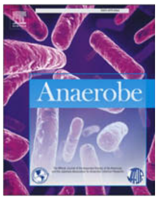
Anaerobe 15 (2009) 173–176

Contents lists available at ScienceDirect

Anaerobe

journal homepage: www.elsevier.com/locate/anaerobe



Veterinary anaerobes and diseases

Dichelobacter nodosus, *Fusobacterium necrophorum* and the epidemiology of footrot

Grant Bennett, Jon Hickford*, Richard Sedcole, Huitong Zhou

Agriculture and Life Sciences Division, P.O. Box 84, Lincoln University, Lincoln 7647, New Zealand

ARTICLE INFO

Article history:
Received 28 July 2008
Received in revised form
3 February 2009
Accepted 12 February 2009
Available online 23 February 2009

Keywords:
Fusobacterium necrophorum
Dichelobacter nodosus
Footrot
Lameness
Sheep

ABSTRACT

Footrot is a debilitating disease of sheep resulting in lameness, production losses and suffering. To study the basic bacteriology of the disease, a survey was initiated across commercial farms and non-commercial research flocks to compare the bacteriology of symptomatic footrot infected sheep with healthy asymptomatic sheep. Of the 80 farmers initially contacted, 14 collected hoof swabs and returned the swabs by post. Following DNA extraction, species-specific PCR was used to identify if *Dichelobacter nodosus* (*D. nodosus*) or *Fusobacterium necrophorum (F. necrophorum)* species were present on each swab. Of the 42 swabs taken from symptomatic footrot infected sheep, 17 were positive for both *F. necrophorum* and *D. nodosus,* two were positive for *F. necrophorum* only, two for *D. nodosus* only and 23 swabs were negative for both *F. necrophorum* and *D. nod osus*. Of the 50 swabs received from healthy asymptomatic sheep, one was positive for *F. necrophorum* only and 49 were negative for both *D. nodosus* and *F. necrophorum*. This suggests that both *F. necrophorum* and *D. nodosus* are linked to footrot in the field in a pastoral farming system. If these bacteria are linked together and collectively cause footrot, this may need to be considered when managing a footrot outbreak, or maintaining a quarantine.

1. Introduction

Ovine footrot is a highly contagious disease that results in lameness, production loss and suffering. The primary pathogen was first identified as *Dichelobacter nodosus* (*D. nodosus*, formally known as *Bacteroides nodosus* and *Fusiformis nodosus*) by Beveridge in 1941 [1].

D. nodosus is however unable to fulfil Koch's postulates of disease as it is unable to replicate the symptoms of disease on its own as a second pathogen *Fusobacterium necrophorum* (*F. necrophorum*) is reported to be required to induce footrot symptoms in sheep during small pen trials [2]. Strict application of Koch's postulates to footrot would mean that the disease causing agent must be able to be isolated from a diseased host, be culturable on media independently of the host and if another un-diseased host was inoculated with the culture, cause disease.

Despite failing to meet Koch's postulates, *D. nodosus* is commonly considered the primary pathogen causing footrot since elimination and quarantine of virulent strains of *D. nodosus* appear to prevent footrot [3], vaccination against *D. nodosus* reduces disease prevalence [4] and *D. nodosus* is found within foot-rot lesions [5].

D. nodosus is a rod shaped, gram negative, obligate anaerobe bacterium that has proteases and keratinases that are able to dissolve sheep hooves. The central role that these enzymes play in the biology of *D. nodosus* and footrot is demonstrated by how they are still used today as a measure of strain virulence and historically dried hoof powder was considered a requirement for the reliable culture of *D. nodosus* [6].

F. necrophorum has been considered as a secondary pathogen in ovine footrot. It is a gram-negative anaerobic bacterium associated with many different diseases and disorders in both animals and humans. These range from Lemierre's syndrome in humans [7–9] to calf diphtheria [10] as reported by Ref. [11–13], ovine footrot [5], bovine rumenitis-hepatic abscesses complex [14,15], abscesses in animals [16], bovine hoof abscesses, toe abscesses and various soft tissue infections in the hooves of both cattle and sheep [17–21].

The etiology of ovine footrot is complex, involving infection by multiple bacterial species [15] modulated by environmental conditions [22,23], host genetics, host immunity [24], nutrition and stocking rates. Some management practices affect footrot allowing disease control through quarantine, selective breeding, foot paring combined with zinc sulphate foot baths, vaccination [4,24] and

---

* Corresponding author. Tel.: +64 3 325 2811; fax: +64 3 325 3851.
E-mail addresses: bennetg2@lincoln.ac.nz (G. Bennett), hickford@lincoln.ac.nz (J. Hickford), sedcole@lincoln.ac.nz (R. Sedcole), zhouh@lincoln.ac.nz (H. Zhou).

1075-9964/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.anaerobe.2009.02.002

antibiotic use. However, using these practices, establishing and maintaining quarantine are expensive, difficult and there is no guarantee that footrot may not become re-established at a later date.

Due to the difficulty of reliably isolating and anaerobically culturing *D. nodosus* and *F. necrophorum*, a PCR-based strategy was used to detect the microbes thought to be associated with the disease. PCR is able to detect non-viable cells, dead cells, live cells and difficult to culture cells, and when combined with specific primers [25] is a very precise way of ascertaining the presence of specific genetic material. It should be noted that PCR cannot prove the absence of genetic material, so care must be taken as to what conclusions are drawn from such results.

2. Materials and methods

2.1. Footrot samples

Of the eighty sheep farmers who were initially contacted, fourteen returned swabs from both/either healthy symptomatic sheep and/or symptomatic sheep with severe under-running footrot. These farmers had received instructions to take swabs from the skin–horn junction on the axial wall of the hoof and to return these swabs in tubes by post. Twelve sheep with severe footrot from the Lincoln university research farm were swabbed and swabs were processed in an identical manner to farmer collected swabs. Once received, swabs were stored at −80 °C till processed.

2.2. DNA extraction

DNA was extracted from swabs using a previously published protocol with minor modifications [25]. Briefly swabs were placed in sterile 1.5 ml tubes with 400 μl of sterile TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and shaken for 20 s. The swab was removed and 40 μl of 10% SDS was added with 220 μl of Tris-buffered phenol (pH 7.8) and 220 μl of chloroform. Tubes were shaken to lyse cells and frozen over night at −20 °C. After thawing, suspensions were briefly mixed by inverting and centrifuged at 5000×*g* for 5 min. The aqueous layer was aliquoted into a new tube and precipitated with 40 μl of 3 M Sodium Acetate (adjusted to pH 5.2) and 500 μl of ice cold iso-propanol. Precipitated DNA was centrifuged at 14 500×*g* for 15 min and the supernatant removed. The DNA pellet was air dried before being suspended in 50 μl of sterile dH_2O. The DNA in solution was stored at 4 °C until used.

2.3. PCR amplification

The *lktA* gene encoding the leukotoxin of *F. necrophorum* was amplified using PCR primers lkt-up (5′-acaatcggagtagtaggttc-3′) and lkt-dn (5′-atttggtaactgccactgc-3′). The PCR was performed in an iCycler (Bio-Rad, CA, USA) with an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 59 °C for 30 s and 72 °C for 30 s. A final extension of 5 min at 72 °C was performed.

The *fimA* gene of *D. nodosus* was amplified using the method described previously [25]. The thermal profile consisted of denaturation at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 62 °C for 30 s and 72 °C for 50 s, with a final extension step at 72 °C for 5 min.

PCR products from both the *lktA* PCR and *fimA* PCR were separated electrophoretically at 10 V/cm on a 1 × TBE (89 mM Tris–borate, 89 mM boric acid, 2 mM Na_2EDTA [pH 8.9]) gel containing 1.0% agarose and 0.5 μg/ml of ethidium bromide and visualised using a transilluminator. If high concentrations of genomic DNA were visible on the gel, extracted DNA was diluted 1/10 and the PCR repeated to improve PCR consistency. If high quantities of genomic DNA were still seen at this concentration, the DNA was further diluted to 1/100 and the PCR repeated.

2.4. Statistical analysis

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

3. Results

Of the 50 swabs taken and received from healthy asymptomatic sheep, one was positive for *F. necrophorum*, none were positive for *D. nodosus* and 49 were negative for both *F. necrophorum* and *D. nodosus*. Forty-two swabs were taken and received from footrot infected sheep with under-running footrot. Of these, two were positive for *F. necrophorum*, two were positive for *D. nodosus,* 17 were positive for both *F. necrophorum* and *D. nodosus* and 23 were negative for both *F. necrophorum* and *D. nodosus*. Statistical analysis showed that *D. nodosus* and *F. necrophorum* are significantly linked to footrot ($p < 0.001$), and that these organisms are found together at a significantly higher rate than would be expected by a random assortment ($p < 0.025$).

The *fimA* and *lktA* PCRs from footrot swabs were found to work more reliably with the addition of 400 ng/μl of BSA and additional $MgCl_2$ to final concentration of 5.25 mM (Fig. 1). The *fimA* PCR was also improved by reducing the primer concentration to 100 nM (Fig. 1).

4. Discussion

This survey shows that in a pastoral farming system *D. nodosus* and *F. necrophorum* tend to be found on the feet of symptomatic sheep with under-running footrot compared to healthy asymptomatic sheep. We also showed that *D. nodosus* and *F. necrophorum* occur together at a significantly higher rate than if they distributed

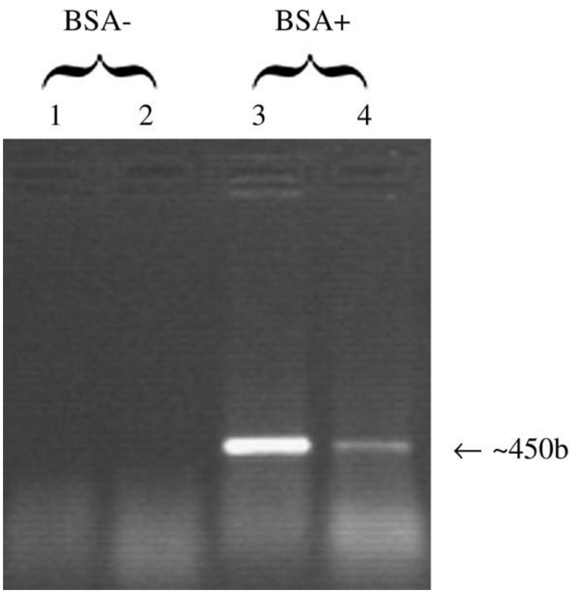


Fig. 1. Optimisation of *fimA* PCR amplification from footrot samples using the same template DNA and various PCR conditions. 20 μl of PCR product was loaded into each lane. Lane 1: 2.75 mM $MgCl_2$, 100 nM of each primer, without BSA; lane2: 2.75 mM $MgCl_2$ 250 nM of each primer, without BSA; lane 3: 5.25 mM $MgCl_2$, 100 nM of each primer, with 400 ng/μl BSA; lane 4: 5.25 mM $MgCl_2$, 250 nM of each primer, with 400 ng/μl BSA.