AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2014 – 2016

TITLE:

Development of a milk-based rapid field test for the high-sensitivity detection of *Salmonella* dublin infected dairy cattle to expedite disease control in endemically-infected dairies

RESEARCH LEADER:

Elain Fu, PhD, Assistant Professor, School of Chemical, Biological, and Environmental Engineering, Oregon State University

COOPERATORS:

Claudia Häse, PhD, OSU Department of Biomedical Sciences, Joy Waite-Cusic PhD, OSU Department of Food Science and Technology, Keith Poulsen, DVM, PhD, DACVIM, formerly in the OSU Department of Clinical Sciences

SUMMARY:

Salmonella dublin infection of dairy cattle can lead to significant animal mortality and reduced dairy production. Current testing, using laboratory-based ELISA, is expensive and has a long time to result that does not allow for easy identification of diseased animals. A rapid, sensitive, and easy-to-use field test for *Salmonella* dublin that is compatible with milk samples would enable better management of diseased animals and could reduce animal mortality and economic loss due to the disease.

OBJECTIVES:

1) Design and fabricate a paper-based *Salmonella* dublin antibody test.
2) Characterize the response of the paper-based test using bovine milk samples for performance comparison of the paper-based test to that of the ELISA test.

PROCEDURES:

The paper-based test has a similar assay format to that of the laboratory-based ELISA for *Salmonella* dublin detection, namely capture of bovine antibody (IgG) to *Salmonella* dublin using purified lipopolysaccharide. The detection species is an anti-bovine antibody that is conjugated to a gold nanoparticle label.

Multiple strains of *Salmonella* dublin were cultured and the LPS extracted in purified form using standard protocols. Inactivated whole bacteria were also prepared and its use as a capture species was assessed, but found to not be robust.

Test cards were fabricated using a CO_2 laser to cut the components to the desired sizes, and the components were then assembled. Materials consisted of nitrocellulose for the main channel of the test including the detection region, cellulose for the wicking pad, glass fiber for the source pad, and plastic laminate served as the overall substrate. A small volume of the capture species, at high concentration, was deposited into the detection region using a pipette and allowed to dry overnight in a dessicator. The patterned nitrocellulose was then treated with a “blocking” solution containing nonspecific proteins to coat the remaining bare nitrocellulose and dried overnight in a dessicator. The addition of surfactant was found to be a critical component of the running buffer.

Milk samples were diluted ten-fold to achieve a viscosity within the acceptable range for on-device processing and another ten-fold to achieve an acceptable level of background. Test performance was characterized using bovine colostrum samples collected from an endemically-infected Oregon dairy and independently analyzed using laboratory-based ELISA for *Salmonella* dublin content. The paper-based assays were run using multiple negative and positive samples of colostrum. Image data of the detection region of each test was collected using a desktop scanner for further analysis of signal intensity.

SIGNIFICANT ACCOMPLISHMENTS:

Sample acquisition and characterization. Seventy-six colostrum (and blood) samples were collected from cattle on an Oregon dairy farm that was endemically-infected with *Salmonella* dublin. These samples were characterized using laboratory-based ELISA. We have six positive samples and 66 negative samples banked for future work.

Paper-based test development. A paper-based test for *Salmonella* dublin detection was designed, implemented, and evaluated using the bovine colostrum samples described above. Test development included sourcing and charactering appropriate reagents, defining the protocol for the assay, and evaluating the positive and negative bovine colostrum samples using the current version of the paper-based assay. The paper-based test results were in agreement with the ELISA results for a subset of the positive and negative bovine samples tested. However, for some samples deemed negative for *Salmonella* dublin by ELISA, the paper-based test produced a high level of nonspecific background which would be interpreted as positive results. Additionally, for some samples deemed positive by ELISA, the paper-based test response was not significant and would be interpreted as a negative result. Thus, further work is needed to improve the accuracy of the paper-based assay.

BENEFITS & IMPACT:

A rapid, sensitive, and easy-to-use field test for the detection of *Salmonella* dublin infection of dairy cattle from milk samples could have multiple benefits. Use of the test to identify diseased cattle could reduce (1) the spread of infection (animal-to-animal transmission and transmission to humans) and (2) the economic burden of this disease with respect to decreased dairy production and loss of dairy cattle.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

None

FUTURE FUNDING POSSIBILITIES:

We plan to apply for follow-on funds using the preliminary data generated on this project. Potential funding agencies include the USDA.