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Incidence of *Candidatus* Piscichlamydia salmonis and *Candidatus* Clavochlamydia salmonicola in the farmed Brown Trout (*Salmo trutta*) in Ukraine.

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ABSTRACT

The Rainbow trout (*Oncorhynchus mykiss*) and the Brown trout (*Salmo trutta*) are the most frequently farmed among fish of the *Salmonidae* family in Ukraine. The main cause of cultured *Salmonidae* fry mortality is gill disease of various etiologies. *Chlamydiae* species associated with epitheliocystis in the fish of *Salmonidae* family are *Candidatus* Clavochlamydia salmonicola and *Candidatus* Piscichlamydia salmonis. This research is dedicated to the study of farmed Brown trout (*Salmo trutta*) from a small private enterprise in the Lviv region. 80 samples were randomly taken from 8 ponds, 10 specimens from each reservoir in May 2019. Samples were examined by PCR for the presence of *Ca.* Clavochlamydia salmonicola and *Ca.* Piscichlamydia salmonis bacteria DNA. The studies were carried out in two stages: at the first stage, *Chlamydia*-positive samples were identified, at the next stage it was determined to which species they belonged to. As a result, first in Ukraine, the *Ca.* Clavochlamydia salmonicola and *Ca.* Piscichlamydia salmonis bacteria DNA were detected in samples taken from Brown trout. 12 out of 80 samples (15%) were *Chlamydia*-positive, among them, 11 samples (13.8%) contained DNA of *Ca.* Piscichlamydia salmonis, two samples (2.5%) – DNA of Ca. Clavochlamydia salmonicola, one sample (1.25%) contained DNA of both *Ca.* Piscichlamydia salmonis and *Ca.* Clavochlamydia salmonicola. The prevalence of *Chlamydia*-like organisms in samples of various age groups was different, most frequently (9 out of 12) they were found in fish sizing up to 7 cm. Prominent clinical signs of epitheliocystis were only observed in two individuals (2.5%) sizing 2 cm (exophthalmos, eye damage) and 14.3 cm (gills and skin damage). In case of coinfection with the presence of both *Ca.* Piscichlamydia salmonis and *Ca.* Clavichlamydia salmonicola, the most severe course of epitheliocystis was observed.

INTRODUCTION

As the human population grows and the consumption of fish products increases, the number of fish raised under aquaculture conditions is also increasing. The share of fish grown in aquaculture conditions is increasing and reaching 46% in 2018, up from 8% in 1990 **(FAO, 2020).** According to the data of State agency of the fishing agriculture of Ukraine, aquaculture is a growing industry in Ukraine, in 2019, 18.6 thousand tons of marketable aquaculture products were grown, in the previous year this figure was 15.9 thousand tons **(Public Report of the State Agency of**

Fisheries of Ukraine 2019). Among fish of the *Salmonidae* family, Rainbow trout (*Oncorhynchus mykiss*) and Brown trout (*Salmo trutta*) are most frequently farmed in Ukraine.

Brown trout (*Salmo trutta*) is one of the most valuable free-living species of fish in Ukraine, the number of which has decreased by 13.4 times compared to the 1940s, and the biomass by 42 times (15 specimens per km.). In the rivers of the Carpathians, the number of this species of fish continues to decline sharply due to anthropogenic influence (industrial and domestic pollution of rivers, unauthorized fishing). The only and effective method of struggle to restore the trout population is the cultivation of this fish species with the subsequent release of young stock into the system of mountain rivers. To facilitate this process, a number of trout farms are engaged in artificial reproduction of the population (catching males and females from the natural environment, selecting fertilization and incubating eggs, raising young animals and releasing them into water bodies) (**Terpay, 2019**).

It is a well-known indisputable fact that among the higher density of cultivated populations, there is an increased frequency of viral, bacterial and parasitic diseases with which the owners of such farms are forced to struggle. In salmonids cultivation, the main cause of fry mortality is gill disease of various etiology. Epitheliocystis is one of the diseases that, in conditions of fish congestion during fish farming, can pose an economic risk for the industry (**Sood *et al.*, 2019**). Chlamydia-like organisms, associated with epitheliocystis, affect more than 90 marine and freshwater wild and farmed fish species. (**Pawlikowska-Warych & Deptuła, 2016; Blandford *et al.*, 2018; Zezekalo *et al.*, 2019a; 2019b**). The number of fish species that are susceptible to epitheliocystis is probably underestimated and, over time, will expand significantly (**Blandford *et al.*, 2018; Wang *et al.*, 2020**). This is due to the fact that in free-living populations of different fish species, epitheliocystis is very difficult to track, since, in most cases, it is not a life-threatening disease (**Stride *et al.*, 2013a; 2013b; 2014**). Species of the Chlamydiae associated with fish disease, epitheliocystis are *Candidatus* Piscichlamydia salmonis, *Candidatus* Clavochlamydia salmonicola, *Candidatus* Parilichlamydia carangidicola, *Candidatus* Actinochlamydia clariae, *Candidatus* Similichlamydia laticola, *Candidatus* Similichlamydia labri, *Candidatus* Similichlamydia latridicola, *Candidatus* Renichlamydia lutjani, *Syngnamydia venezia*, *Neochlamydia-like* bacterium (**Guevara Soto *et al.*, 2016; Sellyei *et al.*, 2017; Blandford *et al.*, 2018; Zezekalo *et al.*, 2019c**). Among fish affecting Chlamydial species, *Ca*. Piscichlamydia salmonis *Ca*. Clavochlamydia salmonicola can be distinguished in territory of Ukraine since their hosts Atlantic salmon (*Salmo salar*) and the Brown trout (*Salmo trutta*) are species bred in Ukraine (**Zezekalo *et al.*, 2019d**).

Epitheliocystis is a disease of fish gills and skin, which cause inflammation and hypertrophy of the gills, occasionally accompanied by the presence of specific white nodules the gill tissue, in some cases, the inflammation spreads to other types of tissues causing exophthalmos, lens prolapse, ulcerative skin lesions, spinal lesions. Mortality occurs as a result of respiratory distress in connection with inflammatory and proliferative lesions of the gills, which leads to fusion and necrosis of the gill filaments. Mortality in farmed fish populations can vary greatly, ranging from no mortality to100% mortality in young. (**Fehr *et al.*, 2013**).

Clinical manifestations of epitheliocystosis in conditions of fish farms and in free-living species are similar, but are observed less often, perhaps this is due to the early death of the fry, or the acquisition of immunity by adult fish. (**Stride *et al.*, 2014; Pawlikowska-Warych & Deptuła, 2016**). Inconsistency between reports by different authors highlights the prospect of an unknown determinant of susceptibility to infections associated with chlamydia-like organisms.

The main method for studying fish with epitheliocystis is microscopy of the gills to identify specific inclusions and subsequent DNA sequencing of samples in which these inclusions were found (**Rey *et al.*, 2020**). This is a laborious and expensive study that is not suitable for routine monitoring in the difficult economic situation in Ukraine. Therefore, our study is based on the PCR method with the detection of the DNA of *Ca.* Piscichlamydia salmonis, *Ca.* Clavochlamydia salmonicola in samples from brown trout grown in a farm.

In 2018, through the efforts of the laboratory of animal health and the laboratory of genetics at the Institute of Pig Breeding and Agro-Industrial Production, NAAS, PCR test-kits were developed for identification and species differentiation of *Ca.* Clavochlamydia salmonicola and *Ca.* Piscichlamydia salmonis, epitheliocystis agents in commercially important aquaculture species of Ukraine (**Zezekalo *et al.*, 2019c; Zezekalo *et al.*, 2019d**).

The purpose of this study was to examine Brown trout from family-owned enterprise using PCR test-kits for identification and species differentiation of *Ca*. Clavochlamydia salmonicola and *Ca.* Piscichlamydia salmonis. There is limited information about epitheliocystis in scientific papers in Ukrainian or Russian, and this is the first study on that topic performed in Ukraine.

MATERIALS AND METHODS

All manipulations with animals were carried out in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, "General Ethical Principles for Animal Experiments". The permit for the use of animals is approved by the Academic Council of the Institute of Pig Breeding and Agro-Industrial Production, NAAS, and the Commission on Bioethics of the Poltava State Agrarian Academy.

80 brown trout (*Salmo trutta*) samples were taken in May 2019 from a private fish farm in Lviv region. Samples were randomly selected from 8 reservoirs, 10 specimens from each reservoir. Selected fish of various lengths from 2 cm to 25cm were raised in flow-through tanks. Fish less than 10 cm in length were raised in tanks with a volume of 1 m^3 (400 specimens / 1 m3), exposed to a natural day/night light cycle. After the fish reached 10 cm, they were transferred in tanks of 50 m^3 (7000-12000 specimens per tank, depending upon a size of the fish). The fish were fed three times a day with a high-protein compound feed from Spezialfuttermittelwerk Beeskow GmbH. Water was taken from a capturing source, the water temperature at the time of intake was 8 ° C, and the water exchange rate was 2 m / s. The content of oxygen dissolved in water was not less than 7.5 g / m3. The fish has been humanely killed with clove oil. About 400 mg of clove oil per liter of aquarium water, the fish remained in the solution for 20 minutes. The collected samples were placed with

sterile forceps or scissors into Eppendorf tubes with 96 % ethanol solution. After that, the test tubes with samples were placed in thermal containers for transportation.

Further studies, molecular genetic analysis, performed using polymerase chain reaction (PCR) method, were carried out at the laboratory of animal health and the laboratory of genetics at the Institute of Pig Breeding and Agro-Industrial Production, NAAS, which was certified for genetic analysis at the DNA level (Compliance certificate ‘‘state of the measurement system’’ number 021-19 from 01/31/2019), using the self-developed PCR test-kits for indicating DNA of the gene encoding 16S rRNA of *Ca*. Clavochlamydia salmonicola and *Ca.* Piscichlamydia salmonis .

As positive control DNA samples of *Chlamydia*-like organisms pathogenic for fish: *Ca*. Clavochlamydia salmonicola and *Ca.* Piscichlamydia salmonis, kindly provided by Dr. Heike Schmidt-Posthaus, (Center for Fish and Wildlife Health, Bern, Switzerland) were used. As negative control deionized water (Thermo Fisher Scientific, USA) added to the mastermix instead of a DNA sample were used.

DNA was extracted from clinical material using the ‘‘DNA express’’ reagent kit, LITECH (Russia), according to the manufacturer's protocol and stored at −20°C until use. PCR reactions were performed using reagents (Thermo Fisher Scientific, USA), reactions were performed in a final volume of 25 μL containing deionized water 16 μL, PCR buffer 2,5 μL, $MgCl_2$ 1μL, solution of deoxyribonucleoside triphosphates (dNTP) 2 μL, Taq polymerase *(Thermus aquaticus*) 0,5 μL, 1 μl each primer and 1μL of a DNA sample. Oligonucleotide primers used in our study were synthesized by Metabion international AG (Table 1).

Table1. List of primers used in this study

Species	**Sequence**	**Product size**
Ca. P. salmonis and *Ca*. C. salmonicola	PCSALF: GCTAACGCGATAAGTGTGCC PCSALR: CCATGCAGCACCTGTGTAGT	197 b.p
Ca. Piscichlamydia salmonis	PICHSF: CTAGACTAGAGTTCAAGGGGG PICHSR: GCTAGGGTTGAGACTAGCTAC	207 b.p.
Ca. Clavochlamydia salmonicola	CLACHSF: GAGTTCGTTAAAGCGGGGGA CLACHSR: CAGGTCTTTCTTGTCCCCAAG	276 b.p

Amplification was performed using a "Tercyc-2" multichannel thermo cycler (DNA technology, Russia). The cycling conditions were 120 sec at 95 ° C, followed by 35 cycles of 15 seconds at 95° C, 1 minute at 60° C and 72 °C, 45 seconds, after 35 cycles 1 minute of final extension at 72 °C.

PCR products were separated using 2 % agarose gel electrophoresis in 1 × TBE buffer for 2 hours at a current of 50 mA in an electrophoresis chamber (Cleaver Scientific Ltd). Plasmids *pUC*19 hydrolyzed with *Msp* I endonuclease (Thermo Fisher Scientific) were used as a molecular weight marker. After the electrophoresis process was over, the gel was stained with a solution of ethidium bromide (10 mg / cm3) and the results of electrophoresis were documented by gel

documentation system (Cleaver Scientific Ltd, UK).

At the first stage, all DNA samples were examined using oligonucleotide primers PCSALF and PCSALR for simultaneous detection of two epitheliocystis agents (to amplify the common fragment of 16S *rRNA* gene for *Ca*. Clavochlamydia salmonicola and *Ca.* Piscichlamydia salmonis). Then all positive samples from the first screening were selected for further study using primers PICHSF and PICHSR to amplify a fragment of *Ca.* Piscichlamydia salmonis, 16S *rRNA* gene and primers CLACHSF and CLACHSR to amplify a fragment of *Ca.* Clavochlamydia salmonicola 16S *rRNA* gene in the DNA, in order to determine the pathogens species.

The identity of obtained PCR products was further verified by selective sequence recognition by endonucleases restriction. The composition of the reaction mixture for the hydrolysis of amplified fragments: 10 × restriction buffer, deionized water, restriction endonuclease (Thermo Fisher Scientific, USA) (Table 2). The reaction mixture was incubated at 37–65 ° C for 1–3 h, depending on the manufacturer's recommendations. After restriction, electrophoresis was performed in vertical polyacrylamide or horizontal agarose gels depending on the size of the DNA restriction fragments.

Table 2. List of restriction endonucleases used in this study

Chlamydia species	**Restriction endonuclease**		**Restriction fragments, b.p.**	
Ca. Clavochlamydia salmonicola (276 b. p.)	*Alu I (10 U/µL)*	AG↓CT	196	61
Ca. Piscichlamydia salmonis (207 b. p.)	*Tas I (10 U/µL)*	↓AATT	178	29
Ca. Cl. salmonicola *&* *Ca.* P. salmonis (197 b. p.)	*Tas I (10 U/µL)*	↓AATT	(13)40	44
			40	57

RESULTS

The farm staff reported that there is a disease of the gills of fish observed, after rains in summertime, which manifests itself as death, which is preceded by lethargy, slow movements, gasping air on the surface of the water, whirling, lethargy, some individuals remain at the bottom of the tank for a long time and show a reduced response to external stimuli. The lethal outcome is typical for the fry, in which, in addition to lesions of the gills, a significant number of individuals have lesions of the eyes and skin. In full-grown fish, swelling and inflammation of the gills, mucus secretion, loss of appetite and growth retardation are observed. Upon necropsy, no visible lesions, other than those described above, are presented. The outbreak usually lasts several weeks, after which the clinical manifestations gradually subside. The main losses are caused by high mortality among fry as well as growth retardation of fish. According to the farm staff, at the time of sampling, there was a low mortality rate, as well as absence of signs of fish gill diseases.

The first stage of research was focused on identifying the DNA of both *Ca*. Piscichlamydia salmonis and *Ca*. Clavochlamydia salmonicola, a chlamydia-like bacteria that has been associated with epitheliocystis in brown trout. As a result of the first stage of research, 4 out of 10 fish samples of the first group, the length of which did not exceed 2 cm, turned out to be Ca. Piscichlamydia salmonis and / or *Ca.* Clavochlamydia salmonicola positive (Table 3). In this group, only one of the fries swam on the surface of the water, and had noticeable damage to the eyes, gills, and exophthalmos. In fish from the second group, the length of which did not exceed 5 cm, no clinical signs characteristic of lesions of the gills were observed. Nevertheless, 2 out of 10 samples turned out to be *Ca.* Piscichlamydia salmonis and / or *Ca.* Clavochlamydia salmonicola positive. In the third group, up to 7 cm in size, no visible changes in the gills were observed, however 3 out of 10 samples were *Ca.* Piscichlamydia salmonis and / or *Ca.* Clavochlamydia salmonicola positive. In the fourth group of individuals up to 10 cm in size, clinical signs were absent; DNA *Ca.* Piscichlamydia salmonis and / or *Ca.* Clavochlamydia salmonicola was not found. In the fifth group, individuals of which were 10-15 cm in size, one of the fish showed lesions of the gills and skin, lethargy, decreased response to external stimuli, and this individual was found to have *Ca.* Piscichlamydia salmonis DNA and / or *Ca.* Clavochlamydia salmonicola DNA. In the sixth group, the size of the individuals from which the samples were taken was 15-20 cm, no clinical signs of gill disease were found, and during the subsequent examination of the gill samples, no chlamydia-like organisms were found. In the seventh group (20-25 cm), no clinical signs of the disease were observed; in one sample, DNA of chlamydia-like organisms associated with gill disease was detected. In the eighth group, samples were taken from fish more than 25 cm, no signs of gill disease, as well as DNA of chlamydia-like organisms associated with gill disease, were found. After the first stage, 12 samples were taken, in which the DNA of chlamydia-like organisms associated with gill disease was found (Table 3).

Table 3. Prevalence of *Ca*. Clavochlamydia salmonicola and *Ca*. Piscichlamydia salmonis in diferent groups of farmed Brown Trout

Group	**Fish size, cm**	**Total amount of samples/ Number of positive samples**	**Presence of clinical signs**	**Presence of *Ca.* Clavochlamydia salmonicola *DNA***	**Presence of *Ca.* Piscichlamydia salmonis *DNA***
1	1-2	10/4	1	0	4
2	2-5	10/2	0	0	2
3	5-7	10/3	0	0	3
4	7-10	10/0	0	0	0
5	10-15	10/2	1	1	2
6	15-20	10/0	0	0	0
7	20-25	10/1	0	1	0
8	25-35	10/0	0	0	0
Total	80	80/12	2	2	11

At the second stage, these samples were examined in order to determine the type of chlamydia-like organisms. As a result, it turned out that in the first, second and third groups, all chlamydia-like organisms belonged to the species Ca. Piscichlamydia salmonis. In the fifth group, in a sample from the same individual were found both DNA Ca. Piscichlamydia salmonis as well as Ca. Clavochlamydia salmonicola. A chlamydia-positive sample from the seventh group was found to have DNA of Ca. Clavochlamydia salmonicola (Table3).

Restriction analysis of PCR amplification products using endonucleases AluI, TasI was carried out, as a result of which the expected spectrum of DNA fragments determined by the nucleotide sequence was obtained.

Chlamydia-like organisms, which are associated with epitheliocystis, were found in 12 of 80 trout samples. Only 2 out of 12 brown trout had clinical signs characteristic of epitheliocystis (2.5 %). Out of 80 samples of Brown trout (*Salmo trutta*) randomly collected from the private fisheries of the Lviv region only 12 (15%) were chosen for further study after examination using oligonucleotide primers PCSALF and PCSALR. A subsequent study using primers specific for each species showed the presence of *Ca.* Piscichlamydia salmonis DNA in 11 samples (13.8 %), Ca. Clavochlamydia salmonicola (2.5 %) in two samples. In one sample (1.25%) DNA of both *Ca.* Piscichlamydia salmonis and *Ca.* Clavochlamydia salmonicola was found. The compliance of the obtained PCR product was confirmed by restriction analysis.

Therefore, for the first time in Ukraine, DNA of Piscichlamydia salmonis as well as Ca. Clavochlamydia salmonicola, chlamydia-like organisms associated with epitheliocystis, were detected in farmed Brown trout' samples.

DISCUSSION

This paper is dedicated to the Brown trout study at a private farm in the Lviv region for the presence of *Ca*. Piscichlamydia salmonis and *Ca*. Clavichlamydia salmonicola DNA, a chlamydia-like bacteria that has been associated with epitheliocystis. The purpose of our work was to conduct a study of Brown trout in fisheries in Ukraine for the detection of DNA of chlamydia-like bacteria *Ca*. Piscichlamydia salmonis and *Ca*. Clavochlamydia salmonicola, and determine the presence or absence of clinical signs characteristic of epitheliocystis.

According to European studies, epitheliocystis of brown trout caused by the chlamydia-like bacteria Ca. Piscichlamydia salmonis and Ca. Clavochlamydia salmonicola and is found both in the wild and on farms (**Blandford *et al.*, 2018**). Brown trout (*Salmo trutta*) was chosen for the study, because it is more sensitive to environmental factors and more susceptible to epitheliocystis infection (**Schmidt-Posthaus *et al.*, 2001**). Moreover, the study was planned in such a way that sampling of the material took place in the summer, since the increase precipitation and water temperature is presumably contributing to the development of this disease (**Mitchell& Rodger, 2011**).

Currently, there is no standard method for diagnosing epitheliocystis, especially in the early stages of the disease. In case of outbreaks of this disease, the diagnosis is made on the basis of

changes in fish behavior (respiratory distress, lethargy, weak swimming behavior, growth retardation) and the presence of macroscopic tissue damage (hypertrophy and inflammation of the gills, lesions of epithelial tissues (**Sellyei *et al.*, 2017; Blandford *et al.*, 2018**), followed by microscopy of gill samples to detect cysts, then, if necessary, follow-up studies are carried out using molecular genetic methods to determine the type of specific etiological agent. Various methods have been used to detect these agents such as transmission electron microscopy (TEM), polymerase chain reaction (PCR), immunohistochemistry (IHC), in situ hybridization (ISH), but none of epitheliocystis agents have been cultured yet (**Nowak & LaPatra, 2006; Seth-Smith *et al.*, 2016; Blandford *et al.*, 2018**). Unlike previous studies, in which the main method was histological followed by the use of molecular genetic methods, our study is based exclusively on the PCR method, which is more affordable due to the difficult economic situation in Ukraine. The study was carried out with a previously developed diagnostic test kit, using primers based on 16S rRNA, which limit the conserved DNA region of chlamydia-like bacteria associated with salmon epitheliocystis, followed by species identification of the identified chlamydia-like bacteria using species-specific primers. A distinctive feature of this study is that at the first stage, primers are used, aimed to identifying not common chlamydial DNA, but DNA of chlamydia-like bacteria that infect fish, in our case, trout. The reason for the choice of such primers was that there is a screening out of chlamydia-like species, endosymbionts of protozoa that are not agents of epitheliocystis, but together with protozoa can be found in reservoirs.

The prevalence and degree of infection of fish populations indicated by different authors is different and ranges from 16% to 100%, as well as clinical signs are present in varying degrees. Mortality from epitheliocystis in different age groups ranges from 0 to 100% (**Seth-Smith *et al.*, 2016; Guevara Soto *et al.*, 2017; Guevara Soto *et al.*, 2016**). This is the first such study in Ukraine to detect *Chlamydia*-like bacteria in fish samples, therefore, we could only compare our results to the data previously obtained in Europe. In histological studies of farmed Brown trout in Europe for the detection of epitheliocystis performed before, the prevalence of epitheliocystis was 0-60 %, and in the rivers of the same region – 4-18 %. In another study of wild Brown trout in tributaries (Venoge and Boiron) of the Rhone flowing into Lake Geneva during the summer, the infection rate was 14-16 %, distribution in the Rhine and the Rhone rivers was 8.29-18.4 % Comparing the infection of wild trout and brown trout grown in farms in the respective river sections showed that the probability of detecting infected fish in the farm is slightly higher than that of detecting one in the river (**Guevara Soto *et al.*, 2017; Guevara Soto *et al.*, 2016**). As a result of our study, 15 % of samples were chlamydia-positive, which does not contradict with previously obtained data.

Epitheliocystis is not evenly distributed among different age groups, so juvenile fish suffer more than older individuals do. Disease in most cases has a chronic course and benign. Clinical signs that may be rarely observed include gills hypertrophy and inflammation, white nodular lesions in epithelial tissues of gills or skin, in severe cases respiratory distress, exophthalmos, lens lesion, phacocele, corneal clouding and blindness, as well as skin ulcers could be observed. (**Mitchell & Rodger, 2011; Pawlikowska-Warych & Deptula, 2016; Blandford *et al.*, 2018**). Data obtained

as a result of our study are consistent with the previously obtained data, so 9 out of 12 selected chlamydia-positive samples were taken from fish up to 7 cm in size. Clinical signs of epitheliocystis were revealed in two individuals, sizing 2 cm (exophthalmos, eye damage) and 14.3 cm (gills and skin damage).

Regarding *Chlamydia*-like bacteria associated with epitheliocystis, Ca. Piscichlamydia salmonis was most frequently detected in the course of our study in 11 out of 12 positive samples, in one sample DNA of Ca. Piscichlamydia salmonis and Ca. Clavichlamydia salmonicola were found, and only in one sample DNA of Ca. Clavichlamydia salmonicola was detected. In the case of coinfection, lesions of the fish gills and skin were found. Our data correlate with the data previously obtained by European researchers in the study of wild and farm fish, where DNA of Ca. Piscichlamydia salmonis was isolated in samples from fish with epitheliocystis more frequently than that of *Ca.* C. salmonicola. Also, in case of coinfection with the presence of both *Ca.* Piscichlamydia salmonis and *Ca.* Clavichlamydia salmonicola, the most severe course of epitheliocystis was observed in both wild and cultured Brown trout (**Blandford *et al.*, 2018)**.

CONCLUSION

The world's population is growing rapidly from year to year, and therefore the demand and consumption of fish and fish products is also increasing. Industrial fishing is gradually being replaced by fish farming. Growing fish under the conditions of fish farms is associated with an increase in the concentration of the number of fish individuals in comparison with the wild, which leads to the appearance of a greater number of infectious diseases, and their more severe course. The main cause of mortality in fish fry is a disease of the gills; epitheliocystis is one of the representatives of such diseases.

It is the first such study conducted in Ukraine, as a result, DNA of *Chlamydia*-like organisms *Ca.* Piscichlamydia salmonis and *Ca.* Clavichlamydia salmonicola were found in samples of farm-raised Brown trout. Further studies will be focused on broader screening of farms and rivers and including histological studies. Further studies of fish from other farms and wild fish are needed to include more individuals to find out how widespread these bacteria are. More detailed disease registration and comparison of data from other researchers are needed to understand the impact that epitheliocystis has on the aquaculture industry. Further research is needed using a broader range of techniques to identify new etiological agents to better understand the disease, which in turn will help reduce infestation and improve fish welfare. Since there is a problem of diseases of fish, which are the object of aquaculture, the study of this disease and its prevention in the future will lead to an increase in the profitability of fish farms.

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