A Laboratory Module for Host-Pathogen Interactions

America's Next Top Model

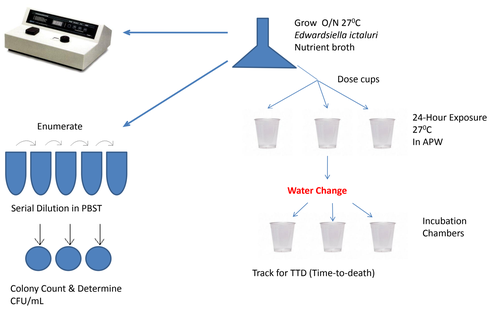
ABSTRACT

While pathogenesis is virtually universally discussed in microbiology and related course lectures, few undergraduate laboratories include experiments, primarily because of logistical issues. Hypothesizing that active learning will give students a better understanding of concepts in pathogenesis, a novel virulence assay has been developed for use in labs which is simple, flexible, inexpensive, and safe for students. For a host this model utilizes the Western Mosquitofish (*Gambusia affinis*), an invasive species broadly distributed across the U.S. These freshwater fish are hardy and maintenance is easy. A positive control for virulence has been established using *Edwardsiella ictaluri*. Being an Enterobacteriaceae, appropriate culture media and equipment are common in microbiology labs. The core bath infection protocol results in time-to-death proportional to the infectious dose, and can be completed in one week. Data indicates a wide variety of experiments can be performed, effectively demonstrating and visualizing the important concepts in pathogenesis. Application modules include antibiotic treatments, virulence screening of enteric isolates, chronic vs acute infections, transmission study, comparison of routes of entry, and immunity to reinfection. A Koch's postulates reenactment by culturing bacteria from liver of infected fish has also been developed. After performing the virulence screen module in a nursing microbiology course, student attitudes were assessed utilizing a Likert scale questionnaire. Responding to how much did the lab component help their learning, students answered positively 83 11%, with a similar 82 14% rating on "enjoying the lab experience." Interestingly, students answered a rather low 40 26% on being concerned about suffering of the fish, yet also a contrasting low and diverse 59 30% over how ethical the usage of animal experiments in medical research is in general. Further study will be performed on how this experience impacts student perceptions of animal research. The developed module protocols will be shared with instructors interested in adding virulence studies into their courses.

INTRODUCTION

A new model for the study of host-pathogen interactions is being developed for the educational laboratory setting. Educators of infection-related course laboratories (medical microbiology, general microbiology, immunology, pathophysiology) can use this model to enable students to study, through experimentation, host-pathogen interactions. Although the concept of a host-pathogen model for the laboratory is not new, an inexpensive, simple, flexible, and student-safe model is not widely available. Thus, while host-pathogen interactions are commonly discussed in lecture as an important topic, few students have the chance to experience and learn for themselves in the lab setting. The use of *Gambusia affinis*, a readily accessible, invasive, robust species of fish offers an appropriate host which can be easily maintained with little caretaking requirements and inoculated through a bath protocol, as opposed to a more difficult and time consuming injection or feeding method. The Western mosquitofish is prevalent across the entire Southern United States, and is easy to catch. To provide a safe (to humans) and easy-to-use pathogen as a positive control and a proof-of-principal, *Edwardsiella ictaluri* was chosen. Edwardsiella, a Gram negative Enterobacteriaceae, can be cultured on commonly available lab media, does not survive at 37°C, and therefore cannot cause systemic infection in humans. Further, Edwardsiella tends to be broadly drug sensitive. Within the gamma Proteobacteria, Edwardsiella is divergent from *E. coli* (only 20% identical in 16S gene) and identified within the cluster containing Proteus, Yersinia, and Morganella. There is a literature base on pathogenesis of *E. ictaluri*, as it is the major agent of enteric septicemia in catfish, one of the most significant causes of economic loss in the cultured catfish industry.

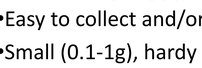
Core Infection Protocol

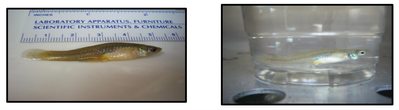


Fish were infected via the bath method individually in 12 oz clear plastic drinking cups with 130 ml sterile artificial pond water. APW is 0.111 mg/ml $CaCl_2$, 0.111 mg/ml $MgSO_4$, and 0.04 mg/ml $NaHCO_3$.

The Host: *Gambusia affinis*

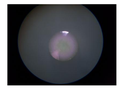
- Easy to collect and/or breed
- Small (0.1-1g), hardy freshwater fish
- Abundant invasive species
- Survives from 4 to 39°C
- Susceptible to infection with *Edwardsiella ictaluri* via bath protocol (contrary to literature)

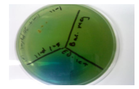




The Positive Control Pathogen: *Edwardsiella ictaluri*

- Gram negative enterobacteria
- Known pathogen in catfish
- Causes hemolytic septicemia
- Core bath infection protocol can be completed in one week





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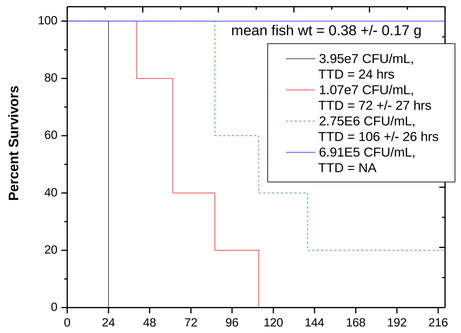
Dose Response Survival Curve

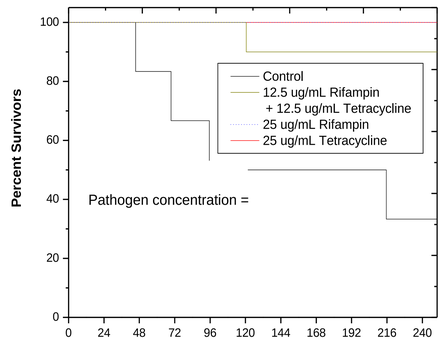


Groups of fish (n=5) exposed to four-fold varying doses of bacteria exhibit time to death proportional to pathogen dose. After bath infection period of 24 hours at 27°C, water is exchanged then fish are observed for mortality.

Koch's Postulates

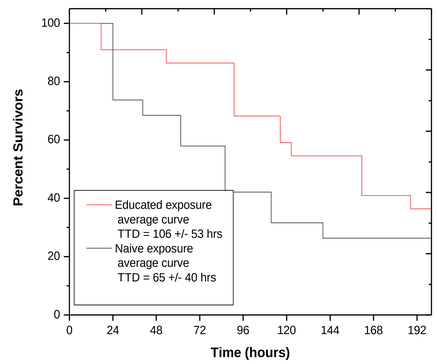
Host can be infected with pathogen and death results in 2-7 days. Bacteria can then be isolated in pure culture from liver, kidney, or whole body using a selective, differential media, and identity confirmed. Reinfection of a healthy fish can be performed after isolation.

Single and Dual Antibiotic Treatment



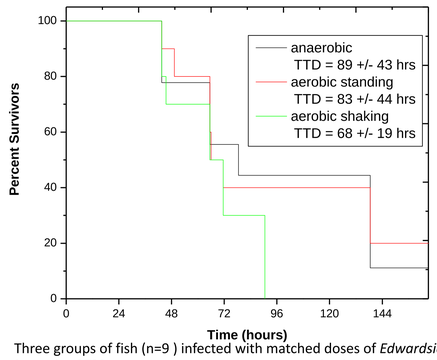
Four groups (n=6 – 10) treated with a single or dual antibiotic therapy following a 24 hour period of 27°C exposure to pathogen at a concentration of 9.32e6 CFU/mL . Bacteria and antibiotic administered via bath protocol.

Protective Response to Reinfection



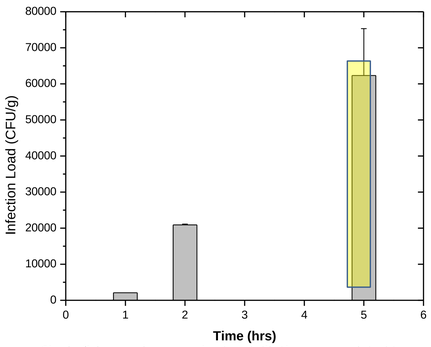
An experimental group (n=20), represented by the red curve, was exposed to pathogen for 24 hours at 27°C, followed by antibiotic therapy (25 µg/ml Rifampicin, 24 hours)and then rested a week. The educated experimental group and a naïve control group was again challenged by bacteria, then monitored for mortality.

Effects of Oxygen on Virulence



Three groups of fish (n=9) infected with matched doses of *Edwardsiella ictaluri* at 27°C, where cultures were grown standing in an anaerobic chamber, standing in atmospheric oxygen, or atmospheric shaking at 150 rpm, all in 250 ml glass flasks .

Penetration and Replication of Pathogen *In Vivo*



Single fish were homogenized and dilutions were plated to monitor for whole body CFU/g of fish at indicated time points. Highlighted column is from a fish exposed for 2 hrs, then incubated in clean water until sacrificed. Data collected exhibits penetration (1 & 2 hr) and replication (5 hr) of the pathogen in the host.

DISCUSSION

The ease, safety, flexibility, and inexpensive nature of the core infection protocol allows it to provide a set of practical lab modules which can be molded to fit almost any lab where concepts in host-pathogen interactions are taught. Screening of enteric isolates for virulence has been performed in an undergraduate pre-nursing microbiology lab for 3 semesters successfully. Fish can be infected by simple addition of bacteria to water, and mortality is proportional to infectious dose. Infection can be treated with two antibiotics (rif and tet), and partially protects against a future infection. Student attitude assessments indicate that students feel that the lab module is helpful to their learning (see attached results). It is the goal of this project to disseminate the information gathered in our preliminary studies to microbiology educators to offer these modules as a flexible tool.

POSSIBLE APPLICATIONS

- ✓Dose response
- ✓Antibiotic and probiotic therapy
- ✓Transmission
- ✓Virulence and physiological status
- ✓Vaccination
- ✓Comparing host and/or pathogen strains

REFERENCES AND ACKNOWLEDGEMENTS

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