Abstract of proposed student project

Mycoplasmas are important pathogens of human and veterinary medicine, causing both acute and chronic infections. Infection with most *Mycoplasma* spp. is characterized by low mortality but high morbidity, most commonly presenting as a chronic, and often clinically silent, disease. Current methods to determine virulence potential and host-pathogen interactions of mycoplasmas have relied on *in vivo* experimental infection of the natural host or surrogate murine hosts or *in vitro* infection of cell lines. *Mycoplasma* spp. are increasingly isolated from a range of wildlife hosts. Sampling of wild caprine, avian, rodent, and marine mammal hosts has identified the emergence of novel *Mycoplasma* spp. as well as known pathogens of domestic animals. Therefore, there is a critical need for an alternative model that has a functioning immune system and is amenable to testing the virulence potential of *Mycoplasma* species isolated from wildlife.

In recent years, there has been an increased focus on the use of invertebrate models in microbial pathogenicity research. The invertebrate waxworm (*Galleria mellonella*) has been used to determine pathogenic potential and specific virulence factors of both bacteria and fungi. *Galleria* shares common innate immune system features with vertebrates, including toll-like receptors, microbial killing pathways, C-lectins, and apoptotic pathways. We showed that infection of *G. mellonella* was able to recapitulate the virulence potential and mirrored the survival curves seen alligators experimentally infected with *M. alligatoris.* Our data from defined waxworm models using reptilian and caprine pathogens support that *G. mellonella* is an excellent surrogate model system for the natural host and has potential to expand virulence testing to other newly described *Mycoplasma* spp. isolated from wildlife where infection studies in the natural host are not feasible. We predict that our model has broader impacts for virulence testing of mycoplasmas and have high confidence that the current proposal will be successful.

Our **overall objective** is to fill the critical gaps in knowledge as to the association of disease etiology, pathogenicity, and virulence potential of *Mycoplasma* spp. isolated from wildlife, especially those that are ecologically at risk and/or understudied (e.g., marine mammals, reptiles). We propose that *Galleria mellonella* is a highly tractable model system to overcome the inherent limitations of establishing pathogenic potential of mycoplasmas, especially those isolated from hosts that are unlikely to be available for experimental infections. Because of the International Mollicutes Culture Collection housed at UF (D. Brown, Culture Collection Director), we have access to all Type strains as well as multiple clinical isolates of selected species.

Aim 1. To determine the pathogenic potential of Mycoplasma spp. isolated from seals (N=3 spp.), elephant seal (N=2 spp.), walrus (N=1 sp.) and sea lion (N=2 spp.). *Galleria mellonella* fifth instar larvae (N=50/group) will be infected by injection of 10^4 or 10^6 colony forming units into one of the last prolegs. Control larvae receive sterile SP4 broth, sterile phosphate buffered saline, or no treatment. *Galleria* (N=25/treatment group) monitored for 28 days for mortality as well as ability to transition to the next stage (larva to pupal stage; pupal stage to emergence) or has an arrested event (insect remained alive but did not successfully transition to next life stage). The remaining larva will be used to determine microbial load in the hemolymph at days 1, 3 and 5 PI; microbial load will be obtained using culture and RT-qPCR.

The **student's role** will include a background literature review coupled with weekly lab discussions on key papers in the literature, growth of the *Mycoplasma* spp. with assistance from lab personnel; injection of larva; daily monitoring and recording of the life stage and mortality events of *Galleria*; data recording and entry into excel file; assist with statistical analyses, figure and table preparations for manuscript, and presentation of findings.

Our group is well positioned to provide mentorship and guidance to a FVSP scholar to undertake these studies. We have access to the existing *Mycoplasma* spp. from marine mammals. The *Galleria* waxworm model is well established in our laboratory and we routinely perform all of the assays proposed. All needed equipment and supplies are available. The **expected outcome** is that this study will establish the virulence potential of Mycoplasma spp. from marine mammals. The **broader scientific impact** for the field is establishment of *G. mellonella* as an excellent surrogate model system to expand virulence testing to *Mycoplasma* spp., and potentially other newly described bacteria, from exotic and wildlife species where infection studies in the natural host are not feasible. The **expected educational outcome** is that the FVSP scholar will develop a strong understanding of rigorous scientific research and experience a climate of collaborative team science with mentors and other DVM FVSP scholars across the three mycoplasma laboratories.