Introduction
Footrot is a debilitating disease of sheep responsible for substantial production and welfare costs in sheep production around the world. The disease process is complex, involving infection by multiple bacterial species evaluated a number of factors. These include prevalent warm wet weather, management practices such as variations in stocking rates, host genetics, host nutritional status, host immunological history, bacterial genetics, bacterial virulence shifts and interactions between virulent and benign bacterial strains over time.

Current treatment regimens involve zinc sulphate footbaths, hoof trimming, vaccination, selective breeding and topical antibiotic applications combined with rigorous quarantine separating clean sheep from infected sheep. Footrot tends to be unidirectional to breed, and in the long term farmers either live with footrot or practice extremely rigorous quarantine. Research worldwide is primarily focused on making footrot management more sustainable by using selective breeding and regional quarantine programs.

Five bacterial pathogens are thought to be involved in footrot, *Dichelobacter nodosus* (*D. nodosus*) and *Fusobacterium necrophorum* (*F. necrophorum*).

**D. nodosus**
- Gram negative
- Obligate anaerobe
- Highly frangible
- Has powerful proteases able to dissolve hooves
- Highly specialised bacteria, rarely found outside sheep and goats
- Considered the primary agent of footrot

**F. necrophorum**
- Gram negative
- Obligate anaerobe
- Secretes a potent leukotoxin (kills white blood cells)
- Found in a wide variety of hosts

Goal
To elucidate the role that *F. necrophorum* and *D. nodosus* play in footrot singly or together in a pastoral farming system.

Methods

**Initial survey**
A postal survey was undertaken with farmers who were asked to take samples using cotton swabs from the skin horn junction of asymptomatic sheep and footrot infected sheep who presented with under-running footrot. Under-running is a distinctive symptom of virulent footrot where extensive destruction of the hoof wall occurs (see Fig. 1).

**DNA extraction**
A total of 96 cotton swabs were resolved by post and frozen at -80°C. DNA could be extracted using standard phenol-chloroform methods.

**PCRs**
- *D. nodosus* was detected using previously described PCR primers specific to its fimA gene (*Fimbrial, structural gene*).

**Forward**

fimA-f: 5’- AGAGAGGCAmCACAmAAGAGC-3’

**Reverse**

fimA-r: 5’-GCTATTCCAAGAAACAAAACACAT-3’

**Reverse**

D. nodosus was detected using primers designed to detect the IktA gene (*Luxobactin, structural gene*).

**Forward**

IktA-f: 5’-ACCTAGAGAGACCTTAGTGAC-3’

**Reverse**

IktA-r: 5’-AGAGAGGCTTACATAGGAGC-3’

**Reverse**

K. necrophorum was detected using primers designed to detect the IktA gene (*Luxobactin, structural gene*).

**Forward**

IktA-f: 5’-GCACTATTCAAGAAACAAAACACAT-3’

**Reverse**

IktA-r: 5’-AGAGAGGCTTACATAGGAGC-3’

**Statistical analysis**
Statistical analysis of results was performed using a log-linear model and Poisson errors (GenStat version 10, 2007, Lawes Agricultural Trust, Rothamsted).

Fig. 1. A sheep’s hoof infected with footrot showing under running.

Discussion
This study shows that in normal New Zealand pastoral farming systems, that *D. nodosus* and *F. necrophorum* are associated with footrot infected sheep feet compared with healthy sheep. We also showed that *D. nodosus* and *F. necrophorum* occur together at a significantly higher rate than if they were randomly distributed. This demonstrates that not only are these bacteria both associated with under-running footrot, they are also involved in the overall process of infection, suggesting they may both be involved in tandem as causative agents of footrot.

Results
Of the 96 samples analysed, 51 came from healthy sheep and 45 from footrot infected sheep with under-running footrot. Of the swabs taken from healthy sheep only 3/51 was positive for *F. necrophorum*. Of the swabs from footrot infected sheep, 37/45 were both positive for both *F. necrophorum* and *D. nodosus*, 4/45 were positive for *F. necrophorum* only and 2/45 for *D. nodosus* only.

**Data summary**

<table>
<thead>
<tr>
<th>Detection of only F. necrophorum (n=45)</th>
<th>Detection of only D. nodosus (n=45)</th>
<th>Detection of both F. necrophorum and D. nodosus (n=45)</th>
<th>Detection of neither F. necrophorum or D. nodosus (n=45)</th>
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<tr>
<td>4</td>
<td>2</td>
<td>17</td>
<td>23</td>
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**Statistical analysis**
Statistical analysis was performed to test if the association of *D. nodosus* (fimA) and *F. necrophorum* (IktA) with footrot was statistically significant. *D. nodosus* and *F. necrophorum* tend to found together when footrot is present (*P<0.025*). Is the distribution random or not?

**Association of F. necrophorum and D. nodosus**

<table>
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**P value**

- Detection of fimA: *P<0.001*
- Detection of fimA and IktA: *P<0.001*
- Detection of fimA and IktA and D. nodosus: *P<0.025*
- Detection of fimA and IktA and *F. necrophorum*: *P<0.001*

**Conclusion**

- *F. necrophorum* and *D. nodosus* are closely associated with footrot infections in sheep and each other. This suggests that either *F. necrophorum* is a pathogen involved in causing footrot, or that it is a very effective coloniser of footrot lesions. Even if *F. necrophorum* is just a coloniser of footrot lesions, its presence could have clinical consequences for the host due to its pathogenic nature.

References

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