Next Generation Sequencing

New diagnostic testing available to identify bovine respiratory disease complex.

*by **Kasey Brown,** senior associate editor*

Bovine respiratory disease (BRD) is the bane of feedlot managers and cow-calf producers alike. It is one of the most costly diseases plaguing the cattle business today, and results in lost performance, decreased animal welfare and lost profits. Ben Hause, clinical assistant professor in the College of Veterinary Medicine at Kansas State University (K-State), said there has been a widespread use of modified-live virus (MLV) vaccines for more than 20 years, but the incidence of the disease is actually increasing.

He noted many combinations of live or inactivated vaccines are used, including bovine viral diarrhea virus, bovine herpesvirus 1, parainfluenza virus 3, bovine respiratory syncytial virus, *Mannheimia haemolytica, Histophilus somni, Pasteurella multocida* and *Mycoplasma bovis.* However, BRD is incredibly complex in terms of factors relating to the host, environment and pathogen, making it difficult to even identify the source of the problem to combat it.

Hause spoke to attendees of the National Institute for Animal Agriculture conference's bovine committee in Kansas City this April. He explained a new form of testing called next generation sequencing (NGS), which is being researched to identify viruses in feedlot cattle.

Bug hunting

NGS generates a massive amount of DNA sequence data, especially compared to the previously used Sanger method of sequencing. NGS can generate millions of 300-base-pair reads from a sample. In contrast, traditional Sanger sequencing generates a single read of about 800 base pairs. NGS can be sequence-dependent or sequence-independent, which means that researchers don't necessarily have to know what they are sequencing. This is unique to this new technology, and Hause called it "bug hunting." For research, this means we may find things for which we didn't know we needed to look.

Compared to traditional diagnostic investigations, NGS can be more cost-effective. For $300 it can identify and characterize viruses and bacteria in a sample. Traditional diagnostic testing such as bacteriology, virology, pathology and molecular testing can often exceed this amount. It also has the ability to detect multiple viruses or quasispecies present in a sample. Additionally, samples can go straight from the animal, like a nasal swab, to the lab for sequencing.

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To put this into practical use, Hause shared data from a study that looked at whether NGS could be used to identify pathogens associated with BRD. The experiment looked at five feedlots in Mexico and five feedlots in the United States with 500- to 800-pound (lb.) calves. Nasal swabs were collected from five animals with acute respiratory disease and five healthy penmates at each feedlot and then submitted for viral metagenomic sequencing.

The results identified about 25 viruses, which he likened to "virus soup." Bovine rhinitis virus A was the most common virus present, and bovine coronavirus was also prevalent. However, Influenza D virus was the only virus that was even moderately statistically correlated with BRD, Hause noted.

The research team used a polymerase chain reaction (PCR) test to verify the influenza D virus results. He added that the sensitivity of NGS was on par with PCR.

He noted that another group in California also used NGS to examine pathogens involved in BRD. The California team found bovine adenovirus, bovine rhinitis A virus and bovine influenza D virus had significant association with BRD. These were detected either alone or in combination in 62% of the animals with BRD.

What these two studies show is that NGS using metagenomic-sequencing methods is a powerful assay for veterinary diagnostics. Besides identifying viruses, it can often determine the complete genetic code of the virus, Hause said, for an isolated virus and directly from clinical samples with a sufficient viral titer. It can be used for cases with unknown etiology or unusual clinical presentation, like clinical symptoms with an absence of the usual suspects.

It can be used to profile pathogens in individual animals or herds and is being used by the swine industry to examine samples used for live exposure to rotavirus and porcine epidemic diarrhea virus. It is a more affordable option than multiple PCR tests, and Hause recommended more widespread use of NGS in the future.

More information on this study can be found in the May 5, 2016, issue of the *Journal of General Virology.*

Even newer option

Hause introduced an even newer possibility, admitting that it is much farther down the road in terms of implementation. AmpliSeq is a hybrid between metagenomic sequencing and PCR panels. It is a highly multiplexed PCR coupled to NGS that is widely used for cancer/inherited disease panels in humans. The question is whether it can be adapted to use for pathogen detection and characterization, Hause noted. There may be potential for an assay to detect and characterize BRD viruses and bacteria.

A challenge for this technology is that the targets are highly variable, requiring redundancy in assay design. Targets may often be present in low amounts, making detection more difficult.

AmpliSeq benefits include low cost — a sample run on AmpliSeq would cost the laboratory about $50 — with a quick turnaround time of one or two days. Data analysis is also much simpler than with metagenomic sequencing. However, being a targeted approach, it could miss divergent pathogens, pathogens not targeted or novel agents.

Hause noted that their goal is to create a highly multiplexed assay for BRD detection and genetic characterization.

The proof of concept is successful, though more development and characterization is required in terms of ability to detect all viruses, ability to detect variant viruses, sensitivity, specificity, and efficiency and user friendliness.