Available online at www.sciencedirect.com
ScienceDirect
Research in Veterinary Science 83 (2007) 141–144
RVS
www.elsevier.com/locate/rvsc

Isolation and characterization of *Dichelobacter nodosus* from ovine and caprine footrot in Kashmir, India

S.A. Wani *, I. Samanta, S. Kawoosa

Bacteriology Laboratory, Division of Microbiology & Immunology, Faculty of Veterinary Sciences & A.H., S.K. University of Agricultural Sciences and Technology of Kashmir, Shuhama (Alusteng), Srinagar 190006, India

Accepted 13 November 2006

Abstract

Footrot is a highly contagious and economically important disease of sheep and goats, caused by *Dichelobacter nodosus*, a slow growing anaerobic Gram-negative rod. The current Australian antigenic classification system, based on variation in the fimbriae, classifies *D. nodosus* into at least 10 serogroups (A–I and M) and 18 serotypes. This investigation was intended to determine the serological diversity of *D. nodosus* in this region of Kashmir, India. Exudates of footrot lesions were collected from 24 naturally infected sheep and 42 goats located in the Kashmir valley. Of these 66 samples, 24 yielded evidence of *D. nodosus* by PCR using 16SrDNA specific primers. Multiplex PCR using serogroup specific primers revealed the presence of serogroup B in all the samples except two, which showed the presence of serogroup E *D. nodosus.* This study also documents the isolation of *D. nodosus* and detection of serogroup E for the first time in India.
© 2006 Elsevier Ltd. All rights reserved.

Keywords: *Dichelobacter nodosus*; Footrot; Sheep; Goat; Isolation; PCR

1. Introduction

Footrot is a highly contagious and economically important disease of the feet of sheep and goats, characterized by the separation of keratinous hoof from the underlying epidermal tissue resulting in severe lameness, loss of body condition and reduced wool production (Egerton et al., 1969). The disease is dependent on a mixed bacterial infection, but the essential causative agent is *Dichelobacter nodosus*, a slow growing anaerobic Gram-negative rod (Billington et al., 1996). The current Australian classification system, based on antigenic variation in the structure of fimbriae, classified *D. nodosus* into at least 10 serogroups (A–I and M) and 18 serotypes (Claxton, 1986; Ghimire et al., 1998).

Traditionally the identification of *D. nodosus* has relied on the isolation of the causative agent from footrot lesion and biochemical tests on the resultant isolates (Pitman et al., 1994). However, recently PCR based methods have been used for detection (La Fontaine et al., 1993) and characterization (Dhungyel et al., 2002) of *D. nodosus* without need to culture. The objective of the present investigation was to expand our earlier findings (Wani et al., 2004) for better understanding of the serological diversity of *D. nodosus* in this part of India.

2. Materials and methods

2.1. Collection of clinical samples

Exudates of footrot lesions were collected during November 2003 to May 2004 from 24 naturally infected sheep and 42 goats of private owners from the Kangan (Srinagar) and Kawoosa (Budgam) area of the Kashmir valley. The site sampled was at the apex of the cleft that develops between the horn of the hoof and the sensitive underlying tissues. Samples were collected on cotton swabs, transported to the laboratory on ice, anaerobically cultured and stored in sterile tubes at −20 °C until further use.

* Corresponding author. Tel./fax: +91 194 2262211.
E-mail address: microlab@sancharnet.in (S.A. Wani).

0034-5288/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rvsc.2006.11.006

2.2. DNA extraction

Suspensions of the material present on the swabs were prepared in 1.5 ml microcentrifuge tubes in 100 μl of sterile phosphate buffered saline (PBS) by gentle vortexing. After removing the swabs, the samples were boiled for 5 min, cooled on ice for 10 min and centrifuged at 10,000*g* for 1 min. Similarly from the culture plates suspected colonies with characteristic morphology were directly suspended into 100 μl of sterile PBS and processed for the extraction of DNA as before. Two microlitres of the supernatant was used as the template for each PCR reaction.

2.3. Detection of 16S rRNA gene of D. nodosus by PCR

PCR amplification was performed in 25 μl in 0.2 ml thin walled PCR tubes (Tarson, India). The PCR mixture contained a final concentration of 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 3 mM $MgCl_2$, 0.01% gelatin, 0.5 μM concentration of each primer, 0.2 mM concentrations of each 2′-deoxynucleoside 5′-triphosphate and 1U of Taq DNA polymerase (Bangalore Genei Pvt. Ltd, Bangalore, India). Oligonucleotide primers (Table 1) were also procured from Bangalore Genei Pvt. Ltd, Bangalore, India. The amplification cycles in a GeneAmp PCR System 2400 Thermal cycler (Applied Biosystems, USA) consisted of 94 °C for 2 min, followed by 5 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s, and 25 cycles of 94 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s for 25 cycles, and final extension at 72 °C for 4 min (La Fontaine et al., 1993). Positive control DNA samples kindly supplied by Dr. O.P. Dhungyel, Faculty of Veterinary Medicine, University of Sydney, Cambden, NSW 2570, Australia, were included in the PCR. Sterile distilled water was used as negative control. The PCR products were analysed in 0.8% agarose gels, stained with ethidium bromide and visualized under ultraviolet illumination and photographed with Gel Documentation System (GDS 8000 system, UVP, UK).

2.4. Serogrouping by multiplex PCR

The samples positive for *D. nodosus* as revealed by the amplification of 783 bp gene products were subjected to serogrouping by multiplex PCR using A–I serogroup specific primers (Dhungyel et al., 2002). The Oligonucleotide primers (Table 1) were procured from Bangalore Genei Pvt. Ltd, Bangalore, India. Positive control DNA samples kindly supplied by Dr. O.P. Dhungyel, Faculty of Veterinary Medicine, University of Sydney, Cambden, NSW 2570, Australia, were included in the PCR. Sterile distilled water was used as negative control. The PCR products were analyzed in 2.0% agarose gels, visualized and photographed as discussed above.

2.5. Isolation of D. nodosus from clinical samples

For the isolation of *D. nodosus*, lesion material from affected sheep and goats was streaked on Nutrient agar (HiMedia, India) plates with addition of 0.15% Serine, 0.5% arginine and 4% hoof powder. The hoof powder was prepared from dried hooves collected from healthy slaughtered sheep. The plates were placed in an anaerobic jar with Gaspacks (HiMedia, india) and incubated at 37 °C. After 4 days of incubation, suspected colonies (Thorley, 1976) were subcultured on the same medium (except the concentration of hoof powder was only 2%) for obtaining a pure culture of *D. nodosus.* Confirmation of the colonies as *D. nodosus* was by detection of species-specific 16SrDNA by PCR as described above except the hoof medium was used as a negative control.

3. Results

Out of 24 samples collected from naturally infected sheep, 12 (50%) yielded the amplified product of the expected size of 783 bp (Fig. 1). The multiplex PCR assay for serogrouping was successfully applied to all the 12 specimens. Ten samples yielded a single band of 283 bp characteristic of serogroup B, whereas the remaining

Table 1
Details of the primers used in PCR assays

Primer name	Nucleotide sequence (5′ → 3′)	Target gene	Size (bp)	Reference
Forward	CGGGGTTATGTAGCTTGC	16S rRNA	783	La Fontaine et al. (1993)
Reverse	TCGGTACCGAGTATTTCTACCCAACACCT	–	–	
FP	CCTTAATCGAACTCATGATTG3′	*fimA*	–	Dhungyel et al. (2002)
RA	5′AGTTTCGCCTTCATTATATTT3′	*fimA*	415	
RB	5′CGGATCGCCAGCTTCTGTCTT3′	*fimA*	283	
RC	5′AGAAGTGCCTTTGCCGTATTC3′	*fimA*	325	
RD	5′TGCAACAATATTTCCCTCATC3′	*fimA*	319	
RE	5′CACTTTGGTATCGATCAACTTGG3′	*fimA*	363	
RF	5′ACTGATTTCGGCTAGACC3′	*fimA*	241	
RG	5′CTTAGGGGTAAGTCCTGCAAG3′	*fimA*	279	
RH	5′TGAGCAAGACCAAGTAGC3′	*fimA*	409	
RI	5′CGATGGGTCAGCATCTGGACC3′	*fimA*	189	

Download English Version:

https://daneshyari.com/en/article/2456301

Download Persian Version:

https://daneshyari.com/article/2456301

Daneshyari.com