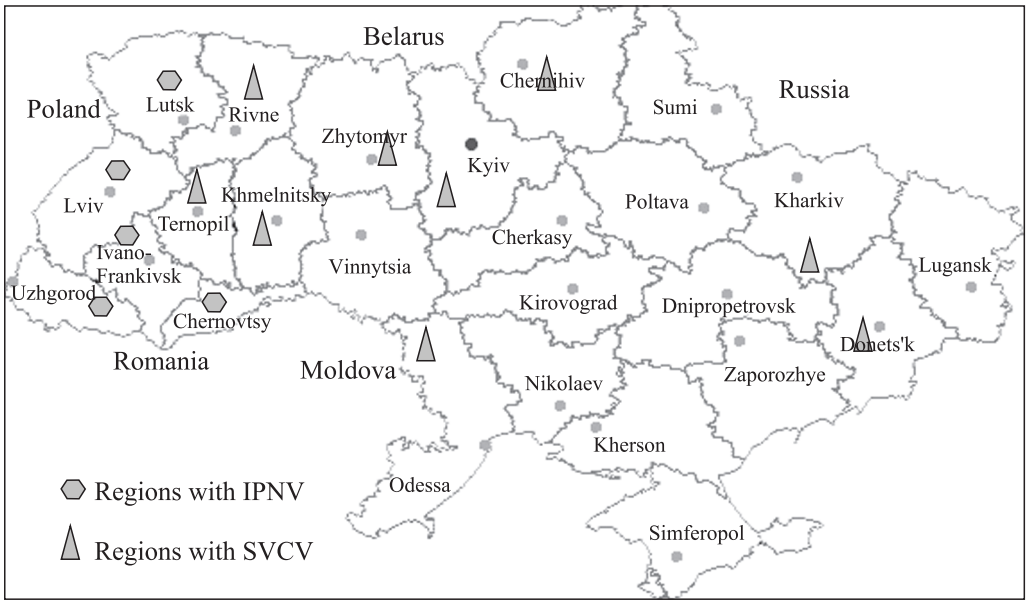
Numerous factors influence the transfer and spread of viral infections, including the impairment of the breeding technology, the mechanic transfer of the agent using the fishing tackle and ectoparasites, ignoring the disinfection techniques while transporting fish and fish breeding materials, *etc*. The fisheries, which are the subjects of selection breeding activity, *i. e*. engaged in cultivating fish seed, are of special interest. They may be an important link in the chain of spreading a viral infection in the territory of Ukraine. First and foremost, the analysis of fisheries started with determining the type of the pond, where fish was bred, and the water supply sources for incubation departments. The literature data [21] and our observations demonstrated that the agents of viral infections may remain in silt and ground ponds for a long time, creating sources of infection. Brood stock and stock groups of fish were studied, as there are data, proving the vertical transmission of salmon viruses and long-term persistence of a virus in the organism of males. While analyzing the situation with viral diseases of rainbow trout in Ukraine's neighboring countries, in particular, Poland, Slovakia, Czech Republic, Russia, we have noticed the circulation of the hemorrhagic septicemia virus of trout and infectious pancreatic necrosis virus in the specialized fishe-ries in the territory of the mentioned countries [4, 6]. Both viruses belong to the group of RNA-containing viruses. Infectious pancreatic necrosis virus is considered to be the most serious viral disease in the cultivation of common Atlantic salmon in the specia-lized fisheries of the European Union [22]. This disease is wide-spread in four continents. IPNV is known for many decades.

Our study demonstrated the presence of infectious pancreatic necrosis virus in some private fish farms of Chernivtsi, Transcarpathian, L'viv, and Volyn regions and in the ponds of water supply for these fisheries. The IPNV was isolated from the larval and juvenile rainbow trout. The hemorrhagic septicemia virus of trout was not detected in the studied fisheries and ponds.

As for SVCV, it was established that this virus was found in all the European countries with developed carp cultivation [23]. It was registered in the USA in 2002, and in 2004 it was found in China [24]. As for the former USSR, the SVCV agent was first isolated from sick fish by N. I. Rudikov. SVCV is circulating in the fisheries of Russia, Ukraine, Moldova, Belarus, Lithuania, and Georgia, where the virus causes the death of up to 30–57 % of carp – the main cultivation object in the freshwater aquaculture [26]. Recently there has been a tendency towards the globalization of this infection. The main way of the spread of the disease is the exchange of alive fish between the fisheries, including decorative carp species [27].

As for the fisheries, specialized in breeding carps, the situation is still complicated. The fisheries of different regions of Ukraine were studied, in particular, L'viv, Rivne, Khmelnytsk, Ternopil, Vinnytsia, Kyiv, Chernihiv, Mykolaiv, Kherson, Donetsk regions. The integrated diagnosis was made taking into consideration the clinical, postmortem and laboratory examinations. While studying fish, the isolates of spring viraemia of carp (SVCV) were found in samples collected in spring summer and during the stress period, when fish is taken from the wintering holes into finishing ponds. Carps of different age groups were infested, but the clinical symptoms of the viral infection were usually registered for one- and two-year-old fish only. The map of Ukraine with the indicated regions of the outbreaks of viral fish infections is presented in Figure.

The analysis of the situation testifies to potential danger of the outbreak of viral infections of salmon and carp in the specialized fisheries of Ukraine. There is an



The map of Ukraine with the indicated regions of the outbreaks of viral fish infections

urgent issue of preventing these infections and elaborating the early diagnostics methods to avoid considerable economic losses of fisheries. This problem is of special relevance due to the import of stocking material and fish breeders into Ukraine from abroad, which may cause the spread of viral infectious diseases in the territory of this country.

As for viral diseases of trout, specific hazard for Ukraine comes from the import of fish and stocking material from Poland, Slovakia, Hungary, Romania, where the majority of trout fisheries are located, and Turkey (the neighbor of the AR of Crimea across the Black sea), where outbreaks of infectious trout diseases both in specialized fisheries and in natural surface waters took place every year [20].

The organic pollution of water bodies, temperature fluctuations, pH and other factors promote the growth and development of bacteria, affecting their virulence and pathogenicity.

The bacteriological examination of fish samples demonstrated a wide generic spectrum of microorganisms of family *Enterobacteriaceae*: *Aeromonas, Acinetobacter, Asomonas, Edwardsiella, Citrobacter, Hafnia, Proteus, Yersinia, Flavobacterium, Pseudomonas, Micrococcus, Renibacterium salmoninarium.* The percentage ratio for the species abundances is as follows: *Aeromonas* – 35 %, *Pseudomonas* – 27 %, *Flavobacterium* – 8 %, *Yersinia* – 6 %, *Proteus* – 2 %, *Micrococcus* – 2 % out of the total amount of isolated microorganisms.

The representatives of genus *Aeromonas* are the most common microflora isolates, detected from water and fish samples. The bacteria of this genus were isolated from carp, salmon, sturgeon species of L'viv, Kherson, Chernihiv, Odesa and Rivne regions, showing straight rods with round ends and spheric dimensions (0.3–1.0) × (1.0–3.5) μm (which are usually single, seldom coupled and in short chains). Gram-negative, mobile, facultative anaerobes were positive to oxidase, catalase, arginine dehydrolase, DNAse and gelatinase, but negative to ornithine, decarboxylase, urease, phenylalanine deaminase and fermented carbohydrates. The diseases, caused by bacteria of genus *Aeromonas*, are characterized by skin inflammation, focal hemorrhages, edema, crumpled scale, hydration of muscle tissue and all the internal organs.

Pseudomonosis is remarkable for carp, hybrids of carp and *Cyprinus carpio*, Prussian carp, and bighead. The enzootic outbreaks are observed most often among one- and two-year-old fish of these species in the aquaculture of Odesa, Kyiv, Kherson, Sumy and L'viv regions. The bacteria isolated from water and fish were cultivated under aerobic conditions and found to be gram-negative, belonging to the genus *Pseudomonas*. These are straight or slightly bent rods of (0.5–1.0) × (1.5–5) μm. Catalase-positive, mobile pseudomonads formed a tender film on meat infusion broth of transparent colonies with yellowish pigmentation; bright pink colonies grew on Endo agar and were easy to extract therefrom.

The family *Enterobacteriaceae* was presented with different genera and species, which are gram-negative, oxidase-negative, catalase-positive, mobile rods, capable of remarkable growth on Endo agar.

The isolates from trout and sturgeon species from Chernivtsi, Odesa and L'viv region, were related to the genus *Proteus*. These are gram-negative, mobile rods, facultative anaerobes, forming a film on the surface of solid nutritious medium; they are oxidase-negative, catalase- and urease-positive, forming hydrogen sulphide; lysine decarboxylase and ornithine dehydrolase are absent.

The bacterial fish diseases are a restraining factor in the development of the aquaculture. These diseases are rather wide-spread in the specialized fisheries of the European countries, Belarus and Russia [6, 11, 28]. The results of our research of bacterial fish diseases of freshwater aquaculture demonstrated the decrease in the epizootic situation and considerable extension of the range of species of bacterial pathogens of fish.

The results of investigations were used to elaborate a complex scheme of laboratory diagnostics and the methods of preventing the spread of viral agents in the specialized fisheries. The establishment of an efficient scheme of conducting investigations includes the examination and analysis of the epizootic situation in the fishery, the estimation of possible risks of being infested with this agent, the consideration of clinical and postmortem changes in fish, the sampling of biological material in accordance with the rules of aseptics and antiseptics, the analysis of the pathological and morphological condition of the selected fish, transportation, selection, systematization and classification of samples using the age criteria and selection place as well as complex diagnostics, which presupposes the application of different investigation methods. The system of treatment and prevention measures has been suggested, involving the application of immunomodulating preparations while keeping strictly to the technology of fish breeding [29] .

CONCLUSIONS

The IPNV isolates of rainbow trout were first isolated in the fisheries of different forms of ownership in the western regions of Ukraine (Volyn, L'viv, Transcarpathian, Chernivtsi regions).

Modern virological, molecular and biological methods were used to study the spread of spring viraemia virus in freshwater aquaculture. It was demonstrated that different age groups of carp are infested with the virus in the fisheries of L'viv, Donetsk, Chernihiv, Kyiv, and Odesa regions which testifies to a wide spread of the virus in Ukraine.

The red spot-like bacterial disease, the swim bladder inflammation of carp, the bacterial diseases of young trout and sturgeon were detected in the investigated fisheries of Ukraine.

400 isolates of different microorganisms were isolated from clinically healthy and sick fish and surface waters; further study allowed identifying and referring them to their corresponding families and genera.

Результати моніторингових досліджень інфекційних хвороб риб у прісноводній аквакультурі України

Н. М. Матвієнко, А. В. Ващенко,
І. О. Циганок, Л. П. Бучацький

e-mail: mnarine73@mail.ru

Інститут рибного господарства НААН
Вул. Обухівська, 135, Київ, Україна, 03164

Мета. Обстежити епізоотичний стан рибницьких господарств України. Дослідити біологічні особливості вірусних і бактерійних ізолятів риб прісноводної аквакультури. **Методи.** Для вивчення поширення вірусних хвороб риб за умов прісноводної аквакультури застосовано сучасні вірусологічні та молекулярно-біологічні методи. Епізоотичний стан господарств визначали згідно з планом обстеження рибницьких господарств. Патогенність виділених бактерій досліджували у біопробі. Ідентифікацію проводили з використанням довідника Берджі. Експрес-ідентифікацію бактерій здійснювали із застосуванням стандартизованої тест-системи API 20E «Bio Merieux» (Франція). **Результати.** Представлено результати моніторингових досліджень епізоотичного стану рибницьких господарств України. Вперше в Україні виділено ізоляти IPNV від райдужної форелі в господарствах різних форм власності західних регіонів України (Волинська, Львівська, Закарпатська, Чернівецька області). Показано ураження вірусом різновікових груп коропа у господарствах Львівської, Донецької, Чернігівської, Київської та Одеської областей, що свідчить про широке розповсюдження вірусу в Україні. В обстежених рибницьких господарствах України з бактерійних хвороб риб реєстрували краснухоподібне захворювання у коропа, запалення плавального міхура коропа, захворювання у молоді форелі та осетрових. Проаналізовано біологічні особливості вірусних і бактерійних ізолятів риб прісноводної аквакультури. **Висновки.** На основі епізоотичних даних зроблено оцінку стану рибницьких господарств України щодо вірусних захворювань риб. Виявлено новий для нашої країни вірус панкреатичного некрозу, що уражує райдужну форель (*Oncorhynchus mykiss*, Wal-

baum, 1792) та показано розповсюдження SVCV у коропових господарствах. Стосовно бактерійних хвороб риб показано погіршення епізоотичної ситуації та значне розширення видового різноманіття збудників бактерійних патогенів риб. Проведення щорічного систематичного моніторингу, а також створення заходів щодо попередження заносу збудників інфекційних захворювань є запорукою благополуччя спеціалізованих рибницьких господарств України.

Ключові слова: риби, моніторинг, бактерії і віруси риб.

Результаты мониторинговых исследований инфекционных болезней рыб в пресноводной аквакультуре Украины

e-mail: mnarine73@mail.ru

Н. Н. Матвиенко, А. В. Ващенко,
И. А. Циганок, Л. П. Бучацкий

Институт рыбного хозяйства НААН
Ул. Обуховская, 135, Киев, Украина, 03164

Цель. Обследовать эпизоотическое состояние рыбоводческих хозяйств Украины. Исследовать биологические особенности вирусных и бактериальных изолятов рыб пресноводной аквакультуры. **Методы.** Для изучения распространения вирусных болезней рыб в условиях пресноводной аквакультуры применены современные вирусологические и молекулярно-биологические методы. Эпизоотическое состояние хозяйств определяли согласно плану обследования рыбоводческих хозяйств. Патогенность выделенных бактерий исследовали в биопробе. Идентификацию проводили с использованием справочника Берджи. Экспресс-идентификацию бактерий осуществляли с применением стандартизированной тест-системы API 20E «Bio Merieux» (Франция). **Результаты.** Представлены результаты мониторинговых исследований эпизоотического состояния рыбоводческих хозяйств Украины. Впервые в Украине выделены изоляты IPNV от радужной форели в хозяйствах различных форм собственности западных регионов Украины (Волынская, Львовская, Закарпатская, Черновицкая области). Показано поражение вирусом разновозрастных групп карпа в хозяйствах Львовской, Донецкой, Черниговской, Киевской и Одесской областей, что свидетельствует о широком распространении вируса в Украине. В обследованных рыбоводческих хозяйствах Украины из бактериальных болезней рыб регистрировали краснухоподобное заболевания у карпа, воспаление плавательного пузыря карпа, заболевания молодых особей форели и осетровых. Проанализированы биологические особенности вирусных и бактериальных изолятов рыб пресноводной аквакультуры. **Выводы.** На основе эпизоотических данных сделана оценка состояния рыбоводческих хозяйств Украины относительно вирусных заболеваний рыб. Обнаружен новый для нашей страны вирус панкреатического некроза, который поражает радужную форель (*Oncorhynchus mykiss*, Walbaum, 1792) и показано распространение SVCV в карповых хозяйствах. Относительно бактериальных болезней рыб показано ухудшение эпизоотической ситуации и значительное расширение видового разнообразия возбудителей бактериальных патогенов рыб. Проведение ежегодного систематического мониторинга, а также создание мер по предупреждению заноса возбудителей инфекционных заболеваний являются залогом благополучия специализированных рыбоводческих хозяйств Украины.

Ключевые слова: рыбы, мониторинг, бактерии и вирусы рыб.

REFERENCES

1. *Plumb JA*. Health maintenance and principal microbial diseases of cultured fishes. Ames, Iowa State Univ. Press. 1999;328 p.
2. *Shchelkunov IS, Shchelkunova TI.* Rhabdovirus carpio in herbivorous fishes: isolation, pathology and comparative susceptibility of fishes. *Viruses of lower vertebrates*. Eds W. Ahne, E. Kurstak. Berlin, Springer. 1989;333–48.
3. *OIE.* Diagnostic manual for aquatic animal diseases. 3rd ed. Paris, World Organization for Animal Health. 2009;Chap. 222 p.
4. *Ahne W, Negele RD.* Studies on the transmission of infectious pancreatic necrosis virus via eyed eggs and sexual products of salmonid fish. *Fish and shellfish pathology.* Ed. A. E. Ellis. London, Acad. Press. 1985;262−70.
5. *Woo PTK, Bruno DW.* Fish diseases and disorders. CABI. 2011;Vol. 3:944 p.
6. *Antychowicz J.* Choroby ryb srodladowych. Warszawa: Panstwowe Wydawnictwo Rolnicze i Lesne. 2007;447.
7. *Matvienko N, Buchatsky L, Rud' Yu.* Methodological recommendations on laboratory diagnosis of fish diseases. Kyiv, IRG NAAN. 2009;19 p.
8. *Makarov VV.* The epizootic methodology. Moscow, Peoples' Friendship University. 2001;224 p.
9. *Musselius VA.* Laboratory equipment workshop on diseases of fish. Moscow, Light and food industry. 1983;296 p.
10. *Manual* of methods for general bacteriology. Ed. P. Gerhardt. Moscow, Mir. 1983;Vol. 1:536 p.
11. *Skurat EK, Asadchaja RL, Sivolotskaya VA, Govor TA.* Prevention and treatment of bacterial infections in fish pond. *Veterinary Science – into Production: scientific research of the S. N. Vyshelessky.* Institute of Experimental Veterinary Medicine, NAS of Belarus. Minsk. 2005;(38):482−5.
12. *OIE*. Manual of diagnostic tests for aquatic animals. 4th ed. Paris, World Organization for Animal Health. 2003;Chap. 2.1.8:358 p.