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THE INVESTIGATION OF THE PRESENCE OF SOME BACTERIAL AND VIRAL AGENTS IN PEARL MULLET (*CHALCALBURNUS TARICHII,* PALLAS 1811) BY REAL-TIME PCR AND THE HISTOPATHOLOGICAL EXAMINATION

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ABSTRACT

The aim of this study was to identify some bacterial (*Vibrio cholerae, Listonella anguillarum, Yersinia ruckeri, Aeromonas hydrophila, Aeromonas sobria* and *Aeromonas caviae*) and viral (infectious pancreatic necrosis virus, viral hemorrhagical septicemia virus and infectious hematopoietic virus) pathogens by comparing the bacteriological culture and molecular methods with histopathological examination of various tissues seen in Pearl mullet from freshwater rivers inflowing to the Van lake. Accordingly, bacterial and viral agents were detected in a total of 180 pearl mullet samples by bacteriological culture and Real-Time PCR methods. The samples were collected from six different freshwater rivers that flows to Van lake by random sampling. In the study, *Vibrio cholerae, Listonella anguillarum, Yersinia ruckeri*, Infectious Pancreatic Necrosis Virus, Viral Hemorrhagic Septicemia Virus and Infectious Hematopoietic Necrosis Virus could not be isolated. However, *Aeromonas* spp. was identified by bacteriological culture and Real-Time PCR methods at 22.22% and 53.33% respectively. The most dominant species was observed to be *Aeromonas hydrophila* among all identified *Aeromonas* spp. In histopathological examinations of *Aeromonas* spp. positive samples, disorders were detected in gill, liver, spleen and kidney tissues. Telangiectasia, edema, hyperplasia and adhesions were observed on the seconder laminates of gills. In liver, the order of the Remak cords were disarranged, and degenerative changes formed in hepatocytes. Melano-macrophages loaded with hemosiderin were intensively detected in all *Aeromonas* positive samples. As a result, the risk of motile *Aeromonas* infections in the pearl mullet living in freshwaters flowing into the Van lake is still highly and this could lead to serious adverse effects on human health. To prevent these effects, attention must be paid to hygiene conditions and cold chain in fish served for consumption, the and rivers must be protected from pollution.

KEYWORDS:
Bacteria, Virus, *Chalcalburnus tarichii,* Real-Time PCR, Histopathology, Freshwater rivers flowing into Van lake

INTRODUCTION

Pearl Mullet (C*halcalburnus tarichi*, Pallas 1811) is the only endemic species in the Van Lake, the largest lake in Turkey [1].

Specific media are used for the isolation of disease factors in fish diseases [2, 3]. Individual and collective fish deaths resulting from various bacterial and viral infections in the life cycle of fish are occasional [4].

Most bacterial infections causing massive deaths in fish are septicemic. In septicemic infections, the agent can be isolated from anywhere in the host's bloodstream. In fish, the anterior kidney is known to be the most suitable site for septicemic infections [5].

Pearl mullet is an endemic fish species in the Van lake and it enters the rivers flowing into Van lake for reproduction.

Van lake basin is a closed basin and the rivers in the basin have high potential in terms of fishing and aquaculture. In addition to determining whether the pearl mullet is a disease reservoir for cultured fish, this study also investigated infectious agents that contaminated pearl mullet in rivers where the aquaculture has been initiated. In addition, zoonosis agents such as *Vibrio cholerae*, *Aeromonas hydrophila* and fish pathogens threatening human health were investigated. These bacterial agents mainly cause poisoning, gastroenteritis, watery

diarrhea, vomiting, fever and epigastric pain in children and elderly.

The aim of this study was to identify some bacterial (*Vibrio cholerae*, *Listonella anguillarum*, *Yersinia ruckeri*, *Aeromonas hydrophila*, *Aeromonas sobria* and *Aeromonas caviae*) and viral (infectious pancreatic necrosis virus, viral hemorrhagical septicemia virus and infectious hematopoietic virus) pathogens by comparing bacteriological culture and molecular methods with histopathological examination of various tissues seen in Pearl mullet from freshwater rivers flowing into the Van lake.

Accordingly, a total of 180 fish samples were collected by sampling from six rivers flowing into the Van Lake and bacterial and viral agents were investigated.

MATERIALS AND METHODS

Sample collection. Sample collection was carried out in Bendimahi, Karasu, Deliçay, Zilan, Enginsu and Güzelsu rivers flowing to the Van Lake during hunting season (Figure 1). In this period, a total of 180 fish samples were collected from six sources. Thirty fish from each river source were freshly caught by fishermen by random sampling method. The samples were brought to Van Yüzüncü Yıl University, Faculty of Aquaculture, Department of Aquaculture Research Laboratory in the short time under cold chain [6].

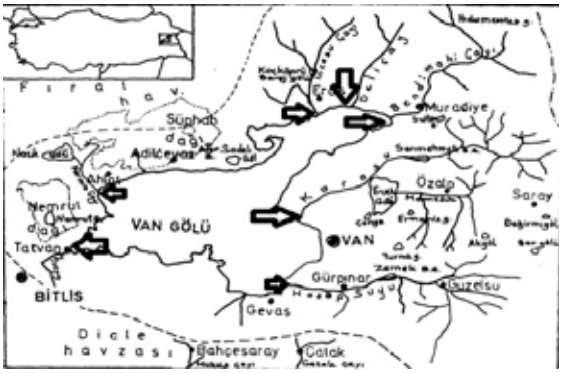


FIGURE 1
The Van lake basin and sample collecting rivers [6]

In this study, the names of the rivers from which the samples were taken were divided into zones and each river source was named as a zone. Accordingly; Region 1 refers to Bendimahi, Region 2 refers to Karasu, Region 3 refers to Deliçay, Region 4 refers to Zilan, Region 5 refers to Enginsu and Region 6 refers to Güzelsu river.

Necropsy and isolation material of fish specimens. The outer surface of the fish samples was disinfected with 70% ethyl alcohol and necropsied aseptically using some sterile forceps and bistouries on a steel tray. Samples were taken from the liver, spleen, kidney, intestine and gills of the fish for bacteriological culture, molecular bacteriological analysis, molecular viral analysis and histopathological examinations. Samples were thoroughly homogenized in sterile phosphate buffer (PBS) containing 20% glycerin for bacteriological assays and viral analysis and stored at -70 °C until the time of culture and real-time PCR procedures. Tissue samples taken for histopathological examination were fixed in 10% formalin and blocked in paraffin. All the sample sections taken from each block at 4-6 µm were stained with Hematoxylin-eosin (HE) and some samples, where deemed necessary, were stained with Brown-Brenn Gram for bacterial examination, and examined under the light microscope [13].

Bacterial isolation. Tissue samples obtained after the necropsy of the fish samples were placed in Eppendorf tubes containing 1 ml sterile 20% glycerin phosphate buffer and homogenized using ultra turrax homogenizer. Then, 200 µl homogenate from five different tissues of each fish sample were collected in sterile Eppendorf tubes. The samples were taken from the solution containing the tissue samples by swab method and inoculated in Shotts-Waltman (SW) for *Y. ruckeri*, Tryptic Soya Agar (TSA) for *V. cholerae* and Thiosulfate Citrate Bile Sucrose (TCBS) for *L. anguillarum* and incubated for 24 hours at 30 ºC. For Aeromonas isolation, 1 ml sample was taken from tissue homogenates and added to 10 ml 0.1% Alkaline Peptone Water (APS, pH 8.4-8.6) for pre-enrichment and incubated for 24 hours at 28 ºC. At the end of 24 hours, 50 µl suspension taken from pre-enrichment in APS were inoculated in Aeromonas Selective Agar (Aeromonas Agar Base-Oxoid CM833 + Aeromonas Selective Supplement-Oxoid SR136E) and Blood Agar (Merck 1.10886.0500) by streak plate method and incubated at 28 ºC for 24 hours [2, 3, 4, 17].

DNA isolation. In the study, *A. hydrophila* ATCC 7966, *A. caviae* ATCC 15468, *Y. ruckeri* ATCC 29473 and *L. anguillarum* ATCC 68554 reference strains were used for positive control. For DNA isolation, broth media prepared at McFarland 0.5 dilution were centrifuged at 5000 x *g* for 10 minutes. The obtained pellet was used for total DNA isolation using Gene Jet Genomic DNA isolation Kit (Thermo) according to the protocol recommended by the manufacturer [21].

RNA isolation for virological analysis. The tissue material removed after the necropsy was centrifuged at 3000 rpm for 15 min after homogenization in the tissue disruptor at +4 ºC in antibiotic PBS (phosphate-buffered saline). The resulting supernatant was used for viral RNA isolation. RNA

TABLE 1
Oligonucleotide primers of bacterial species used in the study

The name of the bacterium	Primer sequence
Aeromonas spp.	F-GGGAGTGCCTTCGGGAATCAGA R-TCACCGCAACATTCTGATTTG
A. hydrophila	F-GCCGAGCGCCCAGAAGGTGAGTT R-GAGCGGCTGGATGCGGTTGT
A. sobria	F-TAAAGGGAAATAATGACGGCG R-GGCTGTAGGTATCGGTTTTCG
A. caviae	F-GAGCCAGTCCTGGGCTCAG R-GCATTCTTCATGGTGTCGGC
Y. ruckeri	F-CGAGGAGGAAGGGTTAAGT R-AAGGCACCAAGGCATCTCT
V. cholerae	F-CAGCCACACTGGAACTGAGA R-TTAGCCGGTGCTTCTTCTGT
L. anguillarum	F-CCAGCAAGAGATCCAAGAGG R-GTCCGCAAGATGGAATGAAT

TABLE 2
The oligonucleotide primers of the examined virus species

Virus	Primer sequence	Gene
VHSV	F-CCAGCTCAACTCAGGTGTCC R-GTCACYGTGCATGCCATTGT	G
IPNV	F-CGCAACTTACTTGAGATCCATTATGC R-GTCTGGTTCAGATTCCACCTGTAGTG	VP2
IHNV	F-GTTCAACTTCAACGCCAACAGG R-TGAAGTACCCCACCCCGAGCATCC	N

isolation was performed in accordance with the Gene Jet Viral DNA/RNA Purification Kit [14].

cDNA Synthesis. For cDNA synthesis in each sample, as the first step, the mixture-1 was prepared using 3 µl sterile distilled water, 0.5 µl random hexamer primer and 3 µl RNA. After keeping at 70 ^{o}C for 5 minutes, the tube was quickly immersed in ice. For the second step, 3.5 µl of mixture-2 containing 2 µl 5x reaction buffer, 1 µl 10 mM dNTP mixture and 0.5 µl M-MuLV reverse transcriptase was added to the tubes containing mixture-1 and incubated at 48 ^{o}C for 45 min [14].

Primers used in the study. Oligonucleotide primers were verified and synthesized using Genbank database based on specific regions of DNA encoding 16S and/or 23S rRNA genes. The primer sets used in the study are given below (Table 1 and 2).

Real-Time PCR. In real-Time PCR procedure, positive control DNAs were used for *Yersinia ruckeri*, *Listonella anguillarum*, *Aeromonas* spp., *Aeromonas caviae*, *Aeromonas sobria* and *Aeromonas hydrophila*. Maxima SYBR Green qPCR Master Mix (Thermo) was used for the amplification step. Preparation of the mixture: 2 µl DNA of the examined bacteria and 1 µM primers were placed in a 25 µl master mix. Then, the mixture was completed to 25 µl with DNase and RNase free water. In the PCR steps, denaturation was performed at 96°C for 10 minutes and then a total of 35 cycles were performed. Procedure was carried out as 30 seconds denaturation at 94 °C, 60 seconds ligation at 60 °C, 60 seconds elongation at 72 °C and 10 seconds final elongation at 72°C [21, 22]. For the viral RNA identification, 30 µl PCR master mix was prepared consisting of 3 µl DNA, 75 mM Tris-HCl (pH 8.8), 20 mM $NH_4(SO_4)_2$, 1.5 mM $MgCl_2$, 10 pmol primers, 0.2 mM dNTP and 0.5 U Taq DNA polymerase (MBI, Fermentas, Lithuania). The PCR reaction was performed on separate conditions for each viral disease agent using the primers indicated in the table. The heating process for VSHV (Viral Hemorrhagic Septicemia Virus) was a 2-minute-denaturation step at 95 °C followed by 40 seconds at 60 °C, 40 seconds at 72 °C and 40 seconds at 95 °C. This procedure was repeated for 30 cycles and the procedure was completed with a final elongation step at 72 °C for 5 minutes. The heating process for IPNV (Infectious Pancreatic Necrosis Virus) was 2-minute-denaturation step at 94 °C followed by 45 seconds at 45 °C, 120 seconds at 68 °C and 45 seconds at 94 °C. This procedure was repeated for 45 cycles and the procedure was completed with a final elongation step at 68 °C for 7 minutes. The heating process for IHNV (Infectious Hematopoietic Necrosis Virus) was 4-minute-denaturation step at 94 °C followed by 30 seconds at 60 °C, 90 seconds at 72 °C and 30 seconds at 94 °C. This procedure was repeated for 40 cycles and the procedure was completed with a final elongation step at 72 °C for 10 minutes [9,12]. Products generated after the amplification were confirmed graphically in the real-time PCR instrument software and also using 1% agarose gel elec-

trophoresis containing GelRed using 1000 bp markers [8, 9, 12].

RESULTS AND DISCUSSION

Bacteriological Findings. As a result of the biochemical identification of Gram negative rod shaped oxidase positive suspect colonies isolated as a result of the bacterial inoculations of the tissue samples taken from the fish using Microgen® GID A+B bacteria isolation kit, *Aeromonas spp*. was not identified in Region 1 whereas nine of the samples in Region 2 were identified as *A. hydrophila* and four as A. *caviae*. Five of the samples in Region 4 were identifed as *A. hydrophila*, nine of the samples in Region 5 were identified as *A. hydrophila* (Figure 2).

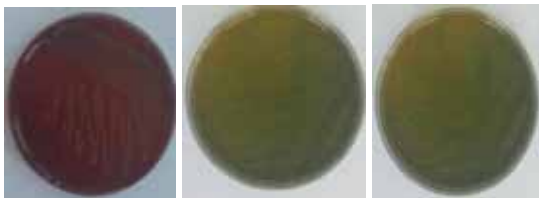


FIGURE 2
Results for Aeromonas in blood agar and Aeromonas selective agars

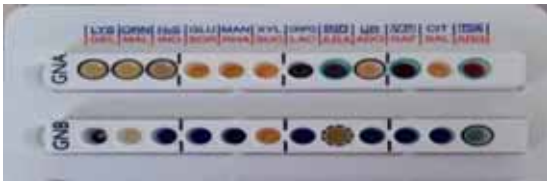


FIGURE 3
Results for Aeromonas in Microgen GNA + GNB bacteria identification panel

FIGURE 4
Results for *Y. ruckeri* in SW agar, *L. anguillarum* in TCBS agar and *V. chloreae* in TCBS agar

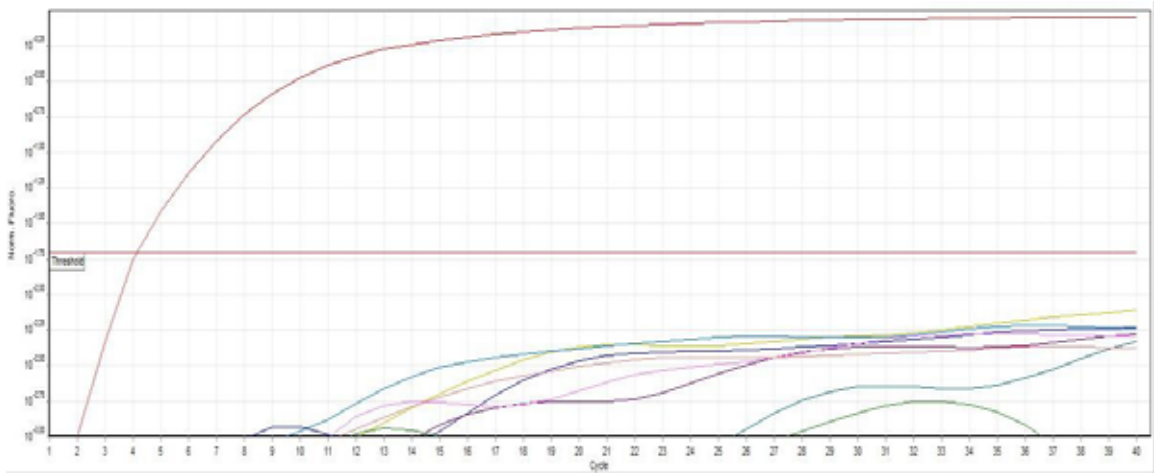
A. sobria was not identified in any of the samples by Microgen GNA + GNB bacteria identification panel (Figure 3).

In the study, *Y. ruckeri, L. anguillarum* and *V. cholerae* were not detected by the culture method (Figure 4).

Molecular Genetic Results. Virological findings. Viral factors were not detected by real-time PCR in the study (Figure 5).

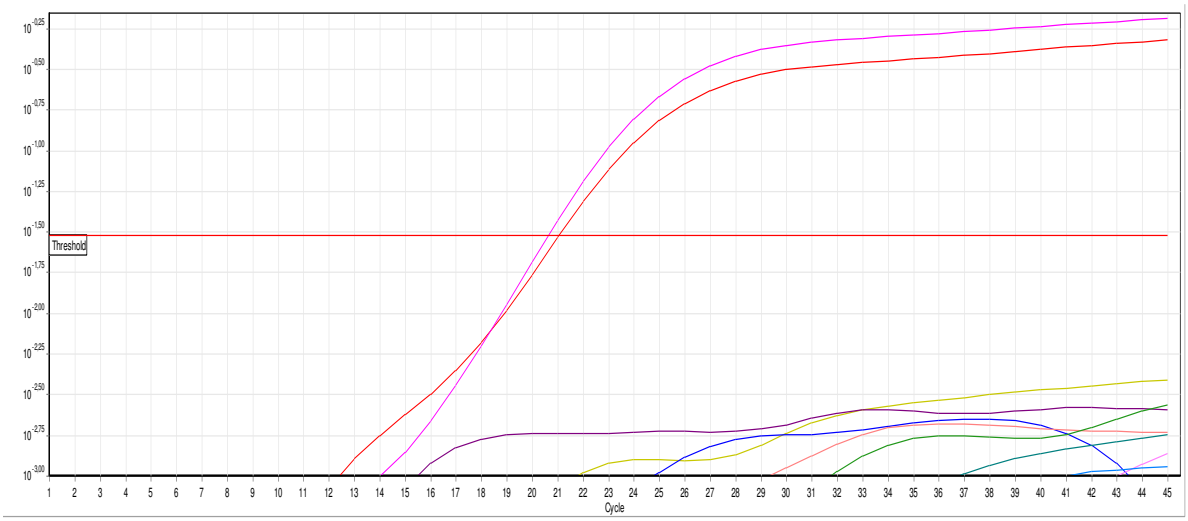
The samples examined in the study were found to be Y. *ruckeri, L. anguillarum* and *V. Cholerae* negative by Real-time PCR method. Real-Time PCR analysis results for the mentioned factors are presented in Figures 6, 7 and 8.

FIGURE 5
Image of a scan for viral agents by real-time PCR



Positive control 1. Group DNA's 2. Group DNA's 3. Group DNA's 4. Group DNA's 5. Group DNA's 6. Group DNA's
Negative control Non-template control

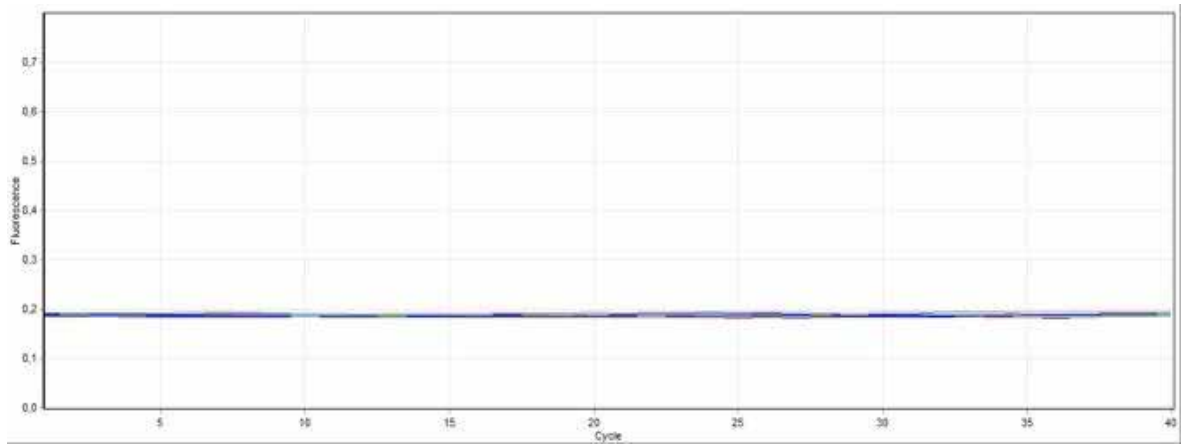
FIGURE 6
***Y. ruckeri* real-time PCR result image**



Positive control-1 1. Group DNA's 2. Group DNA's 3. Group DNA's 4. Group DNA's 5.Group DNA's 6. Group DNA's Non-template control Negative control Positive control-2

FIGURE 7

***Listonella anguillarum* real-time PCR result**

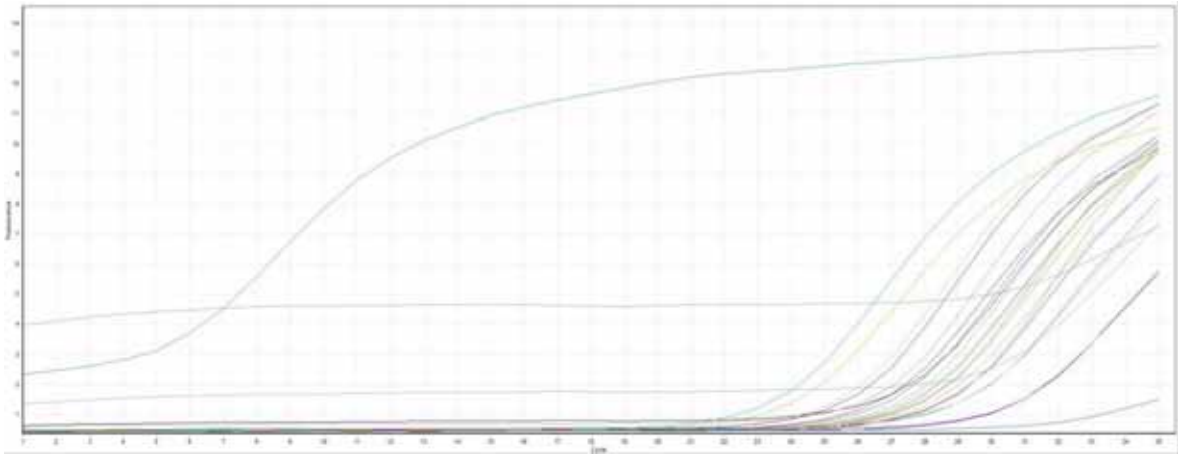


1. Group DNA's 2. Group DNA's 3. Group DNA's 4. Group DNA's 5. Group DNA's 6. Group DNA's Negative control

FIGURE 8

***V. cholerae* real-time PCR result**

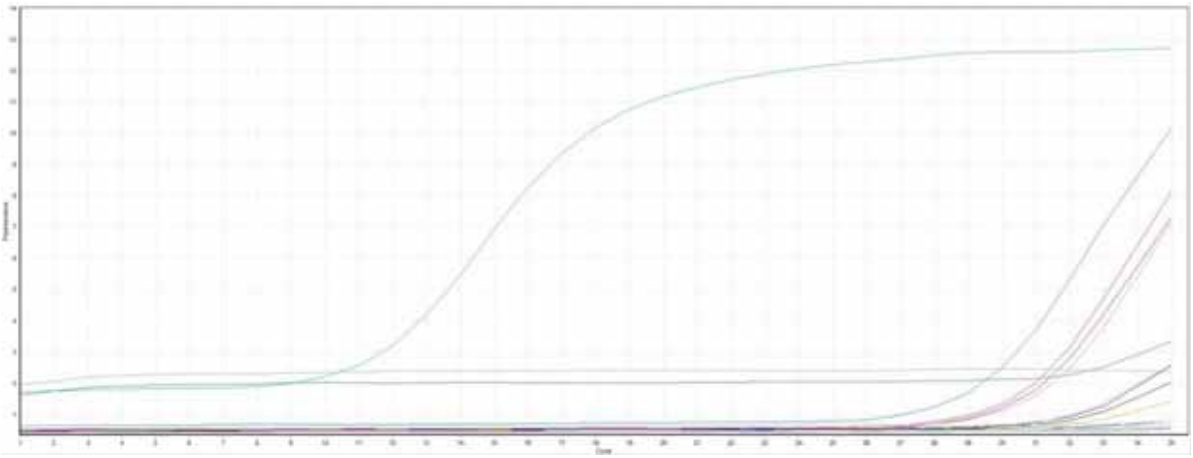
***Aeromonas* spp. findings.** In the study, among the samples collected from six areas in the Van Lake basin, 21 of the samples taken from the first region was found to be *Aeromonas* spp. positive. In 13 of the 21 specimens identified as *Aeromonas spp*. were found to be positive for *A. hydrophila* using species-specific primers while five of them were positive for *A. sobria* and three of the samples could not be identified using species-specific primers. Thirteen of the samples taken from Region 2 were *Aeromonas* spp. positive and five of these were identified as *A. hydrophila* while the remaining eight samples could not be identified using species-specific primers. In the samples taken from Region 3, no positive results were obtained for *Aeromonas spp*., *A. hydrophila*, *A. caviae* and *A. sobria.* In Region 4, 26 samples were identified as *Aeromonas spp*. positive and 13 of these samples were identified as *A. hydrophila* and 9 were identified as *A. sobria*. Four samples were not identifiable at species level. In the pearl mullet samples taken from Region 5, 18 samples were found as *Aeromonas spp*. positive. Five of these samples were positive for both *A. hydrophila* and *A. sobria*, while five were positive only for *A. hydrophila*. Samples identified as *Aeromonas* spp. could not be identified by species-specific primers. In the samples taken from Region 6, 18 samples were positive in terms of genus. Among these positive samples, 9 were identified as *A. hydrophila*. However, whereas other nine samples were negative with specific primers. None of the fish samples taken by random sampling were positive for *A. caviae* by real-time PCR method.



1-2 1-3 1-5 1-6 1-7 2-1 2-2 2-5 4-1 4-2 4-3 4-5 4-6 4-7 5-1 5-2 5-3 5-5 6-1 6-2 6-5 6-7 *A. hydrophila* ATCC® 7966 Negative Control

FIGURE 9

Aeromonas spp. positive samples images identified by real-time PCR method



A.h 1-2 A.h 1-6 A.h 1-7 A.h 2-5 A.h 4-1 A.h 4-3 A.h 4-6 A.h 5-1 A.h 5-3 A.h 6-2 A.h 6-5 A.s 1-3 A.s 4-3 A.s 4-5 A.s 5-3 *A. hydrophila* ATCC® 7966 Negative Control

FIGURE 10

Aeromonas spp. positive sample images identified by real-time PCR method

Histopathological Findings. In the histopathological examination of *A. hydrophila* positive fish, the liver was hyperemic, the sinusoids were enlarged and the order of the Remak cords was broken. In hepatocytes, hydropic degeneration and cytoplasmic vacuoles (Fig. 11, 12), focal necrosis and increased Kuepfer cells were detected (Fig. 13, 14, 15). Hydropic degeneration and necrotic changes were observed in the tubular epithelia of the kidneys (Figure 16-19). In all cases, mononuclear cell infiltration was detected in the kidney and liver. Hyperemia was observed in the gill vessels. Lamellae were disarranged in the epithelial cells of the secondary lamellae due to hyperplasia, adhesions on the secondary lamellae (Figs. 20-22) and sub epithelial edema. In some cases, it was observed that the secondary lamellae were completely destroyed and the primer lamellae were atrophic. Remarkable telangiectasia was observed in the gills (Figure 23). Hyperplasia was observed in the lymphatic follicles in the spleen. Hematopoietic tissue in the spleen, liver, and kidney showed increased hemosiderin-loaded Melan-macrophages (Figures 16-18, 24). In many cases, bleeding was mild and moderate in severity.

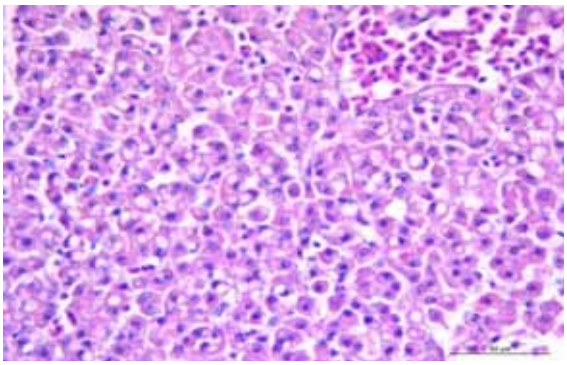


FIGURE 11

Liver Sharp limited fat vacuoles in hyperemia and hepatocytes, HE

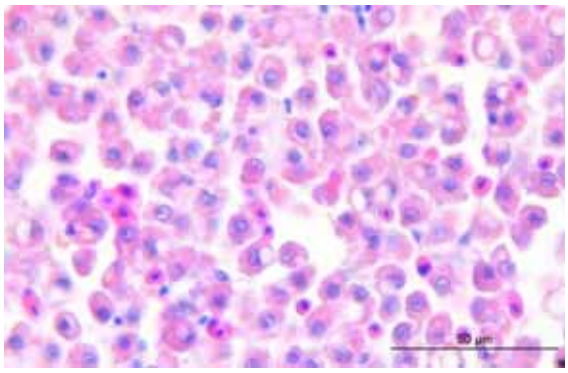


FIGURE 12
Liver Fat vacuoles in hepatocytes, HE

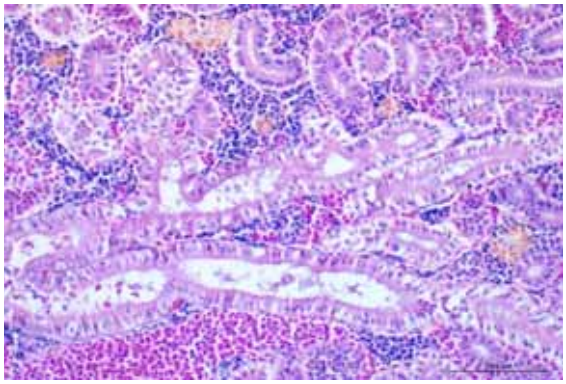


FIGURE 13
Liver Necrosis in hepatocytes, HE

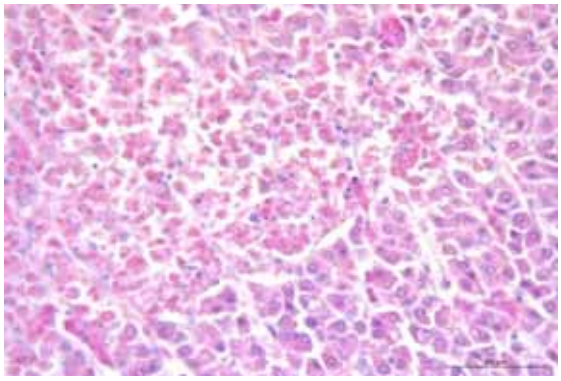


FIGURE 14
Liver Necrosis in hepatocyte, blood vessels hyperemic, HE

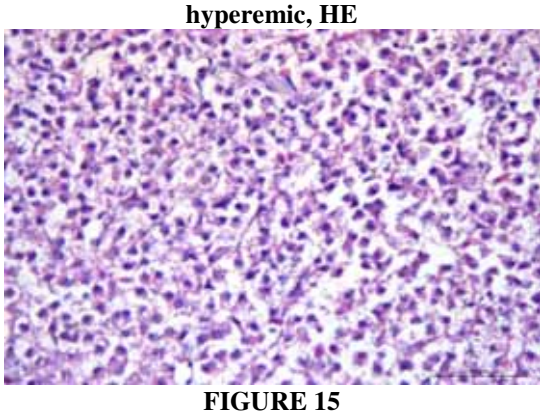


FIGURE 15
Liver Degeneration and necrosis in hepatocytes, HE

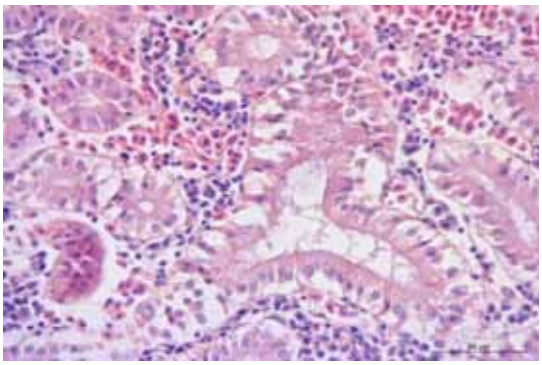


FIGURE 16
Kidney, Degeneration in tubular epithelia

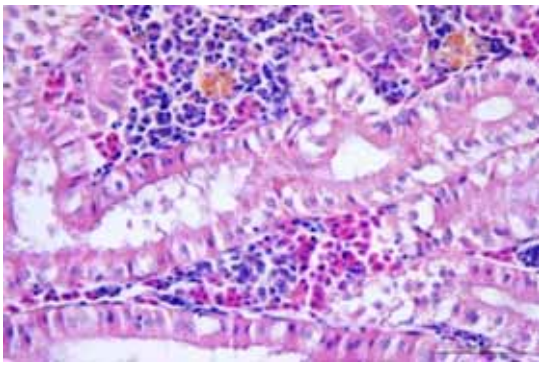


FIGURE 17
Kidney., Degeneration in melanomacrophages and tubular epithelia

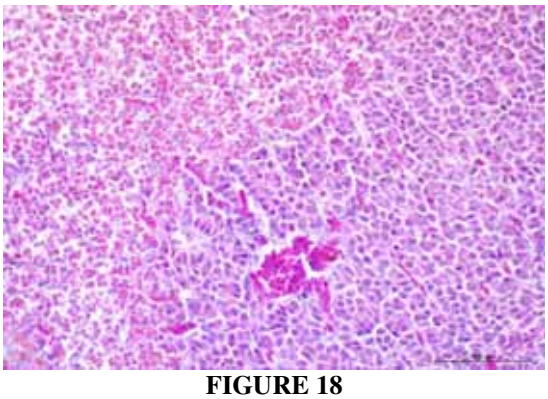


FIGURE 18
Kidney, Degeneration in melanomacrophages and tubular epithelia

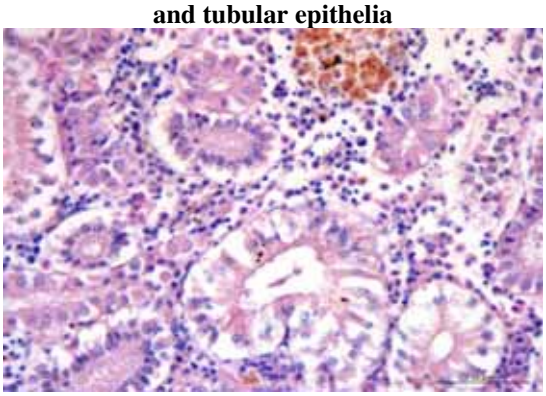


FIGURE 19
Kidney, Degeneration in melanomacrophages and tubular epithelia

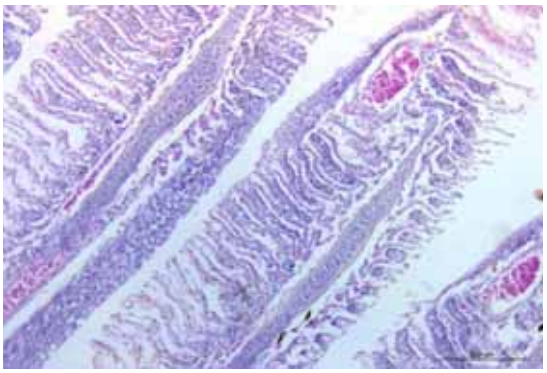


FIGURE 20
Hyperemia in the gills

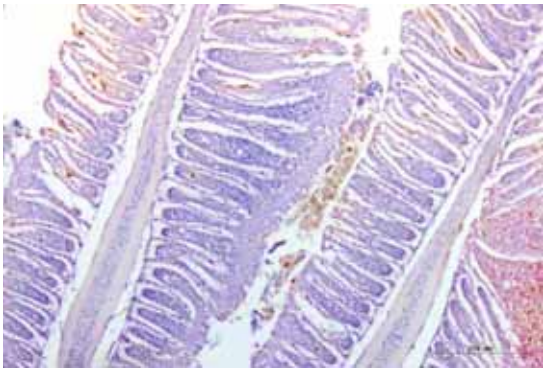


FIGURE 21
Gills, Hyperplasia in the secondary lamellae

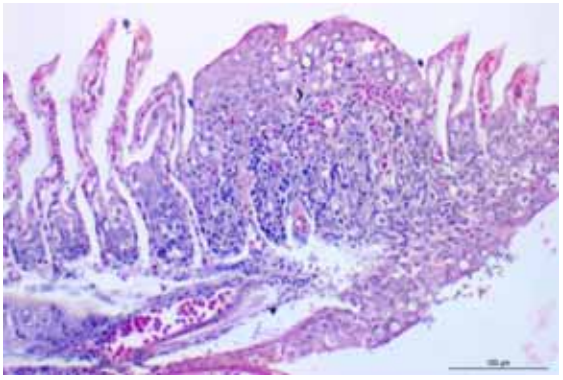


FIGURE 22
Gills, Hyperplasia and adhesions in the secondary lamellae

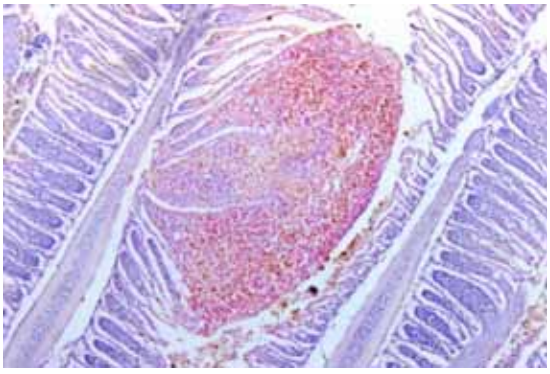


FIGURE 23
Gills, Edema and telangiectasia in the secondary lamellae

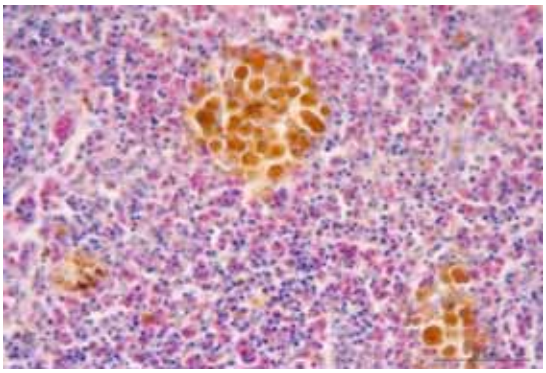


FIGURE 24
Spleen melanomacrophage centers

CONCLUSIONS

Pearl Mullet (C*halcalburnus tarichi*, Pallas 1811) is the only endemic species in the Van Lake, the largest lake in Turkey. This fish species lives only in the Van Lake basin and moves to the freshwater rive mouths for reproduction in early April and enters the rivers when the water temperature exceeds 13 °C. Leaving the eggs onto the gravelly and sandy areas where the flow of the streams slow down and turns back to the lake [1].

Most bacterial infections causing massive deaths in fish are septicemic. In septicemic infections, the agent can be isolated from anywhere in the host's bloodstream. In fish, cranial kidney is known to be the most suitable site for septicemic infection isolation [5].

The living environments of the motile *Aeromonas* species (*A. hydrophila, A. sobria, A. caviae*) that cause significant infections in fish are seas and freshwaters. These microorganisms are found in all kinds of water from sewage to spring water. Due to their widespread presence in the water, they are among the factors of hemorrhagic septicemia in freshwater and marine fish. Since motile *Aeromonas* species are contaminated by contact between animals, the risk of spreading infections in culture fish is higher [18].

In the other study, researchers have reported that they isolated *Aeromonas spp.* in 27 (54%) of the raw pearl mullet samples that they collected from the market. The researchers have reported that 19 (38%) of the isolates were *A. hydrophila*, while 6 (12%) were *A. caviae* positive and 2 (4%) were positive for both *A. hydrophila* and *A. caviae.* Researchers have reported that they did not identify *A. sobria* in any of the samples. In the intestinal contents of the pearl mullets, 26 (52%) were positive for motile *Aeromonas spp.* and 23 (46%) were positive for *A. hydrophila*, 2 (4%) were positive for *A. caviae,* and 1 (2%) was positive for both *A. hydrophila* and *A. sobria* [11].

In another study, researchers have examined 132 fish samples and reported that they isolated a

total of 19 bacterial species, 7 from rainbow trout (4 *A. sobria*, 2 *Y. ruckeri,*1 *H. alvei*), 4 from the common carp (2 *A. sobria* and 2 *A. salmonicida*), 2 from the European chub (1 *A. sobria* and 1 *H. alvei*), 2 from Discus fish and (1 *C. freundii* and 1 *P. aeruginosa*), 1 from sea bass (*L. Anguillarum)*, 1 from Black Sea salmon (*P. Fluorescens),*), 1 from sturgeon (*L. Anguillarum)*) and 1 from dolphin (*A. salmonicida)* [15].

As a result of the biochemical identification of Gram negative rod shaped oxidase positive suspect colonies isolated as a result of the bacterial inoculations of the tissue samples taken from the fish using Microgen® GID A+B bacteria isolation kit, *Aeromonas spp*. was not identified in Region 1 whereas nine of the samples in Region 2 were identified as *A. hydrophila* and four as *A. caviae*. Five of the samples in Region 4 were identified as *A. hydrophila*, nine of the samples in Region 5 we identified as *A. hydrophila*. The results of microbiological inoculation from the samples did not reveal *Y. ruckeri, L. anguillarum* and *V. cholerae*. When the water temperature rises to 13 °C, the infection will occur if the fish carries the disease. Although the treatment with antibiotics was successful, the disease recurs. The introduction of infected fish into the farm is the most common source of infections. Asymptomatic carriers are spread through the bacterial fecal pathway and thus healthy fish are infected. In addition, affected fish may also exert their effects over certain periods [7].

Vaccination is an important factor that reduces profitability as well as protection. The best way of protection is that the disease agent is never transmitted to the farm [5].

A large number of pathogenic fish viruses belong to the *Rhabdoviridae* family. Most of the viral diseases in fish are septicemic. Other general symptoms such as exophthalmos, asities, bleeding and anemia may also be seen [16].

In this study, although the fish samples were collected between April and July, it was observed that the number of motile Aeromonas isolated by both culture (22.22%) and real-time PCR (53.33%) were lower than that found by the researches. Similarly, the researchers have reported that there were higher levels of motile *Aeromonas* species by microbiological culture method, compared to those in the present study. This supports the idea that the fish sold on the market may not be kept undersuitable storage conditions, which could lead to a higher risk of contamination [11].

Previous studies have reported that *Vibrio* and *Listonella* species cannot survive in freshwater for a long time [5, 20]. In this study, it was thought that the sampling from the rivers lowered the chances of survival of the agent and decreased the isolation ratio. However, it was concluded that *Aeromonas* species are found in the natural flora and that isolation from waters can increase the risk of transmission to fish, resulting in a higher isolation rate than other factors [20, 23].

Histopathologic examination of the *Aeromonas* positive cases by laboratory examination with no macroscopic findings taken by random sampling showed evident histopathologic findings, consistent with *Aeromonas*. Hyperplasia and adhesions, telengiectasia and edema, which were reported to be seen in the laminae of the second lamellae in the gills [19] were evaluated intensely and severely in *A. hydrophila*-positive cases in this study. In accordance with the literature [23], Remak cords in the liver lost their order and degenerative changes in hepatocytes were formed. According to the results of the PCR analysis conducted on the tissues, in cases where the agent was not produced in the ID panel although the results were positive for *Aeromonas* spp. and *A. hydrophila*, the histopathological results were consistent with *Aeromonas*. However, necrosis was more severe in the livers of the fish in which Aeromonas spp. and *A. hydrophila* were found to be positive by both real-time PCR and bacteriological culture methods. In many studies [19, 20], haemosiderin-loaded melano macrophages in the liver, kidney and spleen have been reported to be intensively observed in *A. hydrophila* infection. In this study, hemosiderin-loaded melanomacrophages were also observed intensely in all *Aeromonas* positive cases. Researchers have reported that melanomacrophages were not observed in the experimental *A. hydrophila* infection [23].

In this study, parenchymal degeneration, vacuolar degeneration, necrosis and lymphocyte infiltration in the tubule epithelia in the kidney support the literature [19, 20]. Additionally, except for some of the zoonotic *Aeromonas* species (*A. hydrophila* and *A. sobria*), it was determined that the pearl mullet had no reservoir status for bacterial strains such as *Y. ruckeri, L. anguillarum* and *V. cholerae*, which cause serious economic losses in the aquaculture industry and viruses such as IPN, VHS and IHN. This suggests that the regional aquatic ecosystems are untouched for possible aquaculture activities in terms of important fish pathogens. Existing pathogens seem to be of human-origin. Therefore, the use of SPF eggs and rootstocks in aquaculture must be designated as a management strategy and formally followed in order to ensure the healthy development of possible aquaculture activities in the region.

As a result, it was seen that, in the case of pearl mullet, which is consumed quite frequently by people in Van and the region, the risk of motile *Aeromonas* infections is high and it can lead to serious negative effects on human health. To avoid possible risks of zoonotic infections, it was concluded that hygiene and cold chain conditions should be taken into consideration in the fish that are offered for consumption, and that the fishes should be kept clean and protected.

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