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Survival and behavior of silver catfish, *Rhamdia quelen*, submitted to antibiotics and sodium chloride treatments

Sobrevivência e comportamento de jundiá, *Rhamdia quelen*, submetido a tratamento com antibióticos e cloreto de sódio

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- NOTE -

ABSTRACT

*In order to evaluate the use of antibiotics and NaCl on the behavior and survival of silver catfish, **Rhamdia quelen**, infested by **Ichthyophthirius multifiliis** and infected with **Aeromonas hydrophila**, juveniles were treated with chloramphenicol, chloramphenicol + salt, oxytetracycline, oxytetracycline + salt and water alone (control). Fish survival in the treatments with chloramphenicol + salt and oxytetracycline + salt was significantly higher than in the other treatments. The treatment with chloramphenicol presented higher survival than the treatment with oxytetracycline and both showed significantly higher survival than control. Swimming activity was higher in the fish treated with antibiotics and salt compared to control fish. A combination of the studied antibiotics plus salt is more effective to treat both **A. hydrophila** infection and **I. multifiliis** infestation in silver catfish, but since the use of chloramphenicol is not allowed in Brazil, oxytetracycline plus salt seems to be the best treatment option.*

Key words: ***Aeromonas hydrophila**, chloramphenicol, **Ichthyophthirius multifiliis**, oxytetracycline, sodium chloride.*

RESUMO

*Com a finalidade de avaliar o efeito de antibióticos e NaCl sobre o comportamento e a sobrevivência de jundiá, **Rhamdia quelen**, infestados por **Ichthyophthirius multifiliis** e infectados com **Aeromonas hydrophila**, juvenis foram tratados com cloranfenicol, cloranfenicol + sal, oxitetraciclina, oxitetraciclina + sal e apenas água (controle). A sobrevivência dos peixes dos tratamentos com cloranfenicol + sal e oxitetraciclina + sal foi significativamente mais elevada que nos demais tratamentos. O tratamento com cloranfenicol apresentou sobrevivência mais elevada que a do tratamento com oxitetraciclina e ambos tiveram sobrevivência significativamente maior que o controle. A atividade natatória foi maior nos peixes tratados com antibióticos e sal do que nos do controle. A combinação dos antibióticos com sal se mostrou mais eficaz no tratamento de jundiás infectados com **A. hydrophila** e infestados com **I. multifiliis**, mas, como o uso de cloranfenicol é proibido no Brasil, a oxitetraciclina com sal parece ser a melhor opção de tratamento.*

Palavras-chave: *Ae**romonas hydrophila**, cloranfenicol, **Ichthyophthirius multifiliis**, oxitetraciclina, cloreto de sódio.*

Protozoan ***Ichthyophthirius multifiliis*** is one of the most important pathogen of freshwater fishes, leading to lethality or making the animal more susceptible to other diseases. One of these diseases is known as hemorrhagic septicemia, caused by ***Aeromonas hydrophila***, an etiologic agent responsible for substantial economic losses in fish culture. It causes high mortality rate and also increase susceptibility to other diseases (BOIJINK & BRANDÃO, 2001).

Despite of the prohibition of the chloramphenicol use in animals for human consumption in several countries, it has been frequently studied as both bacteriostatic and growth promoter (PALERMO-NETO, 2001). On the other hand, the use of

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oxytetracycline is legalized for aquaculture (PETERSEN & DALSGAARD, 2003). Cultures of ***A. hydrophila*** from silver catfish, ***Rhamdia quelen***, revealed sensitivity to both chloramphenicol and oxytetracycline (PEDROZO et al., 1998). In addition, sodium chloride is effective against ***I. multifiliis*** for this species (MIRON et al., 2003). Thus, this study evaluated the use of chloramphenicol, oxytetracycline and sodium chloride on survival and behavior of silver catfish, infested with ***I. multifiliis*** and infected with ***A. hydrophila***.

Ninety silver catfish juveniles (30 days old and with 0.99 ± 0.05g) infested with ***I. multifiliis*** and infected with ***A. hydrophila*** were equally distributed in 15 continuously aerated 2L aquaria. Infestation with ***I. multifiliis*** was identified by the presence of white spots on the skin (MIRON et al., 2003) and infection with ***A. hydrophila*** was identified by superficial reddening of body surface (ROBERTS, 1993). Bacteriological evaluation was based on methods described by CARTER & COLE (1990). Skin lesions and gills specimens from three fishes were cultured in 5% ovine blood agar and Mac Conkey agar. Morphological, staining and biochemical characteristics were analysed after 48 hours of incubation at 27°C. Cultured reveled significant and pure isolates of ***A. hydrophila***. The isolates demonstrated susceptibility to gentamicin (10µg) and nalidixic acid (30µg) and were resistent to ampicillin (10µg), erytromycin (15µg), lincomycin (2µg), penicillin (10 UI) and sulfazotrim (25µg) and intermediate susceptibility to tetracycline (30µg) by antimicrobial susceptibility test.

Fish were submitted to five treatments (three replicates each) for 96h: chloramphenicol (1mg L^{-1}), chloramphenicol (1mg L^{-1}) + 4g L^{-1} sodium chloride, oxytetracycline (4mg L^{-1}), oxytetracycline + 4g L^{-1} sodium chloride, and a control treatment. Oxytetracycline was used in the commonly utilized concentration in our lab to treat fish infected with ***Aeromonas*** (data not published). The waterborne NaCl concentration used in this experiment is an effective treatment for infestation with ***I. multifiliis*** in silver catfish (MIRON et al., 2003).

In all treatments juveniles were fed once a day until apparent satiety. Remained food, feces and other debris were siphoned 1 h after feeding. Thereafter, nearly 15% of the water was replaced and kept in the same original conditions. The dead fish were also removed and mortality was daily recorded. The water from aquaria with chloramphenicol was renewed at the 48^{th} h, and those with oxytetracycline 6h after application.

At the last three days, behavior observations during the feeding were carried out: swimming activity (absence, slow, slow to moderate, moderate, moderate to fast, and fast), feeding latency (time elapsed until the first food item added was taken) (according to SCOTT et al., 2003), and type of food capture (whether in group or individually, or whether directly at the surface or at the bottom).

Dissolved oxygen, temperature (oxygen meter DM4 - Digimed, Brazil) and pH (pH meter DMPH-2 - Digimed, Brazil) were measured daily, at the beginning of the morning. Homogeneity of variance from survival data and feeding time among the groups was carried out with Levene test. As they were homoscedastic, comparison among different treatments was performed with one-way ANOVA followed by Tukey test, using the software Statistica 5.1.

Values of dissolved oxygen (8.02±0.93mg L^{-1}), temperature (25.13±1.13ºC) and pH (7.14±0.16) did not show significant difference ($P>0.05$) among treatments. Survival in the treatments with chloramphenicol + salt and oxytetracycline + salt was significantly higher than that in the treatments without salt. Fish submitted to chloramphenicol presented higher survival rate than those exposed to oxytetracycline, and both showed significantly higher survival rate than control group, which presented 100% mortality (Figure 1). The used concentrations and time of exposure to oxytetracycline and chloramphenicol are lower than the recommended by ROBERTS (1993) (immersion in 20mg L^{-1} for 6 days and 1h, respectively), but proved to be effective for treating ***A. hydrophila*** infection in silver catfish. The results of the present study are in agreement with the fact that resistance of ***Aeromonas*** spp. to chloramphenicol and oxytetracycline is low (<20%) and does not differ (PETERSEN & DALSGAARD, 2003). Little or no effect on ichthyophthiriasis in rainbow trout (***Oncorhynchus mykiss***) could be attributed to chloramphenicol and oxytetracycline (WAHLI et al., 1993). However, MIRON et al. (2003) verified that 4 g/L sodium chloride reduces ***I. multifiliis*** infestation and increases survival of silver catfish juveniles. Therefore, the lower mortality of silver catfish treated with chloramphenicol and oxytetracycline compared to control indicate that these antibiotics are effective to treat ***A. hydrophila*** infection. Moreover, the combination of antibiotics plus salt caused an even lower mortality, probably because salt (but not the antibiotics, as expected) is effective to treat ***I. multifiliis*** infestation. Another possibility is that this combination is more effective against ***A. hydrophila*** than the antibiotics alone. This last hypothesis may be supported by the following evidences: first, the isolated cultures of ***A. hydrophila*** showed an intermediate susceptibility to tetracycline

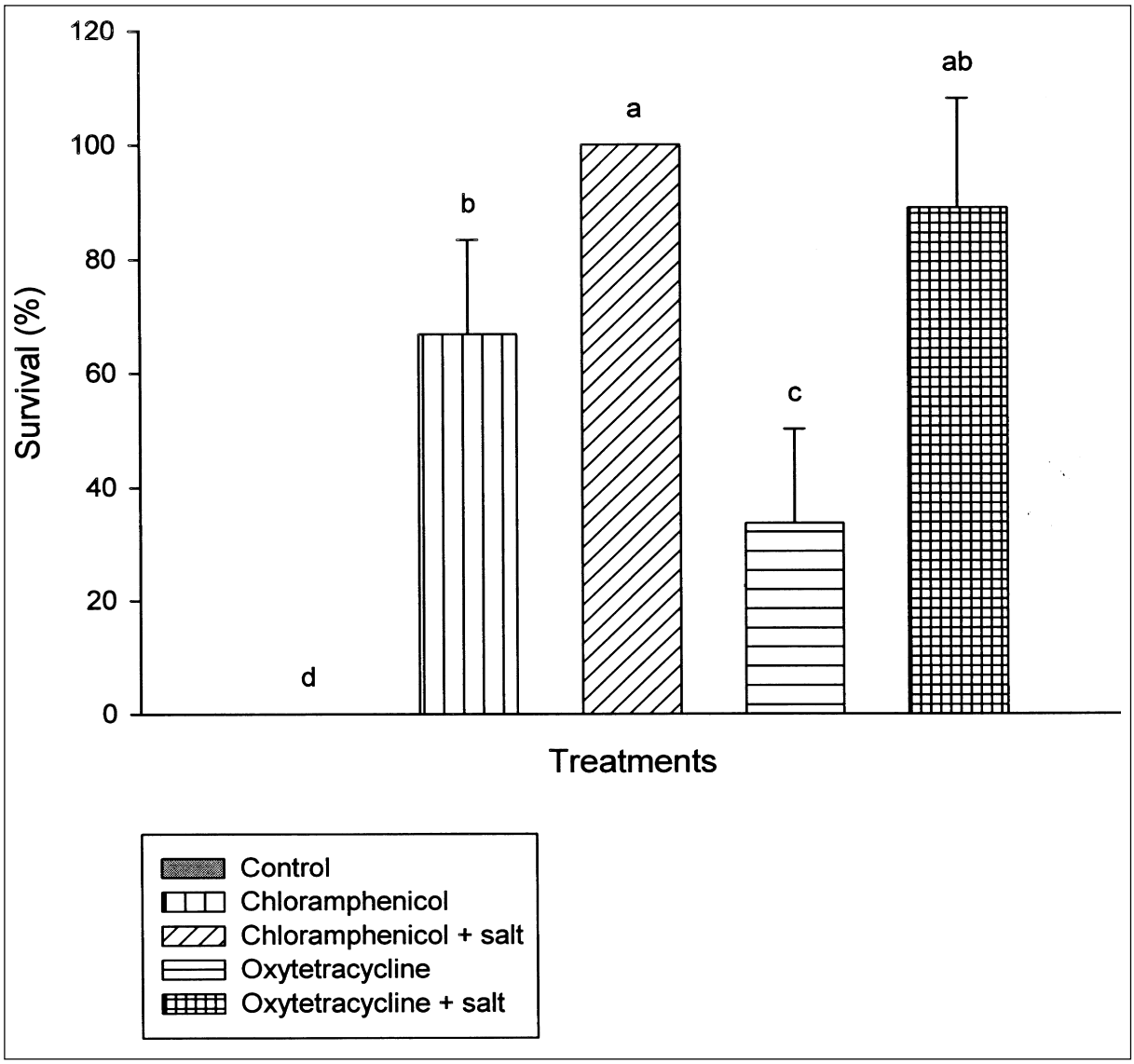

Figure 1 - Survival of silver catfish juveniles submitted to bactericides plus sodium chloride treatments. Means identified by different letters indicate significant difference among treatments ($P < 0.05$). There are no SEM bars in the control and chloramphenicol + salt because SEM is zero.

in the present experiment, and second, according to WHOOLEY et al. (2004) combinations of Tricide (disodium ethylenediaminetetraacetate dehydrate) and oxytetracycline reduced the amount of antibiotics necessary for fish therapy, and render drug-resistant bacteria sensitive to antimicrobial therapy.

Swimming activity of control group was slow in 44.0% of the specimens and 11.11% showed absence of swimming activity through the observation period. BOIJINK & BRANDÃO (2001) also observed reduced swimming activity in silver catfish after inoculation of ***Aeromonas*** sp. In the chloramphenicol and chloramphenicol + salt treatments swimming was moderate in 55.56% of the juveniles and moderate to fast in 27.78% of the observations. In the oxytetracycline and oxytetracycline + salt treatments swimming was moderate to fast in 50% of the observations and moderate in 27.78%. Behavior analyses of common carp and rainbow trout exposed to chloramphenicol (food pellets with 30-50 mg/kg fish weight for the carp and 50-75 mg/kg fish weight for the trout) demonstrate that it causes maximum swimming activity during and after the feeding therapeutic treatments (REIDE & SIEGMUND, 1989).

Feeding latency (8.22±1.58s) and type of food capture (60% at the surface) did not differ

significantly among treatments (P>0.05). Most juveniles fed in group during the observations in the control group (66.67%), chloramphenicol, chloramphenicol + salt (55.56%), and oxytetracycline + salt (77.78%) treatments. Individual food taking was predominant (55.56%) in the oxytetracycline treatment. NIETO & ELLIS (1991) report that fishes infected with ***Aeromonas*** sp. only eat when the food reaches the bottom. Apparently, feeding behavior of silver catfish juveniles was not changed by infection with ***A. hydrophyla*** and infestation with ***I. multifiliis***.

In conclusion, the use of both cloramphenicol and oxytetracycline plus salt were effective in the treatment of the silver catfish juveniles infested with ***I. multifiliis*** and infected with ***A. hydrophila***. However, despite of chloramphenicol being effective, its synthesis, importation and commercialization, as well as of all nitrofurans and products that contain this active principle, are prohibited by law in Brazil. The prohibition is based on the fact that residues of this drug in animal products constitute a risk to health. Therefore, oxytetracycline plus salt seems to be the best option in the treatment of the silver catfish juveniles infested with ***I. multifiliis*** and infected with ***A. hydrophila***.

This study was performed according to the Commission of Ethics and Biosafety from Universidade Federal de Santa Maria.

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