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ISOLATION AND ANTIBIOTIC SUSCEPTIBILITY OF *AEROMONAS HYDROPHILA* IN A CARP (*CYPRINUS CARPIO*) HATCHERY FARM

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Abstract

The aim of this study was to describe a case of skin lesions with haemorrhaging in a carp (*Cyprinus carpio*) hatchery farm and to determine effective antibiotic treatments. In the macroscopic examinations of nine carp with haemorrhagic skin lesions, brown or red spotted skin changes of a varying degree were found, along their bodies. No lesions were observed in the internal organs. Pure cultures of *Aeromonas hydrophila* were isolated from the skin, kidney, heart, and liver of the carp. All of the isolates were found to be susceptible to danofloxacin, enrofloxacin, gentamicin, ciprofloxacin, neomycin, and trimethoprim-sulphamethoxazole and resistant to ampicillin, amoxicillin, amoxicillin/clavulanic acid, oxytetracycline, and streptomycin. Treatment of *A. hydrophila* infection was carried out by applying trimethoprim-sulphamethoxazole for 3 d at 10 g/ton of pool water.

Key words: carp, *Aeromonas hydrophila*, antibiotic susceptibility.

Aeromonas hydrophila is the causative agent of the disease known as "haemorrhagic septicaemia", "ulcer disease" or "red-sore disease" (7, 16). *A. hydrophila* is generally found in the gastrointestinal tract of fish and is considered an opportunistic pathogen (16, 19). Most of bacteria, which are termed "opportunistic" usually, do not cause disease unless other factors are involved. *A. hydrophila* is always capable of producing disease if given the chance (19). Outbreaks of the disease are usually associated with a change in environmental conditions, such as stress, overcrowding, a sudden change of temperature, transfer of fish, mishandling, poor water quality, high nitrite and carbon dioxide levels (2, 5, 6, 12, 19). In Turkey *A. hydrophila* had been reported in eels *(Anguilla anguilla)*, reptiles (crocodile) and grass carp (*Ctenopharyngodon idella*) (17-19).

The aim of this case report is to describe a case of skin lesions with haemorrhaging in a carp (*Cyprinus carpio*) hatchery farm in the Burdur province of Turkey and to determine an effective antibiotic treatment.

Material and Methods

Environmental conditions at the fish hatchery farm. Because of haemorrhages with dermal ulcers along their bodies, nine carp were selected for the investigations. The disease history was obtained from their owner. At the farm, there was not any mortality observed, but there was 20% morbidity. The fish hatchery farm covered an area of 22 960 m^2. In three of 12 pools, the fish were kept at the weight of 600 g, 800 g, and 2 kg, and the capacity was 10-ton of fish per year**.** Environmental conditions were found rather suitable for a carp hatchery. The water, as required for carp hatchery farm, was supplied from a stream and the quality of water was stable during the year. The temperature of water was 10-20°C, and the pH was 8.77.

Sampling and microbiological examination. The samples of the liver, gill**,** kidney, heart, and skin of each of the fish were collected. The samples were placed on 5% sheep blood agar plates (Oxoid) and MacConkey agar (Oxoid) plates and then incubated at 25ºC and 37ºC for 3-4 d under aerobic conditions. After incubation, the pure haemolytic yellow colonies were isolated from skin and internal organs of all the carp. The bacteria were identified as *A. hydrophila* on the basis of colony morphology, Gram-staining, and biochemical characteristics (3, 10). Wet mounts of skin, fin, and gill smears were also examined microscopically as well as a macroscopic examination for parasites.

Antibiotic susceptibility test. The susceptibility of the isolates to the following antibiotics: ampicillin (10 µg, Oxoid), amoxicillin (25 µg, Oxoid), amoxicillin/clavulanic acid (30 µg, Oxoid), oxytetracycline (30 µg, Oxoid), enrofloxacin (5 µg, Oxoid), danofloxacin (5 µg, Pfizer), streptomycin (10 µg, Oxoid), trimethoprim-sulphamethoxazole (25 µg,

Oxoid), neomycin (30 µg, Oxoid), gentamicin (10 µg, Oxoid), and ciprofloxacin (5 µg, Oxoid) (4) were tested using disc diffusion methods.

Results

In the macroscopic examinations of the carp, brown or red spotted skin lesions of a varying degree were found along their bodies (Fig.1). These lesions were mostly scattered over the abdomen, operculum, head, fins, and gills. No lesions were observed in the visceral organs. Gram-negative bacilli were seen in Gram-stained smears, taken from the skin, liver, and kidney. No parasites in the skin, fin, gills, and internal organs were found. *A. hydrophila* was isolated from the liver, gill**,** kidney, heart, and skin of each fish. A biochemical characteristic of *A. hydrophila* was given in Table 1.

A. hydrophila isolates were found to be susceptible to danofloxacin, enrofloxacin, gentamicin, ciprofloxacin, neomycin, and trimethoprim-sulphamethoxazole, and to be resistant to ampicillin, amoxicillin, amoxicillin-clavulanic acid, oxytetracycline, and streptomycin. In the treatment of *A. hydrophila* infection, trimethoprim-sulphamethoxazole was chosen, based on results from an antibiotic susceptibility test. After the fish had been kept in saltwater for 30 min, they were removed and placed in water with trimethoprim-sulphamethoxazole. The antibiotic was used for 3 d in pool water at 10 g/ton. The infection in the carp was treated with trimethoprim-sulphamethoxazole**.**

Fig. 1. The appearance of the brown or red spot lesions on the skins of the carp, from which *A. hydrophila* was isolated.

Table 1
Biochemical features of *A. hydrophila* isolates

Biochemical features	Reactions	Biochemical features	Reactions
Gram-staining	-	ß-haemolysis	+
Catalase	+	H_2S	-
Oxidase	+	Motility	+
Lactose	+	Methyl red	+
Glucose, acid	+	ONPG	+
Glucose, gas	-	Lysine decarboxylase	-
Mannitol, acid	+	Growth at:	
Indol production	+	24ºC	+
Urea hydrolysis	-	37°C	+
Esculin hydrolysis	+	Growth on:	
Nitrate reduction	+	Blood agar	+
Casein hydrolysis	+	MacConkey agar	+

Discussion

As it was mentioned in the introduction, *A. hydrophila* causes a disease known as haemorrhagic septicaemia or ulcer disease in fish, and belongs to the most common bacteria present in aquatic environments throughout the world. The bacterium is naturally found in the intestinal tract of the fish, and does not cause the disease under natural conditions (16). The disease caused by *A. hydrophila* is one of the major disease problems for farmed carp (5, 7). Outbreaks of the disease are usually caused by stress and changes in environmental conditions. Overcrowding, handling, transportation, poor water quality, a sudden change of temperature, low dissolved oxygen, and high CO_2, nitrite, and ammonia levels are the most common predisposing factors associated with this disease (2, 5, 16, 19). In this study, environmental conditions in the hatchery farm were found rather suitable for a carp hatchery. The most common predisposing factors that increase the pathogenicity of *A. hydrophila* were not determined in the carp hatchery farm.

The parasites, such as *Lernaea, Argulus,* and *A. salmonicida* may also be a cause of ulcerative lesions on the skins of carp (1, 13). But, in this study, at the level of macroscopic and microscopic examinations, no parasites were observed. In this study, it is considered that the disease of the fish may be related to *A. hydrophila*, as the same bacteria in a pure culture were isolated from skin lesions and internal organs. It has been reported by several researchers that *A. hydrophila* causes haemorrhagic skin lesions in cultured and fresh water fish (2, 7, 14, 16). In this study, carp is determined as susceptible to *A. hydrophila* infection. Similarly, some researchers reported that most cultured and fresh water fish, such as eel, goldfish, catfish, rainbow trout, ayu, tilapia, were susceptible to *A. hydrophila* infection (2, 11, 19).

A. hydrophila isolated from fish, displays a high degree of resistance to antibiotics (2, 7, 14). Consequently, we performed the antibiotic susceptibility test, in order to determine the susceptibility of *A. hydrophila* to different antibiotics that are used in veterinary medicine in Turkey. *A. hydrophila* strains have been reported to be intrinsically resistant to ampicillin by many researchers (1, 7, 14). In this study, *A. hydrophila* strains were resistant to ampicillin, as predicted. The strains were also found to be resistant to other ß-lactam antibiotics, such as amoxicillin and amoxicillin/clavulanic acid. Resistance of *Aeromonas* sp. to ß-lactam antibiotics was determined by other researchers (7-9, 17, 20). Although some researchers (7, 8) reported that *Aeromonas* sp. were susceptible to streptomycin, the *A. hydrophila* strains isolated in this study were resistant to streptomycin, as found out in several other studies (12, 14). Oxytetracycline is frequently used as an antimicrobial agent in cultured fish hatcheries in Turkey. Therefore, it was expected that the isolated strain was resistant to oxytetracycline.

Turkey has a great potential for carp hatcheries because of its location (8). Economical losses caused by *A. hydrophila* infections have great importance in carp farms (7). At present, the most widely used method of controlling *A. hydrophila* infection in cultured fish is the use of antimicrobial drugs (2, 7, 15). The widespread antibiotic use is associated with an increased antibiotic resistance in aquatic bacteria. Thus, antimicrobial susceptibility tests are important for an effective treatment. The treatment of *A. hydrophila* infection in this study was carried out using trimethoprim-sulphamethoxazole for 3 d, at 10 g/ton pool water. The choice of the antibiotic was based on the results from a susceptibility test.

In conclusion, this case report has exhibited that *A. hydrophila* may cause haemorrhages with dermal ulcers in carp. Uncontrolled and extensive use of antimicrobial agents may cause the frequent occurrence of multiple antimicrobial resistances. Therefore, an antimicrobial susceptibility test in *A. hydrophila* infection, as well as in other bacterial infections, has to be certainly made in cultured fish hatcheries. To prevent the infection water should be regularly changed and overcrowding and stress in the fish population should be avoided.

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