Mathematical modeling of ovine footrot in the UK: the effect of *Dichelobacter nodosus* and *Fusobacterium necrophorum* on the disease dynamics

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ABSTRACT

Dichelobacter nodosus is a virulent, invasive, anaerobic bacterium that is believed to be the causative agent of ovine footrot, an infectious bacterial disease of sheep that causes lameness. Another anaerobe, *Fusobacterium necrophorum*, has been intimately linked with the disease occurrence and severity. Here we examine data from a longitudinal study of footrot on one UK farm, including quantitative PCR (qPCR) estimates of bacterial load of *D. nodosus* and *F. necrophorum*. The data is at foot level; all feet were monitored for five weeks assessing disease severity (healthy, interdigital dermatitis (ID), or severe footrot (SFR)) and bacterial load (number of bacteria/swab). We investigate the role of *D. nodosus* and *F. necrophorum* in the progress of the disease using a continuous-time Markov model with 12 different states characterising the foot. The transition rates between the adjacent states are the (34) model parameters, these are determined using Metropolis Hasting MCMC. Our aim is to determine the predictive relationship between past and future *D. nodosus* and *F. necrophorum* load and disease states. We demonstrate a high level of predictive accuracy at the population level for the *D. nodosus* model, although the dynamics of individual feet is highly stochastic. However, we note that this predictive accuracy at population level is only high in more diseased states for *F. necrophorum* model. This supports our hypothesis that *D. nodosus* load and status of the foot work in combination to give rise to severe footrot and lameness, and that *D. nodosus* load plays the primary role in the initiation and progression of footrot, while *F. necrophorum* load rather increases disease severity of SFR.

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1. Introduction

In the UK, footrot is an endemic infectious disease of sheep. It is present in >90% flocks and causes 80% of foot lameness (Kaler and Green, 2008); approximately 5% of the national flock of 16 million ewes are lame at any one time (Winter et al., 2015). Lameness has considerable impact on animal welfare causing pain, discomfort, and weight loss with consequential reduction in productivity through reduced numbers of lambs per ewe and reduced growth rates in affected lambs (Wassink et al., 2010). The economic impact is estimated to be £25–£80 million per year (Nieuwhof and Bishop, 2005; Wassink et al., 2010). Interdigital dermatitis (ID) and severe footrot (SFR) are two pathological presentations of this disease.

The clinical presentation of ID is inflammation of the interdigital skin whilst SFR presents as separation of the hoof horn from the sensitive tissue of the foot (Winter, 2008).

The primary aetiological agent of footrot is *Dichelobacter nodosus*, an anaerobic bacterium that can be detected on the feet of healthy, ID, and SFR feet (Moore et al., 2005; Bennett et al., 2009; Witcomb et al., 2014). The load of *D. nodosus* is higher on feet with ID and SFR than in healthy feet (Witcomb et al., 2014). All sheep appear to be susceptible to *D. nodosus* and transmission occurs via the surface on which the animals are kept (pasture or pen). If the skin of the foot is damaged or wet, *D. nodosus* can invade the epidermis and causes disease (Beveridge, 1941). Climate is highly influential in the duration of infectiousness of *D. nodosus* off the host (the lifespan of the bacteria on pasture or in soil when infection can pass to another host) with damp, warm conditions aiding spread of disease (Whittington, 1995; Green and George, 2008). *D. nodosus* survives in warm and damp conditions off the
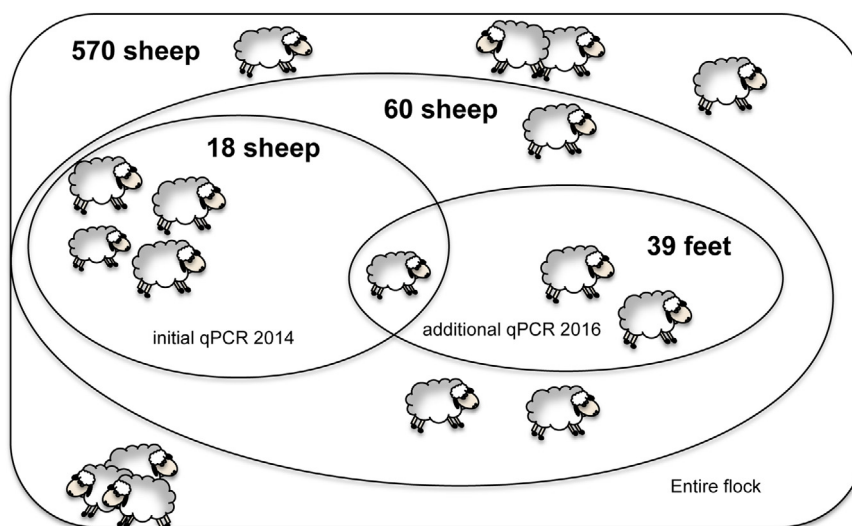
Fig. 1. Study population. The study flock consists of 570 sheep. A subset of 60 sheep was selected for monitoring (Kaler, 2011). 18 sheep were subsequently selected for the 2014 study (Witcomb et al., 2014) (labelled as "initial qPCR 2014") and qPCR was performed to investigate the associations between bacterial load and footrot. Additional foot swabs of 39 feet are selected from the remaining 42 sheep and analysed for *D. nodosus* load using qPCR (labelled as "additional qPCR 2016"), with 4 samples repeated from the initial 18 sheep to test consistency between the 2014 and 2016 qPCR results.

host but cannot be transmitted between sheep in very hot and dry conditions. *Fusobacterium necrophorum*, another anaerobic bacterium, has also been associated with both ID and SFR. It is also detected on healthy, ID, and SFR feet but only detected at a higher loads in feet with SFR (Witcomb et al., 2014), which suggests a secondary or opportunistic role. Hence *F. necrophorum* appears to enhance the disease severity rather than initiate the disease (Beveridge, 1941; Witcomb et al., 2014; Clifton and Green, 2016).

The primary aim of the present study is to quantify the dynamic interaction between bacterial load and disease progression by the development of a mechanistic model; such an approach allows us to speculate on cause and effect more effectively than with a purely statistical model, so can be used to further explore the dynamics of footrot. The model will allow us to establish the relationship between bacterial load and disease progression, and whether a knowledge of the past and future disease states can determine the current load or vice versa.

2. Data aquisition

The data used in this paper is from a subset of data from an 18 month longitudinal study of footrot in England in a flock of 570 ewes (Wassink et al., 2010), see Fig. 1. Footrot had been present in the flock for more than 20 years and the average prevalence of lameness caused by footrot was 6–8% (Wassink et al., 2010). From the 570 sheep, 60 were selected and examined each week for 5 consecutive weeks (Kaler, 2011). Each week, the feet were examined and given a disease severity score. The interdigital skin of each foot was swabbed (see Kaler, 2011; Witcomb et al., 2014 for standardised methodology) and swabs were stored in a transport buffer at $-80\,^{\circ}$C. For the initial part of this study, 18/60 sheep were selected: 3 with no signs of disease throughout the 5-week study; 7 with ID but no SFR; and 8 with at least one foot with SFR (Witcomb et al., 2014). qPCR was used to estimate the loads of *D. nodosus* and *F. necrophorum* (targeting the single copy number of genes rpoD in *D. nodosus*, and rpoB in *F. necrophorum*, in swabs from all feet of the 18 sheep for all 5 weeks). The detailed disease severity scoring and *D. nodosus* and *F. necrophorum* loads used in the previous studies are presented in the supplementary materials.

The initial data therefore comprise a total of 360 observations of 72 feet from 18 sheep. This is characterised by the following set of information: sheep identity, week (from 1 to 5), foot (left front (LF), right front (RF), left hind (LH), right hind (RH)), ID score for each foot, SFR score for each foot (an ID/SFR score of 0 indicates healthy state), *D. nodosus* load on each foot (recorded as number of *D. nodosus* rpoD detected per swab), and *F. necrophorum* load (recorded as number of rpoB detected per swab). The data for the 60 sheep as well as the subset of 18 sheep are shown in the Supplementary Material (data).

2.1. Additional analysis of D. nodosus load

To assess the predictive power of our model, an additional 200 swab samples were analysed by qPCR. Four samples were repeats from the initial 18 sheep (Witcomb et al., 2014) and the rest were selected from the remaining 42 sheep from the original study of 60 sheep (Kaler, 2011). Individual feet, with the greatest variation in disease severity were selected (see Supplementary Material). The qPCR was performed as before (Witcomb et al., 2014).

3. Model formulation

The data for each foot is extremely high-dimensional (with 12 possible states), with a load for the two bacterial species and two scores: one for ID and one for SFR. This high dimensionality precludes the use of complete state-based modelling. We therefore aggregate the values for LOAD (*D. nodosus* bacterial load) and SCORE (disease severity score) for modelling purposes. For LOAD, we use three values (0, 1, and 2): 0 for $<10^4$ bacteria/swab, 1 for between 10^4 and 10^5 bacteria/swab, and 2 for $\geq 10^5$ bacteria/swab. We note in Witcomb et al. (2014), our lowest LOAD was partitioned into undetectable bacterial load and detectable load $<10^4$ bacteria/swab. We believe our aggregation of these two LOAD is justifiable in terms of both practical detection difficulties and mathematical simplicity. For SCORE, we use four distinct values: 0 for healthy, 1 and 2 for ID and severe ID respectively, and 3 for any SFR irrespective of its ID score. This simplification can be justified by the difficulty of characterising the severity of SFR, but the clear distinction between ID and SRF. Throughout we have made the simplifying assumption that each foot acts independently, ignoring the weak positive correlation presumably because of spatial contamination and the small negative correlations that can