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Scotland study evaluates immune responses, disease resistance in clonal lines of Nile tilapia

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Fig. 1: Nile tilapia injected with *Aeromonas hydrophila* shows hemorrhaging on the body surface, base of fins, and operculum.

Intensification of aquaculture systems makes fish more susceptible to stress and disease. Aquafarmers currently treat with drugs such as antibiotics or use vaccines as a means of controlling disease. Another approach is to cull diseased fish and replace them with new stocks after disinfecting the system. However, there are problems associated with these methods.

Antibiotic-resistant strains of bacteria are increasing, and some antibiotics have been shown to be immunosuppressive. Commercially available vaccines have proven very successful in reducing disease outbreaks, but there is still an insufficient number available to cover all the diseases that currently affect the industry.

Genetic selection and disease resistance

Genetic selection as a means of improving disease resistance of stocks is presently receiving much attention. Some strains of fish are naturally resistant to a particular pathogen, and are very useful for studying the underlying mechanisms involved in disease resistance.

Inbred strains

Inbred strains of fish produced in the laboratory using conventional sib-mating are ideal for immunological studies. Phenotypes for different immune traits can be defined in the different strains, and these in turn can be related to disease resistance. However, these strains can take 15-20 generations to develop.

Inbred homozygous clones

Gynogenetic reproduction offers a rapid alternative for developing fully inbred homozygous clones (IC) of fish within two generations. Since the clones have identical genotypes, the genetic variability between clones can be monitored, and clones with favorable or unfavorable genes for disease resistance can be identified.

Immune mechanisms

Although most fish are susceptible to initial infection by an invading pathogen, they appear to differ in their ability to limit the infection through a variety of immune mechanisms. Once a pathogen has breached the physical barriers of its host, it encounters components of the nonspecific immune response.

These include serum lysozyme, the complement cascade, lectins, and C-reactive protein, which attack and help destroy the pathogen. These molecules also coat the pathogen for further destruction by phagocytes, cellular components of the nonspecific immune system, which engulf and destroy invading pathogens.

Clonal line study

A study at the University of Stirling's Institute of Aquaculture in Scotland examined the genetic variation of clonal lines of Nile tilapia (*Oreochromis niloticus L.*) in relation to disease resistance. Mitotic gynogenesis was used to produce a first generation of completely homozygous female Nile tilapia. Three inbred clones (IC A, IC B, and IC C) were then established from mitotic gynogenetic females by meiotic gynogenesis. Three gynogenetic outbred clones (OC) – OC AxB, OC BxC, and OC CxA – were also produced by crossing the inbred clones. A group produced by ordinary crossing was used as an unrelated control.

Nonspecific immune responses

Nonspecific immune responses (serum lysozyme activity, respiratory burst, and phagocytosis of bacteria by head kidney macrophages) were compared between the clones. Differences were found in the number of macrophages containing phagocytosed bacteria. A significantly higher percentage of macrophages containing phagocytosed bacteria were seen in IC A, IC C, and OC AxB compared with IC B. The remaining groups had values intermediate to these.

The percentage of macrophages containing phagocytosed bacteria in OC AxB fish was not significantly different than that of IC A, but was significantly higher than in IC B fish. The results suggested that the inheritance of this immune activity shows dominance toward the stronger phagocytic activity.

The serum lysozyme activity of IC C was significantly higher ($p<0.05$) than in IC A and IC B. Significantly higher ($p<0.05$) lysozyme activity was present in OC BxC and OC CxA fish compared with IC A, IC B, and OC AxB fish, but lysozyme activity was significantly lower than in IC C fish. The OCs showed an intermediate level of lysozyme activity to that of their parents. Their intermediate response suggested additive parental genetic effects in the outbred offspring.

Establishing genetic differences

Ultimately, the best way to establish genetic differences for disease resistance between the clones was to determine their survival rate after artificially infecting them with a pathogen. Fish injected with *Aeromonas hydrophila* started to die within 12 hours after injection. *A. hydrophila* infection was established from kidney swabs taken from dead animals. All dead fish showed typical symptoms of *A. hydrophila* infection, including hemorrhaging on the body surface, base of fins, and operculum (Fig. 1).

Cumulative mortalities in IC B reached 56.7 percent over the six-day experimental period (Table 1). Mortalities were also observed in the other lines, but at a much lower level. Only 3.3 percent of IC A and 6.7 percent of IC C died over the experimental period, with both showing a strong resistance to the pathogen. The outbred clones OC AxB, OC BxC, and OC CxA also showed low levels of mortality (20 percent, 13.3 percent and 10 percent, respectively).

Thompson, Response of clonal lines of Nile tilapia to an artificial challenge with *Aeromonas hydrophila*, Table 1

* Clone	Infected Fish Mortalities	Infected Fish Survivors	Noninfected Fish
IC A[a]	1	0	29
IC B[c]	17	5	8
IC C[ab]	2	0	28
OC AxB[b]	6	0	24
OC AxC[ab]	4	1	25
OC CxA[ab]	3	2	25

* 30 fish injected per clone.

Table 1: Response of clonal lines of Nile tilapia to an artificial challenge with *Aeromonas hydrophila*. Clones with different superscript letters indicate significant differences in levels of infection at $P<0.5$.

Reisolating pathogen

It was also possible to re-isolate *A. hydrophila* from surviving fish at the end of the experiment. Only IC B were still infected with the pathogen (16.7 percent) among the IC groups, while OC BxC (3.3 percent) and OC CxA (6.7 percent) still carried the pathogen among the OC groups. The IC B fish appeared to be the most susceptible to *A. hydrophila*

infection, with the total number of infected fish in this group (mortalities and infected survivors) significantly higher than all other fish groups.

Crossbreeding IC B with the other inbred lines, IC A and IC C, produced more disease-resistant hybrids. These outbred clones, OC AxB and OC BxC, showed significantly higher resistance to *A. hydrophila* than their disease-susceptible parental clone, IC B. The results of the challenge experiment suggested dominance in inheritance of susceptibility to infection in the direction of the more resistant parental clone.

Genetic variation between clones

The significant differences between the inbred clones for nonspecific immune responses and levels of infection with *A. hydrophila* strongly indicated the genetic variation between the clones for these parameters. Cumulative mortalities of fish in the study showed that when an IC susceptible to *A. hydrophila* was crossed with a resistant IC, the resulting progeny exhibited intermediate levels of resistance to that of their parents.

There was some evidence of heterosis in the form of dominance in the inheritance of some of the parameters studied, in that where inheritance appeared to be nonadditive (i.e., not intermediate between the two IC parental clones), the phenotypic values for the OC groups tended to be closer to that of the parental IC with the stronger immune response. However, none of the tilapia OC groups exceeded the highest parental value shown by the two parental lines for any of the parameters measured.

Conclusion

This university study created clones of Nile tilapia in order to evaluate variations in disease resistance due to nonspecific immunity. A positive correlation was obtained between the level of infection with *A. hydrophila* and the parameters measured. Additional work is now being carried out to further phenotype the clonal lines of Nile tilapia to understand the genetic control of the immune response and its role in disease resistance in these fish.

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