AQUACULTURE

EVALUATION OF FACTORS POTENTIALLY AFFECTING VACCINE SAFETY AND EFFICACY

Suja Aarattuthodiyil, Todd Byars, Matt Griffin, Lester Khoo, Terrence Greenway, and David Wise

"ENTERIC SEPTICEMIA OF CATFISH IS A MAJOR PROBLEM CAUSING FISH DEATH. ALTHOUGH VACCINATION COULD IMPROVE THIS SITUATION, THE EFFECTIVENESS OF A VACCINE IS DEPENDENT ON THE CROSS PROTECTION OFFERED AGAINST MULTIPLE ISOLATES. THE CLONAL NATURE OF E. ICTALURI DEMONSTRATED BY DATA NEGATES THE NEED FOR DEVELOPING MULTIVALENT VACCINES OR DEVELOPMENT OF NEW VACCINES TO ACCOUNT FOR ANTIGENIC VARIATION OVER TIME."

David Wise

The channel catfish industry is seriously impacted by Enteric Septicemia of Catfish (ESC), caused by the bacterial pathogen *E. ictaluri*. Traditionally, losses have been controlled by withholding feed from fish to reduce the oral route of infection combined with medicated feeds. Vaccines have been effective in controlling infectious diseases in other aquaculture species and have dramatically reduced antibiotic use. Recently we developed a live attenuated ESC vaccine that is delivered orally to catfish in the nursery production stage. While live vaccines are very effective in providing long lasting immunity against disease, their use represents some level of risk if delivered to animals in suboptimal health or under stressful conditions. In a compromised animal, attenuated vaccines can cause infections leading to morbidity and mortality. Genetic and antigenic variation among strains within the same pathogenic species also presents hurdles for developing effective commercial vaccines.

Under laboratory conditions, the developed vaccine has been shown effective against the parental wild-type strain and safe at 10 times the applied target dose. However, pond production conditions are rarely optimal and many factors could compromise vaccine safety and efficacy. Stress is an unavoidable component of commercial aquaculture and results from overcrowding in a confined environment. Stress lowers immune function making fish more susceptible to infection. In a stressed fish with lowered immune function, application of live vaccine could lower the effectiveness of the vaccine and possible vaccine associated mortality. Another key factor in field vaccinology

	Stressed		Non-Stressed	
Study	**Vaccinated**	**Control**	**Vaccinated**	**Control**
Vaccinate-Stress	4.0	41.3*	2.1	33.6*
Stress -Vaccinate	8.1	74.0*	22.1	68.2*

Table 1. *Mortality of vaccinated and non-vaccinated control fish. Fish were subjected to stress before and after vaccination in two independent studies. In study 1 (vaccinate-stress), fish were vaccinated by oral delivery followed by low oxygen stress. In study 2 (stress-vaccinate), fish were stressed followed by immersion vaccination. In both studies, stress was not shown to cause an appreciable increase in mortality in vaccinated and non-vaccinated fish and vaccination offered significant protection against infection.*
** indicates control mortality was significantly greater than mortality of vaccinated fish within each study.*

The wet lab that is housed in the Thad Cochran National Warmwater Aquaculture Center at Delta Research and Extension Center.

is antigenic variation among pathogen species, where immunization with a vaccine derived from one strain does not provide protection against genetic variants of the same species.

Research was conducted to evaluate possible factors present under field conditions that could potentially reduce vaccine safety and efficacy. Oxygen deprivation is the most common stressor present in commercially raised catfish and laboratory trials were conducted to replicate these conditions before and after vaccination. Additional trials were conducted to determine if the attenuated isolate afforded protection against archived field isolates collected from diagnostic case submissions over a time span of thirty years (1997-2016).

Results

Low oxygen stress: Dissolved oxygen levels were maintained less than 1ppm for approximately 15 minutes until fish ventilation rates exceeded 140 per minute. Blood cortisol levels were elevated shortly after fish were subjected to low oxygen levels, which was indicative of a stress response (increase from 4.3

RPS (2012 challenge)		RPS (2015 challenge)		RPS (2016 challenge)	
Isolate	**% RPS**	**Isolate**	**% RPS**	**Isolate**	**% RPS**
S97-773	89	S97-773	86	S97-773	92
S11-518**	94	S15-394	98	S16-296	83
S99-1634	85	S15-397	82	S16-368	91
S00-1912	95	S15-403	86	S16-374	95
AL-93-75	98	S15-405	86	S16-376	87
		S15-408	100	S16-392	83
		S15-409	97	S16-387	92
		S15-412	100	S16-389	100
				S16-393	100
				S16-395	91
				S16-396	92
				S16-397	68

Table 2: *The relative percent survival (RPS) for each bacterial isolate used in challenge study. With exception to Al-93-75 (Alabama), all isolates were collected from disease fish from commercial catfish operations in Mississippi. The isolate label represents the diagnostic case submission number (Stoneville-year-case #). Challenge conducted in 2015 and 2016 were from E. ictaluri isolate collected from vaccinated populations of fish. RPS is the relative percent survival and used a measure of vaccine efficacy.*

*** E. ictaluri isolate from hybrid catfish. Isolate S97-773 was the parental wild-type strain and used as a positive control in each study.*

nanograms per milliliter to 35.4 nanograms per milliliter cortisol). Thirty days post-vaccination, vaccinated and control (non-vaccinated) fish were challenged with the parental wild type *E. ictaluri* isolate. Under the conditions of this study, low oxygen stress did not induce any post-vaccination mortality or morbidity in any of the vaccinated treatments. Similarly, all groups of vaccinated fish, regardless of stress treatment, were protected against virulent *E. ictaluri* infection (Table 1). These data indicated that acute low oxygen deprivation, before or after vaccination, does not alter vaccine safety and efficacy, however the effects of chronic long-term stress have not been evaluated. Data indicated that short acute stressors are unlikely to influence vaccine safety and efficacy and provides valuable insight in developing commercial vaccination protocols.

Protection against archived field isolates: Vaccination

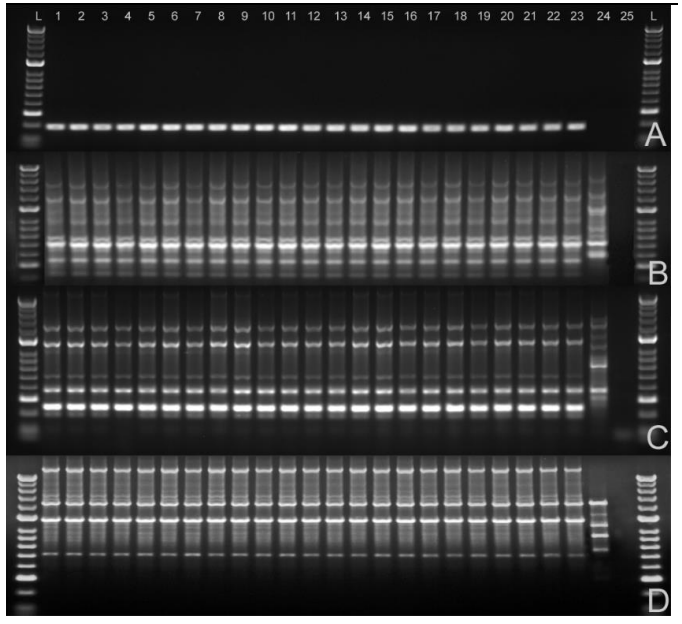

Figure 1: *PCR profiles of the 23 different Edwardsiella isolates used in challenges (2012- 2016). Identical banding patterns strongly support the clonal nature of E. ictaluri which is supported by the literature. Lane 24 contained DNA isolated from a closely related species, Edwardsiella piscicida, which served as a control.*

followed by bacterial challenge with archived isolates were conducted over a three year period. In the first year, vaccinated fish were exposed to archived *E. ictaluri* isolates collected from channel catfish commercially raised in Mississippi over a time span of thirty years. Vaccine efficacy was also evaluated against an *E. ictaluri* isolate collected from hybrid catfish in Mississippi and channel catfish in Alabama. In all trials, vaccination was shown to protect catfish against all challenge isolates, regardless of host species, geographic region (state and farm location) or isolation year (Table 2). In the second and third year of study, *E. ictaluri* isolates were collected from diseased fish from vaccinated populations of fish from farms participating in field vaccination trials. While vaccine efficacy greatly improved survival, yield and fish net-value, limited mortality was observed in vaccinated pond populations. These data demonstrate that mortality observed in on-farm vaccinated fish populations was not related to antigenic variations among isolates. The most likely cause of on-farm mortality was related to unequal distribution of vaccine laden feed to individual fish, an inherent problem with mass delivery of oral vaccines to large populations of animals. In order to differentiate between the isolates, the clonal relation of the 23 different isolates were determined (Figure 1). The PCR profile indicated relative homogeneity among the isolates dating back to 1997. This further confirmed the results which indicated no significant difference between the isolates as all received protection against challenge. The clonal nature of *E. ictaluri* negates the need to develop multi-valent vaccines or construct new vaccines to account for antigenic variation occurring over time.