Indian Journal of Animal Sciences **89**(6): 619–624, June 2019/Article
https://doi.org/10.56093/ijans.v89i6.91100

Detection of chlamydiae from the upper respiratory tract of healthy and diseased draught equines

PRIYANKA[1], R CHAHOTA[2], S GUPTA[3], B BHARDWAJ[4] and M SHARMA[5]

Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh 176 062 India

Received: 25 June 2018; Accepted: 21 February 2019

ABSTRACT

The present investigation was conducted to study the prevalence and molecular characterization of chlamydiae associated with the respiratory infections of equines in the Sub-Himalayan region of India. Equine nasal swab samples (119) from 20 diseased and 99 apparently healthy equines were collected and tested by family *Chlamydiaceae* specific nested PCR based on *ompA* gene, besides 89 serum samples for AGPT. The molecular characterization of chlamydial species/strains was done by analyzing variation in VD II region of *ompA* gene. The chlamydia infections were detected in 48.74% of the nasal swabs (55% in diseased and 47.47% in apparently healthy animals) by nested PCR. Prevalence of two genetically variant strains of *Chlamydia abortus* and a single strain of *Chlamydia psittaci* was detected. AGPT showed 4.49% seropositive equines. High prevalence of chlamydiae was found among equines in the Sub-Himalayan region of India in Himachal Pradesh in both healthy and diseased equines.

Key words: Characterization, Chlamydiae, Equines, Respiratory tract

Equines particularly horses, ponies, mules and donkeys are mainly engaged for transportation of materials in difficult hilly terrains and for joyrides at various hill station tourist places in Sub-Himalayan region of India. Respiratory distress syndrome (RDS) characterized by polypnea, inappetence, pyrexia and lower work tolerance is a persistent problem among draught equines in Himachal Pradesh (Chahota *et al*. 2001, Prasad *et al*. 1992). Chlamydial species are being suspected as important aetiologies in the respiratory infections of equines from long time (Moorthy and Spradbrow 1978, McChesney *et al.* 1982). Chlamydiae are obligate intracellular bacterial pathogens belonging to *Chlamydiaceae* family and were grouped earlier into two genera, viz. *Chlamydia* and *Chlamydophila*, containing nine species (Everett *et al.* 1999). A single genus based classification (*Chlamydia*) with 11 species has been adopted by International Committee on Systematics of Prokaryotes (Greub 2010) and is currently being followed (Kuo and Stephens 2011). Amongst equines, different chlamydial species/genotypes have been reported including *Chlamydia pneumoniae* (Storey *et al.* 1993), *Chlamydia psittaci* (Mair and Wills 1992), *Chlamydia abortus* (Theegarten *et al.* 2008) and *Chlamydia caviae* (Fretz *et al.* 1979). Some chlamydial species like *C. abortus* and *C. psittaci* are also potential agents of direct anthropozoonosis (animals to humans) without the involvement of any intermediate host (Longbottom and Coulter 2003, Rodolakis and Mohamad 2010). Recurrent airway obstruction (RAO) in horses is a naturally occurring dust-induced disease mainly characterized by bronchiolitis, which shows histological and pathophysiological similarities to human chronic obstructive pulmonary disease (COPD).

The investigated clinical condition of equines locally called 'Dhaunkni' meaning laboured breathing and coughing, was having similar clinical pictures as RAO and chlamydial involvement was detected earlier from few animals (Chahota *et al.* 2001). Keeping in view the potential zoonotic nature of the Chlamydia, this study was undertaken to study the role of chlamydiae in respiratory infections in equine species in the Sub-Himalayan state (Himachal Pradesh) of India and identifying the involved strains by isolation and PCR.

MATERIALS AND METHODS

Sample collection: Equine samples (119) were collected from various locations in 3 districts of Himachal Pradesh (Table 1). The nasal swabs were collected from 59 horses and 60 mules, exhibiting respiratory disease manifestations (Nabeya *et al.* 1996) as well as apparently healthy animals

Present address: [1]Research Associate (priyanka7malik @gmail.com), Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, Selesih, Aizawl, Mizoram. [2]Professor (rchahota@yahoo.com), [5]Professor and Head (mandeepsharma289@hotmail.com), Department of Veterinary Microbiology, DGCN College of Veterinary and Animal Sciences. [3]PhD Scholar (shilpi5.biotech@gmail.com), School of Biotechnology, Jawaharlal Nehru University, New Delhi. [4]Veterinary Assistant Surgeon (lucky000051@gmail.com), Indo Tibetan Border Police.

(99). All the samples were collected in sterile vials in duplicate, i.e. in sucrose phosphate glutamate (SPG) containing antibiotics (10 g/l streptomycin, 100 mg/l vancomycin, 50 mg/l nystatin, 50 mg/l gentamicin and 500 mg/l kanamycin) for the isolation of chlamydiae and in phosphate buffered saline (PBS) (pH 7.2) for the PCR based detection of chlamydiae. Serum samples (89) were also collected from 44 horses and 45 mules for conducting seroprevalence studies.

Table 1. Details of samples collected from different equine species

Animal species	Geographical location		Type of sample	
	District	Number of location	Nasal swab	Serum
Horse	Chamba	3[a]	22	22
	Kangra	10[b]	37	22
Sub-total			59	44
Mule	Chamba	5[c]	21	21
	Kangra	7[d]	38	23
	Mandi	1[e]	1	1
Sub-total			60	45
Total			119	89

[a]Three locations include Khajjiar, Pakkatala, Sultanpur. [b]Ten locations of District Kangra include Baijnath, Dadi, Dharamshala, Palampur, Paprola, Paror, Saddu, Shahpur, Sungal, Utrala. [c]Chamba, Mangala, Obri, Pakkatala, Sultanpur. [d]Baijnath, Dharamshala, Kangra, Palampur, Paprola, Sungal, Utrala. [e]Chauntra.

DNA extraction and PCR: DNA was extracted by using phenol-chloroform-isoamyl alcohol DNA extraction method (Sambrook *et al*. 2001). The DNA pellets were suspended in 50 ml of sterile distilled water. All the samples (119 nasal swabs) were screened using family *Chlamydiaceae* specific *ompA* gene based nested PCR. The primers used for the first step were CMGP1F (5'-CCTTGTGATCCTTGCGCTACTT-3') and CMGP1R (5'-GTGAGCAGCTCTTTCGTTGAT-3') while the primers used for second amplification were CMGP2F (5'-GCCTTAAACATCTGGGATCG-3') and CMGP2R (5'-CCACAACCACATTCCCATAAAG-3') (Chahota *et al.* 2006).

Identification and genetic analysis: PCR positive samples (17) were processed further for identification and genetic characterization of chlamydial species and strains by analyzing the nucleotide sequence of VD II region of MOMP. The PCR products were purified by QIAquick PCR purification kit (Qiagen). The purified PCR products were quantified by using Gene Quant. The samples were sent for DNA sequencing to First BASE Laboratories Sdn Bhd, Selangor, Malaysia. The 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 acids sequences. For phylogenetic analysis, *ompA* gene sequences of *C. psittaci* and each representative species of genus *Chlamydia* were retrieved from DNA Data Bank/Gene Bank. Multiple alignment of trimmed sequences were done using Clustal X version 1.83.

Phylogenetic analysis was done using Phylogeny Inference Package Software (PHYLIP) (Version 3.6a3; http://www.evolution.genetics.washington. edu/phylip.html). The distance matrix between species was computed by DNADIST using F84 model and clustering of lineages was done by NEIGHBOR using neighbor-joining method. The phylogenetic tree was constructed by neighbor-joining method. The bootstrap values were calculated to evaluate the branching reliability of tree from a consensus tree constructed by generating 1,000 random data sets using SEQBOOT.

Isolation of chlamydiae: The equine nasal samples found positive for equine chlamydiosis by PCR based screening were processed for isolation in 6 to 8 days old embryonated chicken eggs via the yolk sac route of inoculation. Chlamydial isolates were confirmed by Gimenez staining reaction (Storz 1971), mortality pattern and PCR.

Serological studies: The seroprevalence studies of equine chlamydiosis were conducted using agar gel precipitation test (AGPT). The test was performed as per Ouchterlony (1958) using 1% agarose. The antigen for this test was prepared using locally isolated equine strain.

RESULTS AND DISCUSSION

The chlamydiae have been reported from various disease conditions of equines clinically manifested in the form of pneumonia (Popovici and Hiastru 1968, McChesney *et al.* 1974), rhinitis (Moorthy and Spradbrow 1978, Wills *et al.* 1990), conjunctivitis (Moorthy and Spradbrow 1978), polyarthritis (McChesney *et al.* 1974), hepatoencephalic syndrome (Blanco 1968); and genital chlamydiosis in the form of mild chronic salpingitis (Medenbach *et al.* 1999), reduced reproductivity rates (Herfen *et al.* 1999), low ejaculate quality (Veznik *et al.* 1996) and occasionally abortion (Popovici and Hiastru 1968). Different chlamydial species are being suspected to play important role as an aetiological agent in the respiratory infections of equines.

PCR based detection of the family Chlamydiaceae: In our study, 119 equine samples including 20 (10 horses and 10 mules) with respiratory disease symptoms and 99 apparently healthy were screened for chlamydiosis. The horses and mules had to meet any of the following criteria, either alone or in combination, to be considered as diseased: animal exhibiting clinical manifestations such as respiratory distress, coughing and nasal discharge, as specified by Fretz *et al.* (1979). Animals including those kept for draught and joy riding purpose were randomly sampled in our study.

In the present study, we detected the association of chlamydiae both in apparently healthy as well as diseased animals. The screening of 119 samples of nasal swabs by family *Chlamydiaceae* specific nested PCR targeting the VD II region of *ompA* gene revealed 58 (48.74%) samples positive for chlamydiae. From 20 samples of nasal swabs from the animals showing respiratory disease manifestations, 11 (55%) were positive for chlamydiae.

Whereas, from the 99 nasal swabs collected from apparently healthy animals, 47 (47.47%) were positive for chlamydial infection. This data shows the presence of chlamydiae in apparently healthy equines as well as animals with respiratory manifestations. However, the prevalence was slightly more in animals with respiratory disease symptoms as compared to apparently healthy animals. The high prevalence of chlamydiosis in equines as detected in our study was in accordance with the findings of Szeredi *et al.* (2005), who reported 60% PCR positive cases in horses suffering from recurrent airway obstruction and 45% in clinically healthy controls, based on the screening of lung tissue samples of horses using nested PCR.

The comparative prevalence of chlamydiae in horses and mules was studied and 25 (42.37%) horse samples were positive for chlamydial infections out of 59 tested samples. From the mules, out of 60 samples screened, 33 (55%) were positive for chlamydial infections which was slightly higher as compared to the horses. Among the apparently healthy animals sampled for the screening, 20 out of 49 (40.8%) samples from the horse were positive for chlamydiosis, whereas, 27 out of 50 (54%) samples from mules were positive for chlamydiosis. Out of the 20 samples collected from equines exhibiting respiratory disease manifestations, 5 out of 10 (50%) samples from horses were positive for chlamydial infections as compared to 6 out of 10 (60%) samples positive from mules. Thus, the prevalence of chlamydiosis as detected by the family *Chlamydiaceae* specific nested PCR was higher in mules as compared to the horses in apparently healthy animals as well as in those exhibiting respiratory disease manifestations.

Molecular characterization and genetic variation of chlamydial species and strains: In the present study, out of 58 PCR positive samples including 11 from diseased animals exhibiting respiratory manifestations and 47 from healthy animals, 9 representative samples were selected for the study of genetic variability among prevalent chlamydial species/strains. The genetic analysis of VD II (variable domain II) region of *ompA* genes from 9 representative samples of nasal swabs revealed the presence of two species belonging to the genus *Chlamydia* namely, *Chlamydia abortus* in 6 samples and *Chlamydia psittaci* from 3 samples. Similar results were obtained by Theegarten *et al.* (2008), who found 9 isolates of *C. psittaci* and 13 isolates of *Chlamydia abortus* in the lung tissues of 45 horses which included 25 horses with signs of recurrent airway obstruction and 20 clinically healthy controls.

The nucleotide sequences derived from 9 chlamydiae were genetically analyzed to detect variation at the strain level. The multiple alignment of the representative detected

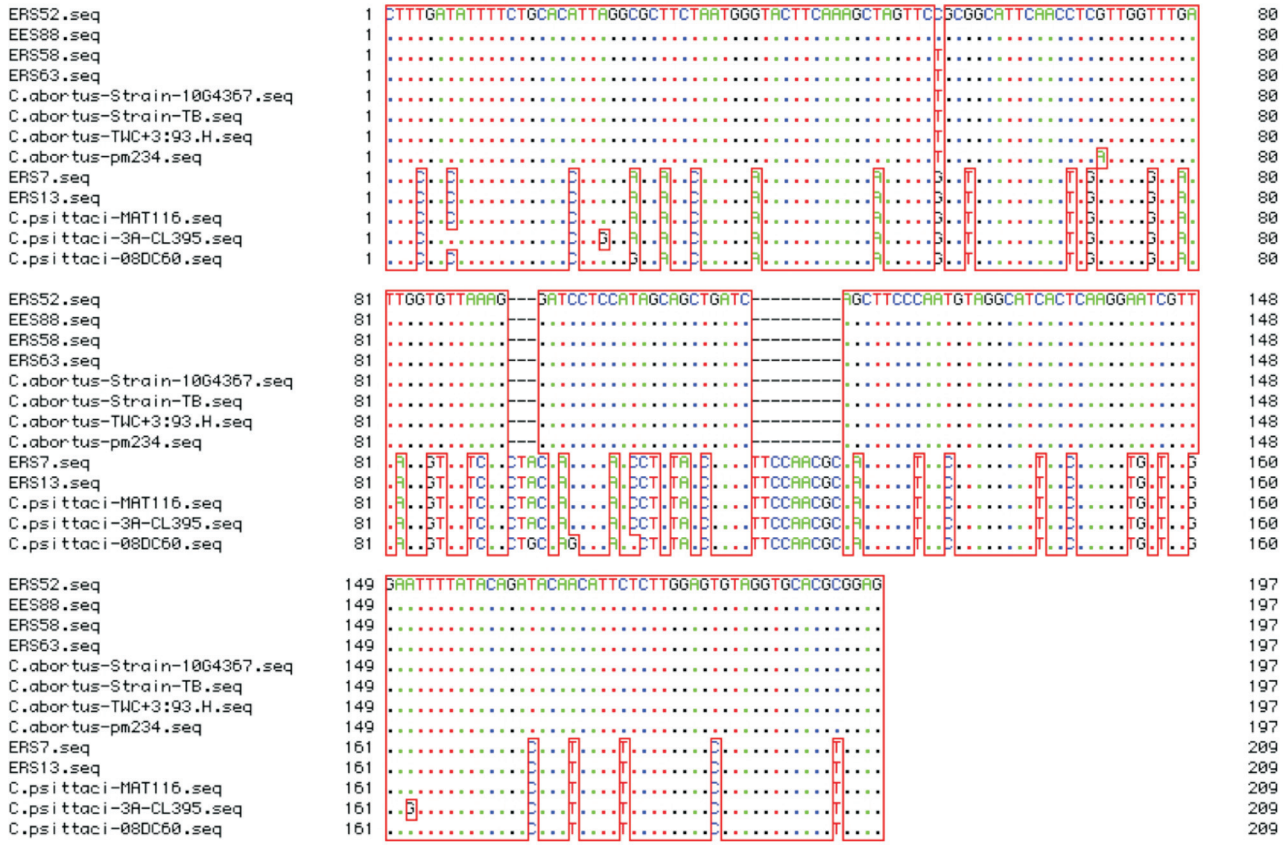

Fig. 1. Comparative nucleotide sequence alignment of representative detected strains of *C. psittaci* and *C. abortus* with identical or closely related previously detected chlamydial strains in the VD2 region and flanking constant domain region of *ompA* gene.

Note: The alignment was done by GENETYX-MAC Ver 13.0.6.

strains is shown in Fig. 1. Out of 6 *Chlamydia abortus* identified from nasal swabs, two genetically variant strains were detected. These two strains were having a difference of one amino acid substitution, whereas, no genetic variation was detected among the detected *Chlamydia psittaci* strains. The strain wise association of chlamydiae belonging to *Chlamydia abortus* and *Chlamydia psittaci* is shown in Table 2. Two strains of *C. abortus*, i.e. ERS-58 and ERS-63 were having 100% homology to 10G4367 strains of *C. abortus* detected from cloacal swab of pigeon in Germany as well as TB strain of *C. abortus* detected from sheep. The similar types of strains were also detected among sheep and goat migratory flocks of Himachal Pradesh responsible for severe pneumonia and abortions among the affected flocks. Whereas, related strain of *C. abortus* detected from nasal swabs (ERS-72, ERS-76, ERS-84, ERS-52) from the equines in this study, had 99% homology with 10G4367 and TB strain of *Chlamydia abortus*. The strain of *Chlamydia psittaci* detected from nasal swabs of equine population had 100% homology with Mat-116 strains of *C. psittaci* detected from various types of psittacine and non-psittacine birds as well as human beings. Taking into consideration the close genetic makeup of detected *C. psittaci* and *C. abortus* strains from equine populations of Himachal Pradesh *vis-à-vis* already known potential zoonotic strains of these two chlamydial species signifies the potential of zoonotic risks involved to the horse and mule handlers as well as other people who come in contact with them.

The phylogenetic analysis of these genetically variant chlamydial species and strains revealed that the detected *C. psittaci* species form a cluster along with other mammalian and avian strains of *C. psittaci*; whereas, *C. abortus* strains detected from equines formed a cluster along with various strains of *C. abortus* detected from various other mammalian species (Fig. 2).

It is also an interesting fact that from equines, multiple species have been reported. Until the end of the 1990s, a number of papers reported clinical cases ranging from respiratory disease to conjunctivitis, polyarthritis, encephalo-hepatitis and abortion in equines, and these cases were ascribed to *Chlamydia psittaci* (old classification), albeit diagnostic testing was often confined to serology. An isolate from a horse with serous nasal discharge (Wills *et al.* 1990) was later reclassified as *Chlamydia pneumoniae* (Storey *et al.* 1993). Investigations of equine abortion cases revealed involvement of *Chlamydia psittaci* (Szeredi *et al.* 2005), which had been redefined as a species in a revised taxonomic scheme (Gaede *et al.* 2009). In a study on horses with recurrent airway obstruction, Theegarten *et al.* (2008) identified both *Chlamydophila psittaci* (old classification) and *Chlamydophila abortus* (old classification) in healthy and diseased horses. Mair and Wills (1992) isolated *Chlamydophila psittaci* from horses. Gaede *et al.* (2009) detected *Chlamydophila caviae* (old classification) in horses with signs of rhinitis and conjunctivitis.

Seroprevalence of equine chlamydiosis: In the present study, out of 89 samples tested by AGPT, 4 (4.49%) were seropositive. Only 1 (12.5%) sample was positive from 8 clinically diseased animals, whereas, from the 81 serum samples collected from apparently healthy animals, 3 (3.7%) were positive for chlamydial antibodies. Two samples each were found positive from 40 horses and 44 mule serum samples.

In this study, 4.49% equines were having anti-chlamydial antibodies by AGPT. Though in other studies, higher seroprevalence has been recorded (Liutkeviciene *et al.* 2007, Nabeya *et al.* 1996), which may be due to low sensitivity of AGPT test used in our study. But these results confirm the role of chlamydiae in precipitation of respiratory disease condition among equines.

Isolation studies for chlamydiosis: Total 24 samples of nasal swabs (5 from diseased and 19 random samples from apparently healthy animals) detected positive for chlamydiosis by family *Chlamydiaceae* specific nested PCR were processed for isolation in 6–8 days old embryonated chicken eggs. DNA was extracted from five samples, passaged three times or more and showing high level of

Table 2. Strain wise association of the chlamydiae in 9 representative samples from different equine species and clinical conditions

Animal species	Type of sample	Sample ID	Clinical status	Chlamydial species and strains detected	Related strains
Horse	Nasal swab	ERS-72	Coughing	*Chlamydia abortus*	99% homology with *C. abortus*
		ERS-76	Nasal discharge and coughing	*Chlamydia abortus*	10G4367 strain from pigeon and TB strain from sheep
		ERS-84	Nasal discharge	*Chlamydia abortus*	
		ERS-13	Respiratory distress and coughing	*Chlamydia psittaci*	100% homology with *C. psittaci* Mat-116 strain from psittacine and non-psittacine birds and human beings
		ERS-14	Coughing	*Chlamydia psittaci*	
		ERS-7	Apparently healthy	*Chlamydia psittaci*	
Mule	Nasal swab	ERS-58	Coughing	*Chlamydia abortus*	100% homology with *C. abortus*
		ERS-63	Coughing	*Chlamydia abortus*	10G4367 strain from pigeon and TB strain from sheep
		ERS-52	Respiratory distress	*Chlamydia abortus*	99% homology with *C. abortus* 10G4367 strain from pigeon and TB strain from sheep

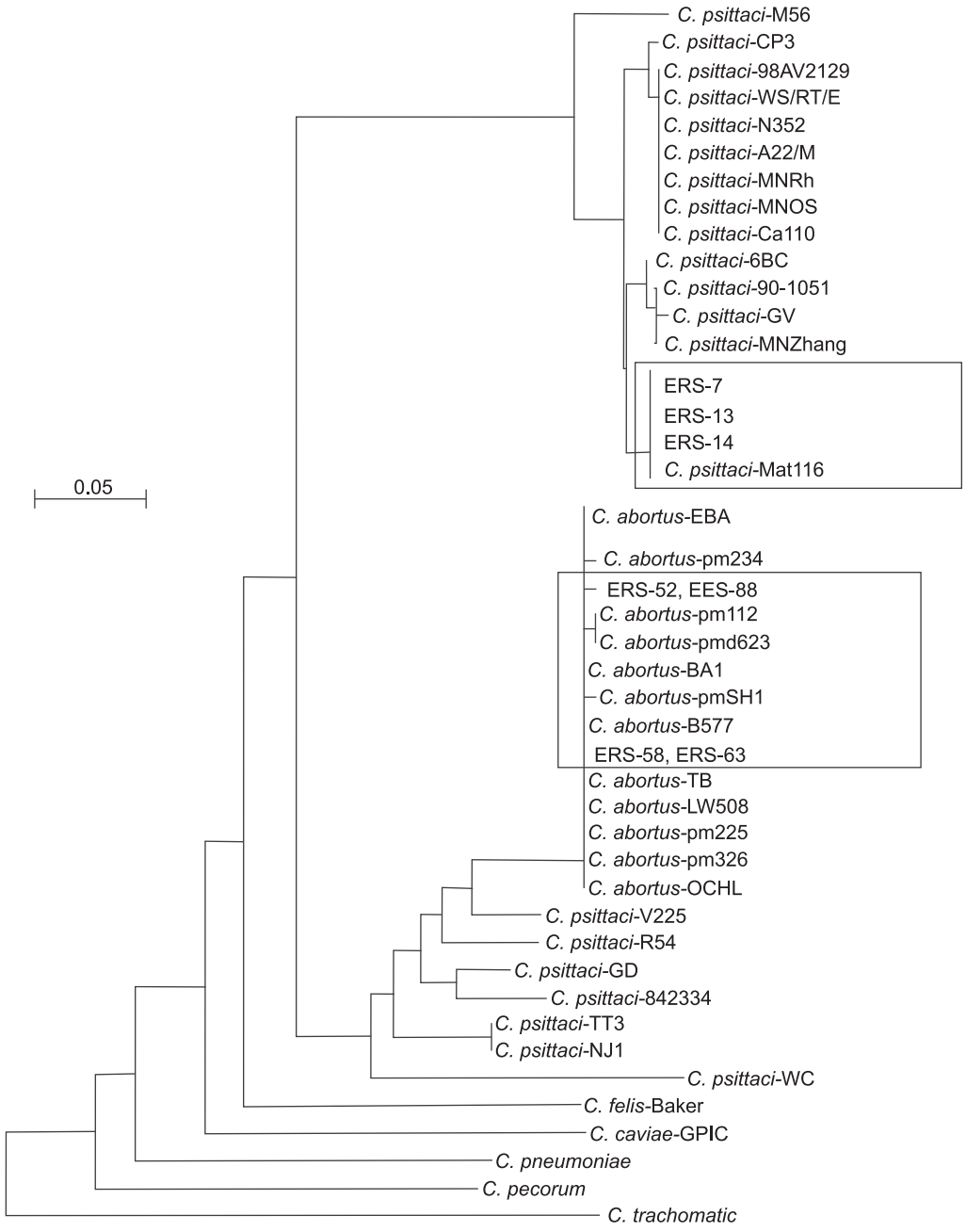

Fig. 2. Neighbor joining (NJ) phylogenetic tree, based on nucleotide sequences of VD2 region of *ompA* gene of different strains of *C. psittaci*, *C. abortus* and other *Chlamydia* species. Note: The strains detected in this study are shown in bold letters and vertical lines mark genetic clusters of *C. psittaci* and *C. abortus*. The genetic distance is indicated in 0.05 units bar. The *C. trachomatis* is used as out-group.

infection (+++ or above) and screened with family *Chlamydiaceae* specific nested PCR. All the five samples were positive by family specific PCR test.

The presence of chlamydiosis in diseased as well as apparently healthy animals as reported in the present study indicates that chlamydiae are important bacteria, which may be a part of the normal flora of the respiratory tract of equines or may be causing inapparent infections, but may play dominant role along with other microorganisms to precipitate the respiratory diseases. Also, it is a known fact that respiratory disease are usually of multiple or mixed aetiologies. Normally present commensals in the respiratory tract of equines may act as pathogens under certain adverse conditions like environmental stress, viral infections, parasitic infestations as well as infections by specific bacterial pathogens. Thus, chlamydiae may be an important bacteria in the multiple aetiology of the equine respiratory infections.

Therefore, the diagnostic, treatment and preventive measures which are being undertaken for the control of respiratory disease in equines should necessarily consider the involvement of chlamydiae so that prompt diagnosis

and effective treatment can be given and thus the respiratory infections can be controlled.

REFERENCES

Blanco Loizeiler A. 1968. Las neo-rickettsiosis animales en Espana. *Revista del Patronato de Biología Animal* **12**: 5–22.

Chahota R, Katoch R C, Verma S, Sharma M, Deswal R S, Asrani R K, Katoch R and Mahajan A. 2001. Aetio-pathogenesis of respiratory distress syndrome (RDS) among equines in Himachal Pradesh. *Indian Journal of Veterinary Pathology* **25**: 38–40.

Chahota R, Ogawa H, Mitsuhashi Y, Ohya K, Yamaguchi T and Fukushi H. 2006. Genetic diversity and epizootiology of *C. psittaci* prevalent among captive and feral avian species based on VD2 region of *ompA* gene. *Microbiology and Immunology* **50**: 663–78.

Everett K D E, Bush R M and Andersen A A. 1999. Emended description of the order Chlamydiales, proposal of *Parachlamydiaceae* fam. nov. and *Simkaniaceae* fam. nov, each containing one monotypic genus, revised taxonomy of the family *Chlamydiaceae* including a new genus and five new species, and standards for the identification of organisms. *International Journal of Systematic and Evolutionary Microbiology* **49**: 415–40.

Fretz P B, Babiuk L A and McLaughlin B. 1979. Equine respiratory disease on the Western Canadian racetracks. *Canadian Veterinary Journal* **20**: 58–61.

Gaede W, Reckling K F, Schliephak A, Missal K, Hotzel H and Sachse K. 2009. Detection of *Chlamydophila caviae* and *Streptococcus equi* subsp. *zooepidemicus* in horses with signs of rhinitis and conjunctivitis. *Veterinary Microbiology* **142**: 440–44.

Greub G. 2010. International Committee on Systematics of Prokaryotes Subcommittee on the Taxonomy of the *Chlamydiae*. Minutes of the Inaugural Closed Meeting, 21 March 2009, Little Rock, AR, USA. *International Journal of Systematic and Evolutionary Microbiology* **60**: 2691–93.

Herfen K, Jager C and Wehrend A. 1999. Genital chlamydiosis infection in mares and their clinical significance. *Reproduction in Domestic Animals* **34**: 20–21.

Kuo C C and Stephens R S. 2011. The family *Chlamydiaceae*. (Eds) Krieg N R, Staley J T and Brown D R. *Bergey's Manual of Systematic Bacteriology—The Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, and Planctomycetes*. Springer, New York.

Liutkeviciene V, Stankeviciene M, Zilevicius G and Stankevicius H. 2007. Investigations on equine chlamydiosis in Lithuania. *Veterinarija ir Zootechnika* **39**: 34–40.

Longbottom D and Coulter L J. 2003. Animal chlamydioses and zoonotic implications. *Journal of Comparative Pathology* **128**: 217–44.

Mair T S and Wills J M. 1992. *Chlamydia psittaci* infection in horses: results of a prevalence survey and experimental challenge. *Veterinary Record* **130**: 417–19.

McChesney A E, Becerra V and England J J. 1974. Chlamydial polyarthritis in a foal. *Journal of the American Veterinary Medical Association* **165**: 259–61

McChesney S L, England J J and McChesney A E. 1982. *Chlamydia psittaci* induced pneumonia in a horse. *Cornell Veterinary* **72**: 9–97.

Medenbach K, Aupperle H, Schoon D, Wittenbrink M M and Schoon H A. 1999. Pathology of the equine salpinx. *Pferdeheilkunde* **15**: 560–67

Moorthy A R S and Spradbrow P B. 1978. *Chlamydia psittaci* infection of horses with respiratory disease. *Equine Veterinary Journal* **10**: 38–42.

Nabeya M, Higuchi R, Hirayama E, Honma H, Ishida H and Toriya Y. 1996. Antibody survey of domestic animals for *Chlamydia psittaci* in Niigata Prefecture. *Journal of the Japan Veterinary Medical Association* **49**: 720–23.

Ouchterlony O. 1958. Diffusion-in-gel methods for immunological analysis. *Progress in Allergy* **5**: 1–78.

Popovici V and Hiastru F. 1968. Izolarea microorganismelor din grupul Bedsonia de la cabaline. *Revista de Zootechnica e Medicinia Veterinaria* **11**: 56–60.

Prasad B, Wadhwa D R and Mandial R K. 1992. Respiratory distress syndrome (Dhaukni) in equines of Himachal Pradesh. *Centaur* **IX**: 18–19.

Rodolakis A and Mohamad K Y. 2010. Zoonotic potential of *Chlamydophila. Veterinary Microbiology* **140**: 382–91.

Sambrook J and Russel D W. 2010. *Molecular Cloning: A Laboratory Manual*. 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Storey C, Lusher M, Yates P and Richmond S. 1993. Evidence for *Chlamydophila pneumoniae* of non-human origin. *Journal of General Microbiology* **139**: 2621–26.

Storz J. 1971. *Chlamydia and Chlamydia Induced Diseases*. (Ed.) Charles C Thomas. Springfield, Illinois, USA.

Szeredi L, Hotzel H and Sachse K. 2005. High prevalence of chlamydial (*Chlamydophila psittaci*) infection in fetal membranes of aborted equine fetuses. *Veterinary Research Communications* **29**: 37–49.

Theegarten D, Sachse K, Mentrup B, Fey K, Hotzel H and Anhenn O. 2008. *Chlamydophila* spp. infection in horses with recurrent airway obstruction: similarities to human chronic obstructive disease. *Respiratory Research* **9**: 14–23.

Veznik Z, Pospisil L, Svecova D, Kummer V, Canderle J, Diblikova I and Maskova J. 1996. Chlamydiae in reproductive disorders and pathology of reproductive disorders in man and animals. *Reproduction in Domestic Animals* **31**: 595–600.

Wills J M, Watson G, Lusher M, Mair T S, Wood D and Richmond S L. 1990. Characterization of *Chlamydia psittaci* isolated from a horse. *Veterinary Microbiology* **24**: 11–19.