The *Flavobacterium columnare* challenge: Host, Genomovar and Virulence

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Abstract

Flavobacterium columnare is pathogenic to most if not all species of freshwater fish. The ubiquitous nature of *F. columnare* makes understanding the disease and disease agent troublesome. The host-pathogen-environment relationship theory (Fig. 1) described by Snieszko [1] can be applied to our understanding of this important fish pathogen. Age, species, strain(s), nutritional and immunological status of the host appear relevant to the establishment of columnaris disease. The pathogen including exposing dose, virulence and genetic type (strain) are also important to disease development. Environmental conditions including temperature and water quality and other stressor(s) (e.g. population density; parasitism) influence the development of columnaris disease in laboratory and field settings. Our experiences and other published examples will be used to illustrate these concepts in relation to *F. columnare* challenge models for studying virulence, pathogenesis, immunity and control strategies (e.g. vaccination and therapeutics).

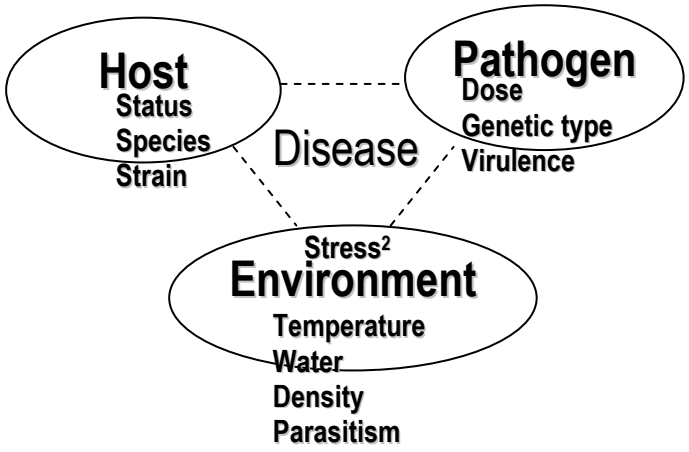

Figure 1. A modification of Sniesko's original host-pathogen-environment disease relationship theory proposed in 1974 [1]. Stress is included and squared because as fish approach physiological limits, stressors increase accumulatively rather than additively [2].

The condition of the host (e.g. age, nutrition and immune status) is an important component to the establishment of columnaris disease. Feed deprivation was utilized as a model to induce columnaris disease in fingerling channel catfish exposed to genomovar I *F. columnare* [3]. Organ weight to body weight ratio and physiological parameters were good indicators of feed deprivation at both 2 and 4 weeks in 36 g channel catfish (*Ictalurus punctatus*). Both gut and hepatosomatic indices significantly decreased in non-fed (NF) fish as compared to fed daily (FD) and fed every other day (FEOD) fish at 2 and 4 weeks. Blood glucose and liver glycogen were significantly lower in the NF fish at 2 and 4 weeks (~ 40 mg/dL and ~2 mg/g) compared to FD

and FEOD fish (65–90 mg/dL and 45–52 mg/g). Cumulative mortality (78 %) was significantly higher ($P<0.01$) in the fish deprived of feed than in those FD or FEOD (0 and 1.7 %, respectively) following *F. columnare* challenge. Similar results were also demonstrated following 7–10 day feed deprivation in smaller sized catfish [4].

Selective breeding of fish is a potential strategy to improve disease resistance to pathogens. Limited research has examined the differential susceptibility of channel catfish families to *F. columnare* due to the difficulty of establishing a reproducible model. Four channel catfish families were tested to examine their innate susceptibility to columnaris disease after challenge with a genomovar II *F. columnare* isolate. Families A and B were more susceptible to *F. columnare* (mean CPM of three independent challenges = 95% and 93%) than families C and D (45% and 48%), demonstrating that there is genetic variation in resistance to *F. columnare* [5]. Spleen index and mannose binding lectin levels could not predict susceptibility or resistance to *F. columnare* [5].

The pathogen including exposing dose, virulence and genetic type (strain) are also important to disease development. Genetic diversity has been demonstrated in *F. columnare* using various methods [6,7,8,9]. In channel catfish, two independent laboratories using different methods established that the genetic type of the bacterium influenced virulence. Shoemaker et al. [10] demonstrated that both fry (~0.5g) and fingerling catfish (~3g) were more susceptible to genomovar II than to genomovar I *F. columnare* isolates that had been typed using restriction fragment length polymorphism (RFLP) analysis of the 16s rRNA gene. In that study, adhesion to whole fry correlated with mortality induced by the different genomovars of *F. columnare* studied and a more recent study demonstrated a higher chemotactic response to catfish skin mucus in the more virulent isolates [11]. Using pulsed field gel electrophoresis (PFGE), Soto et al. [12] demonstrated two types (PFGE profile A and B) and showed higher mortality in channel catfish challenged with profile A than with profile B isolates. Profile A isolates included genomovar II and III *F. columnare*.

To date, all *F. columnare* isolates obtained from rainbow trout (*Oncorhynchus mykiss*) have been reported to be genomovar I based on RFLP typing of the 16s rRNA gene. Within these isolates, 8 different genetic profiles, based on automated ribosomal intergenic spacer analysis, have been demonstrated and shown to differ in virulence at 24°C [9]. In these studies, virulence was correlated with the ability of the isolates to degrade chondroitin sulfate. LaFrentz et al. [13] established an immersion challenge model in rainbow trout held at 16°C. In initial experiments three genomovar I isolates were tested and mortality ranged from 40–100 %. Upon selection of one isolate (051-10-S5), two replicated trials each yielded mortality of ~50%. Following establishment of a challenge model, the hypothesis that genomovar I isolates would induce higher levels of mortality than genomovar II isolates in rainbow trout fingerlings was tested. The hypothesis was rejected as genomovar II isolates exhibited significantly ($P<0.05$) higher mortality than genomovar I isolates at 16°C (Fig. 2). This finding suggests that the introduction of genomovar II isolates into trout populations (farmed or wild) may result in increased death loss.

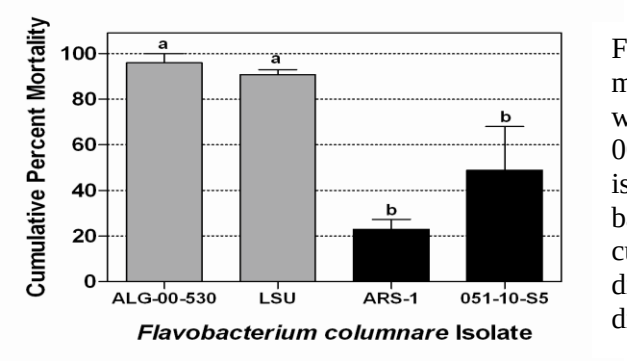

Figure 2. Mean cumulative percent mortality of rainbow trout after challenge with genomovar II *F. columnare* (ALG-00-530 and LSU) and genomovar I isolates (ARS-1 and 051-10-S5). Error bars indicate standard deviation. Mean cumulative percent mortality values with different letters indicate a significant difference at $P<0.05$.

Environmental conditions including temperature, water quality and other stressor(s) (e.g. population density; parasitism [14] are probably the most influential on the development of columnaris disease in the aquaculture environment. Water temperature has been utilized as a stressor to induce columnaris disease in trout [9] and channel catfish [8]. A recent published study suggests that global climate change may be having an impact on the apparent increased prevalence of columnaris disease in Finland [15]. Over a period of about 20 years the authors measured water temperature and showed that mean temperature increased by about 2–3°C over the period (i.e., from ~14 to 16°C). In their study, predicted prevalence of columnaris disease on two farms in two different age classes of Atlantic salmon (*Salmo salar*) increased as the water temperature increased.

Fish density in the farm environment can impact disease development. In laboratory studies with channel catfish (5 replicate 50 L tanks for each treatment ~8 g/fish), we demonstrated a significant effect of density on the outcome of challenge with a genomovar II *F. columnare* isolate. At densities that are probably below those common in hatchery settings significantly ($P< 0.001$) greater mortality (83.8 ± 8 %) occurred in fish held at 16 g/L compared to 59.6 ± 4 % at 8 g/L and 29.6 ± 3 % g/L at 4 g/L.

To illustrate the concept of the host-pathogen-environment theory we summarize a field trial conducted to evaluate the efficacy of a modified live *F. columnare* vaccine in largemouth bass (*Micropterus salmoides*) [16]. The trial was conducted at a fish hatchery with a history of columnaris disease to allow for a "natural" challenge. Largemouth bass fry 7–9 days post-hatch (dph), were immersion vaccinated with AQUAVAC-COL (Merck Animal Health) following the manufacturer's protocol. Sham-vaccinated fry were exposed to modified *Cytophaga* media and glycerol using the same conditions. After vaccination, 55,000 vaccinated and 55,000 sham-vaccinated fish were stocked into each of two 0.24 ha earthen ponds. Fish were allowed to feed naturally in ponds for 33 d (38–40 dph) at which time they were harvested and stocked into two 4914 L fiberglass raceways and trained to accept commercial feed. At 45 d post-vaccination, the sham-vaccinated fish were calcein marked so that sham-vaccinated fish could be distinguished from vaccinated fish during the cohabitation trial. After marking, 1900 sham vaccinated fish (0.94±0.01 g/fish) and 1900 vaccinated fish (0.92±0.03 g/fish) were stocked into each of three fiberglass tanks (378.5 L) for the 44 day trial. Each day at the same time, dead fish from each tank were examined using a SE-MARK detector. Employees who were blinded to the treatment assignments counted the fish and determined if the dead fish was marked (sham-vaccinated) or un-marked (vaccinated). Feed training is stressful and can result in poor nutritional status. Water quality was also affected due to an algae die off in the supply water at day 13 that resulted in

elevated carbon dioxide levels of 21–29 mg/L for 20 days and elevated ammonia levels (1.8 mg/L) for 1 day. Results demonstrated that during every time interval, the probability that a vaccinated fish would survive past time, *t*, was greater than for sham-vaccinated fish and survivor functions were significantly different ($P<0.001$). Overall, vaccinated fish had a 43% lower risk of death during the field trial demonstrating vaccination significantly reduced the risk of death from columnaris disease.

Specific details of all the experiments can be found in the cited references. We hope this brief overview of the host-pathogen-environment interactions as they relate to *F. columnare* disease development will be considered by others studying virulence, pathogenesis, immune response and control strategies (e.g., vaccination and therapeutics) for *F. columnare*.

Acknowledgements

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