

Clinical and Epidemiological Profile of Common Vector-borne and Zoonotic Infections in North West Region of Punjab: A Retrospective Observational Study

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ABSTRACT

Introduction: Infections caused by vector-borne pathogens and zoonotic agents are a great economic burden in developing countries like India because their numbers keep increasing every year. National programs like the National Vector Borne Disease Control Program and National One Health Program for Prevention and Control of Zoonoses have been actively tracking their load through their network of Diagnostic Laboratories.

Aim: To evaluate the clinical and epidemiological profile and seropositivity of vector-borne and zoonotic diseases in the North West region of Punjab, India.

Materials and Methods: This retrospective observational study was conducted in the Department of Virology, Guru Nanak Dev Hospital, Amritsar, Punjab, India from January 2024 to December 2024. It was done on samples of suspected patients of Acute Febrile illness attending the OPD/IPD of Guru Nanak Dev Hospital. A total of 1,308 serum samples were received in the Virology Research and Diagnostic Laboratory and tested for various infections, including Dengue, Chikungunya, *Brucella*, *Leptospira*, Scrub typhus, and *Toxoplasma*, using the Enzyme-

Linked Immunosorbent Assay (ELISA) method. Demographic parameters like age, sex and seasonality were studied from patient records for these samples. Statistical analysis was done using Chi-square and Fisher's exact test and a p-value of <0.05 was considered significant.

Results: A total of 1,308 samples were tested, out of which 108 samples (8.25%) were positive for at least one or more of the infections. *Leptospira* infection was the most common, with 50 out of 248 cases (20.16%), followed by scrub typhus with 18 out of 235 cases (7.65%), chikungunya with 11 out of 171 cases (6.43%), brucellosis with 10 out of 170 cases (5.88%), dengue with 17 out of 410 cases (4.14%), and toxoplasmosis with 2 out of 74 cases (2.7%).

Conclusion: The present study highlights that infections such as *Leptospira*, *Brucella*, and scrub typhus should be considered in the differential diagnosis of acute febrile illness, in addition to dengue and chikungunya. Strengthening diagnostic facilities at both peripheral and tertiary levels is essential for timely treatment, along with coordinated efforts from the community and health sector for effective prevention and control.

Keywords: Chikungunya, Dengue, *Leptospira*, Seropositive, Scrub typhus

INTRODUCTION

Infectious diseases are a continuous threat to the human population, as they always have the potential to turn into a pandemic. Emerging infectious diseases are not only viruses but also drug-resistant bacteria that can cause a major setback to a country's economy [1]. Dengue and Chikungunya are the major vector-borne viruses in India, and their growing rates every year are becoming a difficult challenge to tackle. The global economic burden of Dengue alone is estimated to be about 8.9 billion USD annually. In 2016, India had approximately 53 million symptomatic infections with an estimated cost of 5.7 billion USD [2]. The rising temperatures associated with global warming create favourable conditions for mosquitoes, enhancing the transmission of diseases they carry. India hosts a diverse range of 415 mosquito species, out of which fewer than 20 have been identified as primary or secondary vectors of diseases. The never-ending menace caused by mosquitoes because of changes in environmental factors, socioeconomic factors and their biological genetics all reduce the effectiveness of mosquito control measures and contribute to increasing numbers of mosquito-borne diseases with each passing year [3-6]. So, it is important to continuously monitor and adapt vector control efforts to address changing ecological, social and environmental conditions. National Vector-Borne Disease Control Program (NVBDCP) is a Government of India programme under the Ministry of Health and Family Welfare, which consists of one of the most comprehensive and multifaceted

public health activities, including prevention and control of mosquito-borne diseases [7]. It focuses on prevention and control of malaria, dengue, chikungunya, Japanese Encephalitis, Kala-azar and lymphatic filariasis. The Government of India has also established the Virus Research and Diagnostic Laboratory Network (VRDLN) to strengthen the laboratory capacity in the country for providing timely diagnosis of disease outbreaks [3].

Apart from the above viral infections, the burden of zoonotic diseases like brucellosis, leptospirosis and scrub typhus also needs to be estimated. These diseases are among the neglected zoonotic diseases that have become endemic in the animal population. It is claimed that clinical signs exhibited by zoonotic diseases mimic some major diseases in humans, which is the leading factor for under-reporting of such diseases [8]. Nearly 75% of new or emerging infectious diseases are of zoonotic origin [9]. Presently, our country has a National One Health Program for Prevention and Control of Zoonosis (NOH-PPCZ) scheme for surveillance of zoonotic diseases. The Programme is intended to enhance and strengthen the country's capacity to mitigate the morbidity and mortality caused by prevailing endemic viral, bacterial and parasitic zoonoses and to reinforce the International Health Regulation's core capacities for zoonotic threats with Pandemic potential. One health approach brings together coordinated action between the health care sector, animal husbandry and veterinary services and wildlife and environmental sectors for prevention and control of

these infections. The main zoonotic diseases covered under this program are brucellosis, rabies, leptospirosis, anthrax, plague, nipah virus, avian and emerging and other re-emerging zoonosis [10]. The seroprevalence of diverse viral and bacterial infections, both zoonotic and vector-borne, has long been under-reported and overlooked in this region. Documenting these patterns will enhance the understanding of emerging and re-emerging infections, thereby enabling more effective efforts to control and prevent their spread.

Therefore, the study aimed to assess the clinical and epidemiological characteristics of zoonotic and vector-borne infections occurring in the North West region of Punjab, India.

MATERIALS AND METHODS

This retrospective observational study was conducted in Guru Nanak Dev Hospital, Amritsar, Punjab, India from January 2024 to December 2024. The samples were collected under all septic precautions and sent to Virology Research and Diagnostic Laboratory, Government Medical College, Amritsar, where further processing and interpretation were done. This was a retrospective study based on anonymised patient records. No direct patient involvement occurred. In accordance with institutional policy, IEC approval and informed consent were not required. The study adhered to the principles of the Declaration of Helsinki.

Inclusion criteria: All samples of acute febrile illness of recent onset received during the study period of one year. Patients from all age groups from both IPD and OPD samples were included in the study.

Exclusion criteria: Patients with incomplete clinical history and epidemiological information were excluded from the study.

Study Procedure

The serum from blood samples was tested for serological tests as requested by the clinician, according to clinical suspicion and provisional diagnosis. These samples were accompanied by the requisite forms containing all the necessary demographic and geographic details, like age, sex, address, clinical history of the patients, etc.

About 5 mL of whole blood was received in an ice box with a cold chain maintained. Samples were stored at 2-8° before being processed within 24-48 hours. If required, centrifugation at 3000 rpm for 10 minutes was used to separate the serum. ELISA kits with good sensitivity and specificity were used for testing. For Dengue, NS1 Antigen (Trustwell, sensitivity 100%, specificity 99%), IgM antibody Dengue (Trustwell, sensitivity 91.7%, specificity 95.4%) were used. Similarly, good-quality kits were used for Chikungunya (Trustwell, sensitivity 91.3%, specificity 96.8%), *Leptospira* (Trustwell, sensitivity 96.96%, specificity 99.69%), Scrub typhus (J Mitra, sensitivity 98.58%, specificity 100%), *Brucella* (Calbiotech, 98% agreement), *Toxoplasma* (Qualisa). All the tests were performed as per the manufacturer's instructions. The NS1 antigen is detectable from day 1-7 of illness, and IgM antibodies appear from day 4-5 onwards. So, in patients presenting with day 4-10, a single marker may miss the cases. Therefore, NS1 and IgM for dengue were tested on all samples in which dengue serology was requested. The absorbance readings of the tests were then used to calculate results. Results were calculated only after positive and negative controls were validated. Invalid tests were repeated.

STATISTICAL ANALYSIS

Statistical Package for the Social Sciences (SPSS) version 21.0 was used for data analysis. The data was evaluated using the Chi-square method and Fisher's exact test. A p-value <0.05 was considered statistically significant.

RESULTS

In the present study, a total of 1308 samples were received of which, 108 (8.25%) were positive for at least one or more of the

infections. It was noteworthy that 50 cases of *Leptospira* (20.16%) were found to be the most predominant infection among enrolled patients, followed by Scrub typhus (18, 7.65%), Chikungunya (11, 6.43%), *Brucella* (10, 5.88%), Dengue (17, 4.14%) and *Toxoplasma* (2, 2.7%) [Table/Fig-1].

| Pathogens tested | Cases tested (n) | Positive cases (n) | % of positive cases |
|--------------------------|------------------|--------------------|---------------------|
| <i>Leptospira</i> spp. | 248 | 50 | 20.16 |
| <i>Brucella</i> spp. | 170 | 10 | 5.88 |
| Scrub typhus | 235 | 18 | 7.65 |
| Dengue virus | 410 | 17 | 4.14 |
| Chikungunya virus | 171 | 11 | 6.43 |
| <i>Toxoplasma gondii</i> | 74 | 2 | 2.7 |
| Total | 1308 | 108 | 8.25% |

[Table/Fig-1]: Total distribution of positive cases.

The male group 58 (53.70%) had a higher percentage of positivity overall, but *Leptospira* was reported equally among both males 25 (50%) and females 25 (50%). Similarly, in other infections like *Brucella* and *Toxoplasma*, both males and females were equally infected [Table/Fig-2].

| Pathogens tested | Positive cases n (%) | Positive cases male | Positive cases female | χ^2 (p-value) |
|--------------------------|----------------------|---------------------|-----------------------|--------------------|
| <i>Leptospira</i> spp. | 50 (20.16) | 25 | 25 | 0.00 (1.00) |
| <i>Brucella</i> spp. | 10 (5.88) | 5 | 5 | 0.00 (1.00) |
| Scrub typhus | 18 (7.65) | 11 | 7 | 0.80 (0.37) |
| Dengue virus | 17 (4.14) | 9 | 8 | 0.06 (0.80) |
| Chikungunya virus | 11 (6.43) | 7 | 4 | 0.82 (0.36) |
| <i>Toxoplasma gondii</i> | 2 (2.7) | 1 | 1 | 0.00 (1.00) |

[Table/Fig-2]: Sex distribution of positive cases.

The chi-square test was applied, and a p-value <0.05 was significant

The age group of 21-40, 41 (37.96%) years of young adults was found to be the most common age group affected by these infections, followed by the middle age group of 41-60 years 27 (25%) [Table/Fig-3]. No statistical significance was found among positive cases of different age groups.

| Pathogens | 0-20 years (n) | 21-40 years (n) | 41-60 years (n) | >60 years (n) | p-value |
|--------------------------|----------------|-----------------|-----------------|---------------|---------|
| Chikungunya virus | 2 | 1 | 7 | 1 | 1.000 |
| Dengue virus | 8 | 5 | 4 | 0 | 0.052 |
| <i>Toxoplasma gondii</i> | 1 | 1 | 0 | 0 | 0.572 |
| Scrub typhus | 3 | 6 | 7 | 2 | 1.000 |
| <i>Brucella</i> spp. | 3 | 5 | 1 | 1 | 1.000 |
| <i>Leptospira</i> spp. | 7 | 23 | 8 | 12 | 1.000 |

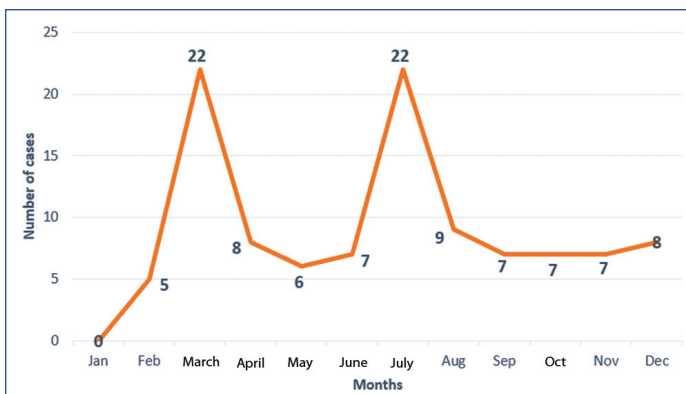
[Table/Fig-3]: Age distribution of positive cases.

P-value <0.05 is significant, calculated by Fisher's exact test

While observing the monthly distribution of positive cases, the maximum of cases (22) was reported in March (14 *Leptospira*, 3 Scrub typhus and 5 *Brucella* cases) and July (11 *Leptospira*, 5 Dengue, 4 Chikungunya and 2 Scrub typhus cases) and the lowest was in January [Table/Fig-4].

DISCUSSION

The transmission of zoonotic diseases into human hosts has led to many pandemics in the past. The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic is the most recent example, highlighting the continued need for improved research and preparedness to better understand zoonotic diseases [11]. Zoonotic diseases are on the rise not only globally but also in India, which is a cause of concern [12]. In the present study, evidence of antibodies to Zoonotic diseases like *Leptospira*, Scrub



[Table/Fig-4]: Seasonal distribution of positive cases over the year 2024.

typhus, *Brucella* and *Toxoplasma* shows that there has been a silent transmission of these agents in this part of India, although very few studies have reported them in the past [13]. Studies from veterinary sciences also support the fact that awareness and surveillance gaps contribute to this unnoticed transmission [14,15]. *Leptospira* was found to be the most common infection among the acute febrile cases. In recent years, there has been an unexpected increase in cases of Leptospirosis. Unlike other states like Tamil Nadu, Kerala, Karnataka, Gujarat, Maharashtra, and Andaman and Nicobar, where Leptospirosis is endemic, it was being under-reported in the present region [13]. Seropositivity of *Leptospira* spp. 20%, which was noted in this study, was comparable to other studies from India [16-18]. A study from the same institute in 2023 also showed an incidence of 8.7% of *Leptospira* [19]. The main risk factors of Leptospirosis in this region may be rice cultivation practices, consumption of contaminated food and water, contact with animals, open defecation, etc. Major factors for the re-emergence of leptospirosis have also been outlined in a review article [20].

Scrub typhus, a vector-borne zoonotic disease, has long been a neglected disease in India, 7.61% positivity was found in the present study. A similar study from the same institute reported a 1.4% incidence among acute febrile cases [19]. In a recent review article, the author has published the overall seroprevalence of 26.4% in our country [21]. Brucellosis, another common zoonotic disease, has been reported in the range of 0.9-18.1% in a seroprevalence study [22]. Being mostly an agricultural state with a large livestock population, Brucellosis in Punjab may have an endemic existence, so seroprevalence in the current study was 5.88%. A similar study from North India reported 4.96% of this infection [23]. The present study shows seroprevalence of *Toxoplasma gondii* as low as 2.7%, although seroprevalence of North India has been seen in the range of 4.7- 51.8% in previous study [24].

Vector-borne diseases like dengue and chikungunya have been a major health burden for many decades. The epidemiology of these vector-borne diseases is complex and ever-changing. As compared to last year, the present region saw a low prevalence of Dengue and Chikungunya [19,25]. The current study shows an overall seropositivity of 4.14%. Decreased rainfall in the past year and high temperatures may have led to such low numbers. Similarly, for chikungunya, numbers were quite low (6.43%) compared to the last year's study (20.65%) from the same institute [25].

Overall, males outnumbered females in the current study, but *Leptospira* was reported equally among both males and females. A number of other authors have also noted such a trend [26,27]. The reason behind this may be that males are mostly exposed to such infections because of occupational behaviour like livestock activities, agricultural farming, paddy field farming, exposure to animal faeces, animal urine, rodents, etc.

The young adult population in the range of 21-40 years was mostly affected in the current study's findings, as this age group is mostly occupationally active or college-going, and involved in

various outdoor activities. Similar findings have been noted by many authors [26,27].

Overall, the present study depicts the seroprevalence of the most common infectious agents found in the region. Some of these infections were not being monitored earlier, so further check has to be imposed on such infections through prevention and control. There is a need for collaborative efforts to create a local model through which community people can also be educated about zoonotic and vector-borne infections.

Limitation(s)

The sample size was relatively small, which may limit the generalisability of the findings. Being a hospital-based study, the results may not reflect the true burden of infections in the community. Although Polymerase Chain Reaction (PCR)/ Immunofluorescence assays (IFA) /culture are considered confirmatory tests for infections like leptospirosis, brucellosis and scrub typhus, ELISA tests with good sensitivity and specificity were used in the present study as recommended by National Centre for Disease Control (NCDC). More clinical epidemiological data could have been collected to establish an association between clinical and laboratory findings.

CONCLUSION(S)

This study emphasises the significant contribution of neglected zoonotic infections to the burden of acute febrile illness in North India. Among the zoonotic infections evaluated, leptospirosis emerged as the most common infection, followed by scrub typhus and brucellosis, highlighting their continued public health relevance. Low clinical suspicion and limited routine diagnostic testing contribute significantly to underreporting of these infections despite their substantial public health concern. Therefore, integration of human, animal and environmental health is critical for early detection, prevention and control of neglected zoonotic infections. Strengthening diagnostic capacity and clinical awareness at peripheral and tertiary healthcare levels will improve case detection and disease outcome.

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