Zebrafish as a model host for *Vibrio parahaemolyticus* virulence

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Vibrio parahaemolyticus is responsible for nearly 50% of Vibrio-related infections in the United States of America. The majority of infections are acquired by ingestion of raw shellfish harboring the bacterium, such as oysters, resulting in a severe but self-limiting gastroenteritis. Oysters can be naturally colonized by hundreds of strains of this bacterium although only a small percentage of the population appears capable of causing human infections. Studies on the genetic mechanisms of virulence have been hampered by the absence of an optimal animal model. Currently virulence of *V. parahaemolyticus* strains is assessed by the characterization of two phenotypes, enterotoxicity and cytotoxicity. Enterotoxicity is determined by measuring fluid accumulation using the rabbit ileal loop model, while cytotoxicity is assessed by the ability of strains to cause cytopathic effects on eukaryotic cell lines. The lack of an easy to use, reproducible *in vivo* infection model for *V. parahaemolyticus* led to the investigation into the use of zebrafish (*Danio rerio*) as such a model to directly compare strain virulence. Our studies show that *V. parahaemolyticus* can establish a lethal infection in adult zebrafish with a reproducible dose-response when challenged by intraperitoneal injection. When virulence was compared between a wild-type *V. parahaemolyticus* strain and an isogenic *pilA* (type IV pilin) mutant, the LD_{50} of the *pilA* mutant was consistently 1 log higher than that of the wild-type strain, Differences in virulence based on rate of mortality were also observed between strains with or without *tdh*, the thermostable direct hemolysin. Histological changes in fish injected intraperitoneally with *V. parahaemolyticus* include degeneration and eventual lysis of erythrocytes, increased vascular plasma proteins, coagulation necrosis of skeletal muscle and hemorrhage in the retroperitoneal region. These conditions become more pronounced as the post-exposure period increases. Future studies using the zebrafish model may provide a method for identifying specific genes involved in virulence as well as elucidating host-pathogen interactions.