Prevalence of ovine footrot in the tropical climate of southern India and isolation and characterisation of *Dichelobacter nodosus*

D. Sreenivasulu [(1)], S. Vijayalakshmi [(1)], D. Raniprameela [(1)], A. Karthik [(1)], S.A Wani [(2)*] & I. Hussain [(2)]

This paper (No. 06122013-00022-EN) has been peer-reviewed, accepted, edited, and corrected by authors. It has no yet been formatted for printing. It will be published in December 2013 in issue **32**-3 of the *Scientific and Technical Review*.

(1) Department of Veterinary Microbiology, College of Veterinary Sciences, Sri Venkateswara Veterinary University, Tirupati-517502, Andhra Pradesh, India

(2) Bacteriology Laboratory, Division of Veterinary Microbiology and Immunology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama (Alusteng), Srinagar-190006, Jammu and Kashmir, India

*Corresponding author: shakilwani@gmail.com

Summary

The present communication records the first determination of the prevalence of footrot in the unexpected situation of the tropical climate of Andhra Pradesh and Tamil Nadu, two states in southern India where the maximum temperature rises to 42°C. In total, 73 outbreaks of footrot in Nellore brown sheep were investigated in 11 districts of Andhra Pradesh and one district of Tamil Nadu during the period March 2009 to March 2011. The overall prevalence of ovine footrot was 15%, with severity scores of 2 to 4 (lesion severity scale 0 to 4). The outbreaks occurred mostly during the rainy season, which is usually from June to December. From a total of 1,050 samples of lesions in naturally infected sheep, 478 (45.5%) were positive for *Dichelobacter nodosus*. Serogrouping of the isolates revealed six serogroups: A, B, C, E, F and I. Among the positive samples, 448 (93.7%) were a single serogroup and 30 (6.3%) carried a mixed infection with two serogroups. Taking single and mixed infections

together, serogroup B was most frequent at 50.4% and was found in all districts, followed by serogroup I in 29.3% of samples, A in 14%, F in 6.7% and C in 5.6%. Serogroup E was detected in only one sample. Serogroups A and F were detected for the first time in India. All of 58 *D. nodosus* isolates in a sub-sample representing –erent serogroups were found to be virulent, based on the production of thermostable proteases and the presence of the integrase A gene *intA*. Thus, the present paper reporting isolation and characterisation of *D. nodosus* confirms the occurrence of virulent footrot in the tropical climate of southern India.

Keywords

Dichelobacter nodosus – Serogroup – Sheep – Southern India – Virulent footrot.

Introduction

Footrot is a highly contagious and economically important disease of ruminants, especially sheep and goats. The disease is characterised by an exudative inflammation followed by necrosis of the epidermal tissues of the interdigital skin and hoof matrix, which results in separation of the hoof from the underlying soft tissue. Affected animals exhibit lameness, loss of body condition, reduced production of wool and meat, and decreased fertility (1, 2). Footrot is mainly considered a disease of temperate climates with moderate to heavy rainfall. Muddy pastures, frequent rains and foot injuries predispose animals to the infection (2, 3, 4).

The disease results from the synergistic action of several bacterial species, of which *Dichelobacter nodosus* is the essential causative agent (2). The bacterium is slow growing and requires a special medium under anaerobic conditions. Currently, *D. nodosus* strains are classified into at least ten serogroups (A–I, M) (5, 6) based on fimbrial antigens. The identification of serogroups of *D. nodosus* in a footrot lesion previously required isolation and purification of the organism by subculture, followed by antigenic analysis using serological methods, which could take three or four weeks. Polymerase chain

reaction (PCR) with 16S rRNA gene-specific primers is now used for identification of *D. nodosus* (1) and multiplex PCR (m-PCR) with serogroup-specific primers for serogrouping, without the need for culture (7). Strains of *D. nodosus* can be categorised as virulent, intermediate or benign (8, 9), based on the production of thermostable proteases and/or the integrase A gene *intA*, and relate to the corresponding forms of the disease (2).

In India, footrot has been reported only from the Himalayan state of Jammu and Kashmir, where the climate is temperate (10, 11). However, there has been strong suspicion of ovine footrot in the tropical climatic extremes of southern India, based on clinical symptoms and lesions but without any laboratory confirmation. Thus, the aim of the present investigation was to determine the prevalence of footrot, with isolation and characterisation of *D. nodosus*, in the unexpected situation of tropical Andhra Pradesh and Tamil Nadu states in southern India.

Materials and methods

Inspection of animals

A total of 73 outbreaks of footrot in Nellore brown sheep (a hair-type meat breed of southern India) reported by local veterinary officers were attended in 11 districts of Andhra Pradesh (Chittoor, Nellore, Kadapa, Kurnool, Guntur, Krishna, Nizamabad, Ongole, Srikakulum, Mehaboobnagar, Nalgonda) and the Tiruvallur district of Tamil Nadu (Fig. 1) during the period March 2009 to March 2011. Overall, a total of 16,889 animal feet in 295 household flocks (Table I) were inspected for signs of the disease and scored on a scale of 0 to 4 according to the severity of the lesions (12).

Collection of samples

Sterile cotton swabs were used to collect 1,050 duplicate samples of footrot exudate (Table II) from among the 2,548 animal feet with lesions of severity 2 (interdigital dermatitis) to 4 (under-running of the hard horn of the hoof) on the scale. The samples were taken from the

apex of the cleft between the horn of the hoof and the sensitive underlying tissues. One of the samples was inoculated onto a solid medium and the other was used for DNA extraction for direct detection of *D. nodosus* as described below.

Isolation of *Dichelobacter nodosus*

Swab samples were immediately streaked onto trypticase arginine serine agar containing 4% hoof powder and placed in anaerobic jars (Hi-Media, India) with gas packs (Becton Dickinson and Company, United States of America [USA]). The jars were transported to the laboratory within 2 h to 3 h and incubated at 37°C. After five days of incubation, suspected colonies were subcultured on the same medium to achieve pure isolates of *D. nodosus*. Plates that did not show any characteristic growth were incubated for a further five days before being discarded as negative. Colonies were confirmed as *D. nodosus* by demonstration of the typical cellular morphology in Gram-stained smears and detection of the species-specific 16S rRNA gene in PCR.

Extraction of bacterial DNA

Material present on the swabs was suspended in 100 µl of sterile phosphate buffered saline in 1.5 ml microcentrifuge tubes by gentle vortexing. After removal of 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, suspected colonies showing characteristic morphology on the culture plates were directly suspended in 100 µl of sterile distilled water. Suspensions were boiled for 5 min, cooled on ice for 5 min and then centrifuged at 10,000 × *g* for 5 min. Portions (2 µl) of the supernatants were used as templates for the PCR reactions.

Detection of the 16S rRNA gene of *Dichelobacter nodosus* using polymerase chain reaction

For detection of the 16S rRNA gene of *D. nodosus*, PCR conditions and primers were essentially the same as described previously (1). The DNA extracted from *D. nodosus* strain JKS-5B (serogroup B) was used as a positive control and sterile distilled water served as a

negative control. Details of the primers used in this study are shown in Table III.

Serogrouping of *Dichelobacter nodosus*

Multiplex PCR with nine (A–I) serogroup-specific primers was used for serogrouping the *D. nodosus* isolates, as described by Dhungyel *et al.* (7). Serogroup-specific DNA supplied by Dr O.P. Dhungyel was used as a positive control.

The PCR products were analysed in 1% to 2% agarose gels, stained with ethidium bromide, visualised under ultraviolet illumination and photographed using an Alphalmager® HP automatic gel imaging analyser (Alphalmager Corporation, USA).

Identification of virulent and benign strains of *Dichelobacter nodosus*

Gelatin gel test

To determine whether a strain was virulent or benign, 58 *D. nodosus* isolates representative of different serogroups (A = 8, B = 25, C = 5, F = 5, I = 15) were screened for thermostable proteases in the gelatin gel test (9), with slight modification. The selection of representative isolates was based on a high score for lesion severity. Briefly, agarose (0.8% w/v) and gelatin (0.6% w/v) (Sigma Chemicals, USA) were solubilised in gel buffer (0.2M TRIS, 0.01M $CaCl_2$; pH 8.8), poured onto a petri plate and allowed to cool for 10 min. Wells were cut at 25 mm intervals and the bottom of each well was sealed with a small amount of melted gel.

For testing the thermostability of the proteases, 1 ml of each 48 h to 72 h broth culture of *D. nodosus* was diluted with 1 ml of HEPES dilution buffer (0.4M HEPES, 0.01M $CaCl_2$; pH 8.5) in standard test tubes and 50 µl portions were used in the gelatin gel test before and after heating at 68°C for 8 min and 16 min in a water bath. After 18 h of incubation at 37°C, the test was read for precipitation of unhydrolysed gelatin with a saturated solution of ammonium sulphate.

A clear zone indicating hydrolysis of the gelatin by the test sample after 16 min of heating was considered positive.

Detection of the integrase A gene *intA*

All 58 isolates tested in the gelatin gel test were screened for the presence of *intA*. Detection of the gene was as described previously (8), with minor modifications. The concentration of magnesium chloride was 2.0 mM. Samples were amplified in 32 cycles, each comprising 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. The PCR products were examined as described above.

Results

During the period of study, outbreaks of footrot were mainly recorded during the rainy (monsoon) season from June to December. Of the total 73 outbreaks, 21 were recorded in 2009 and 52 in 2010.

The prevalence of footrot in the states of Andhra Pradesh and Tamil Nadu during the observation period ranged from 5% to 21%, with an overall prevalence of 15% (Table I). Higher prevalence was recorded in Chittoor, Nellore and Krishna than in the other districts. Among 2,548 affected feet of the naturally infected sheep, lesions of severity 4 were present in 561 (22%) feet, severity 3 (under-running of the soft horn) in 866 (34%) and severity 2 in 1,121 (44%).

In the two states overall, among a total of 1,050 samples of lesions, 478 (45.5%) were positive for *D. nodosus*. Serogrouping of *D. nodosus* isolates revealed the presence of six serogroups (A, B, C, E, F, I) (Table II). Taking samples with single and mixed serogroups together, serogroups A, B, C, E, F and I were detected in 14%, 50.4%, 5.6%, 0.2%, 6.7% and 29.3% of positive samples, respectively.

Among 467 positive samples from the state of Andhra Pradesh, 205 (43.9%) belonged to serogroup B and 124 (26.6%) to serogroup I; this was followed by serogroup A in 58 (12.4%) samples, F in 32 (6.9%) and C in 20 (4.3%). Serogroup E was detected in only one sample. Mixed infections with serogroups A+B, B+C or B+I were present in

Andhra Pradesh in 27 samples. Serogroup B was detected in all the districts of Andhra Pradesh except Srikakulum, serogroup A was detected in six districts, I in five, C in three, F in three, and E in only one district. Serogroups A and I were mostly confined to the two districts Chittoor and Nellore; serogroup F was mainly confined to the Krishna district. Similarly, among 11 positive samples from Tiruvallur in Tamil Nadu, serogroup B was detected in six samples, serogroup C in two samples, and mixed serogroups B+C in three samples.

The gel test demonstrated that thermostable proteases were present in all 58 isolates tested (Fig. 3). The presence of *intA* (Fig. 4) was detected in 45 (77. 6%) of the 58 isolates (Table IV).

Discussion

Footrot is usually considered a disease of temperate climates that have warm moist conditions. Nevertheless, the presence of footrot, together with the isolation and characterisation of *D. nodosus*, is now described in sheep being reared in the tropical climate of southern India, where the maximum temperature ranges between 27°C and 42°C.

The available literature on the occurrence of footrot in the tropics is limited (13). In the present study, footrot in Andhra Pradesh was seasonally associated with rains, the favourable environmental conditions prevailing from July to December; no footrot was reported during the dry period, which is usually from January to June. The onset of the disease in Andhra Pradesh coincided with the start of the south-west monsoons (June to September). The prevalence of footrot increased gradually, reaching a peak during the period of the north-east monsoon (October to December) when the temperature ranges between 16°C and 32°C. Andhra Pradesh experiences a mild winter (December to January), as the temperature does not usually go below 16°C. The summer season begins in mid-April and continues to mid-June, when the temperature reaches 42°C or more. In the period studied, maximum numbers of outbreaks were recorded in 2010 (15 during the south-west monsoon, 37 during the north-east monsoon) compared with 2009 (five during the south-west monsoon, 16 during the north-east monsoon). This could be attributed to the higher rainfall

in 2010 (Fig. 5). It is not known how *D. nodosus* survives during the dry hot season, when there are no outbreaks of footrot; it is possible that the bacteria survive in the hooves of infected animals and later manifest during the favourable monsoon period.

The overall prevalence (15%) of footrot observed in the present study was higher than that reported in other parts of the world; for example, 8% to 10% in the United Kingdom (14, 15) and 3.1% in organised sheep farms in Bhutan (16). Nevertheless, the observed prevalence correlates with a report from Kashmir, India, where the prevalence was 16.4% (17). The distribution of the disease in Andhra Pradesh revealed that the greatest numbers of outbreaks occurred in Chittoor, Nellore and Krishna districts. This could be related to the presence of suitable geographic conditions, particularly the waterlogged vegetation.

In the present investigation, serogroup B was found to be predominant (50.4%), followed by serogroups I, A, F and C. The distribution and predominance of *D. nodosus* serogroups varies from country to country but predominance of serogroup B has been reported in Kashmir (10, 17), Australia (8), Great Britain (18), New Zealand (19) and Bhutan (16). In Kashmir, the prevalence of serogroup E was reported as higher (21.7%) than I (3.6%) (10) and serogroup C was also detected in 1.3% samples (17). Detection of serogroups A and F in India is reported here for the first time. Mixed infection with two serogroups (A+B, B+C, B+I) is in agreement with reports from Kashmir, Australia, England and Wales, among others (17, 20, 21). The range of *D. nodosus* serogroups in the tropical climate of southern India appears wider than that reported for the temperate climate of Kashmir.

The presence of virulent strains of *D. nodosus* confirms the virulent nature of the clinical disease. In the present study, 77.6% of isolates tested carried *intA* and were considered virulent strains, confirming the high correlation between the presence of the gene and the ability of strains to cause virulent footrot (8). This is in agreement with other

reports in India, where the majority of *D. nodosus* isolates were virulent (22).

Conclusion

The identification and serogrouping of isolates of *D. nodosus* in the present investigation has confirmed the presence of footrot in native sheep in the tropical climate of southern India. The observed 15% prevalence of footrot is suggestive of heavy economic losses among sheep farmers, emphasising the need for development of a multivalent vaccine for effective control of this disease. Further studies covering a larger geographical area and with greater numbers of samples would be required before a control programme against this disease could be formulated.

Acknowledgements

Thanks to the National Agricultural Innovation Project of the Indian Council of Agricultural Research, New Delhi, for financial assistance. Thanks also to Dr O.P. Dhungyel, University of Sydney, Camden, NSW 2570, Australia, for supplying the serogroup-specific positive control DNA.

References

1. La Fontaine S.L., Egerton J.R. & Rood J.I. (1993). – Detection of *Dichelobacter nodosus* using species-specific oligonucleotides as PCR primers. *Vet. Microbiol.*, **35** (1–2), 101–117.

2. Stewart D. (1989). – Footrot in sheep. *In* Footrot and foot abscesses of ruminants (J.R. Egerton, W.K. Yong & G.G. Riffkin, eds). CRC Press, Boca Raton, Florida, 5–45.

3. Beveridge W.I.B. (1941). – Footrot in sheep: a transmissible disease due to infection with *Fusiformis nodosus*: studies on its cause, epidemiology and control. *CSIRO Bull.*, **140**, 1–56.

4. Whittington R.J. (1995). – Observations on the indirect transmission of virulent ovine footrot in sheep yards and its spread in sheep on unimproved pasture. *Aust. vet. J.*, **72**, 132–134.

5. Claxton P.D. (1986). – Serogrouping of *Bacteroides nodosus* isolates. *In* Proc. of a workshop, Melbourne, 1985. Commonwealth Scientific and Industrial Research Organisation, Division of Animal Health, and the Australian Wool Corporation, Sydney, 131–134.

6. Ghimire S.C., Egerton J.R., Dhungyel O.P. & Joshi H.D. (1998). – Identification and characterization of serogroup M among Nepalese isolates of *Dichelobacter nodosus*, the transmitting agent of footrot in small ruminants. *Vet. Microbiol.*, **62** (3), 217–233.

7. Dhungyel O.P., Whittington R.J. & Egerton J.R. (2002). – Serogroup specific single and multiplex PCR with pre-enrichment culture and immuno-magnetic bead capture for identifying strains of *D. nodosus* in sheep with footrot prior to vaccination. *Molec. cell. Probes*, **16** (4), 285–296.

8. Cheetham B.F., Tanjung L.R., Sutherland M., Druitt J., Green G., McFarlane J., Bailey G.D., Seaman J.T. & Katz M.E. (2006). – Improved diagnosis of virulent ovine footrot using the *intA* gene. *Vet. Microbiol.*, **116** (1–3), 166–174.

9. Palmer M.A. (1993). – A gelatin test to detect activity and stability of proteases produced by *Dichelobacter* (*Bacteroides*) *nodosus*. *Vet. Microbiol.*, **36** (1–2), 113–122.

10. Hussain I., Wani S.A., Qureshi S.D. & Farooq S. (2009). – Serological diversity and virulence determination of *Dichelobacter nodosus* from footrot in India. *Molec. cell. Probes*, **23** (2), 112–114.

11. Wani S.A., Samanta I., Buchh A.S. & Bhat M.A. (2004). – Molecular detection and characterization of *Dichelobacter nodosus* in ovine footrot in India. *Molec. cell. Probes*, **18** (5), 289–291.

12. Egerton J.R. & Roberts D.S. (1971). – Vaccination against ovine foot-rot. *J. comp. Pathol.*, **81** (2), 179–185.

13. Zakaria Z., Radu S., Sheikh-Omar A.R., Mutalib A.R., Joseph P.G. & Rusul G. (1998). – Molecular analysis of *Dichelobacter nodosus* isolated from footrot in sheep in Malaysia. *Vet. Microbiol*., **62**, 243–250.

14. Grogono-Thomas R. & Johnston A.M. (1997). – A study of ovine lameness. MAFF Open Contract OC 5945 K, DEFRA Publications, London.

15. Wassink G.J., Grogono-Thomas R., Moore L.J. & Green L.E. (2003). – Risk factors associated with the prevalence of footrot in sheep from 1999 to 2000. *Vet. Rec*., **152** (12), 351–358.

16. Gurung R.B., Tshering P., Dhungyel O.P. & Egerton J.R. (2006). – Distribution and prevalence of footrot in Bhutan. *Vet. J*., **171** (2), 346–351.

17. Farooq S., Wani S.A., Hussain I. & Bhat M.A. (2010). – Prevalence of ovine footrot in Kashmir, India and molecular characterization of *Dichelobacter nodosus* by PCR. *Indian J. anim. Sci*., **80** (9), 826–830.

18. Hindmarsh F. & Fraser J. (1985). – Serogrouping of *Bacteroides nodosus* isolated from ovine footrot in Britain. *Vet. Rec*. **116** (7), 187–188.

19. Kingsley D.F., Hindmarsh F.H., Liardet D.M. & Chetwin D.H. (1986). – Distribution of serogroups of *Bacteroides nodosus* with particular reference to New Zealand and the United Kingdom. *In* Footrot in ruminants (D.J. Stewart, J.E. Peterson, N.M. Mckern & D.L. Emery, eds). Commonwealth Scientific Industrial Research Organisation, Division of Animal Health, and the Australian Wool Corporation, Sydney, 143–146.

20. Claxton P.D., Ribeiro L.A. & Egerton J.R. (1983). – Classification of *Bacteroides nodosus* by agglutination test. *Aust. vet. J*., **60** (11), 331–334.

21. Moore L.J., Wassink G.J., Green L.E. & Grogono-Thomas R. (2005). – The detection and characterization of *Dichelobacter nodosus* from cases of ovine footrot in England and Wales. *Vet. Microbiol*., **108** (1–2), 57–67.

22. Rather M.A., Wani S.A., Hussain I., Bhat M.A., Kabli Z.A. & Magrey S.N. (2011). – Determination of prevalence and economic impact of footrot in central Kashmir, India with isolation and molecular characterization of *Dichelobacter nodosus*. *Anaerobe*., **17** (2), 73–77.

Table I
Prevalence (%) of footrot in sheep in the tropical climate of Andhra Pradesh and Tamil Nadu states in southern India

District no.*	District name	No. of footrot outbreaks attended	No. of household flocks examined	No. of animal feet inspected	No. (%) of animal feet affected	
1	Chittoor	18	86	4,583	824	(18)
2	Nellore	22	108	5,326	1,006	(19)
3	Kadapa	8	23	644	32	(5)
4	Kurnool	2	12	523	42	(8)
5	Krishna	6	14	1,020	214	(21)
6	Guntur	1	5	352	42	(12)
7	Prakasam	9	19	2,053	164	(8)
8	Nizamabad	2	8	650	78	(12)
9	Srikakulum	1	3	263	18	(7)
10	Mehaboobnagar	2	8	897	80	(9)
11	Nalgonda	1	6	458	36	(8)
12	Tiruvallur	1	3	120	12	(10)
Total		**73**	**295**	**16,889**	**2,548**	**(15)**

*Districts 1 to 11 are in Andhra Pradesh; district 12 is in Tamil Nadu

Table II

Serological diversity of *Dichelobacter nodosus* in the tropical climate of Andhra Pradesh and Tamil Nadu states in southern India

District no.[a]	District name	No. of samples collected	No. of samples positive for Dn	No. of samples in each serogroup[b]								
				A	B	C	E	F	I	A+B	B+C	B+I
1	Chittoor	264	150	20	51	7	0	0	62	2	0	8
2	Nellore	350	204	31	88	12	0	0	58	5	2	8
3	Kadapa	99	9	0	9	0	0	0	0	0	0	0
4	Kurnool	25	2	0	2	0	0	0	0	0	0	0
5	Krishna	90	52	0	20	1	1	29	1	0	0	0
6	Guntur	30	12	1	9	0	0	1	1	0	0	0
7	Prakasam	80	8	0	6	0	0	0	2	0	0	0
8	Nizamabad	33	15	3	12	0	0	0	0	0	0	0
9	Srikakulum	20	3	1	0	0	0	0	0	2	0	0
10	Mehaboobnagar	25	4	0	4	0	0	0	0	0	0	0
11	Nalgonda	18	8	2	4	0	0	2	0	0	0	0
	Subtotal	**1,034**	**467**	**58 (12.4)**	**205 (43.9)**	**20 (4.3)**	**1 (0.2)**	**32 (6.9)**	**124 (26.6)**	**9 (1.9)**	**2 (0.4)**	**16 (3.4)**
12	Tiruvallur	16	11	0	6	2	0	0	0	0	3	0
	Grand total	**1,050**	**478**	**58 (12.1)**	**211 (44.1)**	**22 (4.6)**	**1 (0.2)**	**32 (6.7)**	**124 (25.9)**	**9 (1.9)**	**5 (1.0)**	**16 (3.3)**

a) Districts 1 to 11 are in Andhra Pradesh; district 12 is in Tamil Nadu

b) Numbers in parentheses indicate percentage

Dn: *Dichelobacter nodosus*

Table III

Primers used for detection and serogrouping of *Dichelobacter nodosus*

Primer name	Primer sequence (5' – 3')	Primer concentration (µM)	Position in *fimA*	Target	Serogroup	References
Forward	CGGGGTTATGTAGCTTGC	0.25	–	16S	–	(1)
Reverse	TCGGTACCGAGTATTTCTACCCAACACCT			rRNA		
FP	CCTTAATCGAACTCATGATTG	0.625	25 to 46	fimA	–	
RA	AGTTTCGCCTTCATTATATTT	0.25	421 to 441	fimA	A	(7)
RB	CGGATCGCCAGCTTCTGTCTT	0.25	286 to 309	fimA	B	
RC	AGAAGTGCCTTTGCCGTATTC	0.25	331 to 351	fimA	C	
RD	TGCAACAATATTTCCCTCATC	0.25	325 to 345	fimA	D	
RE	CACTTTGGTATCGATCAACTTGG	0.25	367 to 389	fimA	E	
RF	ACTGATTTCGGCTAGACC	0.25	250 to 267	fimA	F	

RG	CTTAGGGGTAAGTCCTGCAAG	0.25	283 to 305	fimA	G	
RH	TGAGCAAGACCAAGTAGC	0.25	412 to 435	fimA	H	
RI	CGATGGGTCAGCATCTGGACC	0.25	194 to 215	fimA	I	
intAF	ACATCATGCGACTCACTG AC	0.25	–	intA	–	(8)
intAR	TCT CTG GTC GGT CGT ACA AT	0.25				

Table IV
Characterisation of virulent *Dichelobacter nodosus* isolates from severe footrot lesions in Nellore sheep in southern India

Lesion severity*	Serogroup	No. of isolates	No. of positive isolates	
			Gelatin test	*intA*
4	B	21	21	19
3	B	9	9	7
3	B	2	2	0
4	I	9	9	9
3	I	5	5	2
4	I	1	1	0
4	A	5	5	4
3	A	2	2	0
4	C	4	4	4
Total		**58**	**58**	**45**

*Scale 0 to 4

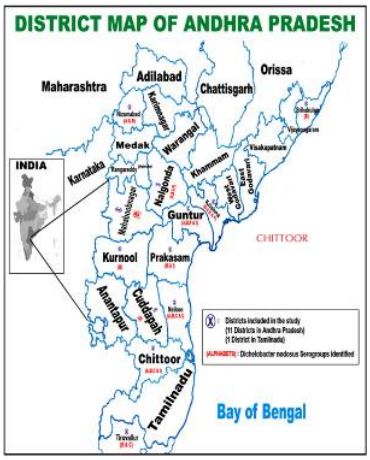
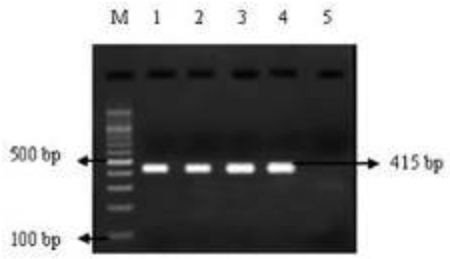


Fig. 1
Distribution of footrot and serogroups of *Dichelobacter nodosus* in districts of Andhra Pradesh and Tamil Nadu states of southern India



Lane M: 100 bp DNA ladder

Lanes 1 to 3: samples positive for serogroup A

Lane 4: positive control

Lane 5: negative control

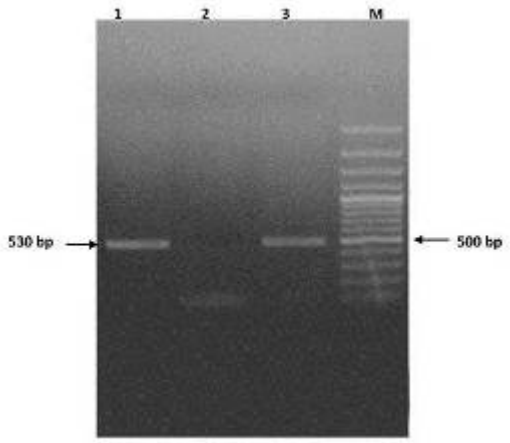
Fig. 2
Serogrouping of *Dichelobacter nodosus* using multiplex polymerase chain reaction

Well 1: unheated culture

Well 2: culture heated for up to 8 min

Well 3: culture heated for up to 16 min. A clear zone of gelatin hydrolysis even after heating for 16 min was considered positive for the test and indicates virulence of *D. nodosus*. Absence of hydrolysis after heating for 16 min indicates a benign strain

Fig. 3
Gelatin gel test of a *Dichelobacter nodosus* isolate (serogroup B)
A clear zone of gelatin hydrolysis even after heating for 16 min was considered positive for the test and indicates virulence of *D. nodosus*. Absence of hydrolysis after heating for 16 min indicates a benign strain



Lane 1: positive control

Lane 2: negative control:

Lane 3: amplified polymerase chain reaction products of 530 bp specific for *intA*

Lane M: 100bp DNA ladder

Fig. 4

Detection of the integrase gene *intA* in polymerase chain reaction

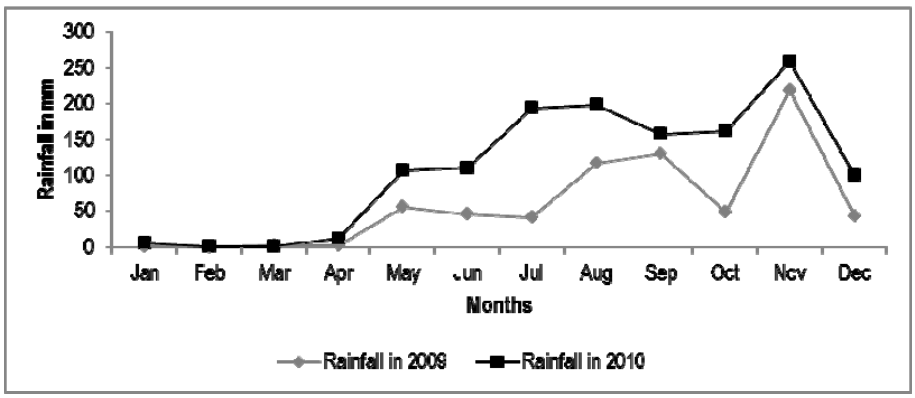


Fig. 5
Average rainfall in 11 districts of Andhra Pradesh state during 2009 and 2010