Development of a New Assay for Diagnosis of Brucella abortus Infections in Wyoming Livestock

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Introduction
Bovine brucellosis is one of the world’s most widespread human diseases and still causes problems to domestic livestock producers in Wyoming and the other two states surrounding the Greater Yellowstone Area (GYA), Idaho and Montana. In the U.S., the Cooperative State-Federal Brucellosis Eradication Plan, initiated in 1934, was successful in eradicating brucellosis from cattle populations. However, the disease still has a reservoir in elk and bison in GYA with multiple instances of spillover into cattle herds on private and public lands within GYA in the last decade. Positive cases in livestock lead to quarantines that can cost the producer upward of $254,000 based on an analysis of a “typical” Wyoming beef cattle operation.

Additionally, producers may elect, and in some cases be required, to cull their herd and submit them to imperfect and time-consuming diagnostic testing. Producers who are located in the Designated Surveillance Area (DSA), which spans Wyoming, Idaho, and Montana, are also required to undertake increased testing requirements prior to shipping cattle to some specific states. This applies to producers not under quarantine, but who conduct their livestock operations within the DSA.

Objectives
The objective of this study was to develop a new, more accurate assay to detect brucellosis in the tissues of affected animals.

Materials and Methods
For this project, we conducted the most in-depth computer analysis of Brucella spp. ever performed using genetic sequences acquired from the U.S. Department of Agriculture’s (USDA’s) National Veterinary Services Laboratories. This analysis revealed potential targets for diagnostic testing. These targets went through an extensive screening process, ultimately leaving eight candidates for full optimization and validation.

In parallel to this process, we wanted to make sure that the methods used to extract Brucella DNA from tissue samples produced the highest yield as the bacterium is presumed to exist in small numbers in these samples. We screened six different commercial extraction kits against blood and its fractions and all standard tissue types. The kits that we are using on field samples were taken directly from this analysis.

Results and Discussion
Through this project, we have been able to acquire samples from 87 suspect/reactor animals (18 cattle and 69 bison). Of these animals, only 42 (48.3%) were culture positive despite all of the animals being suspect or
positive on the standard blood test for Brucella exposure. On these animals, our top polymerase chain reaction (PCR) candidate set is currently detecting Brucella DNA on 77 (88.5%) of the animals tested. This indicates that our PCR assay has the ability to detect almost twice as many animals as the current gold-standard assay, bacterial culture. Thus far, on seronegative cattle, elk, and bison located outside of the endemic area, our assay has 100% specificity (n=51). With our development of vaccine-specific primers-probe sets, we are able to differentiate vaccine-related infections from field infections.

The potential to replace the current gold-standard diagnostic test of culture with a more sensitive test could decrease the cost of an outbreak in livestock. Additionally, PCR testing of a suspect animal is about one-quarter of the cost of culture and can be completed in a few hours, in comparison to 10–14 days with culture. This means producers and veterinarians can have results the same day samples arrive at the lab. This assay is still in the research phase, and validation is expected in the next year.

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