



Economic impact of ovine footrot and serological diversity and virulence of *Dichelobacter nodosus* in north Kashmir, India

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ABSTRACT

The present study was aimed to determine the prevalence and economic impact of footrot as well as to determine the serological diversity and strain differentiation of *Dichelobacter nodosus* in north Kashmir, India, comprising district Baramulla, Bandipora and Kupwara. The overall prevalence was 14.77% with the estimated economic impact to the tune of Rs 41 million annually to the sheep production in north Kashmir. The prevalence was highest (17.41%) in district Kupwara and lowest (12.71%) in district Bandipora while district Baramulla recorded a prevalence rate of 14.44%. Out of 300 samples collected from footrot lesions of naturally infected sheep, 150 detected positive for *D. nodosus* by PCR. Over all prevalence of serogroups B, E and I of *D. nodosus* was 92.7, 32 and 26%, respectively. Infection due to single serogroup was found in 87 samples while, 63 samples revealed mixed infection of 2 or 3 serogroups. Out of 226 isolates of *D. nodosus* from positive samples, 139 (61.50%) belonged to serogroup B, 48 (21.23%) to E and 39 (17.25%) to I. Virulence characterization revealed that 66.80% showed the presence of integrase (*intA*) gene, thus were considered as virulent strains. Serogroup wise *intA* gene was found in 91 (65.46%) isolates of serogroup B, 33 (68.75%) of E and 27 (69.23%) of I. These findings suggested the need for incorporation of serogroups B and E in the formulation of a vaccine to effectively combat footrot in north Kashmir.

Key words: *Dichelobacter nodosus*, Footrot, PCR, Serogrouping, Sheep, Virulence

Footrot, an economically important infectious syndrome of sheep and goats, is also reported in cattle, horses, pigs etc. It is characterised by the separation of keratinous hoof from the underlying epidermal tissue resulting in severe lameness, loss of body condition and reduced wool production and caused by the synergistic action of certain bacterial species of which *Dichelobacter nodosus* (*D. nodosus*) is the main transmitting agent (Thomas 1964). The *D. nodosus* strains were classified into at least 10 serogroups (A–I and M) (Claxton 1986, Ghimire *et al.* 1998). Identification of the *D. nodosus* is being carried out by polymerase chain reaction (PCR; La Fontaine *et al.* 1993), and its serogrouping is being carried out by multiplex PCR (m-PCR; Dhungyel *et al.* 2002). The disease has major economic impact on sheep rearing in Australia and New Zealand; and in United Kingdom (UK) the footrot accounts for an economic loss of £24 million per annum (Nieuwhof and Bishop 2005). *D. nodosus* (Stewart 1989) causes

virulent, intermediate and benign forms of disease. Virulent footrot (VFR) is highly contagious and often the target of expensive control programmes. Differentiation of *D. nodosus* into virulent, intermediate and benign strains was done by elastase (Stewart 1979) and gelatin gel tests (Palmer 1993) and subsequently by ELISA (Stewart *et al.* 1990, Katz *et al.* 1991). Recently, Cheetham *et al.* (2006) described a PCR assay, for detection of a virulent specific genetic element the integrase (*intA*) gene, which showed a high correlation with the ability of the strain to cause virulent footrot. Even though the disease is present in several parts of India, but has become enzootic throughout Jammu & Kashmir. There are several reports of its prevalence and serological diversity (Wani *et al.* 2007, Farooq *et al.* 2010, Rather *et al.* 2011). The present study records the prevalence and economic impact of footrot as well as determines the serological diversity and strain differentiation of *D. nodosus* in north Kashmir

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MATERIALS AND METHODS

Survey: During the period between September 2008 and June 2009, a survey was conducted to determine the prevalence of footrot in north Kashmir, India, comprising 3 districts. A two-stage simple random sampling procedure (Farooq *et al.* 2010) was followed.

The serological diversity of *D. nodosus* was conducted on 300 swab samples one from each sheep affected with footrot.

Collection of samples : Swab samples (300) from foot lesions of the affected sheep with a lesion score of 2 to 4 were collected in duplicate from 100 naturally infected sheep from each of the 3 districts of north Kashmir, viz. Baramulla, Bandipora and Kupwara. One swab from each sample was used for inoculation onto media and another for DNA extraction for direct detection of *D. nodosus*.

Isolation of *D. nodosus*: For isolation of *D. nodosus*, all the swab samples were streaked at the place of collection on tripticase-arginine-serine (TAS) agar containing 4% hoof powder (Thorley 1976). The isolation was done as per Farooq *et al.* (2010). Confirmation of the isolates as *D. nodosus* was done by demonstration of the typical cellular morphology in Gram stained smears and detection of species-specific 16S rRNA gene by PCR as described below.

Extraction of bacterial DNA: The material present on the swabs or bacterial colonies from the culture plates were suspended in 100 µl of sterile phosphate buffered saline (PBS) in 1.5 ml microcentrifuge tubes by gentle vortexing. The samples were boiled for 5 min, cooled on ice for 10 min and centrifuged at 10,000×g for 1 min. Two microlitres of the supernatant was used as the template for each PCR reaction.

Detection and serogrouping of *Dichelobacter nodosus* by PCR: Detection of *D. nodosus* was performed by 16S rRNA gene specific PCR as per La Fontaine *et al.* (1993). Similarly serogrouping by multiplex PCR was carried out by using 9 (A–I) serogroup specific primers (Dhungyel *et al.* 2002). Positive control DNA kindly supplied by Dr O P Dhungyel, Faculty of Veterinary Medicine, University of Sydney, Cambden, NSW 2570, Australia, was included in the PCR. Distilled water served as negative control.

Strain differentiation of *D. nodosus* into virulent and benign: To ascertain the virulent or benign status, all the *D. nodosus* isolates were screened for presence of *intA* gene by PCR (Cheetham *et al.* 2006) with minor modifications. The concentration of the primers was 0.25 µM while concentration of MgCl₂ was 2.0 mM. The amplification was done with 31 cycles, each cycle consisting of 1.5 min at 94°C, 1 min at 60°C and 2 min at 72°C. This was followed by a final extension of 5 min at 72°C.

Analysis of PCR products: The PCR products were analysed in 1–2% agarose gels, stained with ethidium bromide, visualized under ultraviolet illumination and photographed with gel documentation system.

RESULTS AND DISCUSSION

Prevalence of footrot in north Kashmir: Ovine footrot is widespread in the state of Jammu and Kashmir and is one of the economically most important diseases of sheep. The disease demands immediate intervention in terms of an effective vaccine. However, for the development of an efficacious vaccine against virulent footrot, it is essential to know the serological diversity of *D. nodosus* as well as

Table 1. Prevalence of footrot in various districts of north Kashmir, India

District	Number of HHFs in the selected villages	5% of HHFs selected through SRS	Flock strength	Affected	Prevalence of footrot
Baramulla	520	26	1634	236	14.44%
Bandipora	298	15	857	109	12.71%
Kupwara	408	21	867	151	17.41%
Total	1226	62	3358	496	14.77%

HHFs, House hold flocks; SRS, simple random sampling.

virulent status of different serogroups prevalent in the field. In this direction the present study was conducted to cover the areas of north Kashmir which so far remains unexplored with respect to footrot.

The prevalence of footrot as revealed during the period of study in north Kashmir is depicted in Table 1. Considering 10% production loss (Marshall *et al.* 1991), average treatment cost of Rs 20/sheep, 40 kg average body weight/sheep and Rs 90/kg live weight, then economic loss at current prevalence rate of footrot was estimated to amounts to Rs 41 million in north Kashmir.

The overall prevalence (14.77%) of footrot observed in present work is more or less similar to that from other parts of Kashmir (Farooq *et al.* 2010, Rather *et al.* 2011) but is higher than that observed in other parts of world like 8–10% in United Kingdom (Grogono *et al.* 1997, Wassink *et al.* 2003) and 3.1% in Bhutan (Gurung *et al.* 2006). Based on the observed prevalence, the estimated economic impact of ₹ 41 million annually to sheep production in north Kashmir is similar to the observations of Farooq *et al.* (2010) in south Kashmir. Similarly Marshall *et al.* (1991) reported a loss of A\$ 43 million per annum in New South Wales (Australia) and Nieuwhof and Bishop (2005) reported economic loss of £24 million / annum in United Kingdom due to footrot.

Serological diversity of *D. nodosus* in south Kashmir

Out of 300 swab samples collected 150 (50.0%) samples detected positive for *D. nodosus* by the amplification of 783 bp product of 16S rRNA gene of *D. nodosus* (Fig. 2). District wise 52% samples from Baramulla, 54% from Bandipora and 44% from Kupwara were found positive for *D. nodosus*.

Serogrouping of *D. nodosus* positive samples by m-PCR using 9 (A–I) serogroup specific primers revealed the presence of 3 serogroups (Table 2; Fig. 1). Based on individual sample observations 56% samples carried serogroup B, 2% samples carried serogroup E and rest showed mixed infection. Among the samples with mixed infection 12% samples showed infection of serogroup B and I, 5.3% samples yielded mixed infection of serogroup I and E, 16% samples yielded mixed infection of serogroup B and E and 8.7% samples were found positive for

Table 2. District wise serological diversity of *Dichelobacter nodosus* in north Kashmir, India

District	Samples collected	Samples positive for Dn	Serogroup of samples distribution of						Dn isolates	Predominant serogroup	Serogroupwise <i>intA</i> gene		
			B	E	I+E	B+E	B+I	B+E+I			B	E	I
Baramulla	100	52	40	2	-	4	6	-	62	B (96.15%)	30	6	5
Bandipora	100	54	29	-	6	15	4	-	79	B (88.88%)	33	17	8
Kupwara	100	44	15	1	2	5	8	13	85	B (93.18%)	28	10	14
Total	300	150	84	3	8	24	18	13	226	B (92.66%)	91	33	27

Dn, *Dichelobacter nodosus*.

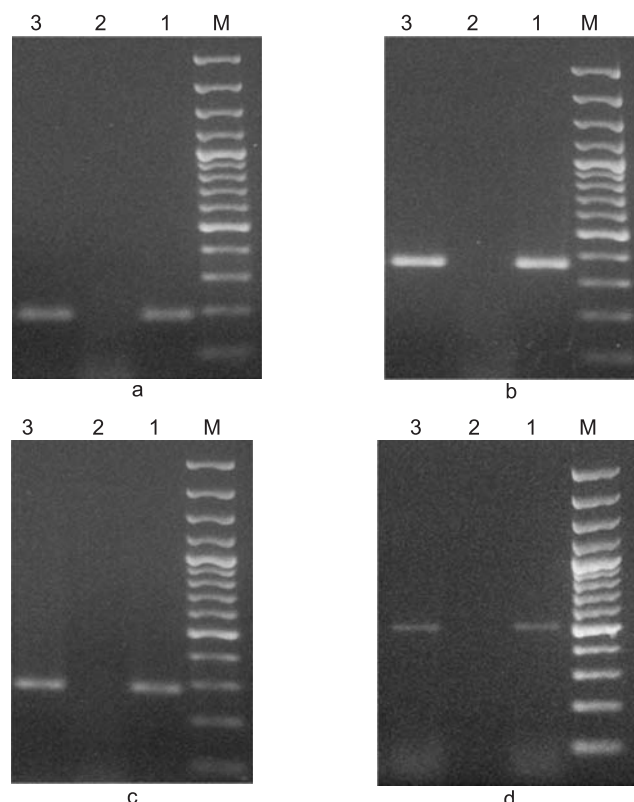


Fig. 1. PCR products of *Dichelobacter nodosus* from clinical samples of sheep affected with footrot, (a) serogroup B specific product, (b) serogroup E specific product, (c) serogroup I specific product, and (d) *intA* gene specific product. M, 100bp ladder; 1, positive control; 2, Negative control; 3, sample.

serogroup B, E and I. Overall 92.7% samples revealed serogroup B, 32% serogroup E and 26% serogroup I.

Serological diversity trend in the present study was similar to that reported by Farooq *et al.* (2010) and Rather *et al.* (2011) from south and central Kashmir, respectively. Similarly, Claxton *et al.* (1983) reported the predominant prevalence of serogroup B in Australia, Hindmarsh and Fraser (1985) in Great Britain, Kingsley *et al.* (1986) in New Zealand and Gurung *et al.* (2006) in Bhutan.

The occurrence of mixed infection with 2 different serogroups in 42% of samples corroborates with the findings of Rather *et al.* (2011) from central Kashmir but is higher than 4.46% observed in south Kashmir by Farooq *et al.* (2010). Hindmarsh and Fraser (1985) reported 13.80% of

sheep had a mixed infection of *D. nodosus* in Great Britain. The significance of mixed infection may be explained by the fact that *D. nodosus* can undergo serogroup conversion as a result of natural transformation (Kennan *et al.* 2003). This also explains why a serogroup specific vaccine fails under these typical field conditions.

Virulent and benign status of *D. nodosus*: Out of 226 *D. nodosus* isolates, 66.80% carried *intA* gene. Serogroup wise 65.46% isolates of serogroup B, 68.75% of serogroup E and 69.23% of serogroup I carried *intA* gene.

The designation of 66.80% *D. nodosus* isolates as virulent strains in the present investigation corroborated with the earlier findings by Rather *et al.* (2011) and Farooq *et al.* (2010) who also recorded that 61.88 and 61.60% isolates from central and south Kashmir, respectively, were virulent.

Thus the present investigation again confirms the heavy economic impact due to high prevalence of ovine footrot and establishes the predominant prevalence of serogroup B of *D. nodosus* followed by serogroup E. Hence the use of bivalent vaccine containing these 2 serogroups is recommended to combat the menace in north Kashmir.

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