Pneumonia in calves

Novel bacteria potentially associated with pneumonia in calves is examined by Dayle Johnston,[1,2] Paul Cormican,[1] Gerard Murray,[3] David Kenny,[1] Sinéad Waters,[1] Mark McGee,[4] Alan Kelly,[2] Matthew McCabe[1] and Bernadette Earley[1]

Currently unknown and non-culturable bacterial species may be involved in calf pneumonia.

Teagasc researchers used a novel molecular diagnostic assay to identify bacteria, including non-culturable bacteria, which were present in the lungs and lymph nodes of calves that died from pneumonia.

Morbidity and mortality (5.3%) rates in Irish calves are high. In the Republic of Ireland, 6.5%, equivalent to circa 132,297 calves, excluding stillborns, die in their first year of life. Pneumonia is the most common cause of morbidity and mortality in pre-weaned calves. It is a disease of the lower respiratory tract that causes the following symptoms: an elevated rectal temperature, an increased respiratory rate, nasal and ocular discharges, coughing, dyspnoea, a decreased appetite and depression (see Figure 1). Pneumonia is normally contracted by calves following periods of stress and/or inhalation of primary pathogens, including viruses such as bovine herpesvirus-1 (also known as infectious bovine rhinotracheitis), bovine respiratory syncytial virus, bovine parainfluenza-3, bovine viral diarrhoea virus, bovine coronavirus, and *Mycoplasma*. They compromise lung function and allow colonisation by secondary bacterial pathogens, many of which are normal flora of the bovine upper respiratory tract, which are generally responsible for the progression of the disease and the ultimate death of the animal.

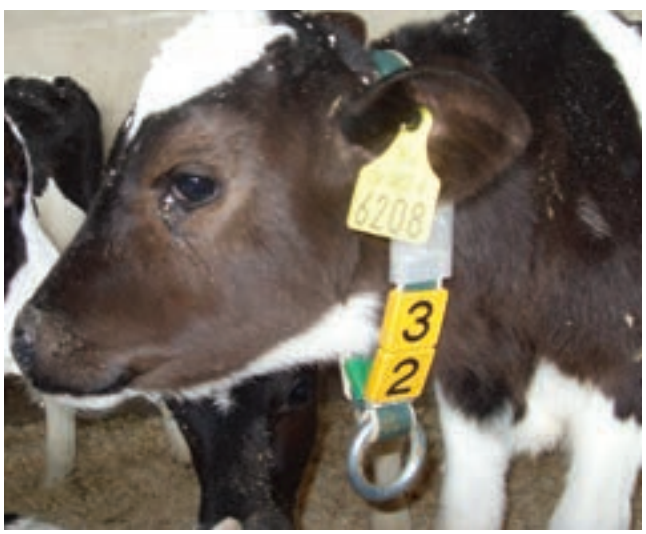

Figure 1: A calf displaying symptoms of pneumonia.

DIAGNOSING BACTERIA CAUSING PNEUMONIA

The diagnostic tools that are used for identification of bacterial species causing pneumonia are culture, and a molecular test, known as the polymerase chain reaction (PCR). However, these tests cannot be used to identify unknown or non-culturable bacteria that may be involved in respiratory diseases. Furthermore, antibiotic treatments and vaccinations are targeted against known bacteria associated with pneumonia. However, unknown bacteria, which may be key players in the development and progression of pneumonia, are escaping detection.

This lack of knowledge about bacteria, which currently cannot be cultured (present in the bovine lungs), may contribute to the poor efficacy of vaccination and antimicrobial treatments against pneumonia-associated bacteria.

Therefore, our group at Teagasc Grange Animal Bioscience department, in collaboration with the Department of Agriculture, Food and the Marine (DAFM) regional veterinary laboratories (RVLs), decided to use next generation sequencing (NGS) of the bacterial 16S ribosomal RNA (rRNA) gene-PCR amplicons, which is a culture-independent, open-reference method, to examine bacteria present in lung and lymph-node samples, from fatal cases of pneumonia, and from clinically healthy calves.

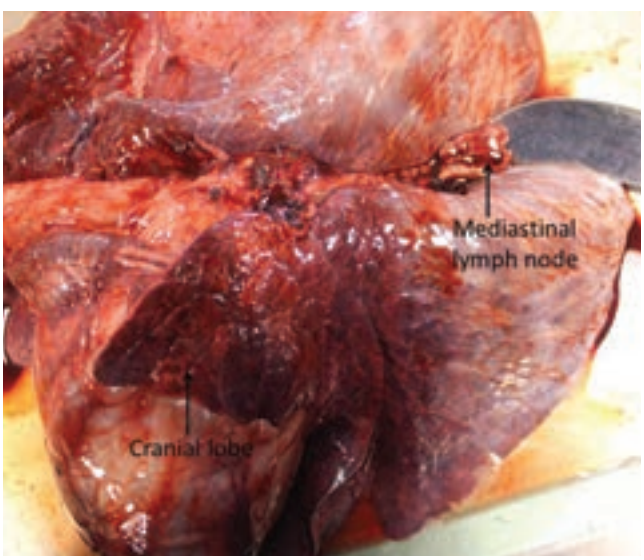

Figure 2: Calf lungs from a fatal case of pneumonia with the cranial lobe and mediastinal lymph node indicated with arrows.

STUDY DESIGN

Vets at Sligo, Athlone and Kilkenny RVLs collected cranial lung lobe and corresponding mediastinal lymph node post-mortem tissue samples (see Figure 2) from 32 beef calves and six dairy calves that had pneumonia as a cause of death. We also collected 20 cranial lung lobe and mediastinal lymph-node tissue samples from clinically-healthy dairy calves that were slaughtered at Teagasc Ashtown.

At Teagasc Grange Animal Bioscience centre, we prepared

bacterial 16S rRNA gene amplicon libraries by extracting DNA from the lung and lymph node tissues and performing two rounds of PCR amplification. Initially, we PCR amplified a small portion of the bacterial 16S rRNA gene, as this gene contains regions that are conserved and regions that are variable between bacterial species. The conserved bacterial sequences enabled PCR amplification and the variable sequences would allow subsequent identification of bacterial genera by comparing the variable PCR amplified sequences (amplicons) with databases containing bacterial 16S gene sequences. In the second round of PCR, we added barcodes to the amplicon targets so each sample could be identified following NGS. Finally, we sequenced the libraries on an Illumina MiSeq and used bioinformatics software to determine which bacterial genera were present in each lung and lymph node sample.

IDENTIFICATION OF *LEPTOTRICHIACEAE* IN PNEUMATIC TISSUE

We found bacteria present in all lung and lymph node samples, including samples from calves that died from pneumonia and from clinically healthy calves. Therefore, like other recent studies, we confirmed that lung tissue from clinically healthy calves is not sterile, as was once thought. However, the frequency of the detection of bacteria associated with pneumonia was lower in the lungs and lymph nodes of clinically healthy calves, compared with fatal pneumonia cases.

The most abundant bacterial genera/families identified in the lungs and lymph nodes of the calves which died from pneumonia were *Leptotrichiaceae*, *Mycoplasma*, *Pasteurellaceae*, and *Fusobacterium*. While the *Pasteurellaceae* family and the *Fusobacterium* and *Mycoplasma* genera contain bacterial species, which are known to cause pneumonia, the *Leptotrichiaceae* family is not currently associated with pneumonia. However, *Leptotrichiaceae* sequences were only found in lesioned tissue in our study and were not present in the lung or lymph nodes from the clinically healthy calves, that had no lung lesions.

Using DNA from one of the pneumatic calf lung samples, we then sequenced longer regions of the *Leptotrichiaceae* genome in order to identify this bacterium to species level. However, despite being identical to an uncultured bacterium obtained from the reproductive tract of cows at University College Dublin, our *Leptotrichiaceae* bacterial genome sequences did not match any known bacterial species in the sequence databases. This suggests that we have identified a novel bacterial species within the *Leptotrichiaceae* family that is potentially involved in bovine pneumonia. It is not possible yet to infer whether this novel *Leptotrichiaceae* species is pathogenic and causing lung lesions or whether it is merely able to grow opportunistically in the lung lesions. However, as we have discovered it in bovine lung lesions and as it has also been identified in cows' reproductive tracts in an independent study, it may be an emerging pathogen in Irish agricultural systems.

REFERENCE

The full paper is available at: Johnston D, Earley B, Cormican P et al. Illumina MiSeq 16S amplicon sequence analysis of bovine respiratory disease associated bacteria in lung and mediastinal lymph node tissue. BMC Vet Res 2017 2; 13(1): 118. doi: 10.1186/s12917-017-1035-2

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