

Hospital-Based Surveillance of Leptospirosis, Scrub Typhus, and Dengue in the Malwa Region of Punjab: A Five-Year Retrospective Analysis

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Short running title: A Retrospective study

Abstract

AIM: The study aims to evaluate the prevalence of acute febrile illness and incidences of co-infection with different laboratory parameters.

METHODOLOGY: The current retrospective study was conducted on 11884 blood samples obtained from patients with acute febrile illness at tertiary care hospital over five years (2019-2023) in Punjab, India.

RESULTS: The study found variable prevalence of leptospirosis, dengue and scrub typhus across the five years with dengue showing the highest prevalence (27.09%) in 2021. However highest rate of prevalence (10.61%) was found in 2020 in case of leptospirosis while scrub typhus showed highest prevalence (19.14%) in 2022. Co-infection incidences data revealed that it was frequent in 2019 and 2021. Gender distribution data indicated the high prevalence of infection among males with age group of 20- 40 years across the five years. Statistically significant changes were witnessed over the five years.

INTERPRETATION: The findings of the present study emphasizes on continuous surveillance, differential diagnosis and integrated diseases management to combat these infections.

KEYWORDS: Co-infection; Dengue; Leptospirosis; Punjab; Scrub typhus; Vector borne diseases

Introduction

Leptospirosis and Scrub Typhus emerge as notable adversaries, both posing significant challenges to public health systems globally [1,2]. Leptospirosis, caused by spirochete bacteria of the genus *Leptospira*, is mainly transmitted through urine excreta of rodents especially rats [1]. Due to their helix from and internal locomotor apparatus, the endoflagellum of leptospirosis show remarkable mobility even in the thickest medium thus infecting human host generally when exposed to contaminated river or lake water or animals in tropical and subtropical region [3]. On the other hand, Scrub Typhus is caused by the *Orientia tsutsugamushi* (obligate intracellular Gram-negative bacterium) which transmits by larval mites commonly known as chiggers, belonging to the *Leptotrombidium deliense* group in rural areas with dense vegetation [4]. These larvae usually feed on wild rats of the *Rattus* subgenus. Human infection arises incidentally when individuals enter mite-infested regions, often termed as mite islands. However, infection can be noticed in diverse habitats such as seashores, rice fields, and semi-deserts [4].

The infection typically presents clinically as a non-specific febrile illness, often characterized by symptoms such as headache, myalgia, nausea, vomiting, diarrhoea, cough, or breathlessness [5,6]. Despite their distinct modes of transmission and clinical presentations, Leptospirosis and Scrub Typhus share common challenge. Together with leptospirosis, malaria, and dengue fever, scrub typhus is one of the differential diagnoses in patients with haemorrhagic fever, particularly if it is accompanied by jaundice and/or renal failure [2]. Understanding the complexities if Leptospirosis and Scrub Typhus is paramount for healthcare professionals, researchers, and policymakers to effectively combat their impact on human populations.

Materials and Methods:

A retrospective study of five years was conducted using blood samples collected from patients with acute febrile illness at the outpatient department (OPD) and inpatient department (IPD) of a tertiary care hospital from a period of 2019 to 2023. A total of 11,884 blood samples collected during the study period from patients with a fever history ranging from 1 to 15 days residing in Punjab and Haryana regions. Serum was separated from the blood samples by centrifugation and stored at -20°C for further analysis.

All the samples from patients with acute febrile illness underwent diagnostic testing for dengue, leptospirosis, and scrub typhus [7]. For patients with a fever history of 1 to 5 days dengue NS1 Antigen testing was conducted using the commercially available Dengue NS1 Antigen ELISA kit (RecombiLISA, CTK BIOTECH, INC, California). For those with a Fever duration exceeding 5 days, dengue IgM antibodies were assessed using the NIVDENGUE IgM CAPTURE ELISA kit (ICMR-NIV,Pune) Additionally, all samples underwent testing for leptospirosis using the Panbio LeptospiralIgM ELISA kit (Standard Diagnostics, INC., Republic of Korea) and for scrub typhus using the InBiOS Scrub typhus Detect IgM ELISA kit (Seattle, WA USA), following the manufacturers' instructions. In these cases, standard laboratory tests (complete blood count, peripheral smear, urine analysis, urea, creatinine, glucose, liver function tests) were also performed. Results were further analyzed using statistical methods.

Results:

Over the course of five years from 2019 to 2023, a series of diagnostic tests were conducted to evaluate cases of dengue, leptospirosis, and scrub typhus. The data, summarized in the table 1, illustrates the total number of tests performed each year and the corresponding positive cases for each disease.

Table 1 shows data from 2019 to 2023, the data reveals a variable pattern in the number of cases tested and positive cases for dengue, leptospirosis, and scrub typhus. From 2019 to 2023, the number of samples tested for dengue varied considerably, the highest number of positive cases 891 was observed in 2023 while low number of positive cases 271 was

observed in 2022. In 2019, 631 positive cases were found, followed by 524 positive case in 2021 and 522 positive cases in 2023.

Leptospirosis testing peaked in 2021 with 301 samples tested and had the lowest number of samples tested in 2023 with only 93. However, positive cases for leptospirosis ranged from a high of 30 in 2022 to a low of 4 in 2023. In the year 2019 only 14 cases were found to be positive among 280 tested. While in 2020 leptospirosis testing decreased to 113, yielding 12 positive cases.

For scrub typhus, the number of samples tested was relatively stable, with the highest number tested in 2022 (94) and the lowest in 2019 (78). Positive cases scenario for scrub typhus showed variability. Highest positivity was observed in 2022 with 18 positive cases while lowest positivity was observed in 2023 with only 5 cases. From the year 2019-2021 it was found that 8, 10 and 11 cases were positive for scrub typhus out of 78, 82 and 90 samples respectively.

Co-infections of dengue and leptospirosis were most frequent in 2019 with 8 cases and were absent in 2023. Co-infections of dengue and scrub typhus were highest in 2021 with 5 cases and were also absent in 2023. Notably, while dengue consistently showed higher case numbers, leptospirosis and scrub typhus exhibited lower but still significant prevalence rates. These findings underscore the importance of continued surveillance and diagnostic efforts to monitor and manage these infectious diseases effectively.

The data in table 2 illustrates the total positive cases of dengue, leptospirosis, and scrub typhus over the years 2019 to 2023 by gender distribution. In 2019, there were 631 positive cases of dengue, with (55.30%) cases among males and (44.7%) among females. Additionally, there were 14 cases of leptospirosis, evenly distributed (50%) between genders, and 8 cases of scrub typhus, with more cases among (62.5%) males than females (37.5%).

The following year, 2020, out of 271 positive cases, (60.14%) positive cases was found in male and (39.86%) positive cases belongs to female category in dengue. For leptospirosis, distribution of positive cases was (66.66%) and (33.33%) between male and female respectively while for Scrub typhus female showed higher number (60%) of positive cases than males with (40%) of positive case.

In 2021, dengue cases rose to 524, with more cases (54.77%) in males than (45.22%) females, while in leptospirosis more number of cases was observed in females (62.06%) than males with (37.94%). Usual trend was observed in scrub typhus with (63.64%) male case and (36.36%) female cases.

The trend continued in 2022, with 522 cases of dengue, more prominent among (64.94%) males than (35.05%) females. Alongside in leptospirosis also, male (70%) showed high prevalence of positive cases than (30%) females. But reverse trend was observed in scrub typhus with (61.11%) positive cases of females and (38.88%) of males.

Finally, in 2023, dengue cases surged to 891, predominantly affecting (73.96%) males population than (26.03%) females, while in leptospirosis it was found that 75% in males and (25%) in females cases were affected. However in scrub typhus (60%) of males and (40%) of females were found to be affected.

The table 3 presents a comprehensive breakdown of positive cases for dengue, leptospirosis, and scrub typhus across different age groups from 2019 to 2023. In terms of dengue, the highest number of cases consistently occurred in the 20-30 years age group ranging from (33% to 55%) across all years, followed by the 30-40 years age group with percentage range from (30% to 25%). While there were fluctuations in case numbers across years, these age groups consistently exhibited the highest incidence. Similar trends were observed in leptospirosis and scrub typhus cases with highest distributions of cases across the age groups of 20-30 years and 30-40 years over all five years of span.

Discussion:

The present study highlights a changing pattern in the incidence of dengue, leptospirosis, and scrub typhus, with dengue showing the highest variability. The relatively low and stable incidence of leptospirosis and scrub typhus suggests a different dynamic of transmission compared to dengue. The presence of co-infections, although not frequent, underscores the importance of differential diagnosis in endemic areas.

The current study has monitored a five-year dataset evaluating Dengue fever cases and alongside other infectious diseases, from 2019 to 2023. In 2019, Dengue fever cases varied significantly as 631 out of 2654 people tested positive for the disease. Subsequent years saw

variations: in 2020, 271 out of 1199 tested positive, followed by 524 out of 1934 in 2021, and 522 out of 2532 in 2022. In 2023, the highest Dengue fever cases were recorded; with 891 out of 3565 individuals tested positive. The unstable trends observed in dengue, leptospirosis, and scrub typhus infections between 2019 and 2023 are consistent with findings from other research on diseases carried by vectors [8,9]. A study conducted in India estimated the prevalence of vector borne infection in 100 patients and found that 39 patients were positive for dengue and 19 were positive for scrub typhus [10]. It is important to comprehend how the environment and socioeconomic factors impact the spread of disease. Co-infection cases emphasize the need of combined surveillance and control measures. Previous studies have revealed that there are many factors contributing to fluctuations in prevalence of dengue transmission over time such as changes in temperature, rainfall, and vector abundance. For instance, a study found that dengue transmission in urban areas is closely linked to temperature and precipitation patterns, with increases in cases often observed during warmer and wetter months [11-13]. Similarly, in another study the impact of urbanization on dengue transmission, with rapid urban expansion leading to increased breeding sites for *Aedes* mosquitoes with higher transmission rates was demonstrated [14]. A study in urban areas of India examined the epidemiology of dengue and identified key risk factors associated with its transmission. The study highlighted the role of rapid urbanization, inadequate sanitation, and water storage practices in increasing dengue incidence rates in densely populated urban settings [15-17].

In case of Leptospirosis the data shows varying numbers of positive cases across the years, with the highest recorded in 2021 (29 cases out of 301 tested) and 2022 (30 cases out of 296 tested), while notably fewer cases were observed in 2023 (4 cases out of 93 tested). Leptospirosis, often associated with flooding events and poor sanitation, also exhibits variable incidence rates influenced by environmental factors. Another research highlighted the role of rainfall and flooding in triggering leptospirosis outbreaks, particularly in urban and peri-urban settings where exposure to contaminated water is more common [18,19]. Additionally, few more studies emphasized the importance of urbanization and changes in land use in shaping leptospirosis transmission dynamics, with urban slum areas experiencing higher rates of infection due to inadequate housing and sanitation infrastructure [20,21].

Similarly, Scrub Typhus cases fluctuated, with the highest incidence in 2022 (18 cases out of 94 tested). Despite their lower prevalence compared to Dengue fever, the presence of these diseases underscores the diverse infectious disease landscape in the region. One more study

investigated leptospirosis outbreaks in coastal regions of India, particularly in areas prone to flooding and water logging [22]. A few more studies examined the burden of scrub typhus in rural agricultural communities in India, where agricultural workers are at increased risk of exposure to chigger mites [23,24].

The detection of co-infections between dengue and other vector-borne diseases, as observed in the data from 2019 to 2023, aligns with previous research indicating the potential for simultaneous infections and overlapping risk factors. More studies have reported cases of co-infections between dengue and leptospirosis or scrub typhus, highlighting the complex interactions between different pathogens and the challenges they pose for diagnosis and clinical management [25,26]. Few case studies also reported the cases of co-infections involving dengue, leptospirosis, and scrub typhus, particularly in regions where these diseases overlap geographically [27,28].

The gender-specific distribution of positive cases for dengue, leptospirosis, and scrub typhus from 2019 to 2023 indicated male predominance among individuals aged 20-30 years aligns with findings from previous studies that have identified gender-specific risk factors and behaviors contributing to dengue transmission [29,30]. Another research in India highlighted the role of gender disparities in water storage practices and outdoor activities, which can increase male susceptibility to dengue infection [31]. Additionally, studies in other regions have pointed to gender differences due to outdoor occupations as key factors driving higher dengue incidence among males [32,33]. It was also reported that occupations such as agriculture, fishing, and construction work as high-risk factors for leptospirosis, which are often male-dominated fields [34,35]. More studies have also focused on the increased risk of infection among younger individuals engaged in outdoor activities such as farming, fishing, and manual labor [36,37]. The more balanced gender distribution observed in scrub typhus cases may reflect differences in exposure patterns and susceptibility between males and females. Another research by has indicated that activities such as farming, camping, and outdoor recreational activities, which are associated with scrub typhus transmission, may be equally common among both genders [38,39].

Conclusion: The analysis of positive cases, gender distribution and age distribution for dengue, leptospirosis and scrub typhus from 2019 to 2023 reveals dynamic patterns that underscore the complex epidemiology of these vector-borne diseases. While dengue consistently remained the most prevalent of the three diseases, fluctuations in positive cases

for leptospirosis and scrub typhus suggest shifting epidemiological dynamics that warrant further investigation. It was also observed that middle aged male population is most affected with varied blood profile.

Overall, the analysis underscores the need for integrated approaches to disease surveillance, prevention, and control that consider the dynamic interplay between environmental, behavioral, and demographic factors.

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Table 1: Annual distribution of positive cases (2019-2023).

Year	Total sample tested for Dengue (no.)	Total positive cases for Dengue (no.)	Total sample tested for Leptospirosis (no.)	Total positive cases for leptospirosis (no.)	Total sample tested for Scrub Typhus (no.)	Total positive cases for Scrub Typhus (no.)	Co infection of dengue and leptospirosis	Coinfection of dengue and scrub typhus
2019	2654	631 (23.77%)	280	14 (5%)	78	8 (10.25%)	8	3
2020	1199	271 (22.60%)	113	12 (10.61%)	82	10 (12.19%)	4	2
2021	1934	524 (27.09%)	301	29 (9.63%)	90	11 (12.22%)	7	5
2022	2532	522 (20.61%)	296	30 (10.13%)	94	18 (19.14%)	3	1
2023	3565	891 (24.99%)	93	4 (4.30%)	93	5 (5.37%)	0	0

Table 2: Gender-specific Distribution of Positive Cases for Dengue, Leptospirosis, and Scrub Typhus (2019-2023)

Year	Dengue (Positive cases)		Leptospirosis (Positive cases)		Scrub Typhus (Positive cases)	
	Male	Females	Male	Females	Male	Females
2019	349(55.30%)	282(44.7%)	7(50%)	7 (50%)	5(62.5%)	3(37.5%)
2020	163(60.14%)	108(39.86%)	8(66.66%)	4 (4%)	4(40%)	6(60%)
2021	287(54.77%)	237(45.22%)	11(37.94%)	18 (62.06%)	7(63.64%)	4(36.36%)
2022	339(64.94%)	183(35.05%)	21(70%)	9 (30%)	7(38.88%)	11(61.11%)
2023	659(73.96%)	232(26.03%)	3(75%)	1(25%)	3(60%)	2(40%)

Table 3: Age-specific Distribution of Positive Cases for Dengue, Leptospirosis, and Scrub Typhus (2019-2023)

Age Group (in years)	Dengue (Positive cases)					Leptospirosis (Positive cases)					Scrub Typhus (Positive cases)				
	2019 N/ %age	2020 0	2021 1	2022 2	2023 3	2019 9	2020 0	2021 1	2022 2	2023 3	2019 9	2020 0	2021 1	2022 2	2023 3
0-10	13/ 2.0 %	11/ 4.1 %	17/ 3.2 %	21/ 4%	33/ 3.7 %	0	0	1/ 3.5 %	2/ 6.6 %	0	0	0	0	1/ 5.5%	0
10-20	52/ 8.2 %	32/ 11. 8 %	28/ 5.4 %	16/ 3.1 %	98/ 10.9 %	1/ 7.1 %	0	3/ 10. 3 %	5/ 16. 7 %	0	0	0	1/ 9.1 %	0	0
20-30	213/ 33.7 %	91/ 33. 5 %	251/ / 47. 9 %	272/ / 52. 1 %	350/ / 39.3 %	6/ 42. 9 %	4/ 33. 3 %	9/ 31. 0 %	11/ 36. 7 %	2/ 50 %	6/ 75 %	5/ 50 %	3/ 27. 3 %	3/ 16. 7 %	0
30-40	195/ 30.9 %	72/ 26. 6 %	114/ / 21. 8 %	131/ / 25. 1 %	211/ / 23.7 %	5/ 35. 7 %	7/ 58. 3 %	14/ 48. 2 %	6/ 20 %	2/ 50 %	1/ 12. 5 %	4/ 40 %	7/ 63. 6 %	7/ 38. 9 %	5/ 100 %
40-50	83/ 13.4 %	45/ 16. 6 %	63/ 12 %	60/ 11. 5 %	154/ / 17.3 %	2/ 14. 3 %	0	1 3.5 %	4/ 13. 3 %	0	1/ 12. 5 %	0	0	5/ 27. 8 %	0
>50	75/ 11.8 %	20/ 7.4 %	51/ 9.7 %	22/ 4.2 %	45/ 5.1 %	0	1/ 8.4 %	1 3.5 %	2/ 6.6 %	0	0	1/ 10 %	0	211 .1 %	0