An Epidemiological Study of *Mycoplasma species* Occurring in Cats From Shelters and Households Utilizing a Novel Pan-Myco Realtime PCR Detection and Differentiation Assay

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**Abstract:**

*Mycoplasma felis* are part of the normal flora of the conjunctiva and upper respiratory tract of one third of cats (Low 2007, Haesebrouck 1991). However, *M. felis* can be associated with feline disease complexes; feline conjunctivitis, and upper and lower respiratory tract infections (Holst 2010). Currently, *M. felis* was considered to be a co-pathogens commonly occurring along with Feline Herpes Virus 1 and *Chlamydia felis*. More recently, clinical data support the observation that *M. felis* is likely one probable cause of feline conjunctivitis and respiratory diseases occurring in 12.8-49% of cats with upper respiratory disease and conjunctivitis (Hartmann 2010 and Low 2007). Moreover very limited data is available on the detection and prevalence of other *Mycoplasma species* occurring in cats with upper or lower respiratory disease and conjunctivitis. A recent study which included a handful of samples (n=40 total) collected from household cats, shelter cats and farm cats with conjunctivitis was able to identify additional *Mycoplasma species* associated with clinical disease. Aside from *M. felis*, this pilot study identified *M. canadense, M. cynos, M. gateae, M. lipophilum* and *M. hyphoargyris* in feline samples. However, the role of additional *Mycoplasma species* play in disease syndromes and their occurrence in the clinically normal cats remains to be determined. This small study (Hartmann 2010) utilized nested PCR for detection of *Mycoplasma species* in clinical samples and various species detected were identified by DNA sequencing. This detection method is costly, time consuming and is not readily amenable to high throughput testing in a large scale epidemiological study to determine the role of other *Mycoplasma species* in feline respiratory disease and conjunctivitis. The this summer project the summer scholar will validate a new PCR method for detection and differentiation of *Mycoplasma species* occurring in cats and investigate the prevalence of these agents in cats with and without disease.

**Introduction:**

To facilitate large scale epidemiological studies to investigate the role of *Mycoplasma species* in various hosts, the mentor, Dr. Trujillo has developed a real time PCR assay to detected and differentiate pathogenic and commensal Mycoplasma species in biological samples (Trujillo 2009). The assay utilizes DNA binding dyes in place of fluorescent probes to detect amplicons generated in real time PCR utilizing PCR primers that bind to a conserved regions of the mycoplasma genome. However, between these conserved regions are sequences unique for each *Mycoplasma species*. Post PCR dissociation curves determine the Melt temperature (Tm) of the amplicons. Additionally this data is utilized in advance software analysis (Precision Melt, High Resolution Melt (HRM), BioRad) to provide a unique spectral signature characteristic of each *Mycoplasma species*. The TM and HRM are utilized to identify the Mycoplasma species present. If necessary further characterization is provided by determination of the amplicons size utilizing electrophoresis and DNA sequencing. This novel Pan-Myco SYBR PCR detection and differentiation assay has been utilized in studies detecting bovine, porcine and avian Mycoplasma species (Trujillo 2010). To date this assay has been validated for detection and differentiation of greater than 25 Mycoplasma species. This assay is designed to rapidly and affordably screen large numbers of samples (assay cost is less than $8 per sample), can detect multiple mycoplasma species within a single sample as well as detect Mycoplasma species occurring in atypical hosts or novel mycoplasma species.

**Project:**

In this study the veterinary student summer scholar will utilize this assay in an epidemiological study to determine the prevalence and diversity of *Mycoplasma species* occurring in the respiratory tract and conjunctiva of normal and clinically affected shelter cats and household cats presenting at the Veterinary Medical Center (VCM) at Iowa State University (ISU). The roles of the summer scholar is in the project is underlined.

This study will address the hypothesis that *Mycoplasma species* in conjunction to *Mycoplasma felis* are associated with feline respiratory disease and conjunctivitis.

Previous research has shown that upper respiratory tract infections are a serious problem within feline shelter populations occurring at rates exceeding greater than 56% in a multi-shelter study (Bannasch 2005). The study hypotheses will be addressed in two specific aims which will be completed by the summer scholar.

**Aim one:** Validation of the Pan-Myco PCR assay for the detection and differentiation of Mycoplasmas species previous reported in cats.
Sequence alignment of the primes utilized in the Pan-myco PCR assay demonstrate that these primers will amplify *M. felis*. For assay validation commercially available strains of *M. felis* and other *Mycoplasma species* list above available from ATCC will be utilized for development of profiles for each *Mycoplasma species*. This aim will be completed by the summer scholar. These profiles will then be utilized in aim two to identify Mycoplasma species detected in clinical samples.

**Aim 2: Determine the prevalence of *Mycoplasma species* occurring normal cats and cats with respiratory disease and conjunctivitis.**

 Conjunctival, nasal and deep oro-phargeal swabs will be collected for cats upon admission in to participating Animal shelters and cats presenting at the VMC at ISU (and potentially other participating veterinary hospitals). Clinical disease scores will be determine at the time of sample collection utilizing the clinical scoring scale developed by (Hartmann et. al., 2010). Cats that are within shelters for longer than seven days will be rescored for clinical signs and repeat samples will be collected. At a minimum 250 cats will be sampled for this study. It is anticipated that at least 60% of these cats will have URD or conjunctivitis. The summer scholar with assist in sample collection and clinical scoring, testing of collected samples, data compiling and preparation of a poster presentation and manuscript.

**Project Significance:**

The study will provide the necessary validation of the Pan-Myco PCR for detection and differentiation of *Mycoplasma species* occurring in cats which will facilitate the translation of this assay as a tool for rapid cost effective etiological diagnosis of feline respiratory disease and conjunctivitis which allow for rapid treatment and management of options for Mycoplasma infections. This is especially important for shelter populations as money and time are often critical components of infectious disease management. The results will aid with creating appropriate risk assessment, management and treatment protocols as well as identify additional *Mycoplasma species* involved in the feline respiratory disease and conjunctivitis. The study is applicable to the One Health initiative to foster and support research investigating transmission of infectious diseases at the human – animal interface. Since some *Mycoplasma species* occurring in cats are reported to be zoonotic (McCabe 1987), this study will aid in determining if felines are carriers of humans mycoplasma pathogens or if they are susceptible to pathogenic Mycoplasma species occurring in humans or other domestic species.

**Project Mentor:**

Dr. Jessie Trujillo from the Department of Veterinary Microbiology and Preventative Medicine, at Iowa State University will serve as a faculty mentor in this project training, overseeing and advising the student and the project. Dr. Trujillo’s laboratory is fully equipped to successfully perform testing proposed in this study and has research dollars cover all costs of this study. Dr. Trujillo is the original inventor of this assay and has overseen multiple epidemiological applications of this assay in various hosts and has extensive experience in diagnostics medicine and epidemiological studies some particularly related to feline medicine (please see the attached biosketch). Dr. Trujillo mentored three summer scholar veterinary students in the summer of 2010, one project validated this assay for use in detection of swine mycoplasma species. Dr. Trujillo also serves as a mentor for two PhD graduate students and two undergraduate students.

**Other Research and Program Opportunities:**

As a member of the ISU Veterinary Summer Scholars Program, a program designed to introduce students to research, critical thinking skills and the scientific process including data collection and analysis, experimental design, and scientific writing. The program will include several opportunities for learning including seminars in experimental design, animal use and care, bioethics/scientific integrity, statistics, and poster preparation. There is also a weekly journal club with both the Summer Scholars program as well as within Dr. Trujillo’s lab. The highlight of the ISU summer scholars program is the CVM research day, where students present their research in poster and awards for the best poster presentation are selected. All of these activities are designed to expose student to biomedical research and improve student’s presentation, communication, and critical thinking skills. I am also encouraged and hope to have the opportunity to present my findings as a peer reviewed manuscript and in further presentation at a national meeting.

**Animal Involvement:** This Study will utilize cats presenting at the VMC and cats at animal shelters. No cats will be infected with pathogens during this study. All samples collected at classifieds non-invasive and non-painful by the ISU Animal care and use committee (project approval pending). Moreover, results of this study will be provide to patient and animals care takers to aid in the medical treatment of the cats, and thus greatly benefiting the health and welfare of participants.
References:


Trujillo, JD., Nara, PL. Novel SYBR real-time PCR assay for detection and differentiation of Mycoplasma species in biological samples from various hosts. Conference of Research Workers in Animal Disease, December 7, 2010
