

TITLE: Outbreak by *Aeromonas* species as causative agents of mortality in farmed *Arapaima gigas* (Arapaimidae) and investigation of virulence factors involved in bacterial coinfection

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ABSTRACT:

The Northern Brazil in which is entirely in the Amazon region, as well as other countries from Amazon, have its aquaculture on based in native fish species as *Arapaima gigas* (pirarucu), for several zootechnical reasons. However, one of the problems in production of *A. gigas* has been the high mortality during the early stages of cultivation caused by bacteriosis, which has not been investigated regard to phenotypical, molecular and virulence determinant factors. In freshwater fish, bacteriosis has been generally linked with *Aeromonas* species, but coinfection caused by such bacteria has been little addressed. The aim of the study was to investigate, through experimental infections, the characterization of virulence factors and phenotypic and molecular differentiation of *Aeromonas* spp. involved in an outbreak of *A. gigas* fingerlings that occurred on newly acquired specimens from commercial fish farming, which resulted in significant financial losses due to the mortality of approximately 1,400 fingerlings. Fish samples were collected with clinical signs of bacteriosis and used to studies of experimental coinfection. Bacterial isolates were characterized phenotypically as *Aeromonas hydrophila* and *Aeromonas jandaei*. For the differentiation of the phenospecies the spectra of each sample were generated in Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF LT Microflex, Bruker) and then analysed using the MALDI Biotyper 3.1 (Bruker) program. Virulence genes were detected by polymerase chain reaction following recommendations of primers and protocols standardized by Enterobacteria Laboratory, Oswaldo Cruz Institute, Oswaldo Cruz Foundation. The isolates were screened for hemolysin (*aerA* and *hlyA*), cytotoxin (*act*), termolabil cytotoxic enterotoxin (*alt*), lipases (*lip*) and *gcat* - glycerophospholipid: cholesterol acyltransferase), serine protease (*ser*) and DNase gene (*exu*). Five of the eight virulence genes assayed were present in the *Aeromonas* isolates: *aerA*, *hlyA*, *lip*, *gcat* and *exu*. The virulence genes *act*, *alt* and *ser* were not detected in any of the strains isolated. Coinfection and the pathogenicity of *A. hydrophila* associated with *A. jandaei* in *A. gigas* established provided experimental support for the role synergistic of such bacteria, indicating several implications related to this coinfection and determinants of virulence.

Keywords: Bacteria, Coinfection, Culture, Virulence, Pirarucu

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