Family association between immune parameters and resistance to *Aeromonas hydrophila* infection in the Indian major carp, *Labeo rohita*

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Abstract   Seven innate immune parameters were investigated in 64 full-sib families (the offspring of 64 sires and 45 dams) from two year-classes of farmed rohu carp (*Labeo rohita*). Survival rates were also available from *Aeromonas hydrophila* infection (aeromoniasis) recorded in controlled challenge tests on a different sample of individuals from the same families. Due to strong confounding between the animal additive genetic effect and the family effects (common environmental + non-additive genetic), reliable additive (co)variance components and hence heritabilities and genetic correlations could not be obtained for the investigated parameters. Therefore, estimates of the association of challenge test survival with the studied immune parameters were obtained as product moment correlations between family least square means. These correlations revealed statistically significant ($p < 0.05$) negative correlations of survival with bacterial agglutination titre (-0.48), serum haemolysin titre (-0.29) and haemagglutination titre (-0.34); and significant positive correlation with ceruloplasmin level (0.51). The correlations of survival to aeromoniasis with myeloperoxidase activity, superoxide production and lysozyme activity were found to be not significantly different from zero ($p > 0.05$). Assuming that the negatively correlated candidate traits are not favourable as indirect selection criteria, the results suggest that ceruloplasmin level could potentially be a marker for resistance to aeromoniasis in rohu. The use of this immune parameter as an indirect selection criterion for increased resistance to aeromoniasis in rohu will, however, require that the parameter shows significant additive genetic variation and a significant genetic correlation with survival. Further studies are therefore needed to obtain

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a reliable heritability estimate for ceruloplasmin and its genetic correlation with survival from aeromoniasis.

Introduction

At present, freshwater aquaculture in India is mainly based on Indian major carps (*Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*) with an annual combined production of 1.8 million tonnes, contributing to more than 80% of the total national aquaculture production of 2.2 million tons [1]. Disease problems represent important constraints to the aquaculture industry and to the culture of Indian major carps. The need to control endemic diseases imposes severe year-on-year costs on producers, and the advances made in the development of vaccines for carp diseases are very meagre. One of the major bacterial diseases causing problems in Indian major carp farming is aeromoniasis caused by *Aeromonas hydrophila*. *A. hydrophila*, a ubiquitous bacterium responsible for primary or stress-associated pathogenicity in warm and cold water fish (e.g. carps, catfish and salmonids) [2], is associated with small surface lesions, local haemorrhaging and septicaemia [3], dropsy, exophthalmia, and fin and tail rot [4,5]. Various vaccination strategies have been employed with different bacterin preparations against *A. hydrophila* in carp and catfish species but with limited success [3,6]. No effective chemotherapeutant or commercial vaccine is currently available to prevent or control this infection on a long term basis, and extensive use of antibiotics is not desirable since it may lead to the development of resistant *A. hydrophila* strains and residue problems.

Selective breeding offers possibilities of protection against a disease on a long term basis. Prerequisites for using this strategy to control aeromoniasis are genetic variation in susceptibility to the disease, and a breeding program where fish are tagged for family identification. As differences in mortality due to aeromoniasis between full-sib families of rohu were noted in a preliminary study [7], the potential to explore resistance through selective breeding seems to be promising. A breeding program for improved growth in *L. rohita* is already in operation at the Central Institute of Freshwater Aquaculture, Bhubaneswar, India [8]. Considerable genetic variation in the mortality patterns against specific pathogens among fish at the inter- and intra-specific level has been demonstrated [9–20]. Genetic transmission of innate resistance to several bacterial diseases was first documented in salmonids and carp [13,21–23] and substantial genetic variation in resistance to furunculosis in Atlantic salmon (*Salmo salar*) [9,11,24], viral haemorrhagic septicaemia in rainbow trout [19] and aeromoniasis in Nile tilapia (*Oreochromis niloticus*) [12] have been reported.

Building on this knowledge, geneticists and pathologists have pursued an interest in determining intrinsic resistance factors that protect fish from various diseases. A strong genetic basis for innate physiological, and/or biochemical mechanisms conferring resistance to micro-parasites have been demonstrated [13], and a series of previous studies indicated that disease resistance in coldwater fish species is correlated with non-specific immune parameters, viz., serum lysozyme, complement and haemolytic activity, phagocytic respiratory burst and bactericidal activities; all likely affect the inherent capacity of fish to resist pathogens prior to a specific immune response [10–12,25–29]. Furthermore, the ubiquitous presence of circulating natural antibodies, i.e. lectins and agglutinins that are of IgM nature, and the variation in levels of these antibodies in lines of common carps of different genetic background, suggested that levels of natural antibodies might be candidate traits for indirect selection for increased disease resistance [30]. However, despite the efforts directed at studying candidate traits for resistance to a number of diseases of salmonids [10–12,25–29], no such trait has until now been identified that fulfils the requirements for a trait suitable for individual selection; i.e. (1) genetic variability, (2) being sufficiently correlated with the primary disease resistance trait, and (3) being simple and rapid to quantify in large groups of live fish [31].

The ultimate goal of this work was to decide whether any one of seven innate, humoral immune parameters fulfils the requirements for a trait suitable for indirect selection for increased resistance to aeromoniasis in the Indian major carp rohu (*L. rohita*). To that end, the present investigation aimed to determine the magnitude of genetic variation in the immune parameters recorded on blood samples of individual fish of full-sib families of rohu and to study the genetic associations between these parameters and the survival of the same full-sib families exposed to *A. hydrophila* in controlled challenge tests.

Materials and methods

Fish

The experimental fish were from 64 full-sib families of two year-classes of rohu; i.e. 30 families of year-class 2003 (the offspring of 19 dams and 30 sires) and 34 families (the offspring of 26 dams and 34 sires) of year-class 2004. The fish had been selected for increased growth over five generations in an Indo-Norwegian collaborative project carried out at the Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, India [8]. The full-sib families of each year-class were produced over a period of 1–2 weeks in June 2003 and July 2004, respectively, using a nested mating design with males nested within females.

The families of each year-class were initially reared in separate equal sized (100 m^2) earthen ponds at CIFA. The water temperature in the ponds varied from 25 to 30 °C during the 45 weeks rearing prior to tagging. At a size of about 45 g (year-class 2003), and 25 g (year-class 2004) fish from each family were brought to 700 L ferro-cement tanks in an indoor wet laboratory. A random sample of 30 fish from each family was individually tagged with passive integrated transponders (PIT) [32]. Following the tagging operations, the fish's recovery was monitored for 2 days before two random samples of 15 fish per family were