DEBORAH MARY MOORE$, T. RYAN WITHERS, HONGWEI YU, and WENDY TRZYNA, Department of Biological Sciences, Marshall University, Huntington, WV, 25755, Biomedical Sciences, Marshall University, Huntington, WV, 25755. **A simple plating assay using Acanthamoeba to evaluate the effect of environmental stressors on growing and dividing cells.**

Acanthamoeba provide a potential alternative to mammalian models for investigation of bacterial virulence. Acanthamoeba, a ubiquitous single celled eukaryotic microbe, feed on bacteria within their environment. Bacteria can be utilized as food, establish endosymbiosis, and virulent strains may result in amoebal death. These properties along with the ease with which Acanthamoeba can be cultured allowed development of a rapid plating assay in this lab. Effects of environmental stress on Acanthamoeba can be evaluated using this as shown in our previous studies. The present study has extended this assay to evaluating bacterial virulence using Acanthamoeba and Pseudomonas aeruginosa strains differing in pathogenicity. Acanthamoeba was co-cultured with either a virulent or non-virulent strain of Pseudomonas spp. on amoeba saline agar plates. To test particular strains of bacteria, a measured volume of bacteria of known density is applied to plates in triplicate lines with a precise number of Acanthamoeba cysts inoculated at the starting end of the bacterial line. If environmental conditions on plates are favorable, cysts will excyst, amoebae consume the bacteria and proliferate. Plates are evaluated over time for advancement of proliferation along the bacterial line, overall numbers of amoebae and the overall effect of the bacteria on the amoebae. The present study tested a panel of Pseudomonas strains of unknown virulence. Amoeba assay results were strikingly similar to those observed in animal trials for the same bacterial strains. This assay has the potential to provide a rapid, cost-effective alternative to studies carried out in animal models for evaluating bacterial virulence.