AQUACULTURE

EDWARDSIELLA PISCICIDA: A NEW CATFISH PATHOGEN

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WE HAVE EVIDENCE THAT SUGGESTS EDWARDSIELLA PISCICIDA IS AN EMERGENT PATHOGEN WITHIN MISSISSIPPI AQUACULTURE. WE DON'T KNOW WHAT IS DRIVING THIS EMERGENCE, BUT WE NOW HAVE MOLECULAR ASSAYS THAT CAN DETECT AND QUANTIFY THESE BACTERIA IN THE POND ENVIRONMENT. THESE ASSAYS WILL BE VALUABLE RESEARCH TOOLS MOVING FORWARD.

A new *Edwardsiella* taxon was recently described from fishes of Europe and Asia. Phenotypically similar to *Edwardsiella tarda*, extensive genetic and phenotypic characterization determined this new strain does not belong to any established *Edwardsiella* taxa, leading to the adoption of a new taxon, *Edwardsiella piscicida*. Concurrent research also identified two genetically distinct taxa within the group of organisms traditionally classified as *E. tarda*. Comparisons of *gyrB* sequences between US isolates and *E. piscicida* from Europe and Asia identified several US isolates with >99.6% similarity to the *gyrB* sequence of the *E. piscicida* type strain (ET883) but <87% similarity to the *E. tarda* type strain from humans (ATCC #15947). A discriminatory PCR was developed for the identification of *E. tarda* and 2 genetic variants of *E. piscicida* (*E. piscicida* and *E. piscicida-like* species). Using these PCR assays, a survey was conducted of 44 archived bacterial specimens from disease case submissions to the Aquatic Research and Diagnostic Laboratory at the Thad Cochran National Warmwater Aquaculture Center between 2007 and 2012. All 44 isolates, originally identified phenotypically and biochemically as *E. tarda*, were identified as *E. piscicida* by PCR. Repetitive sequence-mediated PCR (rep-PCR) analysis of these archived specimens suggests they are largely homogenous, similar to what has been observed for *E. ictaluri*. The *gyrB* sequence data, coupled with the *E. piscicida* specific-PCR and rep-PCR data, confirms that *E. piscicida* has been isolated from fish disease cases in the southeastern USA. Moreover, our survey data suggests *E. piscicida* is likely more prevalent in catfish aquaculture than *E. tarda*, which is a zoonotic pathogen.

This work led to the development of individual real-time polymerase chain reaction (qPCR) assays for *E. tarda, E. piscicida,* and *E. piscicida–like* sp. to provide rapid quantitative confirmatory tests for these phenotypically ambiguous bacteria. The qPCR assays were shown to be repeatable and reproducible, with high degrees of sensitivity. Moreover, each assay was found specific to their respective targets with no observed amplification from non-target organisms, including the closely related *E. ictaluri* and *E. hoshinae.* Under the conditions used in this study, the 3 assays had a quantifiable limit ranging from 10^3 (*E. piscicida*) to 10^2 (*E. piscicida–like* and *E. tarda*) colony forming units (CFU) in kidney tissue biopsies (ap-

proximately 25 mg), pond water samples (35 mL), and broth culture (20 µL). In experimental disease challenges, the assays were able to detect their respective targets in both clinically and subclinically infected channel catfish (*Ictalurus punctatus*) fingerlings. This challenge work also identified significant differences in pathogenicity to channel catfish between these three enteric pathogens (Figure 1), with minimal mortality observed in fish exposed to *E. tarda* and *E. piscicida-like sp.*, even at doses >1x10^7 CFU. In addition to quantifying target bacteria from various substrates, the assays provide rapid identification, differentiation, and confirmation of the phenotypically indistinguishable *E. tarda, E. piscicida,* and *E. piscicida–like* sp., a valuable tool for diagnostic assessments.

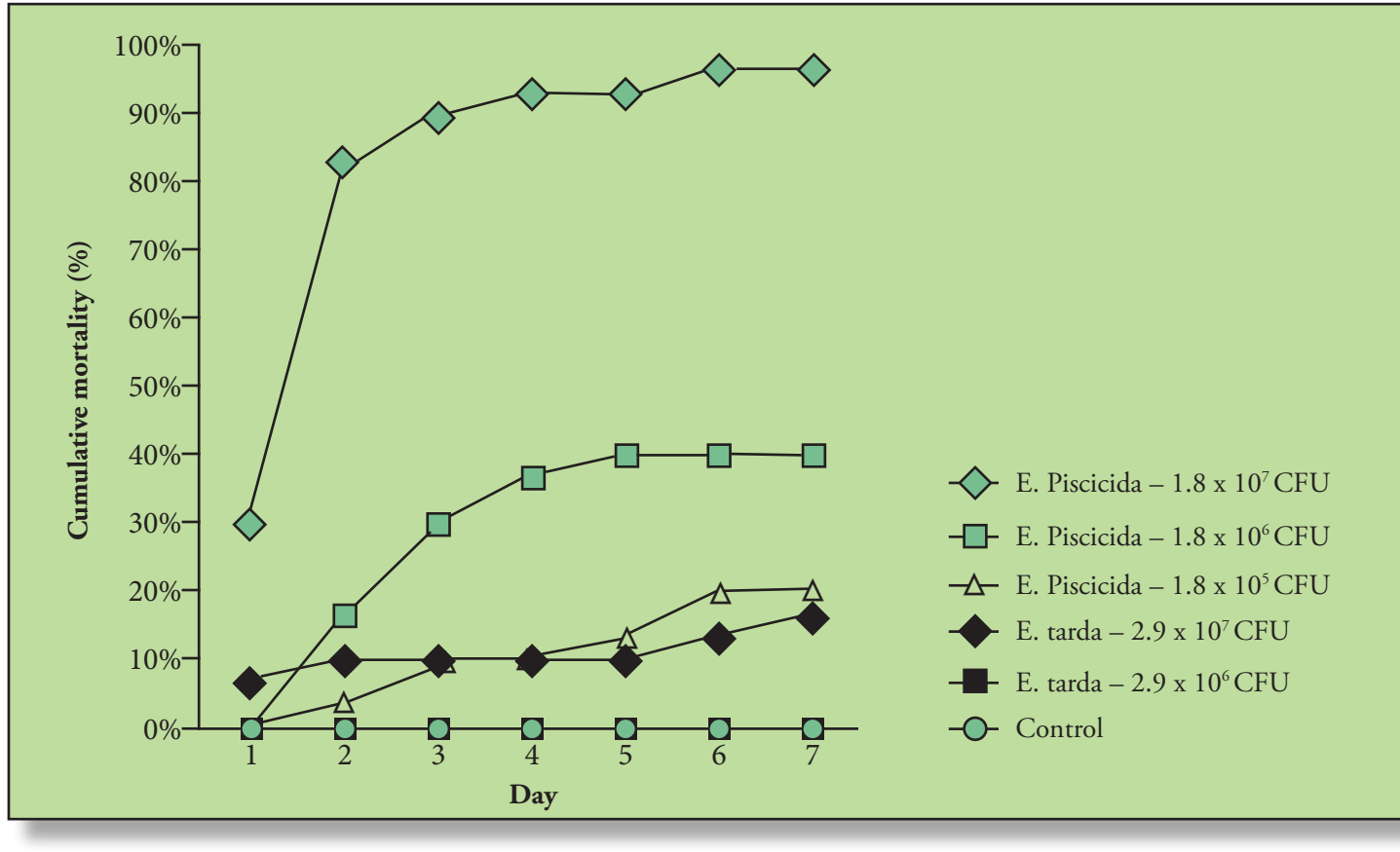

Figure 1: *Nonreplicated cumulative mortality for channel catfish challenged with 3 different doses of* Edwardsiella piscicida, Edwardsiella piscicida–like *sp., and* Edwardsiella tarda. *Thirty fish were challenged with each dose by IP injection. The cumulative percent mortality is reported.*