

Diagnosing bacterial pneumonia in cattle

By John Gill and David Tisdall

When it comes to identifying pathogens, multiple case studies show that PCR is far superior to standard cultures.

Bacterial pneumonia develops in cattle after viral infections, which are initiated by the stress of transport and mixing with other animals, disrupt and suppress the immune system. This allows bacteria that are normally present in low numbers in the upper respiratory tract to take over. The most commonly encountered bacteria are *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni* and *Trueperella pyogenes*.

Until recently, standard cultures were used to identify these pathogens, but they often produced variable results because of poor sample quality. The heavy mixed growth of contaminating bacteria is frequently identified because of contamination during necropsies, the invasion of postmortem bacteria or high environmental temperatures during transit to the laboratory. Bacterial die-off because of antemortem antibiotic treatment is another cause of poor culture results. A reasonably common bacterial pathogen in New Zealand – *Histophilus somni* – is often difficult to isolate in culture because it is a slow-growing bacterium easily swamped by faster-growing bacterial contaminants.

New molecular techniques, like real-time PCR, can provide answers to this culture problem, as they not only are designed to detect specific pathogens but also can be quantitated by the provision of specific quantification cycle (cq) values. The lower the cq value, the higher the concentration of this pathogen and the increased likelihood that the pathogen is significant. PCR is also suitable for testing samples from animals who have been dead for a number of hours and those who have been treated with antibiotics, as PCR is not affected by autolysis, postmortem invaders or non-viable agents.

Multiplexing – combining an array of PCRs from multiple bacteria and viruses from one extraction – also helps to reduce costs.

Sampling

- ▶ **Live affected animals:** pooling dry nasal swabs from several affected cattle and running a pooled test works well, especially for some viral conditions like infectious bovine rhinotracheitis (IBR). Testing fluids from trans-tracheal washes may also be useful in live affected animals.
- ▶ **Necropsy sampling:** dry swabs from the airways of affected areas of lungs or small blocks of affected parenchyma can also be used, even from cattle who have been dead for 24 hours or more, depending on the degree of decomposition.

Preservation methods are not necessary.



Clinical cases of bacterial pneumonia where PCR has been used for identification

Case 1

Thirty-four 12-week-old beef calves from a mob of 300 died in a one-month period. Bacterial cultures of a lung from an initial case grew *Trueperella pyogenes* as the only significant isolate (from a mixed growth) and a bacterial bronchopneumonia was identified on histopathological examination.

Fresh affected lung samples from two more dead calves were submitted for a multiplex PCR for bacterial and viral pathogens. Large numbers of *Histophilus somni* were identified in the lungs of both calves. No other bacterial or viral pathogens were identified.

Case 2

An adult dairy cow was found with severe dyspnoea at morning milking. Within an hour she collapsed and she died shortly after that. Necropsy showed a severe consolidation of the cranioventral part of the lungs and a lot of fluid in the chest cavity. Histology demonstrated a severe bronchopneumonia, and cultures after three days identified *Mannheimia haemolytica* (from a heavy mixed growth); it was also identified by a multiplex PCR on lung tissues. No other bacteria were found by PCR.

Case 3

A dairy heifer presented with severe, acute upper respiratory-tract signs of infection. She became recumbent and was eventually euthanased. A necropsy showed a severe airway inflammation. IBR was confirmed on histology and a positive IBR PCR. Lung cultures identified a mix of *Trueperella pyogenes*, *Pseudomonas sp.* and *Proteus sp.* However, PCR identified a high concentration of *Mannheimia haemolytica* in the same lung several days later. No other bacterial pathogens were detected.

Case 4

A six-month-old calf was euthanased after a week of severe dyspnoea and a failure to improve after two courses of antibiotic treatment. A necropsy revealed extensive lung damage, confirmed as severe bronchopneumonia on histology. A culture, however, revealed a heavy mix of enteric contaminants. PCR for respiratory bacteria was added a week later and revealed a high concentration of *Histophilus somni*.

Case 5

A number of a group of three-month-old calves were found to be in very poor condition with high temperatures. A necropsy of one affected calf revealed an extensively consolidated lung and lungworm. A culture of the affected lung revealed a heavy growth of *Escherichia coli*.

Several days after receiving the culture results, a PCR was added and revealed only significant numbers of *Histophilus somni*.

Case 6

Several six-month-old dairy calves in a large mob of 100 who had been yarded and drenched a week prior were discovered weak and pyretic. A necropsy of one calf revealed extensive lung changes. A culture of the lung grew a heavy mixed growth of organisms, but an added PCR identified significant numbers of *Histophilus somni*.

Discussion

These case examples demonstrate that using multiplex PCRs designed to identify specific bacterial pathogens from tissue samples from pneumonia cases provides more significant results than relying on bacterial cultures alone.

In a survey of a large number of these cattle pneumonias, PCR tests for a number of respiratory viruses were also carried out. Respiratory viruses were identified in only two cases, possibly because although viruses would have initiated these infections, they are only present in the lungs for a very short period.

IBR virus was not found in the affected lungs, but it was identified by PCR in dried nasal swabs from clinical cases of upper respiratory-tract infection.

Histophilus somni was the most common cause of pneumonia in calves. ^{vs}

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