GRANT PROGRESS REPORT REVIEW

Grant: 01352-A: *Detection of Brucella canis DNA in canine urine, semen and vaginal cells via qPCR Analysis*

Principal Investigator: Dr. Lin Kauffman, D.V.M.

Research Institution: Iowa State University

Grant Amount: $12,960.00

Start Date: 8/1/2009 **End Date:** 7/31/2010

Progress Report: 12 month

Report Due: 7/31/2010 **Report Received:** 7/31/2010

Recommended for Approval: Approved

(Content of this report is not confidential. A grant sponsor's CHF Health Liaison may request the confidential scientific report submitted by the investigator by contacting the CHF office. The below Report to Grant Sponsors from Investigator can be used in communications with your club members.)

Original Project Description:

Background: Brucella canis is a bacteria that causes reproductive disorders in dogs. The number of B. canis cases has been on the increase across the US and specifically the Midwest over the past several years with increasing costs associated with reproduction losses due to disease and euthanasia of infected animals. Currently the only available tests for this disease are microbiological cultures and serological testing. Microbiological culture of the blood, vaginal secretions, semen or fetal placenta is the diagnostic gold standard since a positive culture confirms the disease. Unfortunately the organism is hard to culture under the best conditions. Serological tests detect the disease 8-12 weeks postinfection and cannot detect early disease, from the time of infection until when there are detectable levels of anti-Brucella antibodies. At present, laws make canine brucellosis a reportable disease subject to quarantine. In some states this means testing all breeding animals and euthanasia of infected animals. The economical impact of quarantine and repeated testing is such that there is potential for individuals to have their dogs tested out of state to avoid quarantine, sell known infected animals and/or to medically treat potentially infected animals to avoid detection with current tests. Brucella canis has infection potential to humans, especially the very young, elderly and immuno-compromised individuals. Currently there is no early detection testing available for this disease.

Objective: The goal of this project is to use the Polymerase Chain Reaction (qPCR) based assay to detect Brucella sp. in field samples of suspect B. canis urine (male), semen and vaginal cells

and compare how well this test works verses traditional culture and serology. Early detection of disease would shorten the quarantine period and would add a measure of safely for buyers of puppies or breeding stock.

Grant Objectives:
Objective 1: Test suspect B. canis positive field samples of blood, urine, semen, and vaginal cells via our qPCR assay, and whether the dog is symptomatic along with documented clinical signs

Objective 2: Test the same suspect B. canis positive field samples of blood and serum via culture and serology

Objective 3: Statically compare results of qPCR with serology and culture to see if qPCR can detect positive dogs prior to seroconversion or positive culture

Objective 4: Determine the best, least invasive, samples to submit for the male and female in order to diagnose Brucellosis early and with high sensitivity (decrease false negatives)

Publications:

Report to Grant Sponsor from Investigator:
Brucella canis, a bacteria that causes reproductive disorders in dogs, has been on the increase across the US. This goes hand in hand with increasing costs associated with reproduction losses due to disease and euthanasia of infected animals. The potential for human infection from dogs increases concern over this infectious disease. Currently the only available tests for this disease are not great. The bacteria is hard to culture and serological tests detect the disease 8-12 weeks post-infection but cannot detect early disease. Present laws make canine brucellosis a reportable disease subject to quarantine. In some states this means testing all breeding animals and euthanasia of infected animals. Early detection of disease would shorten the quarantine period and would add a measure of safely for buyers of puppies or breeding stock. The goal of this project is to use Polymerase Chain Reaction (qPCR) assay to detect Brucella sp. in samples of suspect B. canis urine (male), semen and vaginal cells (female) and compare how this test works vs. traditional culture and serology.

To find a more specific and timely diagnostic, this study assessed the ability of qPCR analysis to detect B. canis Omp25 DNA in a variety of samples (blood, urine, vaginal swab) and compared those results against current detection methods for B. canis infection in dogs (serology). qPCR analysis identified the presence of B.canis Omp25 DNA in multiple dogs prior to seroconversion. Non-invasive samples from the genito-urinary tract, including vaginal swabs and urine, were found to be the most sensitive for detection of B. canis Omp25 DNA via qPCR. Use of these samples would make collection of diagnostic samples within the ability of some dog owners and breeders.

The results of this study are very encouraging for use of B. canis Omp25-specific qPCR as a diagnostic screening tool for B. canis. The potential of this assay qPCR for early detection could be very valuable for elimination of B. canis from kennels without having to wait for seroconversion. Additionally, Omp25 qPCR could be a valuable screening tool for B. canis in

newly purchased dogs prior to adding these dogs into a new home or kennel. B. canis is a reemerging infectious disease in the canine breeding industry. A better screening and detection method will be very useful to prevent further spread of this insidious disease. B. canis Omp25 qPCR may be this critical diagnostic component to decrease economic effects of canine brucellosis on the canine breeding industry and prevalence of canine brucellosis in the US.