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Case Report - Veterinary Medicine

Infestation of *Argulus* sp. associated with bacteriosis in ornamental carp - case report

[*Infestação de* Argulus *sp. associada a bacteriose em carpas ornamentais – relato de caso*]

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ABSTRACT

The aim of this study was to diagnose the causative agent of carp (Cyprinidae) mortality in an artificial lake used for recreation in Jaraguá do Sul, state of Santa Catarina, in February 2018. The fish showed symptoms, including skin ulceration, hemorrhages on the surface of the body, loss of scale and increased mucus production. Through the proteomic profile, using the MALDI-TOF technique, three bacterial isolates were identified as *Aeromonas veronii*, *A. hydrophila* and *Citrobacter freundii.* In addition, in the mucus scraping, a large number of the ectoparasite *Argulus* was observed. A blood collection was carried out to verify the hematological indices of the animals and a decrease in the the hematocrit value, erythrocyte count, and differential leukocyte count was observed. These findings indicate that *Argulus* infection increases the carp's susceptibility to bacterial diseases and contributes to the suppression of the innate immune system and consequent increase in mortality.

Keywords: Co-infection, diagnostic, fish pathogens.

RESUMO

O objetivo deste trabalho foi diagnosticar o agente causador da mortalidade de carpas (Cyprinidae) em um lago artificial utilizado para recreação em Jaraguá do Sul, estado de Santa Catarina, em fevereiro de 2018. Os peixes apresentavam sintomas, incluindo ulcerações na pele, hemorragia na superfície do corpo, perda de escama e elevada produção de muco. Por meio do perfil proteômico, pela técnica de MALDI-TOF, três isolados bacterianos foram identificados como Aeromonas veronii, A. hydrophila e Citrobacter freundii. *Além disso, no raspado do muco, foi observado grande número do ectoparasito* Argulus. *Foi realizada uma coleta de sangue para verificar o perfil hematológico dos animais e constatou-se uma diminuição na porcentagem de hematócrito, no número de eritrócitos e na contagem diferencial dos leucócitos. Esses achados indicam que a infecção por* Argulus *aumenta a suscetibilidade da carpa a doenças bacterianas bem como contribui para a supressão do sistema imunológico inato e o consequente aumento da mortalidade.*

Palavras-chave: co-infecção, diagnóstico, patógenos de peixes

INTRODUCTION

The genus *Aeromonas* is emphasized in the diagnosis of fish diseases, often appearing as the primary agent causing ulcerative lesions and hemorrhagic septicemia (Hayatgheib *et al*., 2020). Among the bacteria of this genus, the species *Aeromonas hydrophila* is responsible for significant losses in aquaculture production and fish infected by this pathogen have loss of appetite, loss of balance, epidermal lesions, ulcers with muscle exposure, and erratic swimming. Although *A. hydrophila* is the most isolated species, there are other species, such as *A. veronii*, that have been isolated and identified in fish (Beaz-Hidalgo *et al*., 2010). This species is one of the main causes of epizootic ulcerative syndrome in fish (Hoai *et al*., 2019; Skwor *et al*., 2014.

Citrobacter freundii, on the other hand, belongs to the Enterobacteriaceae family, is a facultative anaerobic gram-negative bacillus, which is capable of infecting aquatic animals and causing high mortality (Huang *et al*., 2021). This bacterium has already been identified and understood from different species of fish and is an infectious agent recognized as an opportunistic pathogen, responsible for systemic hemorrhages, causing serious damage to the

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kidney, spleen, gills, and skin. (Behera *et al*., 2022).

In addition to bacteria, parasites are etiological agents of great importance. The genus *Argulus* sp is known worldwide due to the damage they cause in natural populations and commercial production. Fish infected by these parasites show reduced weight, difficulty in breathing, lesions and ulcers, which causes a potential route for secondary invasions caused by bacteria (Pavanelli *et al*., 2002).

Thus, the goal of this work was to diagnose the causative agent of the mortality of ornamental carp (Cyprinidae) in an artificial lake located in Jaraguá do Sul, Santa Catarina.

CASUISTRY

Fish mortality occurred in an artificial lake (47.3 m² and 0.8 m) located in the Jaraguá do Sul, Santa Catarina, Brazil, in February 2018. The carps were scraping or rubbing against the tank walls and showed clinical signs such as erratic swimming. Clinical diagnostics was divided into three steps: 1st) analysis of water quality variables; 2nd) biopsy of animals with clinical signs; 3rd) sending samples to molecular identification.

Dissolved oxygen, total ammonia, non-ionized ammonia, pH and temperature were measured. Eight symptomatic fish weighing between 735g and 1,565g were removed from the tank for diagnosis of the possible causative agent of mortality. During external observation of the animals, it was possible to observe a large amount of mucus on the surfaces of the skin and gills, erosion of the fins, scale loss, and hemorrhagic points throughout the body (Figure 1). In these fish, a mucus scraping was performed to identify ectoparasites. After that, fish were anesthetized with eugenol (75mg. L^{-1}) to collect blood samples and euthanized by cerebral concussion for removal of internal organs and bacterial examination. The blood was withdrawn from the caudal vein with syringes containing of EDTA 10% and used for blood smears stained with May Grunwald/Giemsa/Wright (Rosenfeld, 1947) for white blood cell count using an indirect method by counting the total leukocytes number (WBC) in 2000 erythrocytes in the smears (Ishikawa *et al*., 2008) and total number of thrombocytes and leukocytes (WBC) were calculated by the indirect method (Jatobá *et al*., 2011). Hematocrit percentage was measured by the microhematocrit method and red blood cell count (RBC) in a Neubauer chamber after dilution 1:200 in Dacie solution.

After hematological collection, the animals were necropsied and the kidney, blood, heart and liver were removed and macerated in sterile mortar with sterile saline solution at 0.85% NaCl in the proportion of 1.0 g of sample to 1.0mL. The macerate was inoculated into petri plates containing blood agar (TSA, "triptone soya agar", Himedia, with 5% defibrinated bovine blood), and incubated for 48 h at 30°C. After the isolation and purification procedures by the depletion method, the strains were incubated for 48 h at 30°C in TSB culture medium ("triptone soya broth" HiMedia, Mumbai, India), and sent for molecular identification at the AQUACEN Laboratory of the Federal University de Minas Gerais (UFMG) through the proteomic profile (Deak *et al*., 2015) by the technique of MALDI-TOF (orption Ionization – Time of Flight") Microflex (MALDI Biotyper).

DISCUSSION

The quality of the environment is directly related to the success of any breeding system. Inadequate water quality conditions result in harm to the health and survival of the animals, as they make them more susceptible to disease outbreaks. The water quality parameters analyzed on the day of collection were dissolved oxygen: 4.5m.L^{-1}, total ammonia: 0.11mg.L^{-1}, ammonia: 0.001 NH^3 mg.L^{-1}, pH: 7.2 and temperature: 25.2°C were adequate for the species. However, there was an excessive presence of organic matter at the bottom of the tank, probably caused by the accumulation of feed leftovers due to the absence of adequate feeding management. The fish were fed without considering the biomass present in the tank, as well as adjustments were not made in the feeding management according to the water temperature. In addition, as it is an artificial lake for recreation, visitors could feed the fish with leftover food (such as bread and crackers), which can compromise the health of the animals, as they did not meet their nutritional requirements.

In the fish´s mucus there was a large amount of fish louse, *Argulus* sp. spread over the entire surface (Figure 2). The mechanism that increased the susceptibility to diseases could be due to the direct impact of the ectoparasites that attach themselves to the skin of the fish and facilitate the entry of infectious agents, mainly opportunistic ones, as is the case with many bacteria, or indirectly the parasite can act as a disease vector. In this report, the bacterial strains isolated were: *Aeromonas veronii*, *A. hydrophila* and *Citrobacter freundii*.

Hematocrit shows variations in situations of stress, the carps presented values between 10.1 and 12.3%, 1/3 of the value considered for healthy carp (29.73 – 33.86 %). The same was observed in the erythrocyte counts, while in the leukocyte differential count, the presence of lymphocytes and eosinophils stands out (Table 1), the latter are very prone to parasitic infections (Alvarez-Pellitero, 2008; Shameena *et al*., 2021), corroborating with the observed in this work.

These data indicate that *Argulus* infection increases the susceptibility of carp to bacterial diseases and contributes to the suppression of the innate immune system, consequently increasing mortality.

Figure 1. White arrows show the points with excess mucus on the surfaces, and black arrows shows erosions and scale loss, and hemorrhages presented in the carps.

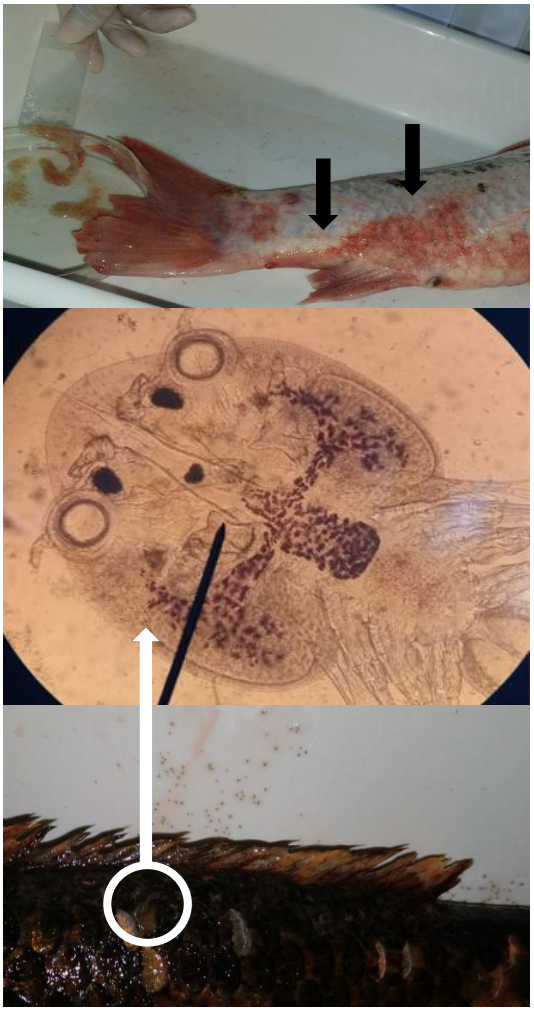

Figure 2. Black arrows show points of scale loss; white circles show *Argulus* sp. seen with the naked eye present in carp.

Table 1. Total and differential blood cell counts of carp (*Cyprinidae* spp.)

Blood cells	Mean ± standard deviation (Max. – Min.)	Healthy carp reference value*
Erythrocytes ($x10^6$)	0.90 ± 0.12 (1.01 – 0.75)	1.69 - 1.91
Thrombocytes ($x10^3$)	1.12 ± 0.52 (1.31 – 0.79)	NR
Total Leucocytes ($x10^3$)	1.92 ± 1.01 (3.31 – 1.04)	19.8 - 28.1
Lymphocytes ($x10^3$)	1.61 ± 0.79 (2.78 – 0.99)	14.7 - 23.5
Monocytes($x10^3$)	0.28 ± 0.43 (0.92 – 0.00)	0.46 - 0.96
Eosinophils ($x10^3$)	2.20 ± 2.17 (4.70 – 0.31)	NR
Basophiles ($x10^3$)	0.21 ± 0.28 (0.61 – 0.00)	0.69 - 1.57
Neutrophils ($x10^3$)	0.44 ± 0.80 (0.16 – 0.00)	1.57 - 3.9

*Tripathi *et al*., 2004. NR: Not reported

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