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UNIVERSITY OF WISCONSIN-MADISON

FRESH Seminar: “Food Safety and Infectious Disease Control in Cultured Shrimp”

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MADISON, Wis. (FRI) – Cultured shrimp farming involves the monoculture of animals grown in high densities in environmentally exposed environments. Farmed shrimp are transported at various growth stages and harvested and shipped internationally. Together, these conditions represent significant risks for serious microbial disease problems for the animals. When compounded with the economic importance of cultured shrimp (a \$9–11 billion industry), the need for effective preventative and therapeutic strategies to control infectious diseases in these animals is evident. At a recent FRESH seminar, Dr. Lyric Bartholomay discussed her laboratory’s work towards developing “vaccines” to combat two viruses that have had devastating effects on the cultured shrimp industry.

Commercial shrimp production begins with brood stock: large (35–50 g), sexually mature shrimp that can produce up to 100,000 progeny at a time. Temperature, humidity, light, and diet must be controlled carefully to optimize spawning and larvae health. As larvae develop, nutritional needs change, with the added complication that many of the foodstuffs (bioplankton, zooplankton) used to feed the larvae also are cultured.

When the post larvae stage is reached, the shrimp (now about 1 cm in length) are packed and shipped to pond facilities to continue growth until harvest. Ponds are typically many meters deep and are clay-bottomed or lined with black plastic. Because of the eventual high value of the shrimp, biosecurity measures (such as physical barriers) are implemented to minimize the contact of birds, crabs, and other animals with the ponds and to reduce introduction of pathogens. However, most biosecurity procedures employed are not totally effective.

The Pacific white shrimp (*L. vannamei*) is the shrimp species most commonly farmed. *L. vannamei* is relatively tolerant of dense living conditions (*i.e.*, they are less likely to attack and eat each other than other shrimp species). In addition, this species also grows fast, requiring as few as 100 days from the time ponds are stocked until shrimp are harvested.

Disease is a particular concern while the shrimp are growing in the exposed ponds. Although few human foodborne diseases arise from cultured shrimp, bacterial, viral, and fungal diseases may all strike shrimp ponds, causing mass mortality or requiring the shrimp be harvested while still very small (“popcorn shrimp”) instead of fully grown (15–20g), when they command a much higher price.



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Bacterial threats to shrimp include necrotizing hepatopancreatitis, and infections from *Streptococcus* and *Vibrio* spp. Antibiotics are used in shrimp culture, and antibiotic resistance in shrimp has been fairly widespread, but it is hard to obtain reliable data on antibiotic usage in shrimp.

Viruses represent the most important pathogens that strike shrimp, especially white spot syndrome virus (WSSV) and infectious myonecrosis virus (IMNV):

- WSSV spreads extremely quickly in ponds and can cause 100% mortality within 3 days. The virus is found worldwide, causing huge (>\$8 billion) economic losses since 1992. The virus is a very large DNA virus of about 280 kB (which appears to be losing genes over time, as it was >300 kB in the past).
- IMNV can result in 40–70% shrimp mortality within 2 months, and is primarily a problem in Brazil and Indonesia. Economic losses from this virus are estimated to be >\$100 million per year. IMNV is a double-stranded RNA virus of 8.2 kB that contains 2 open-reading frames, one of which codes for an RNA-dependent RNA polymerase, and the other encoding the major capsid protein.

No treatment or vaccine is currently available for either of these viruses; biosecurity and chemical disinfection are the only measures that can be employed to help stop disease. Recognizing this need, Dr. Bartholomay's laboratory is developing novel nucleic-acid based antivirals to combat both viruses. The strategy capitalizes on the shrimp's innate immune system, which uses RNA interference (RNAi) to defend against foreign nucleic acid sequences. Introduction of dsRNA triggers a response against those sequences (via the Dicer pathway) which can degrade homologous viral mRNA. Introducing a dsRNA that shares homology with nucleic acid sequences for key viral proteins potentially could inactivate the virus.

In initial experiments against IMNV, dsRNA sequences were designed based on sequences of either the IMNV RNA-dependent RNA polymerase or the major capsid protein. The dsRNA was administered to shrimp via intramuscular injection or reverse gavage (*i.e.*, enema) prior to virus challenge two days later. Animals receiving the RNAi therapy survived much longer (~60–80% survival at day 30) than those receiving saline or heterologous RNA sequences (0% survival by day 15–20). RNAi therapy targeted against the RNA-dependent RNA polymerase was more effective than that targeted against the major capsid protein.

The protection given by the RNAi therapy was not transient; when the treated shrimp were re-challenged with the virus 60 days later with 100-fold the original IMNV dose, animals still survived. In addition to being able to work prophylactically, the RNAi therapy was also effective when administered post-infection, with 50% survival observed when the RNAi therapy was administered 48 hours post-IMNV infection.



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A similar approach was also successful with RNAi therapy targeted against an early gene of WSSV. Doses as low as 0.1 microgram/shrimp afforded more than 74% survival and significantly decreased viral loads. RNAi therapy against a different (late) WSSV gene was less successful, underscoring the importance (and unpredictable nature) of target selection. The utility of this approach against two very different viruses hints at its potential in many different future applications.

Intramuscular or reverse gavage administration of shrimp is, of course, completely impractical in the field. To be commercially viable, the RNAi therapy has to be administered to many animals at once, preferably using a *per os* delivery route. Towards this end, and in collaboration with a group at Iowa State University, a polyanhydride nanoparticle delivery method has been developed. Polyanhydride nanoparticles have numerous qualities that make them good delivery systems: they exhibit surface (rather than bulk) erosion, they protect their payload, and they are internalized by APCs. Importantly, they are already used to deliver human vaccines.

Because the protection conferred by the RNAi therapy is both pathogen specific and long term, this RNAi therapy against shrimp viruses shares similarities to vaccines. The use of this “vaccine” approach could be economical if the cost to treat a pond is low enough and if potential regulatory hurdles regarding the use of a nucleic acid therapeutic in a food animal can be managed. RNAi approaches for mosquito control are further down the development path and may help establish the regulatory landscape for this type of shrimp “vaccine.”

About the Food Research Institute

The Food Research Institute (FRI), a part of the College of Agricultural and Life Sciences at the University of Wisconsin–Madison, operates its own laboratories and administers its own research and service programs. The mission of FRI is to catalyze multidisciplinary and collaborative research on microbial foodborne pathogens and toxins and to provide training, outreach and service to enhance the safety of the food supply. To fulfill this mission, FRI conducts fundamental and applied research, provides accurate and useful information and expertise, delivers quality education and training, and provides leadership in identifying and resolving food safety issues to meet community, government, and industry needs.

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