AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2019 – 2021

TITLE: Variable antigen production in *Chlamydia abortus* from Oregon sheep

RESEARCH LEADER: Dan Rockey

COOPERATORS: None

EXECUTIVE SUMMARY: Ovine Enzootic Abortion (OEA) is a disease associated with infection of pregnant ewes by the bacterial pathogen *Chlamydia abortus*. This pathogen is present in sheep flocks in most countries and can be a very significant burden both in terms of sheep health and economic productivity. The challenge of OEA is felt locally as well: *C. abortus*-induced abortion of sheep is a common problem in the Willamette Valley and throughout most of the western USA. Vaccines are available but there are significant concerns both about their efficacy and, in the case of the live attenuated vaccine, their safety to sheep and to pregnant farm workers. In this project we are examining the hypothesis that poor vaccine efficacy is a function of genomic attenuation during culture of the pathogen *in vitro*. To explore this, we have developed an egg-culture system that will allow full genome sequencing of the pathogen after culture in embryonated eggs and in cell culture. We will compare these sequences and determine if there are differences that are a function of the different culture conditions. If differences are identified, we will examine the specific proteins involved and ask whether they might be important proteins that function in protection against infection and disease.

OBJECTIVES:
1- Generate cultured lines of *C. abortus* taken directly from sheep tissue in hen eggs and cell culture

2- Assess genomic differences between these samples through both gene-specific and genome sequencing technologies.

3- Examine antigenic profiles of chlamydial samples cultured either in eggs, in cell culture, or directly from placentas

PROCEDURES: Although we put a lot of effort into the egg culture work, the results were not impressive, and we focused more actively on cell-culture grown and in-vivo-produced *C. abortus* strains. Therefore, the most important procedures involved culture of chlamydiae and subsequent genome sequence analysis. For this we used the sequencing core facility within the OSU Center for Genomics and Biocomputing to generate and analyze sequence data. We also used immunofluorescence microscopy to examine sheep sera from infected flocks.

SIGNIFICANT ACCOMPLISHMENTS:

Genome sequence analysis of a variety of different *C. abortus* strains. The most noteworthy result is that a strain of *C. abortus* was collected from a fetus that was virtually identical to the vaccine strain used successfully on that farm. This shows that, even in the face of significant protection from disease, virtually identical strain can persist in animals on a specific farm. But, there is no disease on the farm, and thus the vaccine is protecting the sheep. The vaccine strain can be made available to any producer who wishes to use it, or perhaps to a company that wants to work on commercializing the product. There is also a tremendous opportunity here for exploring why this vaccine works and available commercial vaccines do not work so well, even though the preparation of the vaccine is generally similar in both cases.

Creation of clones of individual strains that showed antigenic variation is a constant process and individual unique clones cannot be cloned out of a population.

Established lines of communication among sheep producers that led to the successful creation of a fully sequenced *C. abortus* strains that protects against infection on different farms.

The project helped a graduate student in her thesis work, and two publications addressing *C. abortus* biology are nearing submission.

BENEFITS & IMPACT: The most significant success of this work is the characterization of the novel strain that is protective on the different farms. This has allowed a significant reduction in antibiotic use by the farm, as they do not need to feed pregnant ewes tetracycline to avoid abortion problems.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: We have received two NIH awards that address similar subjects as those covered here. These are R21 AI144865 and R03 AI156514.

FUTURE FUNDING POSSIBILITIES: This is challenging: I have pursued discussions with other farms with goals of trying to test the vaccine in different settings and they are generally not interested. The use of tetracycline is a commonly accepted way to keep abortion losses to an acceptable level. The farm from which the vaccine strain was generated is not interested in pursuing the vaccine as a product. I have not been able to identify other sources of funding to generate and test a vaccine in a case-controlled setting.

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