Travel Medicine and Infectious Disease 43 (2021) 102134

Contents lists available at ScienceDirect

Travel Medicine and Infectious Disease

journal homepage: www.elsevier.com/locate/tmaid

Clinical and laboratory evidence of Lyme disease in North India, 2016–2019

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ARTICLE INFO

Keywords:
Facial nerve palsy
Human granulocytic anaplasmosis
Lyme disease
Radiculoneuropathy
Neuroborreliosis

ABSTRACT

Background: Lyme disease is endemic to parts of the Americas, Europe and Asia. However, only a handful of sporadic cases have been reported from India. In this study, we systematically evaluated the clinical and epidemiological features of Lyme disease in North India.

Method: All samples were tested by using the standard two-tiered testing algorithm (STTA). Paired serum and cerebrospinal fluid (CSF) were used for demonstrating *Borrelia burgdorferi* specific intrathecal IgG antibody synthesis (AI). In addition, a commercial tick-borne bacterial flow chip (TBFC) system and a real-time PCR were also used to detect *Borrelia species* and *Anaplasma phagocytophilum* in patients who were positive by STTA.

Results: The diagnosis of Lyme disease was confirmed in 18 (7.14%) of the 252 clinically suspected cases by STTA. Neurological involvement was reported in 14 (77.78%) patients, whereas joint and heart involvement was reported in five (27.78%) and three (16.67%) patients, respectively. Lymphocytic pleocytosis (median 37.5 cells/mm^3; range 12–175 cells/mm^3) in the CSF was seen in 11 of 14 Lyme neuroborreliosis (LNB) patients. Intrathecal production of *Borrelia* specific IgG antibodies was demonstrated in 9 (64.28%, n = 14) patients, a highly specific finding for neuroborreliosis. Two patients (11.11%) were also found to be co-infected with human granulocytic anaplasmosis.

Conclusions: The results of this study show clinical and laboratory evidence of endemic Lyme disease in North India and thus, highlight the importance for travel medicine practitioners and physicians to evaluate for Lyme disease in patients with compatible symptoms and a history of travel to tick risk areas.

1. Introduction

Tick-borne diseases are a significant public health problem in many countries. Lyme disease is the most common tick-borne zoonosis in the Northern hemisphere. It primarily affects the skin, nervous system, heart and joints, and is caused by the spirochetes in the *Borrelia burgdorferi sensu lato complex* [1–3]. The data on clinical and epidemiological features of Lyme disease in India is limited. In previous surveys, around 106 species of *Argasid and Ixodid* ticks were documented from India [4]. Furthermore, *Ixodes ricinus, Ixodes acutitarsus, Ixodes kaschmericus* (originally described as a subspecies of *I. persulcatus), Ixodes ovatus and Ixodes granulatus* were found to infest domestic and wild animals in the Himalayan region [5–8]. In this study, we investigated clinical cases of Lyme disease, their geographical distribution in North India and presented the first serological and molecular evidence of *A. phagocytophilum* coinfection in North India.

2. Materials and methods

2.1. Study population

A cross-sectional hospital-based study was conducted over a period

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https://doi.org/10.1016/j.tmaid.2021.102134
Received 24 January 2021; Received in revised form 25 June 2021; Accepted 28 June 2021
Available online 30 June 2021

of three years (2016 April −2019 April), whereby 252 suspected cases of Lyme disease were enrolled after taking informed consent. This study was approved by the Institutional ethics committee (IEC-PG-556). All methods were performed in accordance with relevant guidelines and regulations. Informed consents were obtained from all the participants included in the study.

2.2. Case definition

The diagnosis of Lyme disease was made using the surveillance definitions issued by the Centre for disease control (CDC) [9]. Any patient with one or more objective clinical findings of lymphocytic meningitis, cranial neuritis, carditis, radiculoneuropathy (radicular pain in one or more dermatomes along with sensory or motor changes) or joint swelling and serological confirmation by immunoblot was considered as a positive case. Lyme neuroborreliosis (LNB) cases were also classified as definite and possible based on the following case definition given by the European Federation of Neurological Societies (EFNS) [10]: a) definite: neurological symptoms consistent with LNB, lymphocytic pleocytosis and intrathecal *Borrelia burgdorferi sensu lato* (*Bbsl)* antibody production. b) possible: neurological symptoms consistent with LNB, lymphocytic pleocytosis, and *Bbsl* specific antibodies in serum.

2.3. Laboratory method employed for the study

All samples including controls were tested by the standard two-tiered testing algorithm (STTA) using recombinant IgM and IgG ELISA (Nova Tec, GmbH Germany) followed by separate recombinant IgM and IgG Line immunoblot (BLOT-LINE *Borrelia*/HGA IgM and IgG Test Line, Czech Republic). In addition, paired serum and CSF specimens were used to demonstrate *Borrelia* specific intrathecal IgG synthesis (AI).

2.3.1. *Recombinant IgM and IgG ELISA (Nova Tec, GmbH Germany)*

IgM microtiter wells were pre-coated with a combination of selected parts of specific antigen such as OspC of *Borrelia afzelii* and *Borrelia garinii* as well as internal flagellin p41i of *B. garinii*. IgG microtiter wells were pre-coated with OspC of *Borrelia sensu stricto* and *B. garinii*, p100, p18 of *B. afzelii* and p41i of *B. garinii*.

2.3.2. *BLOT-LINE Borrelia/HGA IgM and IgG line immunoblot (Test Line, Czech Republic)*

IgM immunoblot included VlsE Bg, p83 Ba, p41Ba, p39 Ba, OspC (Ba, Bg and Bs), and p44 recombinant antigens. IgG immunoblot included VlsE (Ba, Bg, Bs), p83 Ba, p58, p41Ba, p39Ba, OspB Bs, OspA (Ba, Bg, Bs), OspC Bg, p17 B g, NapA Bs, p44 and TpN17 recombinant antigens. Manufacturers have included the p44 band of *Anaplasma phagocytophilum* for detecting coinfection and the TpN band of *Treponema pallidum* for checking the cross-reactivity. Testing was performed according to the manufacturer's instructions and evaluation of band intensity was done using Test Line immunoblot software (v.1.6.5). IgM and IgG immunoblot results were interpreted using the German Society for Hygiene and Microbiology (DGHM) MIQ-12 guidelines for borreliosis [11].

2.3.3. *Intrathecal production of borrelia specific IgG antibody (AI index)*

To identify a true pathogen-specific synthesis of intrathecal antibodies, this study used the IgG antibody index (AI) described by Reiber et al. [12]. An AI index of >1.5 indicated true pathogen-specific synthesis of intrathecal antibodies. It was calculated as the ratio of the CSF/serum quotient of specific antibodies (Qspec) to the corresponding CSF/serum quotient of total IgG antibodies (Qtotal IgG).

2.3.4. *A. phagocytophilum IgG indirect micro immunofluorescence assay (MIFA)*

The samples that reacted with p44 antigen of *A. phagocytophilum* were further tested with IgG indirect micro immunofluorescence assay (Fuller Laboratories, Fullerton, CA, USA). This test employs semi-purified elementary bodies and morulae from cell culture propagated organisms. Titres of ≥1:80 were considered positive.

2.3.5. *Blot-line A. phagocytophilum IgG line immunoblot assay*

Specific antibodies to specific antigens (Asp62, OmpA) of *A. phagocytophilum* were demonstrated using confirmatory Blot-Line *Anaplasma* IgG Line immunoblot assay (Test Line, Czech Republic).

2.3.6. *Tick-borne bacterial flow chip*

A tick-borne bacterial flow chip (TBFC) system (Master Diagnostica, Granada, Spain) was also used for the qualitative molecular detection of *Borrelia* species and *A. phagocytophilum* from STTA positive samples. Deoxyribonucleic acid (DNA) was extracted from blood and CSF using a QIAamp DNA mini kit (Hilden, Germany) according to the manufacturer's instructions with a starting volume of 200 μl of sample. DNA was eluted in a final volume of 50 μl elution buffer. TBFC allows the detection of 7 tick-borne bacterial pathogens, including *Borrelia* and *Anaplasma* by multiplex PCR, followed by reverse dot blot hybridization based on DNA flow technology with specific probes [13]. The assay included two internal controls; one amplifies the human beta-globin gene and the other amplifies a synthetic DNA control incorporated in the assay as an exogenous amplification control.

2.3.7. *Real-time PCR*

The samples that were positive during an initial screening by TBFC were then subjected to TaqMan real-time PCR assays using previously described primers and protocols with a step one plus real-time PCR detection system (Applied Biosystems, USA) [14–16]. The assays were designed to amplify and detect the gene segments of the *Borrelia* genus (16 S rRNA), *Borrelia burgdorferi sensu lato* (OspA) and *A. phagocytophilum* (Ank A) respectively. The DNA of *B. burgdorferi* B31 and *A. phagocytophilum* was used as a positive control and nuclease-free water was used as a negative control. A blank (nuclease-free water) was included in each batch of DNA extraction to confirm the absence of cross-contamination during the extraction procedure.

2.4. Statistical analysis

The difference in clinical characteristics of Lyme disease and non-Lyme disease patients were determined using a contingency table and analysis were performed with Pearson Chi-square or Fisher's exact test. P values (two-tailed) below 0.05 were considered to indicate statistical significance and analysis was performed using SPSS version 25.

3. Results

3.1. Study population

Of the 252 patients included in the study, 218 (86.51%) were adults (≥18 years), and 34 (13.49%) were children (<18 years). The study included 160 (63.49%) males and 92 (36.51%) females. Exposure history to potential tick habitats (i.e. having been in wooded, brushy, or grassy areas within the last 30 days) known to harbour *Ixodes* ticks were reported in 166 (65.87%) patients. Of 138 (54.76%) patients with neurological involvement included in the study, 42 (30.43%) patients had lymphocytic meningitis, 54 (39.13%) patients had radiculoneuropathy, 30 (21.74%) patients had cranial neuritis, and 25 (18.11%) patients had neuroretinitis. Of 119 (47.22%) patients with joint involvement included in the study, 34 (28.57%) patients had monoarticular arthritis, 64 (53.78%) patients had oligoarticular arthritis, and 21 (17.65%) patients had polyarticular arthritis. Ten (3.97%) patients with cardiac conduction defects were also included in the study. The breakdown of patients included in the study has been depicted in the Venn diagram (Fig. 1).

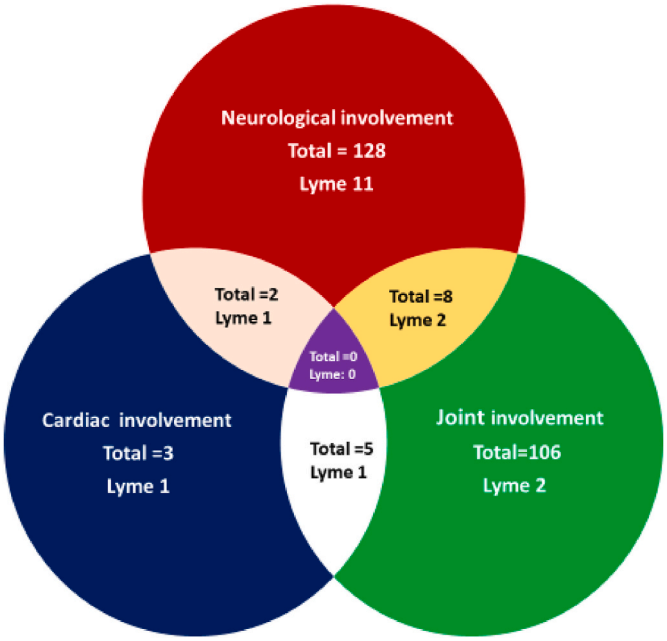

Fig. 1. Venn diagram showing break-up of clinical findings in the study population.

3.2. Identification of Lyme disease cases

Overall, on STTA (ELISA followed by immunoblot) 18 (7.14%) out of 252 clinically suspected patients were found to be positive (Fig. 2). A total of 13 (72.22%, n = 18) patients were positive by both IgM and IgG immunoblot and three (16.67%, n = 18) patients were only positive for IgG immunoblot, showing a duration of symptoms >30 days and IgM antibodies only to p41 antigen. The remaining two samples from patients with arthritis were subjected only to IgG immunoblot and were positive.

A lumbar puncture was performed in 11 (78.57%, n = 14) patients with neurological symptoms. The CSF findings of patients with LNB are summarized in Table 4. Lymphocytic pleocytosis (>5 lymphocytes/mm^3) along with raised protein level and normal glucose in CSF was reported in all 11 patients. Of the 11 patients, quantitative estimation of antibodies to *Bbsl* in serum and CSF by calculating AI could be done in only 9 (81.82%, n = 11) patients. The IgG AI index was found to be positive (>1.5) in all nine cases indicating intrathecal production of *Borrelia* specific antibodies and these were defined as definite LNB (Table 1). Mild to moderate blood-brain barrier dysfunction was noted in 5 (55.56%, n = 9) LNB patients (increased Q_{alb} value ranging from 5.50 to 8.9) while severe blood-brain barrier dysfunction was noted in 4 (44.44%, n = 9) of the AI index positive cases (increased Q_{alb} value ranging from 15.11 to 18.57). In 2 (14.28%, n = 14) cases, CSF quantity was insufficient to perform the analysis. The five (35.71%, n = 14) patients in whom the IgG AI index could not be calculated due to absent or insufficient CSF were classified as possible LNB (Table 1). Further testing of these 18 STTA positive samples by tick-borne bacterial flow chip system detected *Borrelia* spp. DNA simultaneously with *A. phagocytophilum* DNA, indicative of co-infection in 2 (14.28%, n = 14) of the CSF samples of LNB patients. These results were further tested by TaqMan real-time PCR assays and confirmed the presence of *B. burgdorferi sensu lato* and *A. phagocytophilum*. Sequencing was not done due to an insufficient amount of DNA eluate. Table 1 also compares the reactivity of antibodies of the Lyme disease cases with different recombinant proteins of *B. burgdorferi sensu lato*.

All the patients who were suspected for Lyme disease showed negative testing for the following pathogens/diseases: Herpes simplex virus, Human immunodeficiency virus, Varicella Zoster virus, *Mycobacterium tuberculosis*, Cryptococcus spp., scrub typhus, leptospirosis, malaria, dengue and bacterial pathogens (Pneumococcus and meningococcus). All the ELISA positive samples were found to be non-reactive to TpN band of *T. pallidum* of Line immunoblot.

3.3. Demographic and clinical features

Of these 18 immunoblot positive patients, 16 (88.89%, n = 18) were adults and 2 (11.11%, n = 18) were children. Nine (50%, n = 18) had flu-like symptoms several weeks before the onset of neurological or cardiac abnormalities. 6 of 18 (33.33%) patients recalled having a uniform erythematous rash before developing these symptoms. They did not receive antibiotic therapy at that time. None of them had visited or travelled to any foreign countries that are endemic for Lyme disease. All 18 (100%) patients had a history of exposure to tick habitat before developing these symptoms. None of the patients recalled any history of the tick bite. LNB confirmed in 14 (77.78%, n = 18) patients, was the most common clinical manifestation in Lyme disease cases. Arthritis and carditis were reported in 5 (27.78%, n = 18) and 3 (16.67%, n = 18) patients, respectively. The clinical manifestations of the 18 Lyme disease cases have been summarized in Table 1. None, except case 14 (Type 2 Diabetes mellitus and hypertension) had co-morbidities. In our study radiculoneuropathy (50%, p = 0.002), facial nerve palsy (27.77%, p = 0.031) and atrioventricular block (16.66%, p = 0.027) were significantly more common in Lyme disease patients (Table 2). The most frequent neurological symptoms were radiculoneuropathy, which was reported in 9 (50%) cases. Other neurological symptoms in the neuroborreliosis group were summarized in Table 3. The Geographical location of the 18 Lyme disease cases in North India has been represented in Fig. 3.

3.4. Lyme disease and A. phagocytophilum coinfection

During the testing for *Bbsl*, we also found that 2 (14.29%, n = 14) LNB patients had concurrent antibodies against p44 (major surface protein-2) antigen of *Anaplasma phagocytophilum* (Table 1) on BLOTLINE *Borrelia*/HGA Line immunoblot. These two samples were further tested with IgG indirect micro immunofluorescence assay for *A. phagocytophilum,* and a titre of >1280 was obtained. We confirmed the MIFA results by IgG line immunoblot assay using purified recombinant antigens [MSP-2 or p44 (major surface protein), Asp62 (membrane transporter protein) and OmpA (virulence marker)} of *A. phagocytophilum*. These results were then confirmed by a commercial tick-borne bacterial flow chip system (16 S rRNA and MSP 2) and a real-time PCR assay (Ank-A) of *A. phagocytophilum*. Both the patients had a history of fever, mild respiratory complaints, bicytopenia and mildly elevated liver enzymes.

3.5. Treatment details and outcome

The treatment details and response to treatment has been summarized for each case in Table 1. Two (11.11%, n = 18) patients were lost to follow-up before the treatment could be initiated. In the rest of the 16 (88.89%, n = 18) patients, antibiotics (ceftriaxone or doxycycline or both) were initiated. Ceftriaxone was prescribed for 5 (27.78%, n = 16) patients (duration of 2–3 weeks), doxycycline was prescribed for 9 (50%, n = 18), and both ceftriaxone and doxycycline were prescribed for 2 (11.11%, n = 18) patients. During follow up, 9 (56.25%, n = 16) patients had full recovery in response to antibiotic treatment, and 6 (37.5%, n = 16) patients had experienced significant improvement of symptoms. One (6.25%, n = 16) patient (case 10) still had persistent symptoms (radiculoneuropathy) on follow up. Of the 2 LNB patients co-infected with *A. phagocytophilum,* one patient was treated with doxycycline alone and the other one with both doxycycline and ceftriaxone.

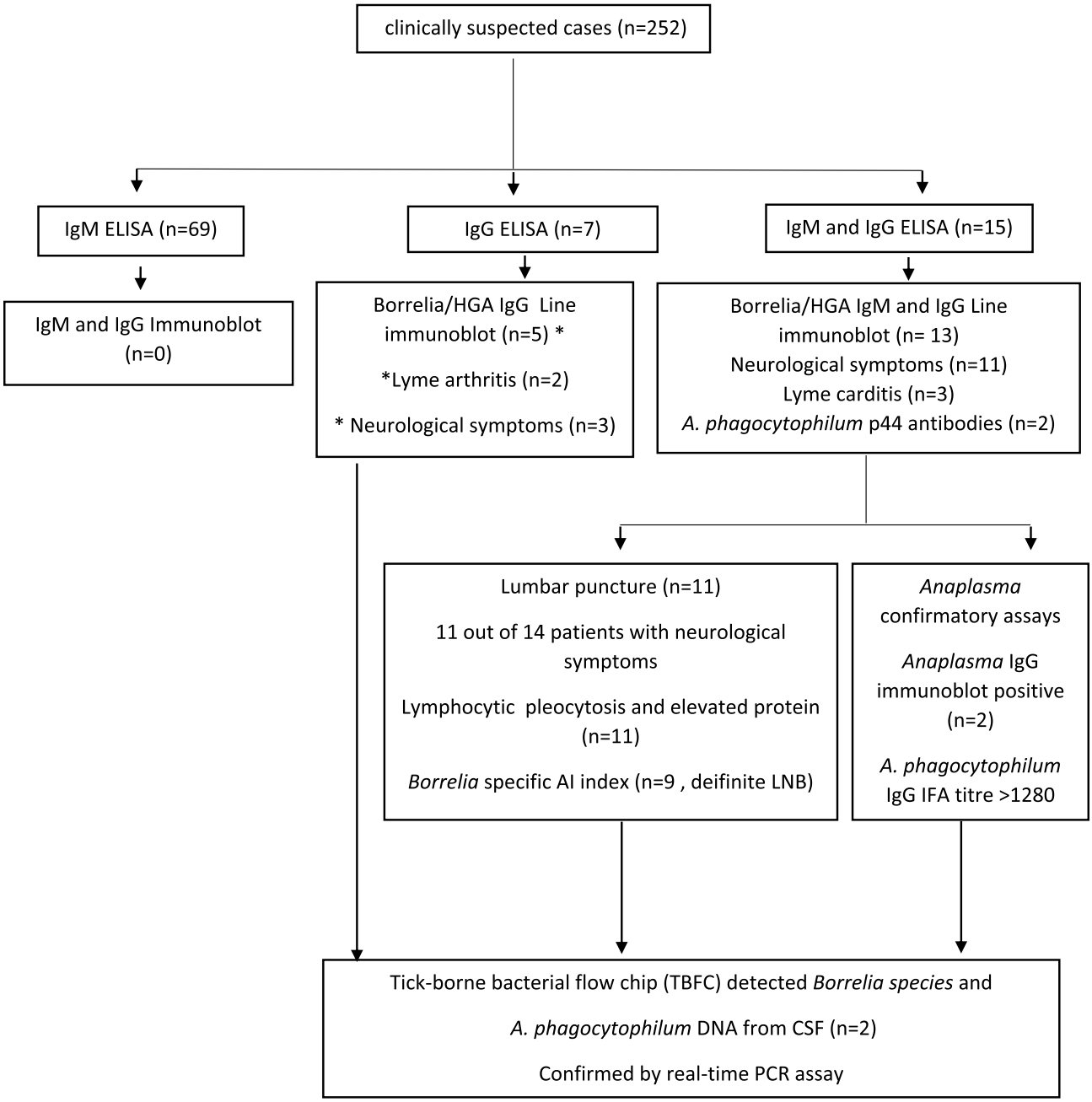

Fig. 2. Description of the laboratory evaluation done in Lyme disease patients (n = 252) who participated in this study in a referral hospital, North India (2016 April −2019 April).
Abbreviations: HGA, Human granulocytic anaplasmosis; LNB, Lyme neuroborreliosis.

4. Discussion

Lyme disease is endemic in the Northern hemisphere including North America, Europe and Northern Asia. The prevalence of Lyme disease among the North Indian population is unknown. In this study, we report 18 cases of Lyme disease with extra-cutaneous objective manifestations involving the neurological, cardiac or musculoskeletal system and demonstrate the presence of specific antibody response to *Bbsl* infection in our cohort of patients from a North Indian population (7.14%; n = 252) by STTA. We also provide molecular evidence of *Borrelia burgdorferi sensu lato* and *A. phagocytophilum* in neuroborreliosis patients in this study. In India, the first case of Lyme disease was reported from Shimla, Himachal Pradesh in 1990. That patient presented with a clinical picture of meningitis, carditis and arthritis [17]. Praharaj et al. reported a high *B. burgdorferi* IgG seroprevalence among military personnel in the North-Eastern states of India (13%; n = 500) [18]. Subsequently, new cases of Lyme disease are being reported every year based on clinical observations, serological methods or both [19–28]. However, most of these cases have been reported based on clinical findings and ELISA without immunoblot confirmation. The review of geographical distribution and clinical manifestations of previously reported cases from India is summarized in Table 5. *Ixodes himalayensis* was reported on rodents and shrews in the hilly regions of Himachal Pradesh and Jammu Kashmir. There are reports of *Ixodes acutitarsus* from Arunachal Pradesh, Sikkim, upper Assam, Himachal Pradesh, Uttar Pradesh, West Bengal and Uttarakhand. *Ixodes granulatus* were reported from Nagaland, Arunachal Pradesh, Assam, Himachal Pradesh, Uttar Pradesh and West Bengal. A recent study conducted by Sadanandane et al. reported *Ixodes*

Table 1
Summary of clinical manifestations and reactivity of antibodies of 18 Lyme disease cases with different recombinant proteins of *B. burgdorferi*.

S. n	Age/Gender, Clinical presentation	Duration of symptoms	IgM/ IgG ELISA	IgM line immunoblot	IgG Line immunoblot	[a] LD. specific IgG AI index	Treatment (duration)/ Follow up
1	3 y/F, Lymphocytic meningitis	<30 days	+/+	**p41, OspC Bs**	**p83, p41, p39**, OspB, OspA Ba, Osp Bg	**2.52**	Ceftriaxone (3 weeks)/Recovered
2	6 y/F Meningoencephalitis	>30 days	+/+	**VlsE Bg,** p83, **p41, OspC**, Ba, Bg, Bs	**p83, p41, p39, OspC**, OspB, OspA Ba, OspA Bg, NaP A	**3.94**	Ceftriaxone and Doxycycline (2 weeks)/Recovered
3	30 y/F, Recurrent Facial nerve palsy Polyarthritis	<30 days	+/+	**p41, Osp C**	**p83, p41, VlsE Bg, p39**	ND #	Doxycycline (2 weeks)/Recovered
4	50 y/M, Bilateral facial nerve palsy, Radiculoneuropathy, AFI	>30 days	+/+	**OspC, p39** p44* (*A. phagocytophilum*)	**VlsE Bg, p41**, **OspC**, OspB, OspA Bg, p44 (+ve for both *Borrelia and A. phagocytophilum*)	**2.38**	Ceftriaxone and doxycycline (2 weeks)/Recovered
5	27 y/F, Facial nerve palsy Lymphocytic meningitis	<30 days	+/+	**Osp C Ba, OspC Bs, VlsE Bg**	**VlsE Bg** (BL), **p83, p41**, OspB, OspA Ba, OspA Bg,	**2.78**	Doxycycline (2weeks)/Recovered
6	21 y/M, Radiculoneuropathy, facial nerve palsy, Bilateral knee arthritis	2 months	+/+	**p41, OspC Bs**	**p83, p41, VlsE Bg**, OspB, OspA Ba, OspA Bg	ND #	Doxycycline (2weeks)/Recovered
7	27 y/M, Radiculoneuropathy, AFI	>30 days	+/+	**p39**, p83, **OspC**	**p83, p41**, **OspC**, OspA Ba, OspA Bg, p44 (+ve for both *Borrelia and A.phagocytophilum*)	**1.74**	Doxycycline (6 weeks)/Recovered
8	23 y/M, Radiculoneuropathy	<30 days	+/+	**p39, OspC**	**p83, VlsE Bg, P41,** OspB, OspA Bg	**2.57**	Ceftriaxone (2weeks)/ Recovered
9	63 y/M, Facial nerve palsy	<30 days	+/+	p41, OspC	p83, VlsE Bg, P41, OspB, OspA Ba	2.15	Doxycycline (2weeks)/Recovered
10	49 y/M, Lymphocytic meningitis, radiculoneuropathy	>6months	+/+	**p41, OspC**	**VlsE Bs, p83, p41, OspC,** OspB, Osp Ba, Osp Bg, NaP A	**3.04**	Ceftriaxone (2 weeks)/Persistent symptoms
11	35 y/M, Radiculoneuropathy	3 months	−/+	**p41**	**VlsE Bg, p83, p41, OspC,** OspB, OspA Ba, OspA Bg	**2.11**	Doxycycline (2weeks)/Recovered
12	32 y/M, Radiculoneuropathy	>6months	−/+	**p41**	**p83, p41, p58**, **OspC**, OspB, OspA Bg, NaP A	ND #	Doxycycline (2weeks)/Recovered
13	16 y/M, Radiculoneuropathy	4 months	−/+	**p41**	**p83, p58, p41,** OspB, OspA Ba, OspA Bg	ND #	Ceftriaxone (2 weeks)/Recovered
14	33 y/F, DM, HTN, Myocarditis with variable AV blocks, Radiculoneuropathy	<30 days	+/+	**p41, OspC Bs**	**VlsE Bg, p83, p39** OspA Bg, NaP A	ND #	Lost on follow up
15	46 y/F, 3rd degree AV block	<30 days	+/+	**OspC (Ba, Bg, Bs)**	**P83, VlsE, p41,** OspB, OspA Ba	ND	Ceftriaxone (2weeks)/ Recovered
16	61 y/F, Variable AV blocks Bilateral knee arthritis	>30 days	+/+	**p41, Osp C**	**p83, p41, p39**, OspB, OspA Ba, OspA Bg, p44 (BL)	ND	Ceftriaxone (2weeks)/ Recovered
17	49 y/M, Bilateral knee arthritis	>30 days	+/+	ND	**VlsE Bg, p83, p41,** OspB, OspA Bg, NaP A	ND	Doxycycline (2weeks)/Recovered
18	37 y/M, Bilateral hip arthritis	>30 days	+/+	ND	**p83, p41, p39,** OspB, OspA Ba, OspA Bg, NaP A	ND.	Lost on follow up

Abbreviation: y-years old, M-male, F-female, AFI-acute febrile illness, DM-diabetes mellitus, HTN-hypertension, AV-atrioventricular, ND-not done, P44-major surface protein (MSP-2) of *A. phagocytophilum, Bg-Borrelia garinii, Ba-Borrelia afzelii, Bs-Borrelia burgdorferi sensu stricto,* AI-antibody index, ELISA- Enzyme-linked immunosorbent assay.
*Antibody index of <1.5- negative for intrathecal antibody synthesis.
* IgM was considered as positive if > 2 of the following were present: p39, OspC, p41 internal, DbpA, VlsE or strong presence of OspC alone.
*IgG positive if ≥ 2 bands of the following are present; p83/p100, p58, p39, OspC, p41 internal fragment, DbpA(OspC17), VlsE.
*Bands meeting criteria are highlighted as bold. Additional specific bands recommended by the manufacturer are also included in the table.
possible LNB as per EFNS criteria.

ticks (0.31%) population in the forest areas of Western Ghats indicate the presence of competent vector in India [29]. *Ixodes ricinus* has been recorded on sheep in the Kangra valley, Western Himalaya and Almora. *Ixodes kachmericus* from Himachal Pradesh, Jammu Kashmir, Sikkim, Uttar Pradesh and West Bengal. *Ixodes petauristae* and *Ixodes ceylonensis* were also reported from Shimoga, Karnataka. *Ixodes vespertilionis* was reported from Rajasthan.

LNB was diagnosed in 14 patients in this study with 9 fulfilling EFNS diagnostic criteria for definite LNB. The remaining 5 cases fulfilled the criteria for possible LNB. All the patients showed exposure to tick risk areas four weeks before developing the symptoms. Radiculoneuropathy was the most common presenting symptom followed by lymphocytic meningitis and facial nerve palsy (50%, 27.78% and 27.77% respectively). Among these 14 cases, 12 (85.71%) patients were classified as early LNB (<6 months), and 2 (14.29%) patients were classified as late LNB (>6 months). CSF analysis found pleocytosis (dominated by mononuclear cells) in 11 of 14 (78.57%) patients (median 37.5 cells/mm^3; range 12–175 cells/$mm^{3)}$. Calculation of quotients and AI index from CSF and serum data of LNB patients are shown in Table 4. LNB-involving CNS usually manifests as aseptic meningitis and appear to be similar to viral meningitis. Lymphocytic pleocytosis occurs due to the production of CXCL13, a chemokine that causes the proliferation of *Borrelia* specific B cells. These events lead to the intrathecal synthesis of *Bbsl* specific antibodies, which is required formally to diagnose LNB in Europe but not required in the US. Antibodies to *Bbsl* were present in the CSF of 9 of the 14 (64.29%) patients with confirmed LNB. We detected elevated levels of Qalb (ranging from 5.57 to 18.57) in 9 out of 14 (64.29%) patients, indicating the dysfunction of the blood-CSF barrier in LNB.

While LNB occurs in approximately 10–15% of people infected with *Bbsl* strains in Europe and the USA, meningoradiculitis is the most common clinical manifestation of LNB observed in Europe [30]. In our study radiculoneuropathy (50%, $p = 0.002$), and facial nerve palsy (27.77%, $p = 0.031$) were significantly associated with LNB (Table 2). The clinical triad of lymphocytic meningitis, cranial neuritis and radiculoneuritis is a well-described entity in literature. Unifocal or

Table 2
General characteristics of LD. positive patients and other non-LD patients.

characteristic	N (%) 252	LD positive cases N = 18	Non-Lyme disease N = 234	*P*-value
Gender				
Male	160 (63.49%)	11 (61.11%)	149 (63.68%)	
Female	92 (36.51%)	7 (38.88%)	85 (36.32%)	0.827[a]
Age group				
Children	34 (13.49%)	2 (11.11%)	32 (13.67%)	
Adults	218 (86.51%)	16 (88.89%)	202 (86.32%)	0.758
History of exposure (≤ 30 days) in wooded, brushy or grassy areas before developing symptoms	166 (65.87%)	18 (100%)	148 (63.24%)	**.001** [b]
Neurological manifestations				
Radiculoneuropathy	54 (21.42%)	9 (50%)	45 (19.23%)	**0.002**[a]
Facial palsy	30 (11.9%)	5 (27.77%)	25 (10.68%)	**0.031**[a]
Lymphocytic Meningitis	42 (16.66%)	5 (27.78%)	37 (15.81%)	0.189[a]
Neuroretinitis	25 (9.92%)	0	25 (10.68%)	0.231[b]
Atrio ventricular block	10 (3.96%)	3 (16.66%)	7 (2.99%)	**0.027** [b]
Joint involvement	124 (49.20%)	5 (27.77%)	119 (50.85%)	0.168[a]

[a] Pearson Chi-square.
[b] Fisher's exact test.

Table 3
Clinical features of patients who presented with neuroborreliosis.

Symptom	Number of patients with neuroborreliosis	
	Early (<6months) n = 12	Late (>6months) n = 2
History of Fever in the past	7	1
Fever at presentation	2	1
Headache	2	0
Neck stiffness	1	0
Fatigue	3	1
Analgesic resistant pain	0	0
Paresis	4	2
Paraesthesia	2	2
Myalgia	2	1
Cranial nerve involvement (other than facial nerve)	0	0
Concentration difficulties	0	1
Memory impairment	0	1

multifocal inflammation of peripheral nerves leads to the development of painful radiculopathy, cranial neuropathy, or mononeuritis multiplex. Rarely brain or spinal cord parenchymal inflammation may also occur. The majority of LNB research data comes from the European region due to infections with *B. garinii* or *B. bavariensis* neurotropic species of *Bbsl complex*. *B. burgdorferi sensu stricto* and recently *B. mayonii* are the only infecting species found in the US [31,32]. Furthermore, the occurrence of LNB and the specific reactivity with VlsE *B. garinii* antigen in five LNB patients indicate that *B. garinii* may be involved in the clinical expression of LNB in North India. The frequency of radiculoneuropathy, facial nerve palsy and lymphocytic meningitis observed in this study indicate that screening of LNB should be considered in this group of patients. The diagnosis should be based on the clinical findings, epidemiological history and must be combined with CSF analysis. Since frequent blood CSF barrier dysfunction occurs in LNB patients, it is always recommended to perform intrathecal production of antibodies in CSF which improves the diagnosis of LNB instead of performing a single absorbance value in serum or CSF.

Oligoarthritis is a crucial late manifestation of Lyme disease in most western studies [33]. In a study from the USA, Lyme disease was diagnosed in 13% of the patients with oligoarthritis [34]. Arthritis develops as a result of an intense immune-mediated inflammatory response to infection with *Borrelia* spp. It usually presents with oligoarticular involvement of large joints of lower limbs (most common-knee) [35]. In our study, 5 (27.78%, n = 18) patients had joint involvement, usually affecting one or both knees; in 2 (11.11%) patients, arthritis was accompanied by neurological symptoms (radiculoneuropathy, facial nerve palsy) and in 1 (5.56%) patient along with variable AV node block. In our study, a broader spectrum of IgG antibodies was observed in arthritis patients and was commonly directed to p41, p83, Osp A, Osp B, p39, Nap A and VlsE antigens. As with many previous studies, asymmetric oligoarthritis involving the large joints of the lower limb was the most common presentation in our study. In literature, there was only one reported case of ELISA positive Lyme arthritis from India [36].

We also report 3 (16.67%, n = 18) patients with cardiac abnormalities of Lyme disease. Two patients presented as acute onset of AV node conduction defect resulting in complete heart block. One patient developed myocarditis followed by fluctuating AV block and radiculoneuropathy. The clinical course of Lyme carditis is variable and reversible with effective antibiotic treatment. It may present with various conduction defects that commonly involve AV node and may progress to complete heart block. The first case of Lyme carditis was described in 1980 in the USA by Steer et al. According to Lyme disease surveillance in the USA, 4–10% of patients with untreated Lyme disease may develop carditis [37]. In a review of 84 patients with Lyme carditis, palpitation was noted in 69% of the patients, conduction abnormalities in 19%, myocarditis in 10% and left ventricular systolic dysfunction in 5% of the patients [38]. The investigation of this study showed comparable findings.

In this study, we observed that IgM antibodies were commonly directed to p41 and Osp C recombinant antigens. OspC and p41antibodies were mainly involved in the early immune response to *B. burgdorferi,* and similar findings were observed in European and US studies. In contrast, antibodies to OspA (p31) and OspB (p34) were found more frequently in IgG immunoblot in our geographical areas along with p41, p83 and VlsE antigens. Similar findings have also been observed in a study from China [39]. OspA and Osp B antigens were immunodominant for late immune response in the US and rarely detected in the European population. *Wilske* et al. reported the lowest potential of OspA and p100/p83 for cross-reaction and considered these as highly specific antigens in their study [40]. The frequency of NapA and OspC was less common in IgG immunoblot (33.33% and 27.77% respectively).

Ticks involved in Lyme disease may also transmit other infectious agents to humans. Another important finding in this study was that two of the LNB patients were co-infected with *A. phagocytophilum,* which presents the first serological and molecular evidence of this intracellular tick-borne pathogen in patients in the Indian subcontinent. Tick-borne bacterial flow chip system (16 S rRNA, MSP 2) and a real-time PCR (Ank-A) assay confirmed the presence of *A. phagocytophilum* DNA in CSF of these patients using three different gene targets. There are reports of seroprevalence (4.71%) of *A. phagocytophilum* among pet dogs in Northeast India by *Borthakur* et al. [41]. Recently *Ranju* et al. confirmed the presence of *A. phagocytophilum* DNA among stray dogs and ticks in Tamil Nadu [42]. High-grade fever and respiratory complaints were noted, along with radiculoneuropathy and facial nerve palsy in these patients. These patients also had thrombocytopenia, leukopenia and mildly elevated liver enzymes, which are common in human granulocytic anaplasmosis. The presence of *A. phagocytophilum* can be used to argue in favour of the presence of Lyme disease in our region as both of them share the same vector. The frequency of Lyme disease and human

Table 4
CSF clinical chemistry findings of 14 LNB patients and *Borrelia* specific AI index.

Sl. no	Exposure history/neurological symptoms consistent with LNB	Total leukocyte count/Lymphocytes In CSF %	CSF glucose mg/dL	CSF protein mg/dL	QAlb	Qspec IgG $\times10^{-3}$	QIgG $\times10^{-3}$	QLimIgG $\times10^{-3}$	AU CSF	AU Serum	Borrelia specific IgG AI index
1	+/+	175/40	51.2	78.5	8.35	7.71	3.05	6.39	111.93	287.41	2.52
2	+/+	50/95	49	164	18.57	14.10	3.58	15.72	351.36	493.51	3.94
3	+/+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	+/+	25/80	57	134	15.56	8.93	3.75	12.94	244.99	543.41	2.38
5	+/+	60/80	49	110	6.05	9.79	3.52	4.37	348.68	705.4	2.78
6	+/+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	+/+	12/100	32	56	15.11	10.16	5.83	12.54	278.84	543.41	1.74
8	+/+	35/80	67	247	5.86	8.77	3.41	4.21	287.41	648.93	2.57
9	+/+	40/90	33	54	5.50	4.58	2.13	3.90	163.16	705.04	2.15
10	+/+	20/80	45	90	8.9	10.45	3.44	6.88	302.95	574.21	3.04
11	+/+	30/100	54	70	15.38	8.33	3.95	12.79	207.69	493.51	2.11
12	+/+	60/80	64	105	ND	ND	ND	ND	ND	ND	ND
13	+/+	40/90	55	52	ND	ND	ND	ND	ND	ND	ND
14	+/+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

AU- arbitrary units corresponding to the cerebrospinal fluid or serum absorbance values from the calibration curves.
QAlb = concentration of albumin CSF/concentration of albumin in the serum.
QSpec (IgG) = arbitrary units in the cerebrospinal fluid of the IgG class/arbitrary units in the serum of the IgG class.
QIgG = total concentration of IgG in the CSF/total concentration of IgG in the serum.
QLimIgG: upper limit of Q_{Alb} range.

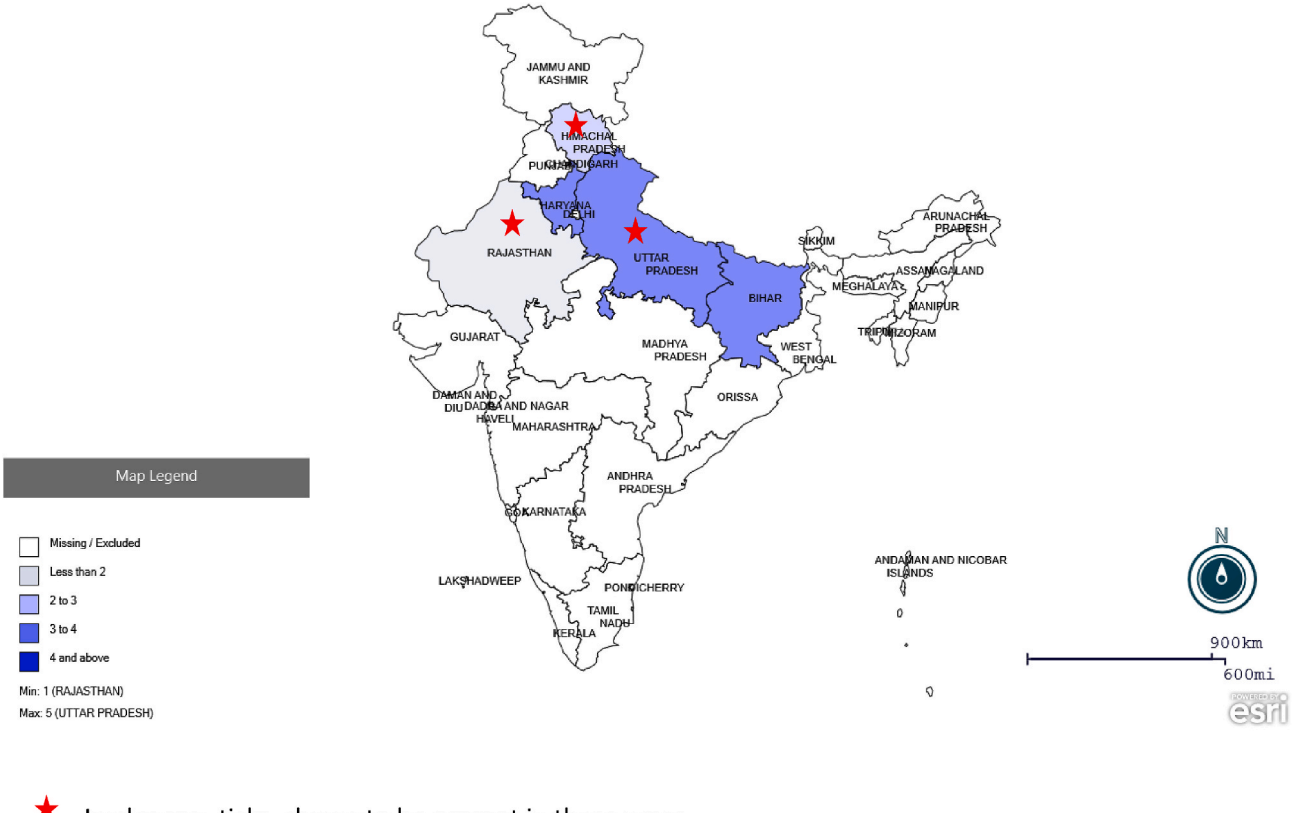

Fig. 3. Geographical location of positive cases and potential Ixodes vectors known to occur in these areas (created using Epi info 7.1.5.2).

granulocytic anaplasmosis coinfections has varied from 2 to 11.7% in other studies [43,44]. To the best of our knowledge, this is the first report of the coinfection of *B. burgdorferi and A. phagocytophilum* in our geographical area.

The Majority of the patients in this study showed an improvement or complete recovery on follow up. Twelve patients (66.67%) including two with *A. phagocytophilum* coinfection were hospitalized. Antibiotic treatment prevented further progression of symptoms, and most of them had experienced significant clinical improvement.

The major limitation of this study is the unavailability of synovial fluids to perform serology or PCR for *Borrelia burgdorferi* in arthritis cases. The numbers that we report could just be the tip of the iceberg and maybe a gross underestimation.

Table 5
Review of Lyme disease cases in India.

Sl. no	Year	Location	Clinical presentation	Laboratory methods employed	Reference
1	2020	Karnataka	19.9% Sero-prevalence in Forest workers, presence of constitutional, neurological (Bell's palsy), migratory joint pains and ocular symptoms	IgM and IgG ELISA	*Babu* et al. [26]
2	2019	West Bengal	A 26-year-old male visited forest area and subsequently developed Erythema migrans, fever and joint pain	ELISA followed by western blot	*Bhanja* et al. [24]
3	2019	Maharashtra	A 9-year-old girl had multiple erythema migrans and early stages of acrodermatitis chronica atrophicans after 6 months with a history of a tick bite	ELISA	*Bhaveja* et al. [27]
4	2018	Uttarakhand	A 20-year-old male presented with quadriparesis and multi-system involvement	Confirmed twice by serology Clinical improvement with doxycycline	*Tevatia* et al. [23]
5	2017	Visit Himalayas	A 25-year-old female visited the Himalayan region and developed neuroretinitis	IgM ELISA followed by western blot confirmation	*Guliani* et al. [28]
6	2017	Himachal Pradesh	A 10-year-old male visited the forested area and developed erythema migrans	IgM and IgG ELISA	*Sharma* et al. [22]
7	2016	Uttarakhand	A 13-year-old girl had a history of insect bite, local erythema, paraesthesia, bilateral lower limb weakness and poly radiculitis	ELISA	*Mritunjay* et al. [21]
8	2014	Haryana	5 cases having a history of Tick bite, erythema migrans and constitutional symptoms	IgM and IgG ELISA followed by Western blot confirmation	*Jairath* et al. [20]
9	2013	Kerala	Group of 6 females involved in coffee plucking job developed acute febrile illness with rash and myalgia (History of tick bite and flu-like symptoms)	ELISA and Western blot	*Sukumaran* et al. [25]
10	2010	Karnataka	A 45-year-old female had a history of Tick bite and developed Neuroretinitis	IgM and IgG ELISA and Western blot confirmation	*Babu* et al. [19]
11	2008	North East	13% Seroprevalence among asymptomatic Military personal (Had a history of rashes, joint involvement)	IgG recombinant ELISA	*Prahraj* et al. [18]
12	1999	Delhi	Mono/ oligoarticular arthritis	IgG ELISA	*Handa* et al. [36]
13	1990	Himachal Pradesh	A 14-year-old boy presented with meningitis, arthritis, carditis, AV block, His father: Erythema migrans and constitutional symptoms	Spirochetes in blood smear	*Patial* et al. [17]

5. Conclusions

The clinical and laboratory findings of this study confirm the presence of *B. burgdorferi* and *A. phagocytophilum* in North India. There is a need for creating awareness amongst physicians and travel medicine practitioners about the possibility of these diseases in relevant clinical settings. Intensive effort to isolate these Spirochetes from clinical specimens and vectors are warranted to understand the antigenic differences in detail. A standardized approach for human surveillance is needed to understand the actual frequency of Lyme disease and Human granulocytic anaplasmosis infections in the Indian population. Active tick surveillance should be conducted in risk areas to understand the expanding foci of the vectors involved in the transmission of these diseases.

Potential conflicts of interest

All authors: No reported conflicts of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

E.V. Vinayaraj: Conceptualization, Writing – review& editing, Resources, Validation. **Nitin Gupta:** Conceptualization, Writing – review& editing, Resources, Validation. **K. Sreenath:** review, Data curation. **Chandan Kumar Thakur:** review, Data curation. **Sheffali Gulati:** Contributed data, revision and supervision. **Vaishakh Anand:** Contributed data, revision, Supervision. **Manjari Tripathi:** Contributed data, revision, Supervision. **Rohit Bhatia:** Contributed, data, revision, Supervision. **Deepti Vibha:** Contributed data, revision, Supervision. **Deepa Dash:** Contributed data, revision, Supervision. **Manish Soneja:** Contributed data, revision , Supervision. **Uma Kumar:** Contributed data, revision, Supervision. **M.V. Padma:** Contributed data, revision, Supervision. **Rama Chaudhry:** Conceptualization, Writing – review&

editing, Resources, Validation.

Acknowledgement

We thank the following, who made an important contribution to this study. Roman Reddy Ganta, College of Veterinary Medicine, Kansas State University, Kelly L. Hawley, Connecticut Children Medical Centre, UConn Health, Szekeres Sandor, Department of Parasitology, Zoology, Budapest, Hungary for their constructive suggestions. The authors acknowledge Dr M. Ramam, Dr Bimal Kumar Das and Dr Nishant Verma, AIIMS, New Delhi for their valuable suggestions.

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