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BEEF CATTLE RESEARCH COUNCIL
RESEARCH & TECHNOLOGY DEVELOPMENT FOR THE CANADIAN BEEF INDUSTRY

Beef Science Cluster

Developing efficient, multi-pathogen tests for common cattle diseases

Project Title:		Project Code:	ANH.13.13
Development of a fully-automated DNA microarray-chip for multiplex detection of bovine pathogens		Completed:	*2016*

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Background

Respiratory and enteric diseases are the most common and costly diseases in beef cattle. Both are multi-factorial disease complexes involving several viruses and bacteria. Effective control of these diseases can benefit from rapid and cost-effective diagnostic tests that can simultaneously detect all relevant pathogens in a single assay. Current diagnostic tests for these diseases are primarily single pathogen tests and are thus inefficient and require separate tests for each pathogen. DNA microarrays, combined with multiplex PCR, are capable of highly sensitive detection and differentiation of multiple pathogens in a single sample.

Objectives

To develop two cost-effective microarray chips that can be used on a new fully- automated technology platform - one for rapid identification of bovine respiratory disease pathogens and one for bovine enteric pathogens.

What they did

These researchers used genetic (DNA or RNA-based) techniques to identify microbes instead of the traditional approach of trying to grow microbes in the lab. The first technique (called polymerase chain reaction, PCR) involves finding one or more genetic sequences that correspond to a particular microbe and selectively duplicating or amplifying them many times so that they can be detected. Many unrelated microbes share similar genetic sequences, so the researchers were careful to design tests that were specific to genetic sequences only found in the targeted microbes. This ensured that the PCR would only amplify genetic

sequences from the specific microbe they were looking for (if they were there) but not microbes that are closely related but harmless. It also allowed them to distinguish between commensal and pathogenic strains of the same microbes. The second step was to develop a system to confirm and further characterize the amplified genetic sequences from the PCR step.

Separate PCR amplification and detection tools were developed and tested for each microbe known to be involved in respiratory disease or diarrhea. Next, the tools were combined into four separate tests to amplify and detect genetic sequences from respiratory disease bacteria (Mannheimia, Histophilus, Pasteurella and Mycoplasma), respiratory viruses (PI-3, BRSV, IBR, coronavirus, BVD types 1, 2 and 3), diarrhea bacteria and protozoa (Clostridium, Salmonella, E. coli, and coccidiosis organisms), and diarrhea viruses (rotavirus, torovirus, coronavirus, BVD types 1, 2 and 3). Finally, the respiratory bacteria and virus assays were combined into one fully automated test, the diarrheal bacteria, protozoa and virus assays were combined into a second test, and both tests were evaluated using archived lab samples.

What they learned

The new tests were highly specific, meaning that they only detected the specific microbes they were designed to detect, so false positives were rare. For instance, the test could distinguish between commensal Mannheimia and pathogenic Mannheimia, between harmless E. coli and enterotoxigenic or enterohemorrhagic E. coli, and between different types of BVD viruses. The new tests were also highly sensitive. This means that if the microbe the test was designed to find was in the sample even in low-numbers, the test found it, so false negatives were also rare. In fact, the new tests found some microbes that the original culture-based lab tests hadn't detected. That can happen when microbe numbers are very low, or when they are very difficult to culture.

What it means

Pending further validation and commercialization, the new tests are expected to cost considerably less than other currently available genetic-based diagnostic tests. They can generate results in six to eight hours, compared to one to two weeks for traditional culture approaches. This isn't fast enough for real-time chute-side treatment decisions but may help veterinarians respond more quickly to herd health outbreaks in the future, and to further refine responsible antibiotic treatment decisions in cattle herds.

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