# Detection of *Fusobacterium necrophorum* and *Dichelobacter nodosus* from cow footrot in the Heilongjiang Province, China

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**Abstract**

Cow footrot in the Heilongjiang Province, northeast China is a problem resulting in lost production in agriculture. In this study, 200 swab samples from footrot lesions of naturally infected cows with odorous exudative inflammation and keratinous hoof separation at 10 farms were examined in the period from May 2016 to May 2017. Twenty cows from each farm were taken for sampling. The samples were examined for detectingthe presence of *Dichelobacter nodosus* (*D. nodosus*) and *Fusobacterium necrophorum* (*F. necrophorum*). Such detection was carried out using polymerase chain reaction (PCR). The PCR primers were designed to identify the *lktA* gene, which encodes a leukotoxin unique to *F. necrophorum*, and the *fimA* gene of *D. nodosus*. Of the 200 samples, 111 (55.5%) revealed the presence of *F. necrophorum* and 11 (5.5%) exhibited *D. nodosus.* The frequent finding of *F. necrophorum* in cow farms of Heilongjiang province, northeast China is noteworthy. The possibility of *F. necrophorum* and *D. nodosus*infection should be an important concern when controlling cow footrot in China.

*lktA, fimA, dairy farm, D. nodosus, F. necrophorum*

Footrot is an acute, highly infectious disease of cattle caused by the synergistic action of several bacterial species. There are two species especially deserving attention: One is *Dichelobacter nodosus* (*D. nodosus*), which is the primary pathogen and essential transmitting agent in footrot (Kumaret al. 2016; Nielsenet al. 2016); the other is *Fusobacterium necrophorum* (*F. necrophorum*), which is required for *D. nodosus* to successfully initiate the infection (Zheng et al. 2016). These bacterial species are present in the rumen and faeces of normal cows and their environment (Tan et al. 1996; Nagaraja et al. 2005; Zhou et al. 2009). The primary aetiology for disease includes the virulence of the infecting *D. nodosus* strain and other factors, such as environmental conditions, the host's genetics, immunity, diet and stocking rates. *Fusobacterium necrophorum* has been suggested as a secondary pathogen in the disease development and could possibly increase its severity (Frosth et al. 2015; Çelebi and Otlu 2016).

Footrot is characterised by the presence of interdigital lesions, swelling, moderate to severe lameness, and a separation of the horny portions of the hoof from the sensitive tissues underneath (Agbajeet al. 2018; Best et al. 2018). It has a serious effect on the production performance of diseased cows. Since footrot was first reported by Adams in the Netherlands in 1960, many treatment and preventive measures have been developed for its control. However, the disease is common, with incidence rates in the range of 10–25% in many countries (Narayanan et al. 2003; Sun et al. 2009; Guo et al. 2010). Recently, the incidence of cow footrot has increased in the Heilongjiang Province in northeast China. The aim of the present survey was to determine the association between *F. necrophorum* and *D. nodosus* in cow footrot and to identify the role of the two bacterial species in the pathogenesis of this disease.

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## Materials and Methods

### Collection of clinical samples

Two hundred foot swabs from cows showing the characteristic clinical sign of foot rot with odorous exudative inflammation and keratinous hoof separation were collected from 10 farms in the Heilongjiang Province, northeast China, from May 2016 to May 2017. Twenty cows were taken for sampling from each farm. One foot of each cow was sampled, and the samples were frozen at –80 °C until DNA was extracted using a sodium dodecyl sulphate (SDS)/phenol/chloroform method, as described previously (Zhou and Hickford 2000).

### Detection of *F. necrophorum* and *D. nodosus*

Identification of *D. nodosus* and *F. necrophorum* was carried out using polymerase chain reaction (PCR) targeting the *fimA* and leukotoxin (*lktA*) genes. All the samples were subjected to the *lktA* gene amplification by PCR for the detection of *F. necrophorum*. The primer sequences were as follows: Forward, 5′-AATCGGAGTAGTAGGTTCTG-3′; Reverse, 5′-TCCAACAGAAACAGAAGCATC-3′. The PCR conditions consisted of an initial denaturation of 95°C for 5 min, followed by 30 cycles of 94°C for 1 min, 53°C for 50 s and 72°C for 1 min. This was followed by a final 10-min extension at 72°C. The *lktA* gene appears to be unique to *F. necrophorum*, as it is reportedly not present in other *Fusobacterium* species (Oelke et al. 2005). The partial DNA sequence of its *lktA* gene bears accession number AF_312861. The specificity of the *lktA* gene PCR was tested using bacterial DNA from the following species: *Bacteroides nodosus*, *Bacillus welchii*, *Staphylococcus aureus*, *Escherichia coli* and*Arcanobacterium pyogenes.*

All the samples were subjected to *fimA*gene-specific PCR for detection of *D. nodosus*. The primer sequences were as follows: Forward, 5′-CGGGGTTATGTAGCTTGC-3′; Reverse, 5′-TCGGTACCGAGTATTTCTACCCAACACCT-3′. The PCR conditions consisted of initial denaturation at 95°C for 5 min followed by 30 cycles of 94°Cfor 1 min, 53°Cfor 50 s and 72°C for 1 min. This was followed by a final 10-min extension at 72°C. The partial DNA sequence of the *fimA* gene bears accession number AF_145217. The specificity of the *fimA* PCR was tested using bacterial DNA from the following species: *Necrobacterium necrophorus*, *Bacillus welchii*, *Staphylococcus aureus*, *Escherichia coli* and *Arcanobacterium pyogenes*.

### Statistical analysis

The chi square ($\chi^2$) test was performed to ascertain the association between *D. nodosus* and *F. necrophorum* in cow footrot.

## Results

### Specificity of the *lktA* and *fimA* PCR

Only *F. necrophorum* and *D. nodosus* produced a correct size amplification. (Figs 1 and 2).

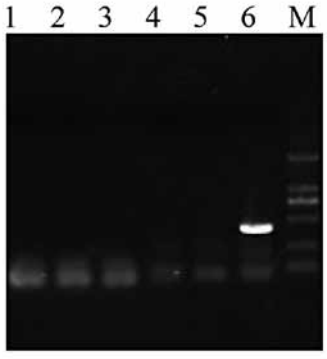


Fig. 1. Specificity of primers to amplify the *lktA* gene of *F. necrophorum*. Lane 1, 2, 3, 4, 5: PCR product of *Bacteroides nodosus, Bacillus welchii, Staphylococcus aureus, Escherichia coli, Arcanobacterium pyogenes*. Lane 6: PCR product of amplificate *F. necrophorum* DNA templates by primers (404 bp). M: DNA Marker DL2000 (2000 bp, 1000 bp, 750 bp, 500 bp, 250 bp, 100 bp).

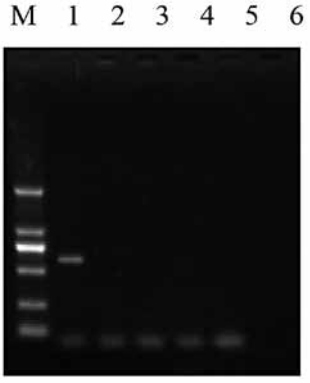


Fig. 2. Specificity of primers to amplify the *fimA* gene of *D. nodosus*. Lane 1: PCR Product of *D. nodosus* DNA templete by primer (621 bp). Lane 2, 3, 4, 5, 6: PCR product of *Necrobacterium necrophorus, Bacillus welchii, Staphylococcus aureus, Escherichia coli, Arcanobacterium pyogenes* DNA templates by primers. M: DNA Marker DL2000 (2000 bp, 1000 bp, 750 bp, 500 bp, 250 bp, 100 bp).

### Detection of *F. necrophorum* and *D. nodosus*

Of the 200 samples collected, 111 (55.5%) revealed the presence of *F. necrophorum* and 11 (5.5%) exhibited *D. nodosus*. Ten (5%) showed the presence of both *F. necrophorum* and *D. nodosus*, while 101 (50.5%) were positive for *F. necrophorum* alone and 1 (0.5%) for *D. nodosus* only. Neither *D. nodosus* nor *F. necrophorum* was detected in 88 (44%) samples (Table 1).

Table 1. The association of *F. necrophorum* and *D. nodosus* in cow footrot.

| | Negative for *F. necrophorum* | Positive for *F. necrophorum* | Total |
|---|---|---|---|
| Negative for *D. nodosus* | 88 | 101 | 189 |
| Positive for *D. nodosus* | 1 | 10 | 11 |
| Total | 89 | 111 | 200 |

## Discussion

In this study, we have used a PCR approach for detecting two bacteria. *Fusobacterium necrophorum* has been reported to be associated with cattle lameness and other diseases (Berg and Loan 1975; Clark et al. 1985; Nagaraja et al. 2005). *Dichelobacter nodosus*, which is described as the most common cause of lameness in sheep (Moore et al. 2005), has been associated with cattle lameness for many years (Egerton and Parsonson 1966). Our survey's results are in agreement with the findings of previous researchers, who also reported detection of *D. nodosus* and *F. necrophorum* in similar proportions in cow footrot (Bennett et al. 2009).

In our study, few positive samples contained both bacteria, so it is not possible to conclude with certainty that both bacteria in combination are required for the manifestation of the disease. Nevertheless, *F. necrophorum* is believed to create an anaerobic microenvironment in hooves due to the necrosis it causes, thereby facilitating the establishment of *D. nodosus* followed by the development of footrot.

A longitudinal study reported that the *F. necrophorum* load was higher in the feet of sheep with severe footrot. The reason for this could be that *F. necrophorum* plays a vital role in creating the anaerobic microenvironment necessary for the establishment of *D. nodosus* in the infected hooves of sheep (Witcomb et al. 2014). This may also be the cause of footrot.

The detection of *F. necrophorum*in a large proportion of the cows and *D. nodosus* at a lower rate suggests that *D. nodosus* is not a major agent of cow footrot in the Heilongjiang Province, northeast China, while *F. necrophorum* could be a major agent of cow footrot. However, it does confirm that *D. nodosus* is present and able to persist on cow hooves, even if the farms are specialised cow farms that do not stock sheep.

As a consequence of the difficulty associated isolating and culturing *F. necrophorum* and *D. nodosus*, we used a PCR technique instead to detect these organisms and report the prevalence of these bacteria on cow hooves. The test results of the limited samples suggest that this technique will have a potential use in confirming the presence of *F. necrophorum* and *D. nodosus* in cows infected with footrot.

In conclusion, *F. necrophorum* is frequently found on the hooves of cows infected with footrot in the Heilongjiang Province, northeast China. *Fusobacterium necrophorum* and *D. nodosus* should be highly considered when controlling cow footrot in China.

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