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Case Report
Olgu Sunumu

Parasitological, bacteriological and virological research of carp (*Cyprinus carpio*) in a case in Hirfanlı Dam Lake in Türkiye

Esat Çilli[1*], Hakan Yeşilöz[2], Ömer Faruk Gökcecik[3], Çağatay Nuhay[4], Abdurrahman Anıl Çağırgan[5], Kemal Pekmez[6], Murat Kaplan[7], Bülent Kafa[8]

[1,2,3,] Department of Parasitology, İzmir/Bornova Veterinary Control Institute, İzmir, Türkiye
[4] Department of Bacteriology, İzmir/Bornova Veterinary Control Institute, İzmir, Türkiye
[5,6,7,8] Department of Virology, İzmir/Bornova Veterinary Control Institute, İzmir, Türkiye

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Abstract: The aim of the present study was shedding light on the disease factors underlying mortality event among common carps inhabiting Hirfanlı Dam Lake in Türkiye in spring of the year 2021. For this purpose various parasitological, bacteriological and virological research techniques were employed. Five parasite genera, *Dactylogyrus* spp., *Myxobolus* spp., *Eimeria* spp., *Trichodina* spp., *Diplostomum* spp. were isolated from 13 common carps. The opportunistic bacterial pathogen of fresh water fishes *Aeromonas hydrophila* was also isolated from wounded fish. No viruses were detected. Fungal infections were encountered in the skin lesions of carps. The source of the encountered morbidity and mortality picture among the studied carps points to the combined action of parasitic, bacterial and fungal agents in these fish. Further investigations on seasonal variations of parasitofauna on the lake are needed for better understanding the dynamics in the ecology of the parasites which are hosted by carps in the Hirfanlı Dam Lake.

Keywords: *Aeromonas hydrophila*, common carp, fish parasites, fungal infections, fish viruses

Türkiye'de Hirfanlı Baraj Gölü'ndeki sazanlarda (*Cyprinus carpio*) bir vakada parazitolojik, bakteriyolojik ve virolojik araştırma

Özet: Bu çalışmanın amacı, 2021 yılının ilkbaharında Türkiye'de Hirfanlı Baraj Gölünde yaşayan sazanlarda görülen ölüm olayının altında yatan hastalık etkenlerine ışık tutmaktı. Bunun için çeşitli parazitolojik, bakteriyolojik ve virolojik araştırma teknikleri kullanıldı. Beş parazit cinsi, *Dactylogyrus* spp., *Myxobolus* spp., *Eimeria* spp., *Trichodina* spp., *Diplostomum* spp. 13 adet sazandan izole edildi. Tatlı su balıklarında fırsatçı bakteriyel patojeni olan *Aeromonas hydrophila* da lezyonlu balıklardan izole edildi. Virüsler tespit edilmedi. Sazanlardaki deri lezyonlarında mantar enfeksiyonlarına rastlandı. İncelenen sazanlardaki hastalık ve ölüm tablosunun kaynağı, bu balıklarda paraziter, bakteriyel ve mantar etkenlerinin birleşik etkisine işaret etmektedir. Hirfanlı Baraj Gölü'ndeki sazanların konaklık ettiği parazitlerin ekolojisindeki dinamikleri daha iyi anlamak için göldeki parazit faunasının mevsimsel değişimleri hakkında daha fazla araştırmaya ihtiyaç vardır.

Anahtar sözcükler: *Aeromonas hydrophila*, balık parazitleri, balık virüsleri, mantar enfeksiyonları, sazan.

Introduction

Fisheries and aquaculture projections predict that diseases of aquatic organisms will continue to be a hot topic due to their impact on global food security through affecting aquaculture production (FAO 2020). Especially when it comes to fish species such as common carp which is the backbone of the sustainable fisheries and aquaculture production in many developing countries in the world (Lucas et al. 2019). Although the contribution of carp species and especially of common carp in the total fisheries and aquaculture production of Türkiye is relatively minimal, it still represents significant part of the diets of the Turkish rural population (Çöteli 2021). Thus, diseases and especially parasites of common carp inhabiting several natural water bodies have been on focus by several research works (Cengizler et al. 2001; Kutlu and Öztürk 2006; Soylu 2009, 2014). As the most aquatic diseases are density dependent when comes to their spreading, it can be expected that with the intensification of aquaculture activities in natural water bodies and dam lakes, there will be a real threat of epizootics both to cultured and native fish populations inhabiting respective places (Çilli 2015).

Current study stresses the importance of the cumulative effect of different pathogens in aquatic

Yazışma adresi / Correspondence: *Esat Çilli, İzmir/Bornova Veteriner Kontrol Enstitüsü Müdürlüğü Erzene mah. Ankara cad. No:172/155 Bornova, İzmir e-mail: esat.cilli@tarimorman.gov.tr*

ORCID IDs of the authors: *[1]0000-0002-4325-7039 • [2]0000-0001-8975-763X • [3]0000-0002-5070-6656 • [4]0000-0002-1475-3041 • [5]0000-0001-7766-3150 • [6]0000-0001-7077-6582 • [7]0000-0002-2634-6478 • [8]0000-0002-1402-894X*

environments exerted on their natural hosts, in this case common carp (*Cyprinos carpio*) from Hirfanlı Dam Lake, Türkiye.

The aim of the present study was shedding light on the disease factors underlying mortality event among common carps inhabiting Hirfanlı Dam Lake in Türkiye in spring of the year 2021. For this purpose various parasitological, bacteriological and virological research techniques were employed.

Materıals and Methods

Upon mortality event, thirteen moribund common carps with ulcerated skins and fin erosions (average weight 700 gr) were captured from Hirfanlı Dam Lake (Ankara, Türkiye) on 5th of April 2021 and on the same day delivered freshly died and moistened with Hirfanlı Dam Lake water, within chilled styrofoam boxes at 4 ˚C to the parasitology, bacteriology and virology laboratories of İzmir Bornova Veterinary Control Instıtute for parasitological, bacteriological and virological examinations.

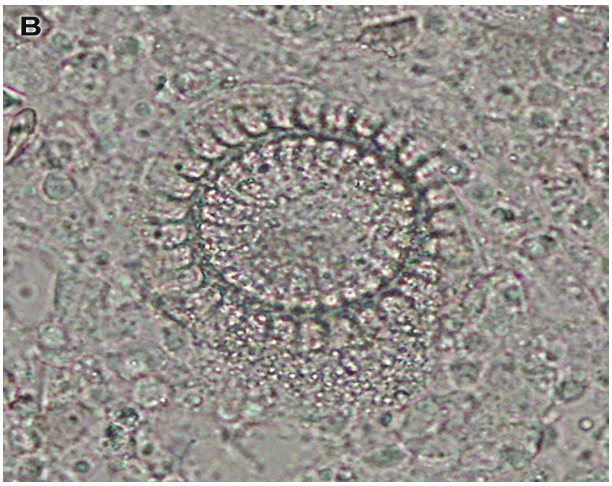
Parasitological examination was done on 1) wet mounts prepared from the eyes, gills, skin scrapings and internal organs, 2) methanol fixed and Giemsa stained kidney tissue smears and finally 3) fecal flotation method for the intestinal content. All parasites were examined under stereo microscope Olympus model BX53F and photographed with Olympus imagining system model D21-CB. All parasites were identified according to Woo (2011).

For bacteriological analysis TSA bacteriological media was used. Biochemical analysis were performed on VITEK®2 System 9.02 (Biomerieux).

For virus detection Eagle's minimal essential medium EMEM (Sigma-Aldrich, USA) was used to homogenize tissue pieces from the fish's spleen, liver, kidney, and heart at a ratio of 1/10 (w/v). This medium was supplemented with 2% fetal bovine serum (FBS) (Biochrom, Germany) and a 10% antibiotic-antimycotic solution (Sigma-Aldrich, USA). Following centrifugation at 4000 x g for 15 min at 4°C with the homogenate, 0.45 m filtrate (Sartorius, USA) was percolated through the mixture. The supernatant was inoculated onto EPC, and BF-2 cells prepared by EMEM supplemented with 10% fetal bovine serum FBS and 1% antibiotic-antimycotic followed by incubation at 15°C for 7 days, respectively. Cell passages were performed two times following the initial incubation.

Results

A metacercaria of *Diplostomum* spp. was isolated from the eye of one fish (Fig. 1a). Inspection of the wet tissue gill slides revealed profound infestation with the protozoan parasite *Trichodina* spp. (Fig. 1b) and monogenean parasite *Dactylogyrus* spp. Subsequently monogeneans were fixed with formalin solution, mounted on glycerin jelly and examined for morphometrics of the opisthaptor (Fig. 1c). Numerous myxospores of myxosporean parasite *Myxobolus* spp. were detected in wet kidney smears, especially densely acumulated in melanomacrophage centers of the anterior kidney (Fig. 1d). Subsequently these smears were fixed in methanol and stained with Giemsa stain. In Giemsa stained slides, phagosytosed *Myxobolus* spp. myxospores were seen inside macrophages (Fig. 1e). Employment of fecal flotation method for the intestinal content revealed infection of the intestines with *Eimeria* spp. based on the presence of oocysts (Fig. 1f). In fresh scrapings from the skin ulcers (Fig. 1g) and eroded fins heavy burden of fungal mycelia with developed sporangia were seen (Fig. 1h).



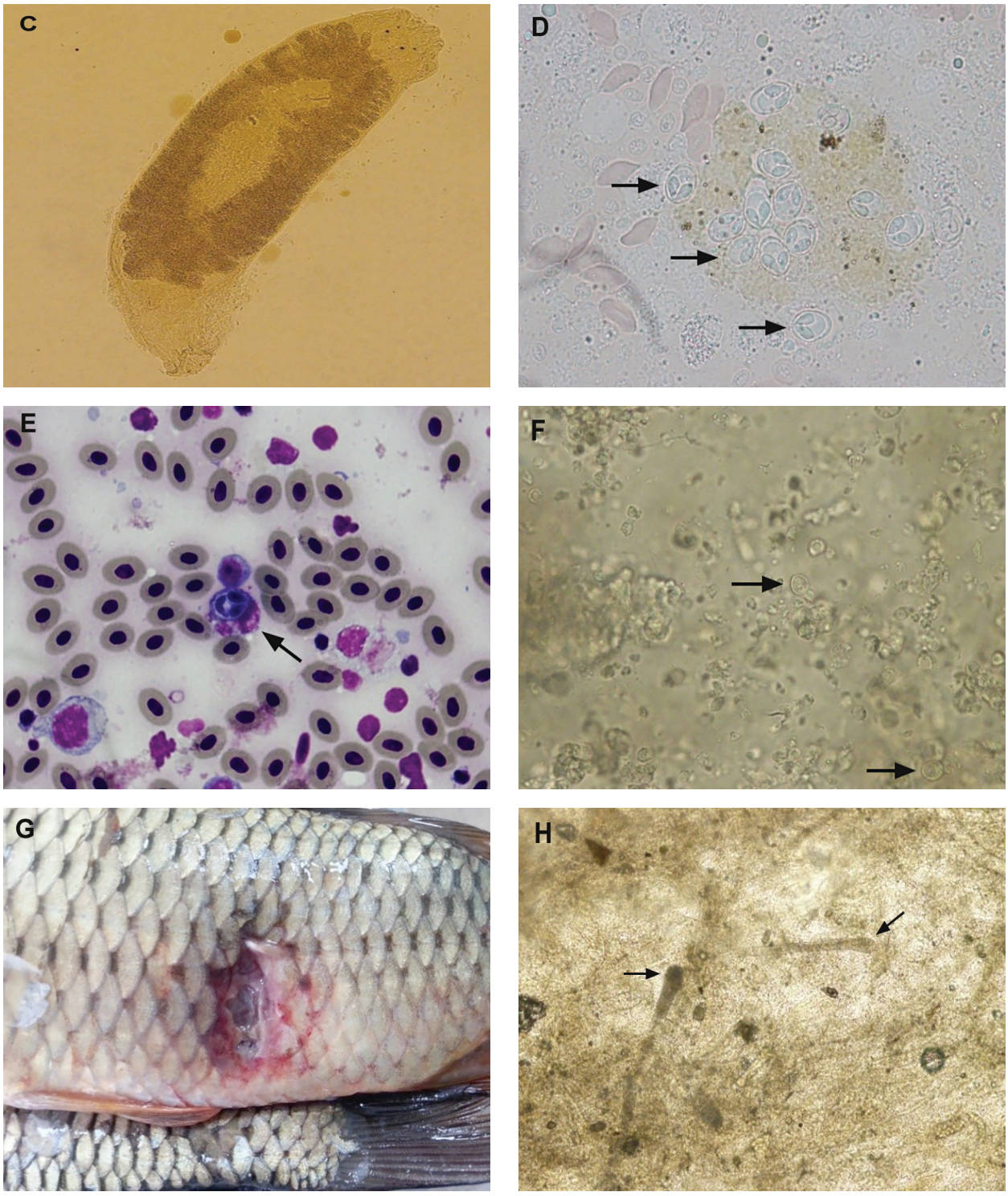


Figure 1. (a) A metacercaria of *Diplostomum* spp. from the eye x100. **(b)** The protozoan parasite *Trichodina* spp. on wet tissue gill slides x1000. **(c)** The monogenean parasite *Dactylogyrus* spp. mounted on glycerin jelly x100. **(d)** Myxospores of myxosporean parasite *Myxobolus* spp. in wet kidney smears (arrows) x400. **(e)** A phagosytosed *Myxobolus* spp. myxospore inside a macrophage (arrow). Giemsa stained slide x400. **(f)** *Eimeria* spp. oocysts (arrows) x400. **(g)** A skin ulcer. **(h)** Fungal mycelia with developed sporangia (arrows) x200.

Inoculation of TSA bacteriological media with kidney and spleen tissue showed bacteriological growth at 25°C after 2 days incubation period. From the shape and colour of the bacterial colonies (white, convex and circular), observation under microscope of Gram-negative, motile straight rods and biochemical analysis performed on VITEK®2 System 9.02 (Biomerieux) the bacteria were identified as *Aeromonas* spp. To distingish between *Aeromonas* species, *A. sobria* and *A. hydrophila*, Voges-Proskauer test was performed which indicated that the bacteria were *A. hydrophila*.

The cytopathogenic effect (CPE) was not observed in EPC and BF-2 cell lines. Thus, no viruses were detected.

The prevalence of all detected pathogens is provided in Table 1.

Table 1. The prevalence of infections in the examined carps (total number of fish, n=13).

Pathogen	Infected organ	Number of fish infected	Prevalence
Diplostomum spp.	eye	1	7.69
Trichodina spp.	gill	8	61.53
Dactylogyrus spp.	gill	13	100.00
Myxobolus spp.	kidney	13	100.00
Eimeria spp.	intestine	5	38.46
Aeromonas hydrophila	liver, spleen, kidney,skin	13	100.00
Fungal mycelia	skin, muscle	13	100.00

Discussion and Conclusion

The encountered morbidity picture among the studied carps points to the combined action of parasitic, bacterial and fungal agents in these fish.

The richness of parasites in the examined carps stays in line with the works of previous authors who investigated the parasitofauna of several fresh water lakes in Türkiye. Although, Kutlu and Öztürk (2006) found three metazoan parasite genera in carps from Karamık Lake (Afyonkarahisar), respectively *Dactylogyrus, Gyrodactylus* and *Bothriocephalus*, only one genus, *Dactylogyrus* was detected in the current study. Hovewer, the list of metazoan parasites on carps from the Turkish fresh water lakes was expanded with the inclusion of *Diplostomum* spp. metacercaria from the carps of Hirfanlı Dam Lake. Other researchers such as Cengizler et al. (2001) also reported several parasite groups from the River Seyhan, among which *Dactylogyrus* spp. and *Trichodina* spp. were the intercepting species with the current research findings.

Special attention deserves the finding of *Diplostomum* spp. metacercaria from the eye of one fish. This finding implicates the presence of the intermediate and final hosts of the parasite in Hirfanlı Dam Lake, whose existence was confirmed in the report of General Directorate of Nature Conservation and National Parks of Republic of Türkiye (Anonymous 2018). In order to combat with *Diplostomum* spp. metacercaria infections, there mıght be need for restocking of Hirfanlı Dam Lake with species such as black carp (*Mylopharyngodon piceus*) which preys on gastropod molluscs. This could be a good strategy for reduction of gastropod populations which serve as an intermediate hosts for Echinostomid metacercaria such as *Diplostomum* spp.

Our results are also relatively similar to other studies carried out abroad. Parallel findings were encountered in the works of Buchmann et al. (1993), Borji et al. (2012) and Panjvini et al. (2016). However, we fortunately have not detected any arthropod parasites such *Lernaea cyprinaceae* and *Argulus foliaceus* and protozoan parasites such as *Ichthyophthirius multifiliis*, which otherwise would have complicated the already present diseased situation of carps in the Hirfanlı Dam Lake. As the Hirfanlı Dam Lake is an artificially constructed water body, it might be speculated that the first carp spawners restocked for the purpose of recreational fisheries were not infected and infested with afforementioned parasite species and the lake remained free of additional exotic carp introductions. However, this cannot exclude the possibility that other cyprinid species are present in the lake and are free of parasites such as *Lernaea cyprinaceae*, *Argulus foliaceus* and *Ichthyophthirius multifiliis* and could serve as reservoir hosts.

Another important aspect of our findings is the presence of numerous myxospores of myxosporean parasite *Myxobolus* spp. in wet kidney smears in contrast to the findings of Dayoub et al. (2007) who

found the spores in the gills of the cultured common carps in Syria. Similar results were obtained in the study carried out by Maftuch et al. (2018) in Indonesia on Koi carp. They found spores not only on gills but also distributed in organs such as intestine, liver and kidney correlated with histopathological alterations. There are also additional records of myxosporean parasites belonging to genus *Myxobolus* from Turkey. *Myxobolus episquamalis* and *Myxobolus ichkeulensis* from *Mugil cephalus* by Özak et al. (2012) and *Myxobolus cyprini* from three fish species (*Barbus lacerta*, *Capoeta trutta* and *C. umbla*) by Ekinci et al. (2022) were recorded. However, in all these studies myxospores were isolated from oval white cysts found on the scales and gills. In the present study, although we were not able to detect cysts on the carps skins and gills, the source of myxospores were plasmodia localised elsewhere in the carp tissues and produced myxospores which were brought by macrophages to the melanomacrophage centres (Fig. 1d) in the anterior kidney for their destruction (Fig. 1e).

Infection with *Eimeria* spp. based on the presence oocysts is in the line with findings of Özer et al. (2014) who reported infection of *E. sardinae* from marine fish from the Turkish Black Sea coast. However, in our study detection of *Eimeria* spp. oocysts in the intestine solely and on its own is not enough argument to claim any disease state due to the *Eimeria* spp. infections. There is a need for further histopathological examination of the intestinal mucosa to reveal if there is any destruction of gastrointestinal mucosa of carps due to proliferating *Eimeria* spp. schizonts.

Typical gross signs of *Aeromonas* septicemia such as ulcerated *Aeromonas* lesions with haemorrhages were seen. This can be attributed to the presence of some environmental stressors such as rising water temperature in spring or poor overwintering conditions of carps. This secondarily might have led to fungal invasion of the lesions and further deepening of the skin damage leading to heavy ulceration. However, the heavy ulceration due to autophagy and apoptosis of the skin cells caused by *A. hydrophila* infection can also be the primary cause of this pathological process which was elucidated by Chen et al. (2020).

Saprolegnia spp. infections in common carp are well documented and almost inevitably lead to heavy pathology (Ashour et al. 2017; Hamad and Mustafa 2018). There was no exception in the findings of the current study, respectively heavy burden of fungal mycelia with fully developed sporangia in ulcers of the carps were seen.

Although the primary focus of the current study was on parasites, detection of facultative bacterial pathogens such *A. hydrophila*, fungal infections and non detection of viruses were important findings from an epidemiological point of view (Ashour et al. 2017; Hamad and Mustafa 2018; Chen et al. 2020).

In conclusion, further investigations on seasonal variations of parasitofauna from the lake are needed for better understanding the dynamics in the ecology of the parasites feeding upon carps in the Hirfanlı Dam Lake.

The source of the encountered morbidity and mortality picture among the studied carps points to the combined action of parasitic, bacterial and fungal agents in these fish. Fish parasites such as *Dactylogyrus* spp., *Myxobolus* spp., *Eimeria* spp., *Trichodina* spp., *Diplostomum* spp. and bacterial pathogen *A. hydrophila* were suspected as the main cause of the pathological changes. Fungal mycelia were thought to proliferate and exaggerate the already present dermal ulcers as secondary invaders. However, histopathology was the missing link in our study. In order to confirm the damage caused by these parasitic, bacterial and fungal agents there is a need for further histopathological examination of vital organs of carps from Hirfanlı Dam Lake exhibiting disease symptoms.

Ethical Statement: No alive animals were used in the present research work. Thus there was no need for ethical approval.

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Author Contributions: Esat Çilli performed the parasitological tests, conceptualised and wrote the manuscript, Hakan Yeşilöz and Ömer Faruk Gökcecik revised the manuscript, Çağatay Nuhay performed the bacteriological tests and revised the manuscript, Abdurrahman Anıl Çağırgan, Kemal Pekmez, Murat Kaplan and Bülent Kafa performed the viral tests and revised the manuscript.

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