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Bacterial and Parasitic Pathogens Isolated from Some Wild Cyprinid Fishes

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Abstract: Parasitic and bacterial fish pathogens present on the wild fish in Deriner Dam Lake in Turkey on the Coruh River were determined in the present study. In total 127 wild fish (6 different species) were sampled between August 2014 and April 2015. Followings were isolated as a parasitic pathogens: Apiosoma sp., Euplotes sp., Gyrodactylus sp., Dactylogyrus sp., Ichthyophthirius multifiliis, Ligula intestinalis, Trichodinella sp., Trichodina sp. and Vorticella sp. present on of the following fish: Alburnoides fasciatus (Transcaucasian spirlin), Squalius orientalis (Chub), Barbus artvinica (Barbell), Capoeta banarescui (Banarescu's barb), Capoeta sieboldii (Colchic khramulya) and Capoeta ekmekciae (Grusinian scraper). Also, Acinetobacter calcoaceticus, Carnobacterium maltaromaticum and Shewanella putrefaciens and six Aeromonas and three Pseudomonas species were isolated. Seasonal prevalence values of parasites were presented. In total, 39 different bacterial strains were isolated. Widespread and massive deaths caused by pathogens were not encountered in the Lake. This study provides preliminary information regarding the wild fish pathogens of Deriner Dam Lake.

Key words : Aquatic system; fish; pathogens, molecular identification.

Bazı Doğal Sazan Türlerinden İzole Edilen Bakteriyel ve Paraziter Patojenler

Öz: Bu çalışmada Türkiye'de ki Çoruh nehri üzerinde bulunan Deriner Baraj Gölü'nde doğal olarak dağılım gösteren balıklar üzerinden paraziter ve bakteriyel balık patojenleri belirlenmeye çalışılmıştır. Ağustos 2014 ve Nisan 2015 tarihleri arasında toplamda 127 doğal balık (6 farklı tür) örneklenmiştir. Alburnoides fasciatus, Squalius orientalis, Barbus artvinica, Capoeta banarescui, Capoeta sieboldii ve Capoeta ekmekciae balıklarından Apiosoma sp., Euplotes sp., Gyrodactylus sp., Dactylogyrus sp., Ichthyophthirius multifiliis, Ligula intestinalis, Trichodinella sp., Trichodina sp. ve Vorticella sp parazitleri ile Acinetobacter calcoaceticus, Carnobacterium maltaromaticum ve Shewanella putrefaciens, 6 farklı Aeromonas ve 3 farklı Pseudomonas türü bakteri tespit edilmiştir. Bakterilerin tanımlanmasında fenotipik ve moleküler yöntemler kullanılmıştır. Balıklardan izole edilen parazitlerin mevsimsel olarak prevelans değerleri sunulmuş ve toplamda 39 farklı bakteri izole edilmiştir. Gölde patojenlerin neden olduğu yaygın ve büyük ölümlere rastlanılmamıştır. Bu çalışma Deriner Baraj Gölü'nün doğal balık patojenleri hakkında ön bilgi vermek amacıyla yürütülmüştür.

Anahtar kelimeler: Sucul sistem; balık; patojen; moleküler tanımlama.

1. Introduction

The Deriner Dam Lake is the longest dam lake in Turkey, constructed on Coruh River located in the Northeastern area of Turkey. It has a 1713 hectare surface area and 90 m water depth. These features of the lake offer a significant potential for fisheries and aquaculture.

Fish pathogens and diseases are very important issues that have been studied by several researchers on wild and reared fish all over the world. Most of these studies generally focused on the reared fish

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species in the aquaculture industry in marine and fresh water systems [1,2]. The other studies deal with the determination of pathogens in wild fish species living in a specific aquatic environment such as natural and artificial lakes, coastal area of the seas, the lagoons, and rivers in Turkey [3,4,5]. The determination of fish diseases and the associated pathogens in the aquaculture systems or wild areas is very crucial for a sustainable aquaculture. However, to prevent fish diseases, physical and chemical qualities of water and micro and macro biological agents present in the aquatic environment must be well-known [6].

There are several studies dealing with the fish pathogens in the different geographic area of Turkey [1]. A comprehensive taxonomical study has been carried on the wild fish in the Coruh River. Different fish species (Ponticola constructor (Caucasian goby), Silurus glanis (Wels catfish), Salmo rizeensis (Rize trout), Salmo coruhensis (Coruh trout), Chondrostoma colchicum (Colchic nase), Phoxinus colchicus, Alburnoides fasciatus (Transcaucasian spirlin), Squalius orientalis (Chub), Alburnus derjugini (Georgian shemaya), Oxynoemacheilus sp. (Loach), Cyprinus carpio (Common carp), Barbus artvinica (Barbell), Capoeta banarescui (Banarescu's barb), Capoeta sieboldii (Colchic khramulya), Capoeta ekmekciae (Grusinian scraper), Seminemacheilus sp. (Anatolian loach), Gobio sp. (Goby)) were reported from the river [7]. However, an investigation about the fish pathogens in the Coruh basin is lacking. The transportation of live fish to different fish farms located in the fresh water and marine areas is an important phenomenon in the aquaculture industry. But in this way, many aquatic systems get contaminated with fish pathogens transported by infected live fish. Therefore, before performing any aquaculture activity in a specific area, fish pathogens of the related aquatic systems must be investigated. The aim of the study was to determine the bacterial and parasitic pathogens of wild fish species living in Deriner Dam Lake. Thus, our results might be useful for determining the suitability of the area for fish culture, in avoiding a threat, and for the comparison of the system in terms of fish pathogens associated with an aquatic activity.

2. Material and Methods

During 2014 – 2015 (12 months), a total of 127 fish samples (Alburnoides fasciatus (Transcaucasian spirlin; n = 31; (8.1-27.7 g weight and 5-13.7 cm total length), Squalius orientalis (Chub; n=30;(45.5-140.9 g weight and 7.9 - 24.1 cm length), Barbus artvinica (Barbell) (n = 8) (8.9 - 47.6 g weight and 6 - 13.5 cm length), Capoeta banarescui (Banarescu's barb) (n = 25) (39.8 - 439.1 g weight and 13.8 - 38.5 cm length), Capoeta sieboldii (Colchic khramulya) (n = 3) (38.8 - 73.8 g weight and 10.5 - 17.5 cm length), and Capoeta ekmekciae (Grusinian scraper) (n = 30) (32.1 - 625.5 g weight and 12.3 - 37.5 cm length) were caught from Deriner Dam Lake in Turkey (41° 10′ 11.0064 "N and 41° 52′ 12.9972 "E) by using different gill nets and electroshock device. The fish species were described according to Bayçelebi et al. 2015 [7]. Temperature and pH values of the Dam water were recorded as seasonally or monthly by Hache-Lange multiparameter.

The live fish were transported to the fish disease laboratory at the Fisheries Faculty of Recep Tayyip Erdogan University. For this purpose, each fish species was placed in separate transport containers with oxygen. In the laboratory, the fish were examined for external and internal parasites. In case of the presence of the parasites, they were fixed with 4% formalin, AFA (Alcohol-Formalin-Acetic acid) and picric acid. The parasites were stained with silver nitrate (2%), Giemsa and carmine dye. The parasite species were identified on the basis of earlier studies [8, 9, 10, 11, 12].

For bacteriological examinations, all fish species were necropsied in antiseptic conditions. Inoculations from liver, trunk kidney, and spleen of the fish were made aseptically on Tryptic Soy Agar (TSA) using sterile lancet [13]. After incubation at 22°C for 48 h, the bacteria isolated from the fish were subcultured on the same medium, and then pure bacterial colonies were biochemically characterized with Analytical Profile Index (API 20NE).

DNA was extracted for the bacterial isolates for their molecular characterization following the boiling method described by Queipo-Ortun˜o et al. 2008 [14]. For this purpose, pure bacterial cultures inoculated on Tryptic Soy Broth (TSB) were incubated at 22°C for 24 h, and then the media were separated by centrifugation at 9000 g for 5 min. The pellets were re-suspended in 40 µL of molecular grade water and boiled at 100°C 15 min, and centrifuged at 15000 g for 5 min, the supernatants discarded, and the final samples were stored at − 20°C .

To identify bacteria, the universal primers (27 F 50 AGA GTT TGA TCC TGG CTC AG - 30, 1492 R 50 GTT TAC CTT GTT ACG ACT T - 30) specific for 16S rRNA gene of eubacteria were used. These primers were used for a PCR amplification using the purified bacterial DNA as template. A 1465 - bp PCR product was purified by using a PCR purification kit (Qiagen) and sequenced with an ABI PRISM 310 genetic analyzer (Applied Biosystems). The derived nucleotide sequences were analyzed and aligned with Macrogen for sequencing (Amsterdam, the Netherlands). The results of the sequencing were used for homology search with the help of BLAST tool (http://www.ncbi.nlm.nih.gov) [15]. The study has been approved by the Local Ethics Committee of Rize University (reference no; 2015/13)

3. Results and Discussion

Seven different protozoan and three metazoan fish parasites were isolated from six different fish species. The parasites, their host fish and prevalence are detailed in Table 1 and Figure 1. According to these results, Trichodina sp. (Prevalence 46.5 %), Ligula intestinalis (Prevalence 37.8 %) and Gyrodactylus sp. (Prevalence 21.3 %) were isolated from all fish species. Ichthyophthirius multifiliis, the causative agent of white spot diseases, was isolated only from two fish species, Alburnoides fasciatus and Capoeta banarescui. The seasonal prevalence of all parasites is shown in Table 2. Multiple hemorrhages were observed in the skin of Capoeta banarescui infested with Trichodina sp. and Gyrodactylus sp. (Figure 2). The water quality parameters and seasonal prevalence of all parasites was shown in Table 2.

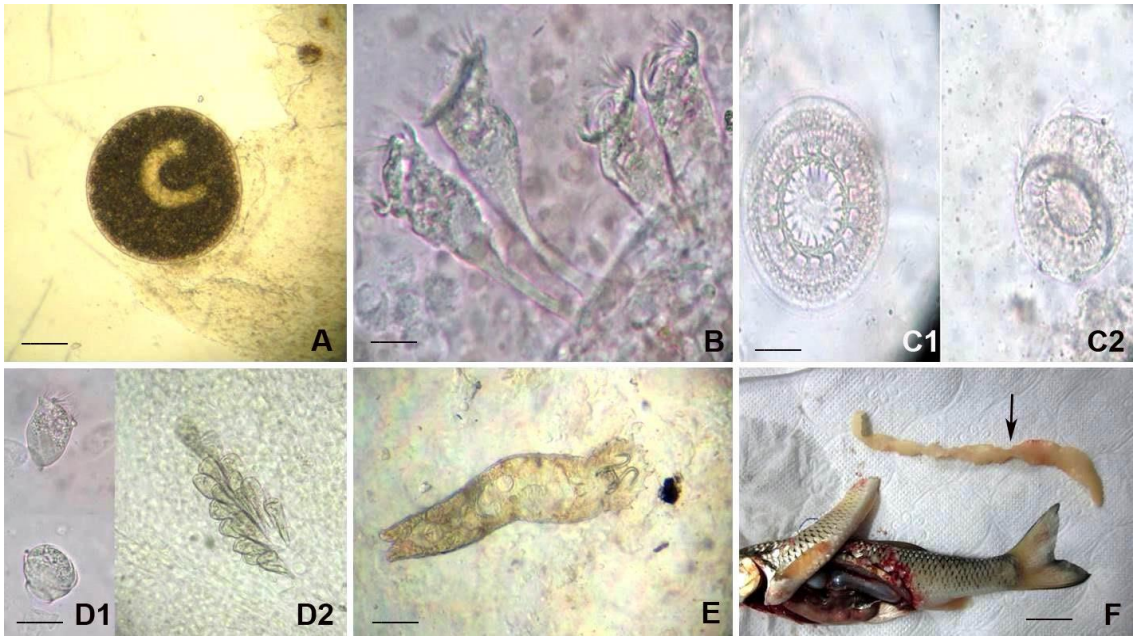

Figure 1. Isolated parasites from fish. A: Ichthyophthirius multifiliis scale bar (0,2mm), B: Apiosoma sp. scale bar (5µm), C1,C2: Trichodina sp. scale bar (20µm), D1: Telotroch form of Vorticella sp. (50µm), D2: Colony of Vorticella sp., E: Gyrodactylus sp. scale bar (0,05mm), F: Ligula intestinalis isolated from Squalius orientalis black arrow scale bar (1.5cm)

Table 1. The isolated parasites and their prevalence, n: number of sampled fish

Fish Species	n	Parasites	Prevalence (%)
Alburnoides fasciatus	(31)	Apiosoma sp.	38.7
		Euplotes sp.	6.5
		Gyrodactylus sp.	12.9
		Ichthyophthirius multifiliis	3.2
		Ligula intestinalis	61.2
		Trichodinella sp.	9.7
		Trichodina sp.	38
		Vorticella sp.	12.9
Barbus artvinica	(8)	Dactylogyrus sp.	12.5
		Gyrodactylus sp.	12.5
		Ligula intestinalis	25
		Trichodina sp.	12.5
Capoeta banarescui	(25)	Ambiphyra sp.	4
		Apiosoma sp.	20
		Dactylogyrus sp.	4
		Gyrodactylus sp.	48
		Ichthyophthirius multifiliis	8
		Ligula intestinalis	32
		Trichodina sp.	88
		Vorticella sp.	20
Capoeta ekmekciae	(30)	Ambiphyra sp.	3.3
		Apiosoma sp.	10
		Dactylogyrus sp.	3.3
		Gyrodactylus sp.	6.67
		Ligula intestinalis	16.7
		Trichodina sp.	23.2
		Vorticella sp.	13.3
Capoeta sieboldii	(3)	Gyrodactylus sp.	33.3
		Ligula intestinalis	33.3
		Trichodina sp.	100
		Vorticella sp.	33.3
Squalius orientalis	(30)	Ambiphyra sp.	10
		Apiosoma sp.	6.7
		Gyrodactylus sp.	23.3
		Ichthyophthirius multifiliis	3.3

	Ligula intestinalis	43.3
	Trichodina sp.	46
	Vorticella sp.	3.3

By bacteriological examinations, 39 different bacterial isolates were phenotypically identified on the fish (Table 3). The pathogenic bacteria of the fish were: Aeromonas hydrophila, Aeromonas salmonicida, Aeromonas sobria, and Pseudomonas fluorescens.

Figure 2: Multiple hemorrhages in the skin of Capoeta banarescui infested with Trichodina sp. and Gyrodactylus sp.

In addition, Aeromonas sp. Pseudomonas sp., Acinetobacter calcoaceticus, Carnobacterium maltaromaticum, Citrobacter sp. Pseudomonas jessenii, Pseudomonas koreensis, Rahnella sp. and Shewanella putrefaciens were also recorded. Aeromonas spp. were also observed as the most common bacterial isolates.

4. Discussion and Conclusion

During 1960s, some studies were carried on fish parasites in Turkey. These studies reported only the parasites on the fish found in their natural aquatic ecosystem [3]. There have been many reports on fish parasites from cultured fish (Oncorhynchus mykiss, Sparus aurata and Dicentrarchus labrax) and different species of ornamental fish in recent years in Turkey [16,17]. Deriner Dam Lake is located in Artvin constructed on Coruh river. Studies on the fish species found in Coruh river mostly address the taxonomic aspects of fish [18]. Studies on the fish parasites found in the basin are scarce. In this context, the present study has the feature of closing a deficit about fish parasites found in the mentioned area.

An examination of the reported fish parasites obtained from the samplings indicated that the existence of Ichthyophthirius multifilis poses a considerable risk for future aquaculture even if it has a low prevalence. Compared to their natural environment, deaths associated with I. multifilis infection have been substantially reported in land-based aquaculture managements under the condition of intensive fish stocks, especially in the hatchery systems and cultured juvenile fish [3,19,20].

Table 2. Temperature and pH values of water sampled from the Deriner Dam Lake (mean±SD), and seasonal prevalence of parasites, n: number of fish, IFS: infested fish, PRV: Prevelance

Water Quality	**Spring**			**Summer**			**Autumn**			**Winter**		
Temperature	6±1.1			26±2.0			7.5±1.2			4.7±0.9		
pH	6.9±0.3			7.4±0.2			6.9±0.4			7.1±0.2		
Parasites	n	IFS	PRV	n	IFS	PRV	n	IFS	PRV	n	IFS	PRV
Trichodina sp.	14	4	28,6	15	9	60,0	43	17	39,5	55	32	58,2
Trichodinella sp.	14	1	7,1	15	-	-	43	1	2,3	55	-	-
I. multifiliis	14	1	7,1	15	-	-	43	1	2,3	55	2	3,6
Gyrodactylus sp.	14	3	21,4	15	3	20,0	43	12	27,9	55	9	16,4
Dactylogyrus sp.	14	1	7,1	15	-	-	43	-	-	55	1	1,8
Euplates sp.	14	-	-	15	-	-	43	3	7,0	55	-	-
Apiosoma sp.	14	4	28,6	15	-	-	43	5	11,6	55	18	32,7
Ambiphyra sp.	14	-	-	15	-	-	43	2	4,7	55	6	10,9
Vorticella sp.	14	-	-	15	1	6,7	43	5	11,6	55	16	29,1
L. intestinalis	14	5	35,7	15	7	46,7	43	13	30,2	55	21	38,2

Trichodina sp. is also present over large areas like I. multifilis and has been reported in regional fishery as a parasite. The protozoan parasites reported in this study have also been reported from aquaculture systems and aquarium fish [3], but there is no report of any serious concern in terms of mortality. The protozoan parasites, especially I. multifilis and Trichodina sp., can be accepted as having a high potential for posing a risk for fish fauna found in the Deriner Dam Lake.
In context of metazoan parasites, it was seen that Gyrodactylus sp. (21.3%) and Dactylogyrus sp. (2.4%) belonging to the monegenean group are commonly reported from the fish found in Turkey. Besides these species, Dactylogyrus cornoides [21], Dactylogyrus distinguendus [22], Dactylogyrus ergensis [23] and Gyrodactylus carassii [24] have also been seen in few samples from different regions of Turkey (Marmara, Aegean, Mediterranean and Western Black Sea).

Ligula intestinalis, belonging to Cestoda group, having a complex life cycle, is a parasite that is generally reported from Cyprinus carpio. The studies deal with the distribution of the parasites, contains about 32 different basins throughout Turkey [25].

Table 3. Isolated bacteria from fish and their molecular and biochemical (API) identification rates. n: number of bacteria. L: liver, S: spleen, K: kidney.

Bacteria species	n	Molecular (%)	%API 20NE	API Profiles	Fish Species	Fish Tissue
Aeromonas sp	12	99	75	7177747 (n=7)	A.fesciatus	L
					B. artvinica	S, K, L
			71	1777755 (n=3)	C. baranescui	S, K,
					C. ekmekcia	S, L
			70	1577755 (n=2)	C. siboldi	L , K
					S. oriantalis	L , S
Aeromonas allosaccharophila	3	99	-	-	S. oriantalis	L , S, K
Aeromonas caviae	2	99	99.8	3575755	C. baranescui	S
			99.7	3577754	C. ekmekcia	S
Aeromonas hydrophila	4	99	99.8	3575755 (n=2)	C. baranescui	S
			98.2	3567755	S. oriantalis	S
					C. ekmekcia	S
			98.2	3577755	C. baranescui	S
Aeromonas salmonicida	4	99	-	-	C. baranescui	S
					S. oriantalis	S
					C. ekmekcia	S
					A. fesciatus	K
Aeromonas media	1	99	-	-	B. artvinica	L
Aeromonas sobria	2	-	99.7	3176755	C. ekmekcia	S
			97.9	3176754	C. baranescui	S
Acinetobacter calcoaceticus	1	99	-	-	A.fesciatus	K
Carnobacterium maltaromaticum	1	99	-	-	B. artvinica	S
Citrobacter sp.	1	99	-	-	S. oriantalis	L
Pseudomonas sp.	1	99	-	-	A .fesciatus	K
Pseudomonas jessenii	1	99	-	-	C. ekmekcia	S
Pseudomonas flourescens	1	99	99.7	0057555	C. ekmekcia	S
Pseudomonas koreensis	1	99	-	-	A. fesciatus	L
Rahnella sp.	1	99	-	-	B. artvinica	L
Shewanella putrefaciens	3	99	62.3	1050345	C. baranescui	L
					A. fesciatus	K, L
					A. fesciatus	L

These basins include Barhal and Cildir basins, which are the nearest basins to the area where this study was carried out. From these two regions, L. intestinalis was isolated only from Barbus plebejus species.

In the present study, L. intestinalis has been isolated from six different species for the first time in this region. On the other hand, earlier studies have reported L. intestinalis mostly from Cyprinus carpio [26]. However, L. intestinalis has not been reported from the fish found in aquaculture environment; and the reason behind this should be investigated. The existence of pathogenic and non-pathogenic bacteria in fish has been investigated for several years. There are many reports in Turkey about the bacteria, isolated from the cultured fish [1]. This case is directly related to the

dissemination of the diseases. This study presents important data about the sampled fish found in their natural environment. In the context of aquatic systems, the data about bacterial contamination mostly include pollution indicator bacteria or the species isolated from the thermophilic areas. In this study, substantial bacterial species, posing a risk for aquaculture, have been reported from six different fish species obtained from Deriner Dam Lake. Aeromonas and Pseudomonas species are most commonly observed ones in this regard; the presence of Aeromonas allosaccharophila, Aeromonas media, Pseudomonas koreensis, and Pseudomonas jessenii is the first report for Turkey. Yersinia ruckeri and Lactococcus sp. are commonly seen pathogens in the aquaculture farms constructed in the rivers and dam lakes of the Eastern Black Sea region of Turkey [27,28]. In last few years, the vaccination improved on fish against these two bacteria has decreased the extensity of the diseases. However, these diseases are still posing a risk in the region. Within the context of this study, Yersinia ruckeri and Lactococcus sp. species have not been recorded from any fish species live in Deriner Dam Lake. For Yersinia ruckeri, this situation can be accepted as a normal. For this bacterium, some mammals, carps, some invertebrate species and the soil of aquatic ecosystems can serve as a reservoir, and can lead to a disease especially for reared salmonids [29]. Y. ruckeri has been reported from reared trout several times in the studies performed in the Eastern Black Sea region and countrywide [3,30,31,32]. Even for the salmonid species found in their natural environment, the rate of the bacterial isolation is at a weak level and this situation can clarify why the bacterium is not isolated from the fish fauna found in Deriner Dam Lake.

Eldar et al. 1995 [33] stated that carp species (Cyprinus sp.) are resistant against Lactococcus infections. In this context, although Lactococcus infections are more common in the sampled fish species than Yersinia infections, in the cases where Lactococcus infections were not isolated can be accepted as a natural result. An absence of these bacterial species observed in the region decreases the risk of an illness. However, while performing future aquaculture activities, the precautions to prevent Deriner Dam Lake from these bacteria should not be neglected.

This study aiming to reveal the risk map of bacterial and parasitic fish pathogens found in Deriner Dam Lake proposes following suggestions: (I). Deriner Dam Lake is a virgin area because aquaculture activities have not started in this area yet, but it has a potential in this sense. In terms of fish pathogens, current situation of the area has been revealed by this study. Before performing future aquaculture activities, precautions should be taken when considering the existence of these pathogens. Risk factors should always be considered, especially in terms of I. multifilis infestations. (II). Besides the pathogens found in the region, the drift of new pathogens by the fish transport for aquaculture to the dam lake should be prevented (III). In terms of the natural fish fauna found in the Deriner Dam Lake, only six different fish species have been used in this study. Apart from that, further studies should be carried out for other fish species found in the lake (IV). This study was carried out in terms of bacterial and parasitic pathogens. The fish fauna found in Deriner Dam Lake should also be studied in terms of viral pathogens (V).

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