Preventive Veterinary Medicine 115 (2014) 48–55

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

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

A longitudinal study of the role of *Dichelobacter nodosus* and *Fusobacterium necrophorum* load in initiation and severity of footrot in sheep

Luci A. Witcomb[a,c,*], Laura E. Green[a,1], Jasmeet Kaler[a,d], Atiya Ul-Hassan[a], Leo A. Calvo-Bado[a], Graham F. Medley[a], Rose Grogono-Thomas[b], Elizabeth M.H. Wellington[a,1]

[a] School of Life Sciences, University of Warwick, Gibbet Hill Campus, Coventry, UK
[b] School of Veterinary Sciences, University of Bristol, Langford House, Langford, UK
[c] UCL School of Pharmacy, University College London, 29–39 Brunswick Square, London, UK
[d] School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Leicestershire, UK

ARTICLE INFO

Article history:
Received 15 May 2013
Received in revised form 3 March 2014
Accepted 5 March 2014

Keywords:
Dichelobacter nodosus
Fusobacterium necrophorum
Footrot
Sheep
Longitudinal study
Quantitative PCR

ABSTRACT

Footrot is an infectious bacterial disease of sheep that causes lameness. The causal agent is *Dichelobacter nodosus*. There is debate regarding the role of *Fusobacterium necrophorum* in disease initiation. This research used an observational longitudinal study of footrot, together with quantitative PCR (qPCR) of bacterial load of *D. nodosus* and *F. necrophorum*, to elucidate the roles of each species in the development of disease. All feet of 18 *a priori* selected sheep were monitored for five weeks assessing disease severity (healthy, interdigital dermatitis (ID) and severe footrot (SFR)) and bacterial load. A multinomial model was used to analyse these data.

Key unadjusted results were that *D. nodosus* was detected more frequently on feet with ID, whereas *F. necrophorum* was detected more frequently on feet with SFR. In the multinomial model, ID was associated with increasing $\log_{10}$ load of *D. nodosus* the week of observation (OR = 1.28 (95% CI = 1.08–1.53)) and the week prior to development of ID (OR = 1.20 (95% CI = 1.01–1.42). There was no association between $\log_{10}$ load^2 of *F. necrophorum* and presence of ID (OR = 0.99 (95% CI = 0.96–1.02))). SFR was associated with increasing $\log_{10}$ load of *D. nodosus* the week before disease onset (OR = 1.42 (95% CI = 1.02–1.96)) but not once SFR had occurred. SFR was positively associated with $\log_{10}$ load^2 of *F. necrophorum* once disease was present (OR = 1.06 (95% CI = 1.01–1.11)). In summary, there was an increased risk of increasing *D. nodosus* load the week prior to development of ID and SFR and during an episode of ID. In contrast, *F. necrophorum* load was not associated with ID before or during an episode, and was only associated with SFR once present. These results contribute to our understanding of the epidemiology of footrot and highlight that *D. nodosus* load plays the primary role in disease initiation and progression, with *F. necrophorum* load playing a secondary role. Further studies in more flocks and climates would be useful to confirm these findings. This study identifies that *D. nodosus* load is highest during ID. This supports previous epidemiological findings, which demonstrate that controlling ID is the most effective management strategy to prevent new cases of ID and SFR.

* Corresponding author at: UCL School of Pharmacy, University College London, 29-39 Brunswick Square, London, UK. Tel.: +44 (0)20 7753 5800; fax: +44 (0)20 7753 5829.
E-mail address: l.witcomb@ucl.ac.uk (L.A. Witcomb).

[1] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.prevetmed.2014.03.004

1. Introduction

Footrot is an infectious bacterial disease of sheep, which causes lameness. It is an important disease in all countries with large sheep industries. Footrot reduces sheep welfare, productivity and profitability (Egerton *et al.*, 2004; Nieuwhof and Bishop, 2005; Wassink *et al.*, 2010a). Footrot is characterised by two distinct pathological presentations: inflammation of the interdigital skin, interdigital dermatitis (ID) and separation of the hoof horn from the sensitive underlying tissue, severe footrot (SFR). Damage to the epithelium of the interdigital skin is a prerequisite for the initiation of disease (Beveridge, 1941). Spread of disease between sheep occurs when environmental conditions are conducive for indirect transmission of bacteria between sheep via pasture or pen floor (Whittington, 1995; Green and George, 2008).

ID and SFR have been treated as separate diseases in many countries in Europe, including the UK (Winter, 2008), with many veterinarians and farmers viewing ID as non-infectious and caused primarily by environmental factors, such as weather and pasture quality (Wassink *et al.*, 2005). In the UK, there is now strong evidence that that ID and SFR are two clinical presentations of the same disease (Wassink *et al.*, 2003, 2010b; Moore *et al.*, 2005). In Australia, ID and SFR have been considered one disease for many years with ID called benign footrot (scores 1–2) and SFR called virulent footrot (scores 3–4) (Egerton and Roberts, 1971; Raadsma and Dhungyel, 2013). There is some, but not complete, correlation between severity of clinical presentation of footrot and virulence traits of *D. nodosus* in Australia (Rood *et al.*, 1996; Cheetham *et al.*, 2006) and between countries (Calvo-Bado *et al.*, 2011a). However, within the UK, 300/305 isolates of *D. nodosus* from cases of ID and SFR were virulent (Moore *et al.*, 2005) indicating that virulence does not correlate with disease severity on commercial farms in the UK. Disease pathogenesis may also be affected by a range of non-bacterial factors, including host immunity and heritability of resistance traits (Escayg *et al.*, 1997) and environmental conditions, such as temperature, rainfall and pasture quality (Whittington, 1995; Wassink *et al.*, 2005).

In 1941, Beveridge produced his seminal work on footrot in which he provided evidence that *D. nodosus*, a Gram-negative anaerobe, was the primary aetiological agent of footrot rather than *Fusobacterium necrophorum*. Several decades later, it was postulated that the presence of *F. necrophorum*, a commensal of the alimentary tract of both humans and animals, was essential for development of footrot (Roberts and Egerton, 1969). Since then, Koch's molecular postulates have provided crucial evidence that the causative agent of footrot is *D. nodosus* (Kennan *et al.*, 2001, 2010). Despite these findings, *F. necrophorum* is still frequently a topic of discussion in footrot literature and is reported to be the cause or associated with both ID and/or SFR (Bennett *et al.*, 2009; Zhou *et al.*, 2009). A number of authors have investigated the presence of *D. nodosus* and *F. necrophorum* in sheep with healthy and diseased feet. *D. nodosus* is recovered more frequently from feet with ID or SFR than healthy feet (Moore *et al.*, 2005; La Fontaine *et al.*, 1993; Bennett *et al.*, 2009). Calvo-Bado *et al.* (2011b) detected *D. nodosus* on all feet of sheep using nested PCR, in a flock that had not had ID or SFR for 10 years. *F. necrophorum* was detected more frequently in feet with SFR (Bennett *et al.*, 2009). However, these studies were cross-sectional and so cause and effect could not be elucidated.

Investigation of bacterial load from uncultured material is common in ecological microbiology because culture can select for certain species of bacteria and so can introduce bias (Amann *et al.*, 1995). Such an approach is currently under-utilised in veterinary epidemiology but it can be used to improve our understanding of the process of infection and disease when culture is unreliable. This approach might inform on aetiology, pathogenesis and control of infectious diseases. *D. nodosus* is difficult to culture and PCR is more sensitive than isolation (Moore *et al.*, 2005). In addition, given that Calvo-Bado *et al.* (2011b) reported that *D. nodosus* was detectable in all feet using nested PCR, irrespective of disease state, load of *D. nodosus* might be a more useful tool to investigate the role of *D. nodosus* and *F. necrophorum* in the pathogenesis of footrot. Quantitative PCR (qPCR) is used to determine bacterial load. Key features that are required for accurate qPCR analyses include a specific sequence (amplicon) present in all strains of only the target bacterial species, a low limit of detection (analytical sensitivity) and no cross reactivity with other non-target microorganisms (analytical specificity).

The aim of this paper was to use sensitive and specific qPCR assays to investigate the change in load of *D. nodosus* and *F. necrophorum* in feet and sheep developing ID and SFR and to elucidate the temporal patterns between bacterial load and disease and so identify the roles of *D. nodosus* and *F. necrophorum* in disease initiation and progression.

2. Materials and methods

2.1. Study population

The study flock comprised of 570 Mule, Suffolk and Roussin ewes. The flock was located on a lowland farm in Oxfordshire, England with a mean rainfall of 10–20 mm per month and a mean daily temperature of 11 °C. The study was done in September/October 2006 when environmental conditions (rainfall and temperature) were conducive for transmission of disease. The flock had had lame sheep with SFR for >20 years, with a prevalence of 6–8% lameness at any one time (Wassink *et al.*, 2010a). During the current study 30.5% of sheep in the flock had ID and 4.7% of sheep had SFR.

2.2. Sample collection and disease severity scoring

From this flock a subset of 60 sheep were selected (Kaler *et al.*, 2011). All 4 feet of all 60 ewes were examined each week for 5 weeks. Each foot was recorded as clinically healthy, having ID or having SFR using a defined system (Foddai *et al.*, 2012) and then the interdigital skin was swabbed by a single trained researcher (JK), in order to standardise the sampling method and to avoid between observer variation. All swabs were collected and stored in transport buffer at −80 °C until required (Moore *et al.*,