Abstract of proposed student project (1 page limit. This should mirror the aims page of a grantand CLEARLY indicate the student's role.)

Bacterial meningoencephalitides (ME) are multifactorial lethal infections for which the adult human mortality rate of 20-30% has not been significantly reduced by modern intensive care, diagnostics or therapy. Fatality ratios are worst among the cases ultimately attributed to atypical pathogens. Sequelae among survivors include paresis, epileptic seizures, cerebral palsy, deafness and mental deficits. The diagnostic uncertainty and empirical treatment failures that precede those adverse outcomes cannot be expected to improve without more comprehensive knowledge of the etiologic agents involved.

Metagenomic surveys showed that the spectrum of bacteria associated with ME is much more extensive than is usually appreciated, including certain *Mycoplasma* spp. among the emerging neuropathogens. We were surprised to find that *Mycoplasma canis*, a commensal of the canine respiratory tract, was culturable from a majority of brain tissue specimens in fatal cases of canine idiopathic necrotizing (N) or granulomatous (G) ME. Our **overall objective** now is to fill gaps in basic knowledge about this organism's candidate virulence factors, the host responses they elicit, and their roles in the genesis of neurological disease. Our **central hypothesis** is that the significant prevalence of *M. canis* in brain tissue of dogs with NME/GME reveals it may be a primary pathogen. Because striking clinical and neuropathological parallels exist between canine and human ME, the **rationale** for this proposal is that new knowledge regarding the pathogenicity of *M. canis* will be generally applicable to delimit more accurately the atypical neuropathogens.

To overcome the exceedingly high barrriers to vertebrate animal experimentation for ME studies, we propose to pilot use of the invertebrate waxworm (*Galleria mellonella*) inoculation model as an alternative. The waxworm has a complete innate immune system, is a proven virulence model for many other bacteria, and can even discriminate intra-species strain virulence. Our focus on the pathogenic potential of *M. canis* represents a distinctly novel extension of the waxworm model in order to achieve the following **specific aims:**

Aim 1. To assess the pathogenicity of *M. canis*, waxworm larvae (n=20/group) will be injected with 10e2 or 10e6 colony-forming units of type strain PG14 from canine respiratory tract, and monitored daily for 1 month. To document colonization and infectious burden, hemolymph will be collected from the larvae until pupation and tested by culture/PCR for *M. canis*. Adverse Effects on larval mortality, pupation, and maturation will be evaluated using standard survival curve analyses versus uninoculated controls.

Aim 2. To assess potential strain differences among *M. canis* isolates, the larvae will be injected with strains LV (a neuraminidase-negative strain), and canine brain isolates G1 through G4, using the methods described for aim 1.

The **student's role** will include: background literature review; *M. canis* strain culture/PCR; waxworm injections; larvae monitoring; data recording; assist with statistical analyses; interpret findings; and present findings.

Our group is exceptionally well-positioned to undertake these studies because we possess *M. canis* isolates from canine brain tissue that are available in no other laboratory. We have already established the waxworm model in concurrent studies of other mycoplasmal diseases, including all necessary equipment, reagents, and biologicals. The **expected outcome** is an initial assessment of the utility of this invertebrate infection model to support better understanding of the virulence potential of *M. canis* and its role in canine NME/GME and other diseases.