RESEARCH

Development of recombinase polymerase amplification (RPA)–based innovation platform for rapid detection of antimicrobial resistant (AMR) bacterial pathogens that cause bovine respiratory disease (BRD)

NEW DIAGNOSTICS TO INFORM ANTIMICROBIAL TREATMENT DECISIONS

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Background: Bovine respiratory disease (BRD) remains the most common and economically important disease affecting feedlot cattle. About 15% of cattle in North America are treated for BRD, and it accounts for about 70% of the illnesses and 40% of deaths. Economic losses to the North American beef industry exceed $1 billion dollars annually.

Bovine respiratory disease is characterized by complex interactions between the host cattle's immune system and bacteria (i.e., Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, and Mycoplasma bovis) and viruses (i.e., Bovine Herpes Virus-1, Parainfluenza-3, Bovine Viral Diarrhea Virus, Bovine Respiratory Syncytial Virus). Suppression of the host immune system as a result of stress or viral infection can allow pathogenic bacteria to proliferate within the upper respiratory tract, spread to the lower respiratory tract and cause BRD.

Clinical symptoms associated with BRD are usually non-specific and generally occur within the first 40 days after arrival at the feedlot. Controlling BRD is one of the primary reasons for the use of antimicrobials in feedlot cattle. Frequently, all high-risk cattle are treated with antimicrobials upon arrival at the feedlot in an effort to control this difficult to diagnose disease. This strategy improves the welfare of the entire lot and can decrease financial losses arising from BRD. The decision to use antimicrobials and the type of antimicrobial selected is often based on the perceived risk of the cattle developing BRD.

However, antimicrobial use can also select for antimicrobial-resistant (AMR) bacteria. If these antimicrobial resistant bacteria persist within the herd or the feedlot environment, they may reduce the ability of antimicrobials to treat or prevent BRD. Our laboratory has isolated BRD-causing bacteria that are resistant to more than 10 different antimicrobials.

Judicious use of antimicrobials can result in a reduction in the development of antimicrobial resistance in bacteria, while increasing their effectiveness at controlling disease. Effective antimicrobial stewardship requires that the right antimicrobial target the right bacteria at the right dosage in the right animal at the right time.

Consequently, the ability to identify the bacteria that is causing BRD and potential antimicrobials that it may be resistant too, is a key step towards more effective use of antimicrobials. Without the ability to quickly, easily and cost-effectively detect which bacteria are already resistant to a particular antimicrobial, feedlot operators and veterinarians run the risk of administering a treatment which will be ineffective due to AMR.

Currently, identifying bacteria and determining whether they are resistant or susceptible to an antimicrobial requires isolation of the bacteria, laboratory culture, and finally, testing for resistance to the various antimicrobials used to

treat BRD. However, traditional culture methods are time-consuming, and it takes several days to determine if they are antimicrobial resistant.

Advancements in microbial genomics now makes it relatively easy to sequence the entire genome of individual bacteria, including those that cause BRD. Bacteria cause disease and become resistant to antimicrobials because they acquire genes that allow them to survive antimicrobial exposure. Using genomic detection methods, it may be possible to detect sequences of DNA that both identify pathogens and the genes which cause AMR. Using genomic technologies instead of culture and resistance testing could reduce diagnostic time from days to minutes. Such a development would enable veterinarians to precisely choose the antimicrobials that will be most effective against BRD bacteria, improving clinical outcomes for cattle at risk of developing or have acquired BRD.

Objectives:

- Design and screen the appropriate DNA primers and optimal temperatures for detection of BRD pathogens and common AMR genes.
- Develop a test that can detect multiple pathogens and AMR genes at the same time.
- Determine the specificity (true positive rate) and sensitivity (true negative rate) of the test under practical conditions in both healthy cattle and those diagnosed with BRD.
- Evaluate the performance of the test's ability to correctly detect BRD pathogens and AMR profiles.
- Develop a portable test kit and validate it under field conditions in a commercial feedlot.

What they did: At Agriculture and Agri-Food Canada in Lethbridge, the research team modified a genomic technique used in human medicine known as recombinase polymerase amplification (RPA). RPA was able to detect the bacteria that cause BRD in less than 30 minutes.

By sequencing the four main bacteria associated with BRD, Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, and Mycoplasma bovis, they were able to design molecular "probes" that target a small unique section of DNA that is specific to each bacterial species, allowing specific identification. As not all types of Mannheimia haemolytica cause disease in cattle, Mannheimia probes were designed to only detect those subtypes responsible for BRD in cattle.

Many of the genes that cause AMR in BRD-causing bacteria are identified, so probes were constructed to detect the genes that make bacteria resistant to several of the antimicrobials used to control BRD, including Micotil, Draxxin, Zuprevo, Nulflor, Trimidox, Terramycin and Aureomycin.

Probes were also designed to detect a region of DNA known as "mobile genetic elements known as ICE." These ICE transfer antimicrobial resistant genes from one bacterium to another, turning bacteria that would normally be killed by antimicrobials into bacteria that are resistant. If these elements are circulating within bacterial populations that cause BRD, it could become much more difficult to treat this disease in cattle.

All of these probes were tested on deep nasal swabs collected from 100 head of feedlot cattle.

What they learned: The RPA assay was able to correctly detect and identify all 4 causative BRD bacterial species in a single assay. The assay was shown to be 100% specific for these causative bacteria and did not detect other bacteria that normally live in the nasal passage of cattle. The assays could also detect seven different genes that code for resistance to the antimicrobials listed above. We could also reliably detect the mobile genetic element responsible for transferring antimicrobial resistance among bacteria. Typically, mobile genetic elements were found in conjunction with the bacteria responsible for BRD, suggesting that BRD-causing bacteria are exchanging genes in order to become resistant to a broad range of antimicrobials. Most of these assays could be performed within a period of 30 minutes.

What it means: Additional improvements will be needed in order to use RPA as chute-side diagnostic tool. Further research into these refinements is already underway in a new project funded by Genome Canada. It is hoped that these assays can be applied as part of a risk assessment program to predict the potential for certain lots of cattle to develop BRD and identify which antimicrobials will be most effective for controlling this disease. As the current assays demonstrated a high level of accuracy in identifying BRD-causing bacteria and AMR related genes, once optimized further, RPA presents a significant opportunity to advance and improve BRD diagnostics.

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