Genome sequence of *Dichelobacter nodosus* JKS-07B isolate from J&K, India associated with virulent footrot of sheep

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Abstract

Introduction: Virulent footrot of sheep caused by *Dichelobacter nodosus* is associated with tremendous economic losses due to recurrent treatment costs and increased culling rates. This organism being a fastidious anaerobe is difficult to isolate on ordinary media that does not support its growth. The *D. nodosus* serogroup B isolate described in the present study has been used in the preparation of the whole-cell killed vaccine against footrot in India. *D. nodosus* serogroup B is the predominant serogroup involved in virulent footrot (lesion score 4) in India as well as in many sheep-rearing countries of the globe.

Methods: Genomic DNA was extracted using wizard Genomic DNA purification kit. The whole genome of the *D. nodosus* strain B was sequenced using an Illumina HiSeq 2500 platform and annotated according to functional gene categories. Annotations were performed using in-house developed Perl scripts using NR/Nt database, uniprot, Pfam, KEGG, Panther DB, and GO database.

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**Result:** The assembled genome size is 1.311,533 Mb and GC content is 44.38. A total of 1215 protein-coding genes, 44tRNA and 7 rRNA were identified. The genome shows 98.63% sequence homology with the reference genome. However, 21 new genes have been identified in this genome. The information will provide insights into the various genes and regulators necessary for *D. nodosus* growth and survival.

**Discussion:** The genome information of this serogroup B of *D. nodosus* isolate involved in 85–90% cases of virulent footrot of sheep in India provides further insights for improvement of the killed vaccine (B serogroup) developed recently in India. For the development of an efficacious vaccine against virulent footrot, it is essential to know the serological diversity as well as the virulent status of the strains of the *D. nodosus*. This serogroup isolate is a potential vaccine candidate to mitigate ovine footrot in India as the majority of virulent footrot cases belong to serogroup B of *D. nodosus*.

**Keywords**

*Dichelobacter nodosus*, serogroup B, whole genome, virulent footrot, sheep

**Introduction**

Ovine footrot caused by a gram negative bacterium *Dichelobacter nodosus*, is a highly contagious disease of feet of ruminants, especially in sheep and goats that causes lameness, significant production, and economic losses worldwide. The disease process begins as an inflammation of the interdigital skin of sheep and may progress to the severe separation of the claw capsule from the underlying soft tissues. The severity of footrot is dependent on the virulence of *D. nodosus* the infecting strain primarily, and on other factors like farm management, sheep breed, environmental/climatic conditions, and the presence of co-infecting bacteria at the site of infection. In some soils this fastidious organism has been reported to survive for two weeks.

The chronicity and endemicity of the disease leads to substantial economic loss by way of loss of body weight, condition and wool growth, decreased lambing percentage, and reduced value at the sale of the affected sheep. The *D. nodosus* organism harbors fimbriae which are highly immunogenic for sheep and are considered to be the major protective immunogens for the host. Based on fimbrial antigens *D. nodosus* isolates are classified into at least ten distinguishable serogroups designated from A-I and M. These serogroups have been further subdivided into 21 serotypes A1, A2, B1, B2, B3, B4, B5, B6, C1, C2, D, E1, E2, F1, F2, G1, G2, H1, H2, I, and M. Serogroup B has been detected in 92.46% of footrot affected sheep with serotype B5 (83.0%) a new subtype of serogroup B as the predominant serotype of *D. nodosus* associated with severe footrot (lesion score 4) in sheep across the state of Jammu & Kashmir (J&K), India. Serogrouping of *D. nodosus* isolates provides valuable epidemiological data on strain diversity which is a prerequisite for effective vaccination programs.

The isolation of *D. nodosus* from a footrot lesion is an extremely cumbersome procedure as the organism is extremely fastidious and ordinary enrichment media are unable to support the growth under anaerobic conditions. Hoof agar media prepared from sterilized sheep hoof capsules supplement with arginine and serine (TAS agar) is required for primary culture, isolation, and purification which could take 3–4 weeks.

Several important virulence factors of *D. nodosus* like extracellular subtilisin-like serine proteases (or subtilases), type IV fimbriae, the vrl, and vap genomic islands have been
incriminated in its pathogenesis. *D. nodosus* strains exist globally in two distinct forms the virulent and the benign. Virulent strains of *D. nodosus* produce three homologous extracellular subtilases, namely AprV5, AprV2, and BprV. The AprV2 protease is thermostable and is responsible for the elastase activity of the organism. A single amino acid substitution (Tyr92Arg) makes AprV2 more virulent than its benign counterpart AprB2. The difference at the gene level, between the protease gene variants aprV2 (accession no. L38395) and AprB2, is due to a 2-bp change from TA to CG at position 661–662. The other two proteases are acidic protease 5 (AprV5 and AprB5) and basic protease (BprV and BprB), respectively. Stauble developed a real-time PCR assay using the protease genes AprV2 and AprB2, to distinguish the virulent and benign strains of *D. nodosus*. The whole genome shotgun sequencing of a large number of *D. nodosus* isolates from different countries has revealed that some of the isolates contain a single nucleotide polymorphism (SNP) in the region of the 3′ end of the forward primer. Previously, the intA gene (integrase) has also been suggested to be associated with virulence.

**Methodology**

The sample had been previously collected from sheep foot (lesion score 4) at the apex of the cleft between the horn of the hoof and the sensitive underlying tissues after scoring the animals for footrot lesion as per the protocol of Stewart and Claxton.

**Isolation of *D. nodosus***

The organism is extremely fastidious and difficult to grow in the laboratory. The swab sample of the isolate used in the present study had been previously streaked on trypticase arginine serine (TAS) agar containing 4% hoof powder under anaerobic conditions (Anaerobic jar, Oxoid, UK) with gas packs (Becton Dickinson, MD, USA) followed by incubation at 37°C. Suspected colonies, post 5 days of incubation, were subcultured on 4% TAS agar again until pure colonies of *D. nodosus* were obtained. The suspected colonies of *D. nodosus* were confirmed by demonstration of typical cellular morphology in Gram-stained smears as well as by species-specific 16S rRNA gene by PCR. The culture was lyophilized for future use.

**Extraction of bacterial DNA and 16s rRNA PCR for *D. nodosus* detection**

The pure colony was suspended into 150 µl of sterile distilled water. Snap chill method of DNA extraction was followed by boiling the suspension for 10 min, cooling on ice for 5 min, and centrifugation at 10,000 × g for 10 min. 2 µl of the supernatant was used as the template for PCR reaction. The extracted DNA was subjected to the amplification of the 16S rRNA gene for the detection of *D. nodosus*. PCR conditions and primer details have been previously described.
Serogrouping of *D. nodosus*

For serogrouping of the *D. nodosus* isolates, the multiplex PCR was carried out by using nine (A–I) serogroup-specific primers as described by Dhungyel et al.19 was followed. Serogroup specific DNA controls were previously provided by Dr O.P. Dhungyel. The PCR products were analyzed in 1–2% agarose gels, stained with ethidium bromide, visualized under ultraviolet illumination, and photographed by Gel Documentation System (UVP, UK).

Virulence characterization

Gelatin gel test20 to detect the thermostable proteases, as well as PCR for detection of *intA* gene (integrase)17 in virulent strains, was carried out with slight modification as described previously.10

Genomic DNA isolation and sequencing

The pure lyophilized culture was revived on TAS agar for extraction of DNA. DNA was extracted using the Wizard Genomic DNA purification kit (Promega Corp; Madison, WI, USA). Whole genome sequence of *D. nodosus* was carried out with paired-end 150 bp multiplex on an Illumina HiSeq 2500 platform (Illumina Inc., San Diego, CA). After the filtering step, clean reads were assembled using SPAdes, ABYSS, and Velvet. The raw reads were quality checked using in-house developed Perl scripts and processed by the cutadapt program21 for removing adapter sequences and low-quality bases trimming towards 3′-end. Reads having quality above Q30 were taken for further analysis. Raw reads were transformed to clean reads using in-house developed Perl scripts, by removing the adapters sequences, low-quality reads and those containing undermined bases. After the filtering step, clean reads were assembled using SPAdes, ABYSS, and Velvet. All clean reads (Phred quality scores >30 and length >25 bp) were mapped to the *D. nodosus* reference genome assembly using Bowtie-v2.2.6 29. We utilized genome assembly from the Velvet program for downstream analysis and better statistical parameters than other assemblers. The final draft assembly contained seven contigs.

Genome annotation

Genes were identified using RAST (Rapid Annotation using Subsystem Technology)22 and the overview of the annotated genome was completed with the SEED viewer.23 This was followed by manual curations by using the NCBI-Blast program against NCBI nr/nt database. UniProt,24 Pfam,25 KEGG,26 Panther DB,27 and GO28 database. The tRNA ScanSE tool29 was used to find tRNA genes.

Results & discussion

We report here the whole genome sequence of *D. nodosus* JKS-07B serogroup B isolate. This strain was isolated from virulent footrot of sheep and produced thermostable
protease in gelatin gel. The G + C content of the genome was 44.38%. Protein-coding gene prediction was performed using the Glimmer program. A total of 1215 protein-coding genes were identified and annotated using homology-based search through the National Center for Biotechnology Information (NCBI) Ref Seq non-redundant database using BLASTX program with an E-value cutoff of $10^{-5}$. A total of 44 tRNA genes, four non-coding RNAs, nine pseudogenes and seven rRNAs genes were identified (Table 1). A graphical map showing the entire genome sequence with sequence feature information is depicted in Figure 1. The functional parts of the metabolic reconstruction of this organism were compared to that of reference *D. nodosus* VCS1703A. We found a total of 21 genes that were unique to this isolate of *D nodosus*, (serogroup B) and have not been reported in the reference strain (Table 2). The genes comprise mainly of oligopeptide transport system permease protein OppB (TC 3.A.1.5.1), Oligopeptide transport system permease protein OppC (TC 3.A.1.5.1), Type IV pilus biogenesis protein PilO, intracellular septation protein IspA etc.

Nucleotide sequence accession No: This whole genome project has been deposited in Genbank as [SRR8820262](https://www.ncbi.nlm.nih.gov/sra) NCBI under accession no [NZ_SRJB00000000](https://www.ncbi.nlm.nih.gov/nuccore/NZ_SRJB00000000). The genome has also been linked to the PubMLST database under ID 142. To fully interpret the biological implication of annotated genes, all genes were mapped to the gene ontology (GO) database. GO analysis revealed a total of 340 terms enriched in biological process, 50 terms enriched cellular components, and 547 terms enriched in molecular function using the FDR corrected *P* value < 0.05.

The first *D. nodosus* genome to be sequenced is strain VCS1703A (GenBank Accession No: NC_009446.1) and has been used in several virulence studies. The genome of *D. nodosus* is 1,311,533 bp, of which one-fifth is believed to have been acquired by lateral gene transfer—an incorporated Mu-like bacteriophage. The *D. nodosus* PubMLST database contains 171 isolates with 115 STs (release date 11

### Table 1. Genome characteristics of *Dickelobacter nodosus* JKS-07 serogroup B

| ID                  | Genome characteristics |
|---------------------|------------------------|
| NCBI Project ID     | SAMN11291012           |
| SRA ID              | SRR8820262             |
| PUBMLST database ID | 142                    |
| Sequencing platform | Illumina HiSeq         |
| Total number of reads | 10,426,863 (PE)       |
| Read length         | 150 bp                 |
| Cleaned reads       | 9,547,762 (PE)         |
| Genome coverage     | >100x                  |
| Estimated genome size | 1,311,533 Mb          |
| GC content          | 44.38                  |
| Protein-coding genes | 1207                  |
| tRNAs               | 44                     |
| rRNA                | 7                      |
| ncRNAs              | 4                      |
| Pseudogenes         | 9                      |
January 2021). The database suggests a high level of diversity with a low level of recombination which is reflected in the grouping of isolates and branch lengths shown in the cgMLST and wgMLST analysis.

Oligopeptide transport system permease protein OppB and OppC are involved in the binding-protein-dependent transport system for the translocation of the substrate across the bacterial membrane. Type IV pilus biogenesis protein PilO has a role in bacterial motility, and its DNA uptake from the surroundings while intracellular septation protein IspA has been shown to have a part in cell division and intracellular septation. This may account for the severity of lesion score (3 or 4) in footrot cases from sheep farms across J&K. *D. nodosus* serogroup B has 1215 coding sequences divided into 190 subsystems for connecting the genes in the new genome to functional roles (Figure 2). Among the various subsystems, protein metabolism (164), cofactors, vitamins, prosthetic groups, pigments (70), DNA metabolism (45), stress response (25) virulence, disease, and defense (17) genes are important.

Among the closest neighbour serogroup B is highly related to *D. nodosus* VCS1703A with a score of 500 followed by *Cardiobacterium hominis* ATCC 15826 with a score of 382 (Table 3). A set of universal proteins plus up to 200 ‘unduplicated’ proteins were used to compare abinitio GLIMMER3 gene candidates to generate the closest phylogenetic neighbors and score them. These gene candidates were also used to help ‘bootstrap’ iterative retraining of GILMMER 3 and are not retained in the final annotation.

A total of 2140706 QC passed reads were mapped with the *D. nodosus* reference genome assembly VCS1703A (GenBank accession No: NC_009446.1) using BWA-MEM version

![Figure 1. Draft genome of *Dichelobacter nodosus* serogroup B. Dark pink circle represent is the open reading frames with purple as the start and pink as the stop site, inner circles are GC-skew plot with black as GC content (magenta portion-GC-skew negative, olive green portion-GC-skew positive).](image-url)
Table 2. Genes associated with a subsystem in *Dichelobacter nodusus* JKS-07B serogroup B isolate from J&K compared with reference genes.

| Category                          | Subcategory                          | Subsystem                                                                 | Role                                                                 | PEG No. |
|----------------------------------|--------------------------------------|---------------------------------------------------------------------------|----------------------------------------------------------------------|---------|
| Amino acids and derivatives      | Aromatic amino acids and derivatives | Common Pathway For Synthesis of Aromatic Compounds (DAHP synthase to chorismate) | 3-dehydroquinate synthase (EC 4.2.3.4).                              | 817     |
| Carbohydrates                    | Central carbohydrate metabolism      | Pyruvate metabolism II: acetyl-CoA, acetogenesis from pyruvate.            | Dihydrolipoamide dehydrogenase of pyruvate dehydrogenase complex (EC 1.8.1.4) | 804     |
| Cell division and cell cycle     | No subcategory                       | Intracellular septation in Enterobacteria                                | Acyl-CoA thioesterase YciA, involved in membrane biogenesis          | 1106    |
| Cell division and cell cycle     | No subcategory                       | Intracellular septation in Enterobacteria                                | Intracellular septation protein IspA                                | 172     |
| Cell wall and capsule            | No subcategory                       | UDP-N-acetylmuramate from Fructose-6-phosphate Biosynthesis              | Phosphoglucomutase (EC 5.4.2.10)                                    | 1091    |
| DNA metabolism                   | CRISPs                               | CRISPRs                                                                   | CRISPR-associated helicase Cas3                                     | 544     |
| Membrane transport               | ABC transporters                     | ABC transporter oligopeptide (TC 3.A.1.5.1)                               | Oligopeptide transport system permease protein OppB (TC 3.A.1.5.1)   | 162     |
| Membrane transport               | ABC transporters                     | ABC transporter oligopeptide (TC 3.A.1.5.1)                               | Oligopeptide transport system permease protein OppC (TC 3.A.1.5.1)   | 163     |
| Membrane transport               | Protein and nucleoprotein secretion system, Type IV pilus | Type IV pilus                                                              | Type IV pilus biogenesis protein PilO                                | 54      |
| Miscellaneous                    | Plant-Prokaryote DOE project         | Iron-sulfur cluster assembly                                             | Putative iron-sulfur cluster assembly scaffold protein for SUF system, SufE2 | 1109    |
| Phages, prophages, transposable  | Phages, Prophages                    | Phage packaging machinery                                                | Phage terminase, large subunit                                      | 623     |

(Continued)
The genome shows 98.63% sequence homology with the reference genome. We found a total of SNP’s at 2933 positions using VarScan and at 92 positions an InDel compared to the reference genome. The pathogenicity of the genome was determined using Pathogen Finder version 1.1. The data identifies 40 genes that correlate with the pathogenicity of this bacteria compared with the reference genome.

This study presents the genomic information of a *D. nodosus* JKS-07B serogroup B isolate from India. The genome information of this strain provides insights for further work on a universal vaccine in Asia as this serogroup has been isolated from >90% cases of virulent footrot in India. The whole genome information pertaining to circulating strains is important in knowing the relationship between virulent and benign strains as well as the relationship of isolates from different geographical regions. The infection due to virulent strains is often difficult to treat and occasionally results in therapeutic.

Table 2. (continued)

| Category           | Subcategory                  | Subsystem                                                                 | Role                                                                 | PEG No. |
|--------------------|------------------------------|---------------------------------------------------------------------------|----------------------------------------------------------------------|---------|
| Potassium elements | No subcategory               | Potassium homeostasis                                                    | Potassium channel protein                                            | 292     |
| metabolism         |                              |                                                                           |                                                                      |         |
| Protein metabolism | Protein biosynthesis         | Programmed frameshift                                                    | Peptide chain release factor 2 programmed frameshift-containing      | 964     |
|                    |                              |                                                                           |                                                                      |         |
| Protein metabolism | Protein biosynthesis         | rRNAs                                                                    |                                                                      | 45      |
|                    |                              |                                                                           |                                                                      |         |
| Protein metabolism | Protein degradation          | Protein degradation                                                       | Dipeptidyl carboxypeptidase Dcp (EC 3.4.15.5)                          | 449     |
|                    |                              |                                                                           |                                                                      |         |
| Protein metabolism | Protein degradation          | Protein degradation                                                       | Oligopeptidase A (EC 3.4.24.70)                                       | 689     |
|                    |                              |                                                                           |                                                                      |         |
| Protein metabolism | Protein degradation          | Proteolysis in bacteria, ATP-dependent                                  |                                                                      | 674     |
| RNA metabolism     | Transcription                | Transcription initiation, bacterial sigma factors                         |                                                                      | 242     |
| Respiration        | Electron donating reactions  | Na(+)—translocating NADH-quinone oxidoreductase and rnf-like group of      | Electron transport complex protein RnfB                                | 265     |
| Stress             | Oxidative stress             | Oxidative stress                                                         | Superoxide dismutase [Mn] (EC 1.15.1.1)                                | 446     |
| response           |                              |                                                                           |                                                                      |         |
failures and complicated outcomes of the disease. The sequenced strain belongs to predominant serogroup B in India which has already been used in the preparation of a killed vaccine with promising results in Jammu & Kashmir (J&K), India.

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Prof Shakil A Wani, PhD (Veterinary Microbiology), PDF (CDC, USA, Boston Tufts University). Retired Director Education SKUAST-K, J&K, India. He has contributed to the understanding of biological properties of an Indian isolate (GP78) of Japanese encephalitis virus (JEV) as well as identification of its host cell receptor. He studied the biological properties of the isolate in tissue-cultured cells and mice and compared it with the JaOArS982 strain from Japan. He has expertise on immunodiagnosis of measles and molecular characterization of measles virus from CDC, USA. He has worked on molecular epidemiology and characterization of rotavirus in lambs, calves and poultry. He worked on virulent footrot – caused by Dichelobacter nodosus (D. nodosus). He has done extensive serogroup surveillance in the filed for identification of the virulent strain as suitable candidate for vaccine production. He developed whole cell killed vaccine against the menace to control the disease in sheep and goats. He worked on other anaerobic bacteria and established the centre for excellence on anaerobic bacteriology. He provided molecular and serological evidence of the presence of a highly fatal viral disease called sheep-associated malignant catarrhal fever (SA-MCF) in cattle in India. He unveiled the presence of Shiga toxin-producing E. coli (STEC) and enteropathogenic E. coli (EPEC) in cattle, sheep and goats in India. He established the presence of enteropathogenic E. coli (EAggEC), diffusely adherence E. coli (DAEC), enterotoxigenic E. coli (ETEC), typical/atypical EPEC and STEC in diarrhoeic human patients. Has worked on Identification and Characterization of Intestinal Colonization factors of the enterohaemorrhagic Escherichia coli (EHEC) using most advanced technique of Signature tagged mutagenesis (STM) at Tufts University School of Medicine, Boston, USA. He has contributed to the understanding of strain differentiation of FMD virus type O and evaluated the methodology used for establishing the same. He has made significant contribution on the isolation, molecular detection of Mycobacterium tuberculosis and M. avium subspecies paratuberculosis (MAP) in poultry and goats, respectively, in local geographical area. He has contributed to the understanding of the enteropathogenicity of Salmonella bareilly. He has published more than 100 research articles in peer reviewed journals.

Dr Shaheen Farooq, BVSc, MVSc, PhD (Veterinary Microbiology). The author is presently working as Assistant Professor, Division of Veterinary Microbiology & Immunology, FVSc &A.H.SKAUST-K, J&K, India. The author has expertise in anaerobic bacteriology and has been working on fastidious anaerobes (viz. Dichelobacter nodosus, Fusobacterium necrophorum, Clostridium perfringens, Trueperella pyogenes, Trepomema, etc.) from last 10 years. Part of the research focused on development on vaccine for footrot.

Dr Zahid Kashoo, BVSc, MVSc, PhD (Veterinary Microbiology), presently working as assistant professor, Division of Veterinary Microbiology & Immunology, FVSc &A.H.SKAUST-K, J&K, India. His research group has been responsible for the development of vaccine for footrot in...
Kashmir. He is presently working on molecular and serological evidence of the presence of a highly fatal viral disease called sheep-associated malignant catarrhal fever (SA-MCF) in cattle in India.

**Dr Basharat Bhat**, PostDoc research fellow from University of Otago. He is currently utilizing Multi-OMICS data to study the disease progression and explore the interaction between drugs and diseases in livestock. Dr Bhat has published more than 20 research articles in various international journals. He is editor in Frontier in Genetics and acting as a reviewer for several international journals.

**Dr Mohammad Isaqul Hussain**, BVSc, MVSc, PhD, (Veterinary Microbiology) is an associate professor, Division of Veterinary Microbiology & Immunology, FVSc & A.H, SKUAST-Kashmir. His research has focused on molecular epidemiology of diarrhoeagenic *E.coli* and *Salmonella*, anaerobes especially *Clostridium perfringens, Clostridium difficile* and the ovine footrot pathogen *Dichelobacter nodosus*. His research group has been responsible for the development of vaccine for footrot in Kashmir. His current research interests are centred upon development of recombinant subunit vaccine against footrot and vaccine against clostridial diseases in sheep. He has published more than 50 research articles in peer reviewed journals. He is a Life Member of the Indian Society for Veterinary Immunology & Biotechnology.

**Dr Aasim Habib** is presently working as a research scientist-I at Viral Research Diagnostic Laboratory (VRDL) in Govt. Medical College Srinagar, India. Dr Aasim has vast experience in anaerobic bacteriology and immunology. He has isolated, identified and characterized anaerobes affecting ovine population, formulating a vaccine and monitoring its immune response. He has worked as research associate in Niche area of Excellence Centre at SKUAST Kashmir. He is the first to report footrot (a disease of sheep and goats) from the state of Himachal Pradesh, India. His research interests include isolation, identification of bacteria, cloning, expression, molecular characterization, vaccine development and monitoring immune responses.

**Prof Mohd Altaf Bhat**, BVSc, MVSC, PhD (Veterinary Microbiology), PDF (Osaka city, University, Japan), presently Professor & Head Division of Veterinary Microbiology & Immunology, FVSc & A.H, SKUAST-K, J&K, India. He is recipient of DBT overseas fellowship award for 2011-2012 and Young Scientist Biotechnology Fellowship 2002-2003 by State Council of Science and Technology, Department of Science and Technology, Jammu and Kashmir. He has more than 89 publications. He has worked on short gun cloning of Buffalopox virus. He has worked on Shiga toxin-producing *E. coli* (STEC) and enteropathogenic *E. coli* (EPEC) in cattle, sheep and goats in India. He established the presence of enteroaggregative *E. coli* (EAggEC), diffusely adherence *E. coli* (DAEC), enterotoxigenic *E. coli* (ETEC), typical/atypical EPEC and STEC in diarrhoeic human patients. His research group has been responsible for the development of vaccine for footrot in Kashmir.

**Dr Shafqut Majeed Khan**, B.V.Sc and A.H, M.V.Sc in animal biotechnology, has worked on optimisation of assisted reproductive technologies especially cloning which involved somatic cell nuclear transfer (SCNT), handmade cloning (HMC), in vitro fertilisation, cell culture etc. He has worked on comparison of efficiency SCNT and HMC in sheep. He has expertise in culture, isolation and molecular characterisation of various anaerobic bacteria like *Clostridium perfringens* and *Dichelobacter nodosus*. Presently working as field Veterinary Assistant Surgeon, catering to the requirements of farming community, wherein his work includes treatment of animals, creating awareness about various infectious diseases, vaccination campaigns, collecting data about
disease outbreaks and management of outbreaks of diseases like Foot and Mouth Disease and Avian influenza, etc.

Dr Arif Ahmad Pandit, BVSc, MVSc, PhD (Animal Biotechnology), Subject matter specilaist, KVK, Leh, SKUAST-K, J&K, India. He has functional expertise in Genomics/Transcriptomics using microarray and RNA Seq technology. He worked alongside the One Health interdisciplinary team of scientists headed by Prof Baljit Singh at the University of Saskatchewan, Canada, as a team-building exercise during an Integrated Training Programme in Infectious Diseases, Food Safety and Public policy. He has attended 5th Annual Meeting of International Cytokine and Interferon Society in Japan, which earned him Kishimoto award supported by the Department of Science and Technology, Govt of India. His research efforts to date have led to the publication of six refereed research articles as first author, two published abstracts, and three presentations and a number of lead papers at professional meetings at the regional, national, and international levels.

Dr Javed A Malla, BVSc, MVSc (Veterinary Virology). During his tenure as senior research fellow in Niche area of Excellence in Anaerobic bacteriology, worked on culture isolation identification of fastidious anaerobes especially *Dichelobacter nodusus* and *Clostridium perfringens*. Presently working as Veterinary Assistant Surgeon, Department of Animal Husbandry, J&K, India where his mandate is disease diagnosis and treatment.

Dr Bilal Ahmad Dar PhD (Zoology). During his tenure as Research Associate in Niche area of Excellence in anaerobic bacteriology, he worked for culture isolation identification of fastidious anaerobes (viz. *Dichelobacter nodosus, Fusobacterium necrophorum, Clostridium perfringens*).