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Identification of chlamydial strains causing abortions and pneumonia in sheep and goat flocks during trans Himalayan seasonal migration in the northern region of India

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ABSTRACT

The involvement of chlamydiae in abortion and pneumonitis cases among sheep and goat flocks was investigated during the trans Himalayan seasonal migration in a northern mountaneous region of India. A total of 243 samples from clinical cases of abortions (n = 104) from sheep (n = 55) and goats (n = 49), as well as pneumonia (n = 139) from sheep (n = 58) and goats (n = 81), were tested using order *Chlamydiales* specific 23S rRNA gene based PCR, and family *Chlamydiaceae* specific *ompA* gene based PCR tests. Sampling was done from 4 different tracks during the seasonal migration between high altitude pastures and grass lands in plains. Involvement of chlamydiae was detected in 30.9% and 16.3% abortion cases, and in 12.0% and 18.5% pneumonitis cases in sheep and goats, respectively. The associated species/strains of chlamydiae were identified initially by PCR-RFLP, and then genetic diversity at species/strain level was analysed depending upon variations in the VD2 region of the *ompA* gene. It revealed that 78.9% abortions and 72.7% pneumonic cases were due to *C. psittaci*, whereas, 21.1% cases of abortion and 27.3% cases of pneumonia were due to *C. abortus,* respectively. Two predominantly prevalent strains of *C. psittaci* and *C. abortus* were also identified. Thus, this study conclusively identified, for the first time, the endemic species/strains of chlamydiae in this region causing frequent abortions and pneumonitis among migratory flocks of sheep and goats. This will help to adopt specific preventive and control measures to curtail the economical losses to farmers and also chances of zoonosis.

Key words: abortions, pneumonitis, chlamydiae, migratory sheep and goats, PCR

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Introduction

Migratory sheep and goat husbandry is an economically important activity of small and marginal farmers of the hilly northern states of India. In winter, flocks of sheep and goats migrate from high altitute Himalayan pastures to grass lands in plains to avoid the harsh winter climate, and in summer they follow reverse migration to highland alpine pastures (CSWRI, 2001; SINGH et al., 2006). Reproductive problems and respiratory infections are frequent disease conditions observed among migaratory sheep and goats, causing production losses in the northern Himachal Pradesh state of India (BATTA et al.,1996; KATOCH et al., 1997; KUMAR et al., 2000). According to the 19th Livestock census-2012 report by the Ministry of Agriculture of the Government of India (dahd.nic.in/dahd/statistics/livestock-census/19th-indian-livestock-census.aspx), there are 0.8 million sheep and 1.1 million goats in Himachal Pradesh, contributing substantially to its economy in the form of mutton, chevon and wool (KUMAR et al., 2012).

Chlamydiae are obligate intracellular bacterial pathogens, that generally parasitize epithelial cells and cause various disease syndromes in animals, birds and humans (STORZ, 1971; STEPHENS, 1999). These genetically diverse organisms belonging to the *Chlamydiaceae* family were grouped earlier into two genera, *Chlamydia* and *Chlamydophila*, containing nine species (EVERETT et al., 1999). But recently a single genus based classification (*Chlamydia*) with 11 species has been adopted by the International Committee on Systematics of Prokaryotes, and is currently being followed (KUO et al., 2011; SACHSE et al., 2015). Amongst ruminants, *Chlamydia abortus, Chlamydia pecorum* and *Chlamydia psittaci* cause clinical or subclinical infections (MOHAMAD and RODOLAKIS, 2010; REINHOLD et al., 2011; KNITTLER et al., 2014). *C. abortus* is responsible for ovine enzootic abortion (OEA), causing abortion, infertility and weak offspring in many countries (OIE 2011), whereas, *C. pecorum* is associated with chronic conditions, such as: pneumonia, polyarthritis, conjunctivitis, enteritis, encephalomyelitis, metritis and mastitis amongst ruminants (BERRI et al., 2009). *C. psittaci* mainly causes "avian chlamydiosis" amongst 471 reported avian species (KALETA and TADAY, 2003; CHAHOTA et al., 2006) but can also infect mammalian species, including ruminants (PANTCHEV et al., 2009; LENZKO et al., 2011; KNITTLER et al., 2014). *C. abortus* and *C. psittaci* are also potential agents of direct anthropozoonosis (animals to humans) without the involvement of any intermediate host (LONGBOTTOM and COULTE, 2003; RODOLAKIS and MOHAMAD, 2010).

The economic impact of chlamydial abortions in small ruminants is reported to be very high since first time exposure to *C. abortus* infection may lead to abortions in one third of the pregnant ewes in a flock, and occasionally twice as many in pregnant goats. This high level of abortions persists for 2 to 3 years until almost all the females abort and later on animals become asymptomatic carriers, leading to economic losses

owing to low productivity and poor health (AITKIN and LONGBOTTOM, 2007; OIE, 2011). Involvement of chlamydiae in reproductive and respiratory infections among ovine and caprines has been documented in this region of India (BATTA et al., 1996; KATOCH et al., 1997; CHAHOTA et al., 2015), but taxonomic identification and molecular characterization of the prevalent chlamydia species/strains responsible for reproductive and respiratory infections in sheep and goats have not yet been undertaken.

Conserved genes, such as 16S rRNA, 23S rRNA and genetically variable genes such as *omp2* and *ompA,* and some other targets, such as GroEL chaperonin, KDO transferase and *ompB,* are used for detection of chlamydiae and analysing species/strain level variations in field studies (EVERETT et al., 1998; EVERETT et al., 1999; CHAHOTA et al., 2006; SACHSE et al., 2009; ABABNEH et al., 2014). Keeping in view the economic importance of reproductive and respiratory diseases among sheep and goats, especially during stressful migratory husbandry practices, the present study was undertaken to identify and genetically characterize the native species/strains of chlamydiae responsible for abortions and pneumonitis. Representative samples from diseased sheep and goats were collected from 4 different migration routes, and tested using 23S rRNA and *ompA* gene based PCR tests.

Materials and methods

Sample collection. A total of 243 samples from sheep (n = 113) and goats (n = 130), having a history of abortions and signs of respiratory distress or pneumonia, were collected from 43 flocks and 19 locations (Table 1) along the 4 migration routes of sheep in 6 districts of Himachal Pradesh, a mountainous northern state of India (Fig. 1). Abortion samples (n = 104), including vaginal swabs (n = 96), placental tissues (n = 5) and aborted feti (n = 3), collected within 2 weeks of abortions, and nasal respiratory samples (n = 139) that included nasal swabs (n = 118) and lung tissues from post-mortem examinations (n = 21), were collected in PBS for PCR based detection, and in sucrose phosphate glutamate (SPG) for isolation of chlamydiae. The respiratory tract samples were collected from the animals with clinical symptoms of nasal discharge, coughing and abnormal lung sounds upon auscultation. All morbid samples, such as vaginal swabs/uterine discharges, aborted fetal contents foetal organs and foetal membranes were collected aseptically, transported on ice to the laboratory and stored at -20 °C, until further processing for confirmation of chlamydiae. In this study, baseline data about abortions and respiratory disease incidence, the locations of the migration routes, average flock size and breeds reared were also collected from all 43 studied flocks.

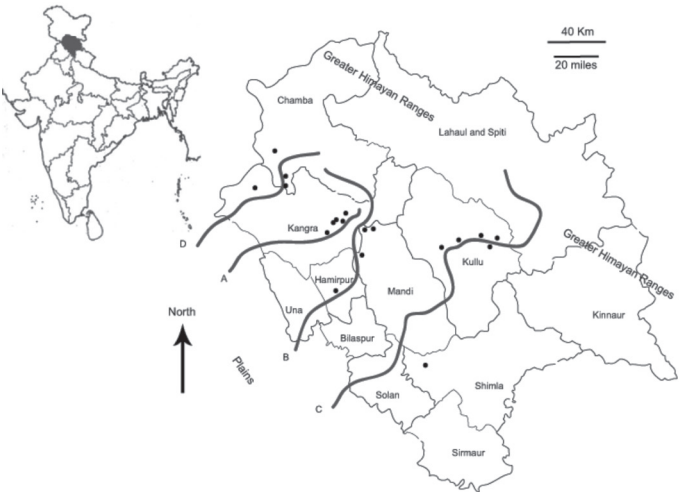

Fig. 1. Map of the Himachal Pradesh state of India, showing approximate geographical locations of migration routes (A, B, C and D). The places of sample collection along the routes are shown in solid dots. Further details about places are shown in Table 1.

Table 1. Location wise details of samples collected from cases of abortion and pneumonia among migratory sheep and goats

Animal species	Districts (no. of locations)	Abortions	Pneumonia	Total
Sheep	Kangra (6[a])	24	18	42
	Mandi (3[b])	8	29	37
	Hamirpur (1[c])	17	nil	17
	Kullu (5[d])	6	3	9
	Chamba (3[e])	nil	1	1
	Shimla (1[f])	nil	7	7
	Subtotal	55	58	113
Goats	Kangra (6[a])	10	16	26
	Mandi (3[b])	6	23	29
	Kullu (4[d])	27	27	54
	Chamba (3[e])	6	2	8
	Shimla (1[f])	nil	13	13
	Subtotal	49	81	130
Total		104	139	243

[a] Places in Kangra district (route A) include Palampur, Maranda, Kandbari, Darang and Paror; (route D) Nurpur. [b] Bir, Utrala and Barot were the places in district Mandi (route B). [c] Tal in district Hamirpur (route B). [d] Kullu, Manali, Sarol, Madhi, Gulaba were the places in district Kullu (route C). [e] Jot, Banikhet and Salooni in district Chamba (route D). [f] Shimla town district Shimla (route D).

DNA extraction and PCR tests. DNA from the samples was extracted by using commercial DNA extraction kits (QiaAmp DNA minikit, QIAGEN, Hilden, Germany). All the samples were screened using order *Chlamydiales* specific 23S rRNA gene based primers U23F and 16SIGR (EVERETT et al., 1998) and family *Chlamydiaceae* specific *ompA* gene based nested PCR, that included firstly an outer pair, CMGP-1F and CMGP-1R, and then an inner pair of primers, CMGP-2F and CMGP-2R, in second steps (CHAHOTA et al., 2006). For both the PCR tests, the same conditions as reported earlier were used. PCR was performed using a Master Cycler (Eppendorf, Hamburg, Germany). For both the PCR tests, electrophoresis was done at 80 to 100V in TAE as running buffer, for 45 to 60 min using 1.5% agarose gel that was stained with ethidium bromide for 30 min to visualize and analyse DNA bands using a UV illuminator Gel Doc Alphaimager (Alpha Innotech, California, USA).

PCR-RFLP. Preliminary identification of chlamydial species was done using the PCR-RFLP method, targeting the 16S-23S rRNA intergenic spacer region, according to the already reported conditions (EVERETT et al., 1999). For PCR-RFLP, primers 16SF2 and 23R were used, producing 600 bp amplicon in the case of the defined genus *Chlamydia* and 585 bp in the case of genus *Chlamydophila* of old classification. Amplicons were further purified using a QIAquick Gel Extraction kit (QIAGEN, Hilden Germany), according to the manufacturer's instructions, and eluted in 50 µL of elution buffer. Purified PCR products were subjected to RFLP studies. The restriction enzymes *AciI, BfaI, SfcI, DdeI, HpaI, BclI* and *RsaI* (Fermentas, Maryland, USA) were used to digest the PCR products. Restriction fragments were separated using 2% agrose gel, and detected by staining with ethidium bromide.

Nucleotide sequencing. By analysing the nucleotide sequence of the variable domain 2 (VD2) region of the *ompA* gene (about 250 bp), native chlamydial strains were identified and genetically characterized. PCR products of positive samples were purified with a Gel Extraction kit (Qiagen, Hilden, Germany) and quantified using Gene Quant (Biorad). Samples having sufficient DNA concentrations were sent for direct sequencing, and those with low DNA concentrations were cloned in a pGEM-T cloning vector (Promega, Madison, USA) and DH5α strain of *E. coli* was used for transformation by the heat shock method. DNA insert containing clones were selected on Luria Bertani (LB) medium plates, containing isopropyl-β-D-thiogalactopyranoside (IPTG) (23.8 µg/mL), ampicillin (50 µg/mL), and 5-Bromo-4-Chloro-β-D-galctoside (X-gal) (40 µg/mL). From each sample, 2-3 clones with an expected size of DNA insert were send for sequencing to First BASE Laboratories Sdn Bhd, Selangor, Malaysia.

Taxonomic and partial ompA gene sequence analysis. The nucleotide sequences were assembled and edited using Genetix-Mac/ATSQ 4.2.3. Chlamydial species and strains were identified by NCBI-BLAST (http://www.ncbi.nlm.nih.gov) search of nucleotide and

deduced amino acid sequences. For partial *ompA* gene sequence analysis, *ompA* gene sequences of *C. psittaci* and each representative species of the genus *Chlamydia* were retrieved from the DNA Data Bank/GenBank. Multiple alignment of trimmed sequences was done using Clustal X version 1.83. The partial *ompA* gene sequence analysis was done with programs from the Phylogeny Inference Package Software (Phylip) (Version3.695; [http://www.evolution.genetics.washington.edu/phylip.html]). The distance matrix between species was computed by Dnadist using the Juke and Cantor model, and clustering of lineages was done by Neighbor using the neighbor-joining method.

Isolation and identification. Isolation of native chlamydial strains was attempted from selected 20 PCR positive samples of abortions (n = 10) and pneumonitis (n = 10) using McCoy cell line. The cells were maintained in Eagle's minimum essential medium (HiMedia, India) containing 5% foetal bovine serum and 10 µg/mL of gentamicin and 1 µg/mL of cycloheximide, and incubated at 37 °C in 5% CO_2. The isolates were later confirmed by observing intra cytoplasmic inclusions after Gimenez staining, and by family specific PCR as discussed above.

Statistical analysis. The hypothesis that the chlamydial infections in the animal populations following different migration routes (geographical locations) are the same was tested using the χ^2 test function in Microsoft Excel software, at 5% ($0.05<P$) significance level and the results of the observed prevalence (experimental hypothesis) were analysed.

Results

Epidemiological analysis. Data from 43 flocks on 4 migration routes in Himachal Pradesh showed incidences of abortions varying from 7.7 to 10.1%, and respiratory infection in the range of 4.6 to 8.7% during seasonal migration excluding occasional outbreaks (Table 2). Abortions were frequently seen during the lambing/kidding season from February to March, when they mated at the beginning of migration from the alpine pastures during September and October, and in the lambing/kidding season of September to October when they mated during March and April. An overall prevalence of 19.3% of chlamydiosis was observed in this study, with 24.0% in sheep and 15.8% in goats (Table 3). On the 4 migration routes, the prevalence of chlamydial infection was found to be: 19.6% (11/56), 22.6% (14/62), 17.9% (17/95) and 16.7% (5/30) on routes A, B, C and D, respectively, in both sheep and goat populations. Statistically no significant variation was found in the prevalence of chlamydial infection on the 4 migration routes investigated.

Chlamydial abortions. Out of 104 abortion samples, overall 24.0% samples were found positive for chlamydiae, including 17 (30.9%) samples from sheep and 8 (16.3%) samples from goats, by either the order or family specific PCR test (Table 3). Maximum 20.2% detection of chlamydiae was possible from vaginal swabs followed by 1.9%

each in aborted foeti and placental tissue samples, indicating that post abortion vaginal secretions are the best source for detection of chlamydiae.

Table 2. Baseline information about migration routes and prevalent reproductive and respiratory disease conditions among migratory sheep and goat flocks in Himachal Pradesh included in the study

Migration Routes	Places around migration tracts	No. of flocks screened[a]	Total animals	Reproductive disorders	Pneumonitis
A	Starting point - lower region of Kangra and Hamirpur district Routes followed-Palampur-Baijnath-Bir-Chhota Bhangal End point-Bada Bhangal (Kangra)	8	2850	220 (7.7%)	130 (4.6%)
B	Starting point - lower region of Kangra and Hamirpur districts Routes followed-Palampur-Paprola-Utrala End point-Holi (Chamba)	10	3510	330 (9.4%)	245 (7.0%)
C	Starting point - lower region of Mandi, Kangra, Baijnath Routes followed-Palampur-Baijnath -Mandi-Kullu-Madhi-Rohtang Pass End point-Lahaul Spiti	16	6120	530 (8.7%)	475 (7.8%)
D	Starting point - lower region of Kangra, Hamirpur and Pathankot districts Routes followed-Nurpur-Jot/ Chuwadi-Chamba End point-Bharmour	9	1775	180 (10.1%)	155 (8.7%)
Total		43	14255	1260 (8.8%)[b]	1005 (7.0%)[b]

[a] The flocks screened during study were mainly keeping Gaddi and Rampur Bushair breeds of sheep and goats, with average flock size ranging between 200 to 400. Horses and dogs were also used during migration long with the sheep and goats and usually 2 to 3 shepherds were accompanying each flock. [b] Numbers include only regular cases at the time of sampling and exlude cases of occasional outbreak of abortions and pneumonitis occurring in flocks as reported by the farmers.

Chlamydial pneumonia. From 139 pneumonia samples tested with both PCR tests, overall, 15.8% were found positive for involvement of chlamydiae by one of the tests.

Higher incidences of chlamydial pneumonia were detected in goats, 15 (18.5%) than sheep, 7 (12.1%) (Table 3).

Table 3. Chlamydial involvement in the cases of abortions and pneumonia in migratory sheep and goats

Disease conditions	Sheep				Goats				Total
	Total samples	PCR positive samples[a]			Total samples	PCR positive samples[a]			
		Simple PCR	Nested PCR	Overall positive[b]		Simple PCR	Nested PCR	Overall positive[b]	
Abortions	55	6 (10.9%)	17 (30.9%)	17 (30.9%)	49	Nil	8 (16.3%)	8 (16.3%)	25 (24.0%)
Pneumonitis	58	2 (3.4%)	7 (12.1%)	7 (12.1%)	81	4 (4.9%)	12 (14.8%)	15 (18.5%)	22 (15.8%)
Total	113	8 (7.1%)	24 (21.2%)	24 (21.2%)	130	4 (3.1%)	20 (15.4%)	23 (17.7%)	47 (19.3%)

[a] The percentage prevalence of chlamydiae among caprine and ovine using different test is shown in parentheses. Sample found positive by either or both tests was considered as positive.

Identification of chlamydiae and genetic variation. Initial identification of the species of chlamydia in 47 positive samples was done by PCR-RFLP analysis of specific restriction patterns, as already reported (EVERETT et al., 1999). All the detected chlamydiae belonged to the genus *Chlamydia* (former genus *Chlamydophila*). A restriction pattern corresponding to *C. psittaci* was observed in 19 and to *C. abortus* in 6 abortion samples, whereas, *C. psittaci* was found in 16 samples and *C. abortus* in 6 pneumonitis cases. The nucleotide sequence analysis of the VD2 region of *ompA* genes (PCR product of nested PCR) from 25 abortion cases showed involvement of *C. psittaci* in 19 (76%) cases, whereas *C. abortus* was seen in 6 (24%) cases. Likewise, from 22 pneumonia samples involvement of *C. psittaci* was found in 16 (72.7%) cases, and *C. abortus* in 6 (27.3%) cases. Phylogentic analysis of all detected strains of *C. psittaci* and *C. abortus* showed all sequence types were grouped into two genetic clusters (Fig. 2): Cluster I containing strains of *C. psittaci,* which shows 100% identity with the avian strain Mat116 (GenBank number CP002744), and CP0319 (AB239892), which is a highly pathogenic strain, isolated from an outbreak among psittacine birds and humans. However, cluster II contained *C. abortus* strains. The detected strains showed 100% identity with the *C. abortus* EBA strain (AF269256) and the B577 strain (KC879303), isolated from bovine and ovine abortion cases respectively. The detected strain showed 99% homology with *C. abortus* strain Pm234 (AJ004875) isolated from abortion cases from swine, which showed that the detected genotype can infect a wide range of animal hosts. We were able to isolate 11 *C. psittaci* and 6 *C. abortus* from abortion and pneuminitis cases for further studies.

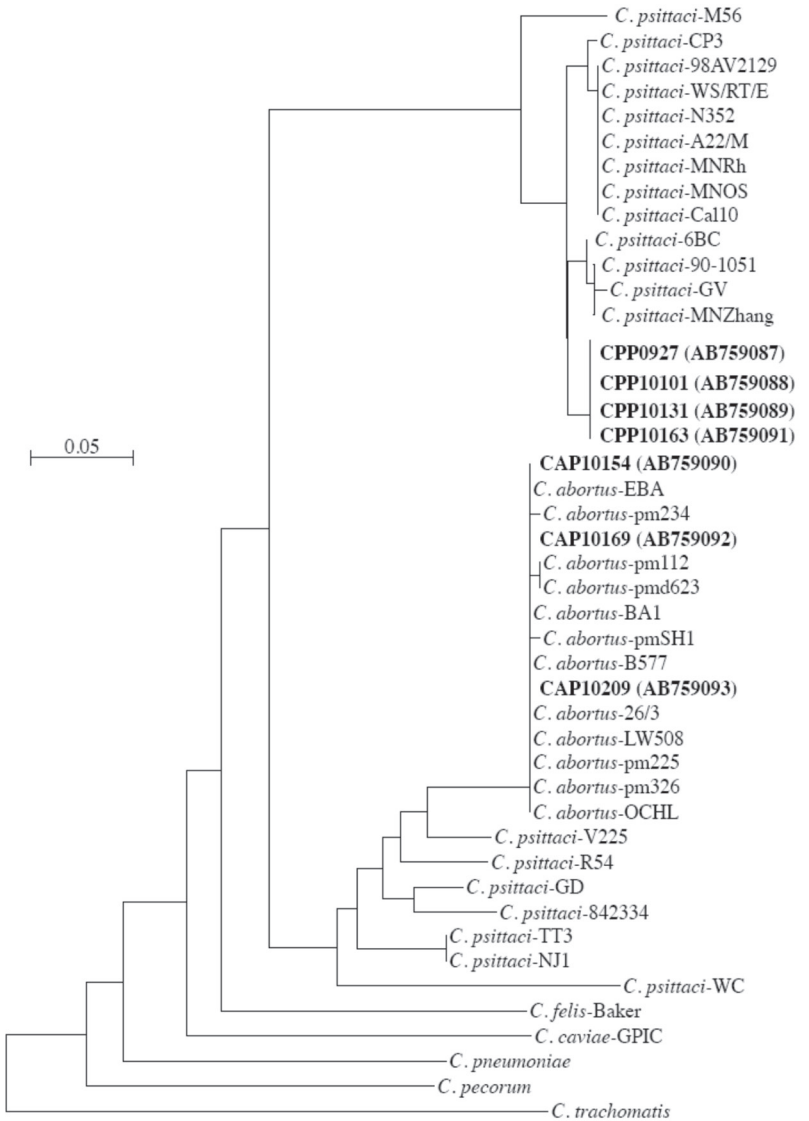

Fig. 2. Neighbour-joining tree of the some of the detected chlamydial species/strains (bold face) vis-à-vis other already known strains. Accession numbers are shown against each detected chlamydial strain. Genetic distance was calculated by DNADIST using the Juke and Cantor method. Relative distance is shown in 0.05 distance bar.

Discussion

Sheep and goats reared by migratory animal husbandry practices are valuable and renewable sources of income for small and marginal farmers, and also for the nomadic people of northern Himalayan regions of India, including the state of Himachal Pradesh. The problem of shrinking sizes of pastures, increasing toxic weed infestations on pastures, coupled with frequent disease outbreaks and harsh climatic conditions, are challenging the sustenance of such farming classes. The state of Himachal Pradesh, known for its biodiversity, is situated between latitude 30° 22' 40'' N and 33° 12' 40'' N and longitude 75°45'55'' E and 79°04'20'' E, with an altitude ranging from 350 m (low valleys) to 6,975 m (snow covered mountains) above mean sea level. It has a total geographical area of 56,673 square kilometres, including 9859 square kilometres of permanent pastureland. The climatic conditions vary from hot and sub-humid tropical in southern low tracts, (450-900 m), warm and temperate (900-1800 m), cool and temperate (1900-2400 m) and cold alpine and glacial 2400-4800 m) in the high northern and eastern mountain ranges. The rainfall varies from 350 to 3800 mm per annum with temperatures varying from -25 °C in January to 42 °C in June. Migration through such varied climatic and geographically stressful conditions exposes flocks of sheep and goats to many infectious and parasitic diseases.

Himachal Pradesh has many migration routes, and sheep and goats share common grazing pastures and routes with wild and other domestic animals, which leads to the exposure of these animals to various infectious agents. Our baseline data also emphasized that reproductive and respiratory infections are important infectious diseases, contributing directly or indirectly to high economic losses.

Chlamydiae are important bacterial pathogens, responsible for serious infections in both animals and humans, including enzootic infections (STORZ, 1971; STEPHENS, 1999). In our investigation, we used Chlamydiae family specific nested PCR, based on the *ompA* gene, followed by nucleotide sequence analysis of its variable domain two (VD2) region. This VD2 region of the *ompA* gene has species and strain specific nucleotide sequence motifs, which ruled out false identification. Our investigation showed that overall 19.3% cases of abortion and pneumonia were chlamydia related in sheep and goats. It also indicated the frequent transmission of chlamydial pathogens between sheep and goat flocks during migration. Different migration routes showed no statistically significant differences in chlamydial infections, indicating the endemic nature of the chlamydial infection in this region. Both *C. psittaci* and *C. abortus* contribute to as many as 30.9% abortion cases in sheep, but comparatively less, 16.3%, in goats. However, in terms of respiratory infection, 18.5% goats and 12.1% sheep showed association with chlamydiae. As sheep and goats are reared together in these flocks and criss-crossing of migration routes are common, inter species/flock transmission of same strains of *C. psittaci* and *C. abortus* may have occurred as reflected in our data. We could not detect any *C. pecorum* positive samples from cases of abortions and respiratory infections. Further studies are needed, including enteric samples to establish the prevalence of *C. pecorum* in this region.

Although *C. abortus* has been considered to be the most economically important reproductive tract pathogen in small ruminants (AITKIN and LONGBOTTOM, 2007; OIE, 2011), recent studies, including the present study, indicate the equally important role played by *C. psittaci* in both reproductive and respiratory infections in sheep (LENZKO et al., 2011) and cattle (KEMMERLING et al., 2009; PANTCHEV et al., 2009; REINHOLD et al., 2011). DEGRAVES et al. (2003) detected *C. psittaci* in 53% cases of genital infection in heifers in Germany. Similarly, KEMMERLING et al. (2009) observed that *C. psittaci* was the most prevalent representative of the family *Chlamydiaceae* i.e. in 56% of vaginal swabs collected from different dairy herds and individual cattle. Similar findings were observed when PANTCHEV et al. (2009) showed the involvement of *C. psittaci* in 11% of abortion samples from cattle, sheep, goats and swine by the more sensitive real time PCR method. The presence of *C. psittaci* in the semen of bulls from Germany and Switzerland has been reported recently (KAUFFOLD et al., 2007; TEANKUM et al., 2007).

Although earlier studies reported chlamydial abortions and respiratory infections in this region (BATTA et al., 1996; KUMAR et al., 2000), this is the first study in India confirming the chlamydial aetiology at strain level in cases of abortions and pneumonitis. We have recently also identified the chlamydial strains frequently causing eye infections in ruminants (GUPTA et al., 2015). Our investigation detected the two predominant strains of *C. psittaci* and *C. abortus* involved in both abortions and respiratory infections during the annual seasonal migration in this region. Although *C. psittaci* and *C. abortus* have both host and tissue specific predilection, *C. abortus* is known as a uteropathic organism of mammals, while *C. psittaci* commonly infects the lungs in avian/mammals. The tissue tropism properties of these isolated strains need to be studied further to understand their pathogenesis. Further, *C. psittaci* and *C. abortus* are well known pathogens frequently responsible for zoonoses among animal handlers that need to be investigated, especially among shepherds and slaughter house workers, owing to their prolonged professional exposure.

Thus, our study conclusively established that only a few strains of *C. psittaci* and *C. abortus* are endemic in this region and circulating amongst migratory flocks of sheep and goats, leading to frequent abortions and respiratory problems, and economic losses. This study highlighted the importance of managing such infections, not only from the animal health angle, but also from the human health point of view, owing to the already reported zoonotic potential of the detected chlamydial species.

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SAŽETAK

Nalaz klamidija u slučajevima pobačaja i upale pluća u stadima ovaca i koza istražen je tijekom sezonske selidbe preko Himalaja u sjevernom, planinskom, području Indije. Ukupno su bila pretražena 243 uzorka kliničkog materijala. Kod pobačaja su bila pretražena 104 uzorka, od ovaca 55 i koza 49, a kod upale pluća 139 uzoraka, od ovaca 58, a od koza 81 uzorak. Uzorci su bili pretraženi testovima lančane reakcije polimerazom uporabom gena 23S rRNA specifičnog za red *Chlamydiales* i gena *ompA* specifičnog za porodicu *Chlamydiaceae*. Uzorci su bili prikupljeni na četirima različitim razinama tijekom sezonske selidbe od visinske ispaše do nizinskih travnjaka. Klamidije su bile dokazane u 30,9% pobačaja u ovaca i 16,3% pobačaja u koza te 12,0% upale pluća u ovaca i 18,5% upale pluća u koza. Vrsta i sojevi klamidija početno su bili identificirani PCR-RFLP-om, a potom je njihova genetska različitost na razini vrste/soja bila analizirana ovisno o varijaciji u VD2 području gena *ompA*. Ustanovljeno je da je 78,9% pobačaja i 72,7% upale pluća bilo prouzročeno vrstom *C. psittaci*, dok je 21,1% pobačaja i 27,3% upale pluća bilo uzrokovano vrstom *C. abortus*. Prevladavala su dva soja *C. psittaci* i *C. abortus*. Time su u ovom istraživanju prviput dokazani endemijski sojevi klamidija na pretraživanom području koji uzrokuju česte pobačaje i upale pluća u selidbenim stadima ovaca i koza. Ta spoznaja pomoći će u poduzimanju preventivnih i kontrolnih mjera za smanjenje gospodarskih šteta i pojava zoonoza.

Ključne riječi: pobačaji, upala pluća, klamidije, selidba, ovce, koze, PCR