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Haemorrhagic septicaemia in the hybrid surubim (*Pseudoplatystoma corruscans* × *Pseudoplatystoma fasciatum*) caused by *Aeromonas hydrophila*

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Abstract

Intensive culture of the hybrid surubim (*Pseudoplatystoma corruscans* × *Pseudoplatystoma fasciatum*) in Brazil is responsible for the occurrence of diseases and consequent economic losses. However, the causative agents are not well known. The objective of this study was to isolate and to characterize the pathogenic agent responsible for mortalities in cultured surubim and to demonstrate its virulence. Ten fish from a fish farm located in the Mato Grosso do Sul State (Brazil) were collected and 14 haemolytic bacteria characterized as *Aeromonas hydrophila* were isolated from the kidneys (eight) and brain (six). As an experimental challenge, fish weighing 98.1 ± 23.6 g were injected with 1 mL of saline solution and 2×10^2, 2×10^4, 2×10^6 and 2×10^8 CFU *A. hydrophila* mL^{-1}. Fish infected with 2×10^8 CFU showed increased external and internal symptoms and mortality of 50 ± 12.5% after 96 h. Increased *A. hydrophila* concentration was responsible for a decrease in haematocrit percentage and erythrocyte number, lymphocytes and eosinophils, as well as an increase in monocytes, neutrophils, serum agglutination titre and serum antimicrobial activity. It was concluded that *A. hydrophila* was responsible for characteristic symptoms of bacterial haemorrhagic septicaemia as well as important haematological and immunological alterations, which led to surubim mortality.

Keywords: *Pseudoplatystoma* sp., motile aeromonads, infection, haematology, immunology

Introduction

The surubim hybrid fish, from the crossing between pintado (*Pseudoplatystoma corruscans* Spix, Agassiz 1829) and cachara (*Pseudoplatystoma fasciatum* Linnaeus 1766) cultured in Brazil is one of the largest freshwater catfish of South America, presenting high commercial value (Godinho, Kynard & Godinho 2007). However, their growth and intensification of production involve stress and excessive nutrient accumulation in the culture environment. Consequently, the bacteria that make up the microbiota of the water and fish digestive tract can increase their pathogenic potential, altering the environmental conditions, which can lead to illness and increased mortality (Woo & Bruno 2003).

Mortalities of bacterial aetiology are the most important cause of economic losses in fish culture. Diseases in *Pseudoplatystoma* sp. cause important losses in culture (Gianquito & Volpato 2005). However, few studies have been carried out to identify the causative agents. Nevertheless, outbreaks of disease in surubim farms during winter have caused bleeding in the intestine and anus, external lesions and fins redness, being responsible for production mortality up to 80% (Campos 2004).

As its recognition as a causative agent of bacterial haemorrhagic septicaemia, the motile aeromonads were considered pathogens that affect a great variety of freshwater fish (Woo & Bruno 2003). Holliman (1993) recognizes that *Aeromonas hydrophila* is the most virulent among the motile aeromonads strains

for fish, also regarded as a zoonotic agent causing diarrhoea and septicaemia in humans (Deodhar, Saraswathi & Varudkar 1991).

Haematological studies in teleost fish are essential for assessment of the physiological, biochemical and pathological fish status. Bacterial diseases cause significant haematological changes in fish, leading to immunological alterations (Clauss, Dove & Arnold 2008).

The objective of this study was to isolate and to characterize the pathogenic agent responsible for the mortality of cultured hybrid surubim. To demonstrate its virulence, an experimental challenge evaluated the mortality rate, clinical symptoms and haemato-immunological parameters.

Materials and methods

Pathogen isolation

During outbreaks of fish mortality throughout the year 2009 in farms located in the Mato Grosso do Sul State, Central-West region of Brazil, a total of 10 diseased hybrid surubim (*P. corruscans* male × *P. fasciatum* female), weighing between 100 and 500 g, were collected to isolate the microorganisms. The mortalities in this region in 2009 reached 20 tonnes of fish, costing approximately US$ 160 000 (data from the owner). Generally, these mortalities were seen after some management in fish when water temperatures fell below 18 °C. These fish showed anaemia, haemorrhage in the intestine and fin base, anorexia, dark skin, erratic swimming, lethargy and epidemic ulceration. Kidneys and brains were harvested and individually macerated using a sterile porcelain mortar at a ratio of 1 g of tissue to 1 mL of 0.65% sterile saline solution. The homogenates were serially diluted (1:10), plated on tryptone soya agar (TSA) supplemented with 5% sheep blood to isolate haemolytic bacteria, and incubated at 26 °C for 24 h. After growth on blood TSA, the strains were selected for each origin (each organ from each fish) that showed different colonies and different morphologies and Gram staining. Individual colonies were isolated from the plates to compose pure cultures.

Haemolytic activity assay

The isolated bacteria were cultured for 24 h at 30 °C in BHI. After incubation, the strains were centrifuged at 1800 *g* for 20 min, and the supernatant was filtered through millipore filter of 0.22 μm. Thereafter, for each strain 3.7 mL of phosphate buffered solution (PBS), 0.8 mL of filtered culture free of bacteria and 0.5 mL of 1% sheep blood were mixed, in triplicates. Broth BHI and a solution of BHI with 0.1% sodium dodecyl sulphate (SDS) were used as negative and positive controls respectively. The assay mixture was incubated at 25 °C for 1 h and the unlysed cells and debris were removed by centrifugation at 400 *g* at 4 °C followed by a absorbance read at 541 nm. The mixture reading with SDS was considered as 100% haemolytic activity, while the reading with BHI was considered 0% of haemolytic activity. Therewith, it was measured the percentage of haemolytic activity of each strain (adapted by Allan & Stevenson 1981).

Biochemistry and molecular identification

Bacteria phenotypic characterization was made using the API identification kit 20E (BioMerieux, Marcy l'Etoile, France), and biochemical tests such as esculin hydrolysis, gas production due to glucose fermentation, were carried out according to the Aerokey II identification key to differentiate among *A. hydrophila*, *Aeromonas sobria* and *Aeromonas caviae* (Carnahan, Behram & Joseph 1991).

The strain that showed the highest haemolytic activity was molecularly identified at the State University of Campinas (UNICAMP). DNA from a strain's single colony was extracted using InstaGene Matrix (Bio-Rad, Hercules, CA, USA) following the manufacturer's instructions. The 16S ribosomal RNA gene amplification was performed with primers p27f and p1401r (Lane, Pace, Olsen, Stahl, Sogin & Pace 1985). Then, amplified fragments of rRNA were purified using a column (GFX PCR DNA and GEL Band Purification Kit, GE Healthcare, Chalfont St Giles, UK) and sequenced by the MegaBACE 1000 automatic sequencer (GE Healthcare) using the primers p10f, 765f, 782r and p1100r (Lane 1991), to cover approximately 1000 bp 16S ribosomal RNA gene. The partial sequences obtained were mounted into one coating (a unique sequence combined from different fragments), which was compared with sequences of similar organisms found in Genbank and the Ribosomal Database Project. From this search, the sequences of the organisms related to our unknown organism were selected to conduct phylogenetic analyses.

Experimental challenge

A bacterial inoculum of the isolated strain was streaked out on TSA plates. After growing at 28 °C

for 18 h, a bacterial colony was inoculated into BHI broth, and the suspension was static incubated at 28 °C for 18 h. After incubation, the culture was serially diluted (1:10) to 10^{-8} and plated on TSA to determine the bacterial concentration of the starting inoculum. To construct a growth curve, inoculums of the bacteria were serially diluted (1:2) in triplicates in 96-well microtitre plates 12 times, and the absorbance of each well was measured at 630 nm using a microplate reader. For experimental infection, a bacterial pure culture grown in BHI broth for 18 h at 28 °C in static incubation was centrifuged for 30 min at 1800 *g*. The supernatant was discarded, and the pellet was resuspended in a sterile 0.65% saline solution to keep the concentration of the bacteria at 2×10^8 CFU mL^{-1}. The bacterial suspension was diluted to the desired concentrations for experimental challenge.

For the challenge, 144 fish with averages of 98.1 ± 23.6 g weight and 26.9 ± 1.7 cm length, size that committed the surubim culture in fish farms causing mortalities, were distributed in 18 tanks (300 L) supplied with continuous aeration, a biological filter and heaters with thermostats to keep the temperature at 26 ± 0.5 °C. Five days after acclimation, the fish were randomly divided into six groups.

The treatments in triplicates consisted of uninjected fish, fish intraperitoneally injected with 1 mL of 0.65% sterile saline solution and fish injected with 1 mL 2×10^2, 2×10^4, 2×10^6 and 2×10^8 CFU mL^{-1}. During the experimental period pH was measured with a YSI model 60 pH meter (YSI, OH, USA) and dissolved oxygen with a YSI 5100 dissolved oxygen meter (YSI), total ammonia concentration by APHA, AWWA and WPCF (1995) method and unionized ammonia according to Emerson, Russo, Lund and Thurston (1975). Twice a day, fish were fed a diet containing 40% crude protein (Douramix Revolution®-fry, Dourados, Brazil) at 3% fish biomass.

Mortality, symptoms and bacterium re-isolation

The experiment was carried out for 10 days after challenge. Dead fish were removed from the tanks every 6 h and samples of the brain, kidney, heart and liver were collected for microbiological diagnosis. The surviving fish were sacrificed (UFSC Ethics Committee number 23080.021883/2009-91) for microbiological diagnosis and clinical signs. At the end of the experiment, samples of the brain, kidney, heart and liver from three animals of each tank were used to re-isolate the bacteria. Next, the tissues were macerated using a sterile porcelain mortar at a ratio of 1 g of sample to 1 mL of 0.65% sterile saline solution and streaked onto TSA plates for incubation at 28 °C for 24 h. After growth in agar plates and colonies were re-isolated, they were phenotypically characterized by biochemical profile using the API 20E identification kit (BioMerieux), supplemented with esculin hydrolysis analysis and gas production from glucose fermentation.

Haematological and immunological analyses

At the end of the experiment, three fish from each replicate were sampled, anaesthetized with benzocaine (0.1 g L^{-1}), and their blood was collected by puncturing the caudal vein using two 3 mL syringes (21G), one of them with anticoagulant EDTA 10% and the other without anticoagulant. The blood with anticoagulant was used to perform a haematological examination, and the blood without anticoagulant was left to coagulate for 1 h at 25 °C and was centrifuged at 1400 × *g* for 10 min to obtain the serum. The serum was stored at − 20 °C for later analysis of serum antimicrobial activity and of serum agglutination titre.

The blood collected in EDTA 10% was used to make blood smears, which were stained with Giemsa/May–Grunwald to obtain the total leucocyte and thrombocyte number, as well as the differential leucocyte count. An aliquot of the blood was used to determine the haematocrit percentage (Goldenfarb, Bowyer, Hall & Brosius 1971) and the remaining blood was stored in glass bottles on ice and used to obtain red blood cell count in a haemocytometer. The total numbers of thrombocytes and leucocytes were obtained from the blood smear using the indirect method described by Ishikawa, Ranzani-Paiva and Lombardi (2008), according to the formula:

$$\text{TL or TT (cells/mL)} = (\text{no.of counted cells in 2000 erythrocytes} \times \text{no.of erythrocytes})/2000$$

where TL is the total leucocytes and TT is the total thrombocytes.

The differential count of leucocytes results were expressed as cell percentage in a total of 200 leucocytes counted in the smears. The agglutination titre used in the experiments was determined by the method described by Silva, Martins, Jatobá, Buglione, Vieira, Pereira, Jerônimo, Seiffert and Mouriño (2009). The test was performed on a U-bottom 96-well microplate onto which fish serum was diluted at

a 1:1 ratio in saline PBS (0.16 M monobasic phosphate, 0.04 M dibasic phosphate, 0.11 M sodium chloride, pH 7.4) in the first well (50 μL PBS solution:50 μL serum), and serially diluted in a 1:2 ratio for the remaining wells until the 12th. After, 50 mL of formalin-inactivated *A. hydrophila* were added to the wells, and the plate was incubated at 25 °C for 18 h in a humid chamber. Agglutination was visually confirmed by observing the bottom surface of the wells. The agglutination titre was considered as the reciprocal of the last dilution in which agglutination was observed.

Serum antimicrobial activity against the bacteria used the method of Silva *et al.* (2009). After 18 h cultured in BHI at 28 °C, the bacteria were adjusted to a concentration of 0.5 on the MacFarland scale and diluted 100 000-fold in poor broth (PB). Then, 150 μL of PB and 50 μL of serum were added to the first row of wells of a 96-well, flat-bottom plate. The serum was serially diluted 1:2 in 100 μL of PB 12 times. For the positive and negative controls, 0.65% sterile saline solution was diluted in PB similarly to serum. Finally, 20 μL of *A. hydrophila* were added to each well containing diluted serum. Twenty microlitres of PB were added as positive and negative controls in every plate. The plates were incubated at 28 °C for 12 h. The concentration of bacterial growth was read at 550 nm using a microtitre reader. The antimicrobial activity of the serum was considered as the reciprocal of the last dilution which exhibited bactericidal or bacteriostatic activity.

Statistical analyses

The significance of the effects of the different concentrations of bacteria used for challenge on the haematological and immunological responses was evaluated by the regression models and one-way ANOVA. Previously, we evaluated the homogeneity of the data using Bartlett's test ($P < 0.05$), and data on agglutination titre and antimicrobial activity were transformed in $\log_2 (x+1)$. The adjustment of the data to the model was verified at 5% significance of the regression coefficients for the *t*-test, the coefficient of determination (R^2 = S.Q. Reg./S.Q. condition), the sum of the squared deviation and the variable used. When necessary, mean significant differences were determined by the SNK test ($P < 0.05$).

Results

Isolation and haemolytic activity assay

Fourteen haemolytic strains of bacteria were isolated, eight from the kidney, six from the brain and four nonhaemolytic strains (two from kidney and two from brain). The haemolytic activity of strains of *A. hydrophila* isolated from hybrid surubim was from 11.7 ± 1.1% to 99.2 ± 1.5%. The strains isolated from the kidney showed an average 48.1 ± 27.9%, while from the brain was 43.1 ± 33.9%, but there was no significant difference ($P = 0.6737$). However, the strain with the highest haemolytic activity was strain 9 (brain) 99.2 ± 1.5% ($P < 0.0001$).

Biochemistry and molecular identification

All haemolytic isolates were biochemically identified as *A. hydrophila* (99.7%) with the same profile and all nonhaemolytic strains as *Pseudomonas aeroginosa* (87%). The strain that showed higher haemolytic activity (228–08 CPQBA DRM) was used in molecular identification and challenge experiments, which exhibited a 99% sequence similarity with 16S rRNA gene of *A. hydrophila*, contained in the consulted databases. Phylogenetic analysis to confirm the results were made by placing the strain into a cohesive group, resulting in a 'bootstrap' value of 82% for the lineage type *A. hydrophila* (ATCC 7966 – X74677), which confirmed the results.

Water quality, mortality and clinical signs

During the experimental challenge, water pH was 7.5 ± 0.2, total ammonia concentration 2.07 ± 1.5 mg mL^{-1}, unionized ammonia 0.04 ± 0.03 mg mL^{-1} and dissolved oxygen 6.4 ± 0.4 mg L^{-1}. Mortalities were observed until 96 h after challenge, but by day 9 the fish still showed an increase in external signs, and sampling were made on day 10. Only fish infected with 2×10^8 *A. hydrophila* mL^{-1} showed mortality (50 ± 12.5%). However, all infected fish also showed clinical signs, whereas the uninjected and saline-injected fish did not present any external and internal clinical signs. Animals infected with the highest concentration of *A. hydrophila* showed an increase in the prevalence of external and internal signs (Table 1).

Clinical evaluation showed depigmentation (Fig. 1a), ventral and dorsal ulceration (Fig. 1b and c), anal bleeding and abdominal swelling (Fig. 1d), pale gills (Fig. 1e) and anal fin deformities (Fig. 1f). We found alterations in liver and kidney, white spots on gallbladder (Fig. 1g) and haemorrhagic spots on heart and brain (Fig. 1h and i). The liver, kidney, brain and heart samples from uninjected and saline-injected fish did not show bacterial growth on TSA. In contrast, the

Table 1 The prevalence of external and internal symptoms in surubim hybrid challenged with different concentrations of *Aeromonas hydrophila*

Treatments	EL	AI	LA	KA	WSG	HSH	HSB
Uninjected	0/24	0/24	0/24	0/24	0/24	0/24	0/24
Saline	0/24	0/24	0/24	0/24	0/24	0/24	0/24
2×10^2 *A. hydrophila* mL^{-1}	6/24	6/24	3/24	5/24	6/24	6/24	4/24
2×10^4 *A. hydrophila* mL^{-1}	2/24	6/24	6/24	6/24	6/24	6/24	6/24
2×10^6 *A. hydrophila* mL^{-1}	4/24	6/24	12/24	6/24	5/24	11/24	10/24
2×10^8 *A. hydrophila* mL^{-1}	10/12	6/12	12/12	8/12	6/12	6/12	10/12

EL, external lesions; IA, anus inflammation; LA, liver alterations; KA, kidney alterations; WSG; white spots on the gallbladder; HSH, haemorrhagic spots on the heart; HSB, haemorrhagic spots on the brain.

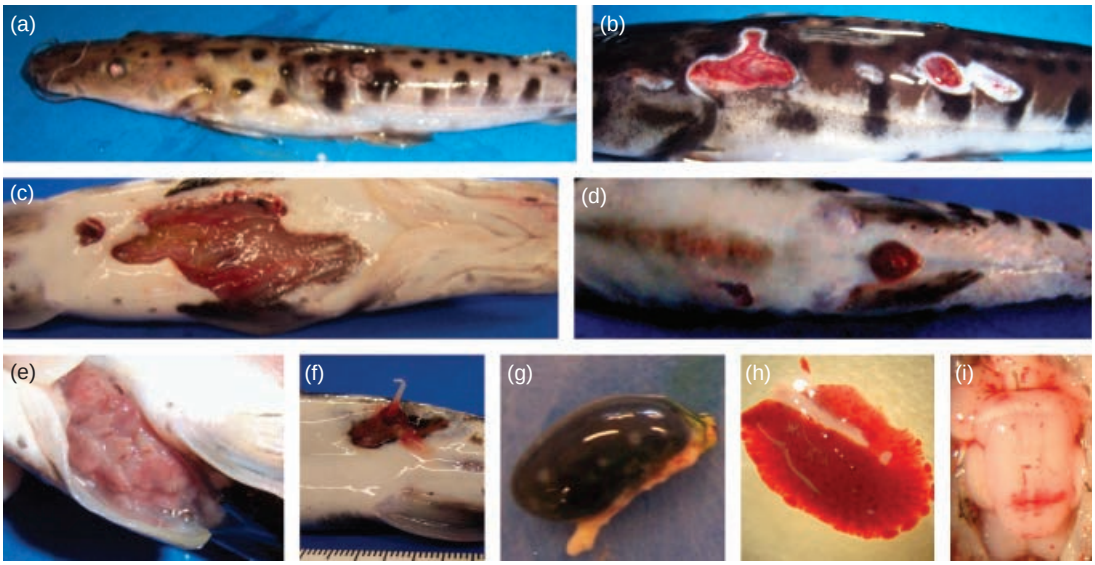

Figure 1 Clinical signs of survivor fish after challenge with *Aeromonas hydrophila*. (a) Epidermis depigmentation, (b) dorsal ulceration, (c) ventral ulceration, (d) abdominal swelling and anus inflammation, (e) pale gills, (f) fin erosion, (g) white spots on the gallbladder, (h) haemorrhagic spots on the heart, (i) haemorrhagic spots on the brain.

tissue homogenates from the survivor infected fish and dead fish showed bacterial colonies on TSA and they were re-isolated for biochemical characterization. We observed that the bacteria from these colonies had the same biochemical profile on an API 20E strip as *A. hydrophila* (99.7%) used for challenge. This result indicates that experimental challenge was efficient and that these bacteria caused damage in the internal organs, even after 10 days post infection. The fulfillment of Koch's postulate was also demonstrated.

Haematological and immunological analyses

The relationship between the agglutination titre, the serum antimicrobial activity and the concentration of *A. hydrophila* was explained by the quadratic and linear regression model respectively. Fish exposed to higher concentrations of *A. hydrophila* (2×10^6 and 2×10^8 CFU mL^{-1}) that survived for 10 days post-challenge showed higher agglutination titre and greater serum antimicrobial activity (Fig. 2a and b). The total number of thrombocyte and leucocyte, as well as the basophil percentage was not influenced by the *A. hydrophila* challenge ($P > 0.05$). The values ranged from 20.12 to 25.03 $\times 10^3$ thrombocyte mL^{-1}, 43.34 to 53.71 $\times 10^3$ leucocytes mL^{-1} and 0.11% to 3.30% basophils. On the other hand, red blood cell count and haematocrit percentage explained by linear regression showed a decrease in infected fish at higher concentrations of *A. hydrophila*, although, between fish infected no difference was observed in the ANOVA results (Fig. 2c, d and e).

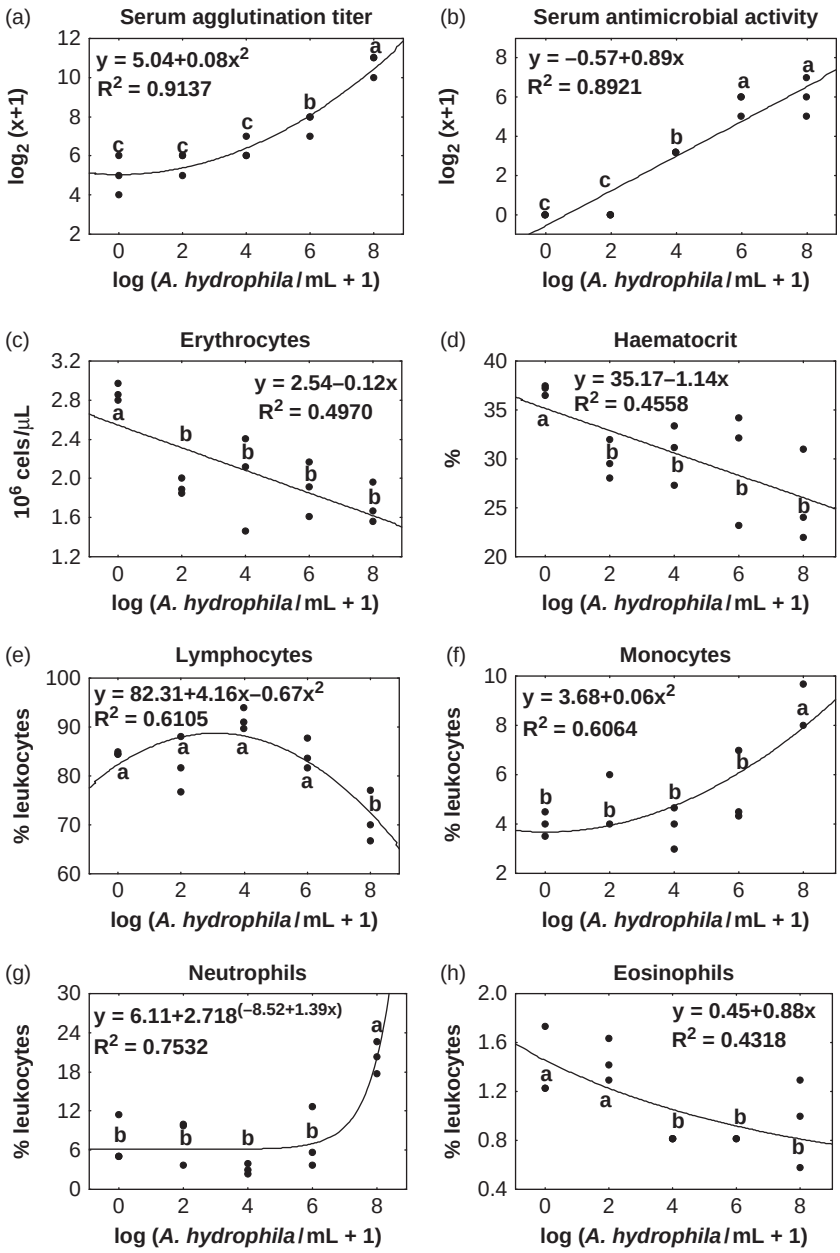

Figure 2 Regression analyses of the (a) agglutination titre, (b) antimicrobial activity, (c) total number of erythrocytes, (d) haematocrit percentage, (e) the percentage of lymphocytes, (f) monocytes, (g) neutrophils and (h) eosinophils in the hybrid surubim after challenge with *A. hydrophila*. Different letters indicate statistical difference in ANOVA ($P < 0.05$).

We observed significant lymphopenia in fish infected with 2×10^8 CFU (Fig. 2e). Neutrophilia and monocytosis were observed in most of the infected fish that survived after exposure to the highest concentration of *A. hydrophila* (2×10^8 CFU mL^{-1}) (Fig. 2f and g). Moreover, the percentage of eosinophils decreased in fish infected with *A. hydrophila* concentrations as from 2×10^4 CFU (Fig. 2h).

Discussion

Clinical signs observed in diseased surubim collected in this study were more characteristic of motile *Aeromonas* (Austin & Austin 2007), and *P. aeroginosa* isolated in this study can be considered as a secondary pathogen. *Aeromonas hydrophila* has been reported as a primary fish pathogen, and diseases attributed to it

have presented great economic importance in freshwater fish farming (Woo & Bruno 2003). As in this study, this bacterium was isolated from several disease outbreaks in salmonid *Oncorhynchus mykiss* (Rehulka 2002), *Oncorhynchus tshawyscha* (Woo & Bruno 2003), carps *Cyprinus carpio* and *Labeo rohita* (Sahoo, Meher, Das Mahapatra, Saha, Jana & Reddy 2004; Selvaraj, Sampath & Sekar 2004) and zebrafish *Danio rerio* (Rodriguez, Novoa & Figueras 2008).

The toxins, haemolysins, proteases, enterotoxins, endotoxins and cholinesterases produced by *A. hydrophila* have been studied by several groups, and they are thought to be responsible for haemorrhagic septicaemia and fish mortality (Cahill 1990). Allan and Stevenson (1981) observed a high correlation between the amount of haemolysin produced by *A. hydrophila* and its pathogenicity for fish. Thus, the strain that showed the highest haemolytic activity was selected for challenge experiments.

Similar symptoms to those described in this challenged are commonly found in fish with bacterial haemorrhagic septicaemia (Woo & Bruno 2003; Austin & Austin 2007), having been observed in zebrafish (Rodriguez *et al.* 2008) and common carp (Schroers, Van der Marel, Neuhaus & Steinhagen 2009).

Antibodies are largely responsible for the agglutination process in fish (Ellis 1999), explaining an increase in this index in fish after challenge. The highest serum agglutination titre was observed in fish infected with higher concentrations of *A. hydrophila*. Rainbow trout (*O. mykiss*) and common carp have also presented an increase in agglutination titre after challenge with LPS from *Aeromonas salmonicida* and *A. hydrophila* respectively (Nakhla, Szalai, Banoub & Keough 1997; Selvaraj *et al.* 2004).

Increased serum antimicrobial activity observed in infected hybrid surubim was similar to that found in rohu carp, which survived an experimental infection with *A. hydrophila* (Sahoo *et al.* 2004; Sahu, Das, Mishra, Pradhan, Samal & Sarangi 2008).

Reduced number of red blood cells and haematocrit percentage in fish infected with higher concentrations of *A. hydrophila* was in agreement with the observations of Rehulka (2002) in rainbow trout infected with *A. sobria* and *A. caviae*. In the present study, this reduction can suggest anaemia in infected fish (Clauss *et al.* 2008). Martins, Shoemaker, Xu and Klesius (2011) found reduced red blood cell count and haematocrit in Nile tilapia *Oreochromis niloticus* infected with *Streptococcus iniae* 18 days post infection.

Differently from the present results, in pacu *Piaractus mesopotamicus* (Garcia, Pilarski, Onaka, Moraes & Martins 2007) and Nile tilapia (Martins, Mouriño, Amaral, Vieira, Dotta, Jatobá, Pedrotti, Jerônimo, Buglione-Neto & Pereira Jr 2008), the total thrombocyte and lymphocyte counts increased after challenge with *A. hydrophila* and *Enterococcus* sp. respectively. It must be emphasized that in the trials by Garcia *et al.* (2007) and Martins *et al.* (2008), blood samples were collected 24 h after infection, whereas in the present assay sampling was made 10 days post infection.

Similar to this study, in pacu, Garcia *et al.* (2007) observed a reduction in the number of lymphocytes after challenge with 6×10^6 CFU mL^{-1} *A. hydrophila*. Shao, Liu and Xiang (2004) reported apoptosis of lymphocytes in goldfish *Carassius auratus* after infection with *A. hydrophila*. These examples may explain the reduction in the numbers of these cells in fish infected with high concentrations of bacteria.

Monocytosis and neutrophilia are normally part of inflammatory processes (Clauss *et al.* 2008) such as those observed in surubim that survived a challenge with *A. hydrophila*. Increased number of monocytes and neutrophils and decreased number of eosinophils observed in this study were in agreement with the findings in pacu following infection with *A. hydrophila* (Garcia *et al.* 2007), and in common carp injected with *A. hydrophila* LPS (Selvaraj *et al.* 2004).

Serum antimicrobial activity of teleost fish is due to several types of proteins and enzymes produced by monocytes and neutrophils, such as lysozyme, transferrin, antimicrobial peptides and antiproteases (Ellis 1999). Consequently, an increase in these cells in infected fish with high pathogen concentrations may have favoured higher serum antimicrobial activity.

Although fish infected with lower concentrations of bacteria did not present clinical signs, situations in the field such as temperature alterations, presence of parasites and inadequate handling may promote or aggravate the disease, culminating in disease outbreaks. Therefore, additional experiments need to be carried out to evaluate the pathogenicity of *A. hydrophila* in relation to other stress factors for surubim.

Thus, it can be concluded that bacterial strain isolated in this study (CPQBA 228-08DRM) characterized as *A. hydrophila* caused mortality, important clinical signs of bacteriosis, anaemia, lymphopenia, monocytosis, neutrophilia and enhanced serum agglutination titre and serum antimicrobial activity. In addition to the alterations in internal organs, there were clear signs of haemorrhagic septicaemia in cultured hybrid surubim (*P. corruscans* × *P. fasciatum*).

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