The role of *Dichelobacter nodosus* and *Fusobacterium necrophorum* in footrot in New Zealand

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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 modulated a number of factors. These include prevalent warm wet weather, management practises 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.[1]

Current treatment regimes involve zinc sulphate foot baths, hoof trimming, vaccination, selective breeding and topical antibiotic application combined with rigorous quarantine separating clean sheep from infected sheep. Footrot tends to be uneconomic to treat, and in the long term farmers either live with footrot or practise extremely rigorous quarantine. Research world wide is primarily focussed on making footrot management more sustainable by using selective breeding and regional quarantine programs.

Two 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 fimbriated
- 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 walls occurs (see Fig. 1).

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

DNA extraction

A total of 96 cotton swabs were received by post and frozen at -80°C till 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-u1: 5'-ATCCCTGCATACAACGACTACAT-3'
  fimA-u2: 5'-GCT ATTCCACAATACCAAAACTACAT-3'

  Reverse
  fimA-d1: 5'-ACTCAAGAGAGAGGCTTTTAAGTAAG-3'
  fimA-d2: 5'-AGAGAGGCTTTCACATTTAAGAGC-3'
  fimA-d3: 5'-GTACCGAAGTACACCTTTGATTG-3'

- *F. necrophorum* was detected using primers designed to detect the *lktA* gene (leukotoxin, structural gene).
  lktA-up: 5'-ACAATCGGAGTAGTAGGTTC-3'
  lktA-dn: 5'-ATTTGGTAACTGCCACTGC-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).

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 1/51 was positive for *F. necrophorum*. Of the swabs from footrot infected sheep, 17/45 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

	Footrot infected sheep (n=45)	Healthy or asymptomatic sheep (n=51)
Detection of only *lktA*	4	1
Detection of only *fimA*	2	0
Detection of both *fimA* and *lktA*	17	0
Detection of neither *fimA* or *lktA*	23	0

Statistical analysis

Statistical analysis was performed to test if the association of *D. nodosus* (*fimA*) and *F. necrophorum* (*lktA*) with footrot was statistically significant and if *D. nodosus* and *F. necrophorum* tend to found together when footrot is present (i.e. is the distribution random or not?).

Association of	P value
Detection of *D. nodosus* with footrot?	P<0.0001
Detection of *F. necrophorum* with footrot?	P<0.0001
Detection of *F. necrophorum* and *D. nodosus* together?	P<0.025

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 where randomly distributed. This demonstrates that not only are these bacteria both associated with under running footrot, they are associating together, suggesting they may both be involved in tandem as causative agents of footrot.

Not detecting *D. nodosus* and *F. necrophorum* on the surface of all sheep hooves infected with under-running footrot does not eliminate *D. nodosus* and *F. necrophorum* as causative agents of footrot, especially considering their anaerobic, transient nature. Once *D. nodosus* and *F. necrophorum* are established on a hoof surface and causing an infection, the possible fate of theses pathogens is varied. Possibilities include spreading to other hosts, persisting in a chronic infection, colonising other reservoirs and removal by oxidative, environmental or immunological stress. Even if these bacteria die out on the surface of the foot, they may still be able to persist in pockets of infection inside the hoof and cause disease at a later date.

Little is understood about the role of *F. necrophorum* as an agent of benign or virulent footrot, what role it might play over time and how it may or may not be interacting with *D. nodosus* during a footrot outbreak.

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

1. Egerton J.R., Yong, W.K., Riffkin, G.G., 1989. Footrot And Foot Abscess In Ruminants. Published by CRC press Inc.

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