Advances in Animal and Veterinary Sciences 2 (5): 302 – 304
http://dx.doi.org/10.14737/journal.aavs/2014/2.5.302.304

Case Report

Rapid Detection of *Fusobacterium necrophorum* as a Main Causative Agent of Foot Rot in Small Ruminants by Polymerase Chain Reaction Assay

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ARTICLE HISTORY

Received: 2014–06–03
Revised: 2014–06–11
Accepted: 2014–06–12

Key Words: *Fusobacterium necrophorum*, goats, lktA gene, foot rot

ABSTRACT

The study was aimed at detection of *Fusobacterium necrophorum* and *Dichelobacter nodosus* directly from field samples of goats and sheep with the aid of PCR assay targeting *lktA* and *16S rRNA* genes, respectively. A total of 24 swab DNA samples from foot lesions suspected for foot rot tested for *lktA* and *16S rRNA*, 19 (79%, Goats– 17 and Sheep–02) were found positive for *lktA* gene of *F. necrophorum* but negative for *16S rRNA* gene of *D. nodosus*. This study shows that *F. necrophorum* alone could cause foot rot in absence of *D. nodosus* in goats.

ARTICLE CITATION: Shivasharanappa N , Gupta VK, Sharma N, Mishra AK, Singh VK, Pawaiya RVS, Gururaj K, Sharma DK, Kumar A, Paul S, Kumar N, Singh SV (2014). Rapid detection of *Fusobacterium necrophorum* as a main causative agent of foot rot in small ruminants by polymerase chain reaction assay. Adv. Anim. Vet. Sci. 2 (5): 302 – 304.

Several studies suggest that over 80% of sheep flocks contain lame animals (Wassink et al., 2003a and Wassink et al., 2003b). Foot rot is a highly contagious bacterial disease affecting feet of small ruminants caused by the *Fusobacterium necrophorum* and *Dichelobacter nodosus* (Beveridge, 1941; Egerton et al., 1969) which is characterized by interdigital tissue necrosis leading to lameness which can result in serious economic losses to small ruminant production. *F. necrophorum* causes most of the inflammation and tissue damage, whereas *D. nodosus* produces enzymes responsible for invasion and separation of the horn (Hodgkinson, 2010). The distinctive lesions and bacterial culture can help to diagnose the disease. The PCR is one of the sensitive assays in detecting presence of these agents from the swabs collected from interdigital necrotic lesions in cattle (Bennett et al., 2009). There are few studies about the role of *F. necrophorum* involvement in the foot rot in goats. Culture and isolation of these causative agents in the laboratory are cumbersome and require specical media and time duration of 3–4 weeks. Therefore, we used a PCR assay targeting *lktA* gene of *Fusobacterium necrophorum* and *16S rRNA* gene of *Dichelobacter nodosus* from goats and sheep with foot lesions suspecting of foot rot.

Table 1: Primers used in the study

Gene	Primer sequence	Amplicon size (bp)	Annealing Temp (°C)	Reference
lktA	F 5'–ACAATCGGAGTAGTAGGTTC–3' R 5'–ATTTGGTAACTGCCACTGC–3'	402	59	Bennett et al., (2009)
16S rRNA	F 5'–GAACGGTGCATGGTTAATAC–3' R 5'–ACATGAGTGTCAGTATTGCC–3'.	312	60	

A total of 24 sterile cotton swabs (19 goats and 05 sheep) of foot rot exudates from animals showing lameness and interdigital necrotic/suppurative lesions were collected and were frozen at –80°C until processed. DNA was isolated using Wizard® Genomic DNA Purification Kit (Promega) and subjected to PCR assay for the detection of *F. necrophorum* and *D. nodosus* using primers for amplification of leukotoxin structural gene (*lktA*) of *F. necrophorum* and *16S rRNA* of *D. nodosus*. Primers used in the study are described in Table 1. The PCR thermal profile consisted of an initial denaturation step at 94°C for 2 min, followed by 35 cycles of 95°C for 30 s, 59°C for 30 s and 68°C for 30 s. A final extension of 5 min at 72°C was performed. Amplification was performed in a 25 uL reaction mixture containing 1uL of DNA, 0.30 uM of each primer, 12.5 uL of FideliTaq PCR Master Mix (2X) (Affimatrix®), 0.4 mM of dNTPs , 3 mM of MgCl and 0.5U of FideliTaq DNA Polymerase (Affimatrix®). Amplification was carried out in a Gradient thermocycler (Eppendorf), and the thermal profile consisted of a denaturation cycle at 95°C for 2 min, followed by 35 cycles of 95°C for 30 s, 60°C for 30 s and 68°C for 30 s, with a final

ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)

extension step at 72°C for 5 min. PCR products from both the *lktA* and *16S rRNA* genes were separated electrophoretically in 1.2% agarose gels containing 0.5ug/mL

Figure 1a and b: Interdigital congestion and necrosis in goats

of ethidium bromide, and visualized under UV light using a transilluminator.

A total of 24 animals (19 Goats and 05 sheep) have shown clinical signs such as lameness and recumbence (5 goats). Clinical examination of feet revealed interdigital necrosis, bleeding, pus formation and sloughing of tissues (Figure 1a and b). Out of 24 DNA swab samples of goats and sheep tested for *lktA* and *16S rRNA* in PCR, 19 (79%, Goats–17 and Sheep–02) revealed amplification of 402bp fragment, which was correspondingto *lktA* gene of *F. necrophorum*. Surprisingly none of the samples revealed amplification of *16S rRNA* gene of *D. nodosus* (Figure 2).This study describes the detection of *F. necrophorum* from swab samples of exudates from foot rot in goats and sheep by PCR assay targeting *lktA* gene without going for isolation and culture. Interdigital necrosis and suppurative lesions in goats and sheep examined in our study may be due to different conditions such as Interdigital dermatitis (ID), benign footrot or very early virulent footrot (Winter, 2004). *F. necrophorum* is an opportunistic pathogen and has been associated with many disease conditions, such as footrot, hepatic abscess and necrotic laryngitis in animals (Nagaraja et al., 2005). It possess a leukotoxin gene (*lktA*) and express a leukotoxin which is considered to be the main virulence factor (Coyle–Dennis and Lauerman, 1979) and is unique to *F. necrophorum*, as it is not present in other *Fusobacterium* species (Oelke et al., 2005; Bennett et al., 2009). *F. necrophorum* may cause inflammation of the interdigital skin (i.e. interdigital dermatitis) and produce a number of toxins which cause necrosis of the superficial layer of the interdigital skin and enable the establishment of other bacteria, including *D. nodosus* (Beveridge, 1941). The diagnosis of footrot is a tedious and time–consuming process, complicated by the fastidious growth requirements and slow growing nature of these organisms (Wani and Samanta, 2006). Therefore, PCR assay was employed for detecting foot rot condition in animals by targeting *lktA* (Bennett et al., 2009), *16sRNA* (Bennett et al., 2009), and *IntA* gene (Cheetham et al., 2006) can be used as rapid, sensitive and specific test for diagnosis of foot rot in animals from swab samples of exudates. When employed, PCR assay vastly improved the accuracy of identification and grouping of *D. nodosus* from footrot lesions (John et al., 1999).

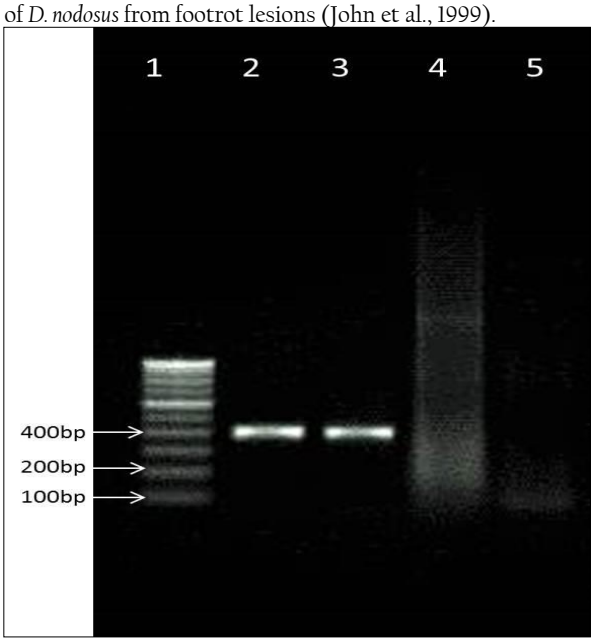

Figure 2: 402 bp amplicon size of Leukotoxin structural gene (*lktA*) of *F. necrophorum* in sheep and goats from foot lesions. Lane 1: Marker of 100bp, 2– Sheep DNA, 3–goat DNA, 4– E.coli DNA and 5 No template control

Our findings suggested that *F. necrophorum* is the main causative agent of foot rot in goats as in 79% of cases no *D. nodosus* detected. All the 79% cases in goats were in advance stages and were showing suppurative and necrotic lesions in feet. However, the presence of *D. nodosus* in lesions cannot be ruled out without bacterial isolation. It is interesting to note that, on the contrary to the available literature, *D. nodosus* seems to be a major player in foot rot in goats. However, further studies are required to establish the fact. Therefore, PCR based diagnosis can be efficiently used in the detection of *F. necrophorum* which are otherwise difficult to culture in common laboratory conditions. The presence of *F. necrophorum* has implications in the management of flock

ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)

health in semi–intensive rearing system in India. Hence PCR assay can be employed to detect the presence of mixed infection by these bacteria in feet lesions of small ruminants. The presence of lktA gene indicated the presence of virulent strain of *F necrophorum* which was responsible for clinical foot rot in goats and sheep. Further studies envisaged on large number of samples collected from different areas are required to ascertain the fact that the *F. necrophorum* is the main causative agent of foot rot in goats.

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ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)