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Original Article

Microbiology Section

Etiological Spectrum of Acute Febrile Illness in a Tertiary Care Hospital in Assam, India: A Cross-Sectional Study

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ABSTRACT

Introduction: Acute Febrile Illness (AFI) encompasses a spectrum of conditions marked by fever, often caused by various infectious agents. The aetiological diversity poses challenges in diagnosis and treatment, particularly in regions with limited diagnostic resources.

Aim: To evaluate the spectrum of pathogens causing AFI with or without rash in patients.

Materials and Methods: A hospital-based cross-sectional study was conducted from January 2024 to December 2024 on 1,544 patients who had attended Lakhimpur Medical College and Hospital, North Lakhimpur, Assam, India. Patients with fever (≥ 37.5°C) with or without rash were enrolled. Serological investigations for Measles, Rubella, Dengue, Varicella-zoster, Chikungunya viruses, Japanese Encephalitis (JE), *Orientia tsutsugamushi*, Leptospira, and Hepatitis viruses were performed using Enzyme-linked Immunosorbent assay (ELISA) and Rapid Detection Testing (RDT) kits. Data analysis was performed using both descriptive and inferential methods. A p-value of <0.05 was considered significant.

Results: Fever was prevalent in all 1,544 (100%) cases, followed by 856 (55.44%) cases of headache, 636 (41.19%) with cough, 476 (30.83%) with vomiting, 347 (22.47%) with abdominal pain, 59 (3.82%) cases with dysentery, 52 (3.37%) with diarrhoea, 46 (2.98%) cases with unconsciousness, and 21 (1.36%) cases presented with rash. Fever was observed in combination with headache in 856 (55.44%), with cough in 636 (41.19%), with bodyache in 481 (31.15%), and with vomiting in 476 (30.83%) cases. The study identified seasonal variations in infection rates, with peaks during the monsoon months. The majority of positive cases were attributed to Scrub Typhus 133/817 (16.28%), followed by Leptospirosis 129/473 (27.27%), Dengue 84/1273 (6.59%), and JE 20/87 (22.99%), while some had co-infection.

Conclusion: The study underscores the importance of integrated clinical and laboratory approaches in diagnosing AFI. Scrub typhus, leptospirosis, and dengue were identified as major causes, emphasising the need for enhanced surveillance and diagnostic capabilities.

Keywords: Co-infection, Enzyme-linked immunosorbent assay, Fever, Seasonal variations, Rash

INTRODUCTION

Fever is a general symptom of many infectious systemic illnesses, often leading to significant morbidity. The majority of unspecified febrile illnesses are treated with generic approaches, typically involving antipyretics and antibiotics [1]. Whereas some febrile illnesses are characterised by rashes, others are not. Fever with rash is commonly observed among patients of all age groups. A common term, "exanthem", is generally used by clinicians to indicate the rashes in patients, which is defined as any eruptive skin rash that may be accompanied by fever or other systemic symptoms [2]. Numerous infectious and non-infectious conditions can manifest as exanthems, but viral exanthems are the most frequent cause of fever with rashes [3]. A wide range of viruses have the ability to cause rashes, which may be localised or diffused. However, approximately 19% of rash cases and 40% of AFI cases exist without a defined aetiology [4]. Some of these rashes may occur with or without associated symptoms such as fever, discomfort, itching, and other systemic signs [5].

The AFI is defined as an illness with fever lasting up to one week, sudden onset, caused by various pathogens without specific organ involvement [6]. This condition is mostly observed in tropical and subtropical regions. AFI can be potentially lethal if the aetiology is not recognised and if not appropriately treated early. As far as the aetiology is concerned, both viral and bacterial pathogens play a crucial role in the development of AFI [7,8].

Factors such as environmental conditions, socio-economic status, and vaccine availability influence the incidence and aetiology of AFI within a region. Diagnosing the cause of AFI with or without rash is challenging for healthcare providers and surveillance systems, particularly in settings where confirmatory diagnostic tests are not readily available [9]. Prodromal symptoms are uncommon in patients, and fever is believed to be the most frequently observed symptom in affected patients [10]. To cope with those patients, a detailed history should be obtained, covering the onset, duration, and nature of the fever, the timing of the rash if present in relation to the fever, the pattern and progression of the rash, accompanying symptoms, the presence of similar lesions in close contacts, recent medication use, and the household's hygiene conditions. A thorough physical examination should include a careful inspection of the rash and assessment for key signs of systemic involvement [11]. Information about the time interval between the onset of fever and the appearance of a rash in patients could be valuable for diagnosis in resource-constrained settings [12].

Furthermore, the common agents responsible for Rash Disease (RDs) include measles virus, rubella virus, dengue virus, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, human herpesvirus 6, human herpesvirus 7, enterovirus, human parvovirus B19, chikungunya virus, and Zika virus [13,14]. Given the overlapping clinical symptoms among these agents, determining the precise cause is crucial not only for selecting appropriate treatment but also for epidemiological purposes, such as infection control and eradication efforts.

However, to the best of our knowledge, no studies have addressed this aspect in this geographical region of India, that is, the north

bank of eastern Assam, India. Therefore, the study was undertaken to determine the prevalence of various pathogens, viz., measles virus, rubella virus, dengue virus, varicella-zoster virus, Chikungunya, *Orientia tsutsugamushi*, Leptospira, and hepatitis viruses, causing AFI in this region.

The aim was to evaluate the spectrum of pathogens causing AFI with or without rash in patients attending Lakhimpur Medical College and Hospital, Assam, India.

MATERIALS AND METHODS

The study was a hospital-based observational cross-sectional study conducted at Lakhimpur Medical College Assam, India, with serological investigations performed in the Virus Research and Diagnostic Laboratory (VRDL), Department of Microbiology, for a period of 12 months i.e., from January 2024 to December 2024. The study was approved by the institutional ethics committee (Human) (Certificate no. LMC/IEC(H)/80). Written informed consent was secured from patients or guardians after explaining the test procedure, and assent from children was obtained if involved.

Inclusion criteria: Patients attending the medicine, dermatology, and paediatrics OPD/IPD with fever (single axillary temperature ≥37.5°C) [15] for three or more days, with or without skin rash, were enrolled after consultation with the concerned clinician. Rashes considered were macular, papular, maculopapular, petechial, vesicular, pustular, or urticarial types.

Exclusion criteria: Patients referred for serology testing but without fever (axillary temperature ≥37.5°C) were excluded from this study.

Study Procedure

A total of 1,544 cases who attended LMCH during January-December 2024 were enrolled in this study. Each case underwent a thorough evaluation involving detailed history-taking and physical examination prior to inclusion. All relevant data were recorded using a standardised Case Report Form (CRF) from VRDL network laboratories.

A 3-5 mL venous blood sample was drawn using aseptic venipuncture technique and placed into a red-capped clotted vial for serological analysis. Samples were refrigerated at 2-8°C and processed with reports issued within 24-48 hours. An aliquot from each was preserved at -80°C to support subsequent quality control measures. This testing included immunoglobulin M (IgM) antibody assessment by the ELISA technique for JE Virus, West Nile Virus, Measles Virus, Rubella Virus, Varicella Zoster Virus (VZV), Chikungunya Virus, Dengue Virus, Hepatitis (A, B, C, E) Virus, Leptospira, and *Orientia tsutsugamushi* based on the case definition and clinician's recommendation [16]. Levels of specific IgM antibodies against the viral/ bacterial pathogens or specific antigen were determined using ELISA method, following the instructions provided by the commercial ELISA kits manufacturers [Table/Fig-1].

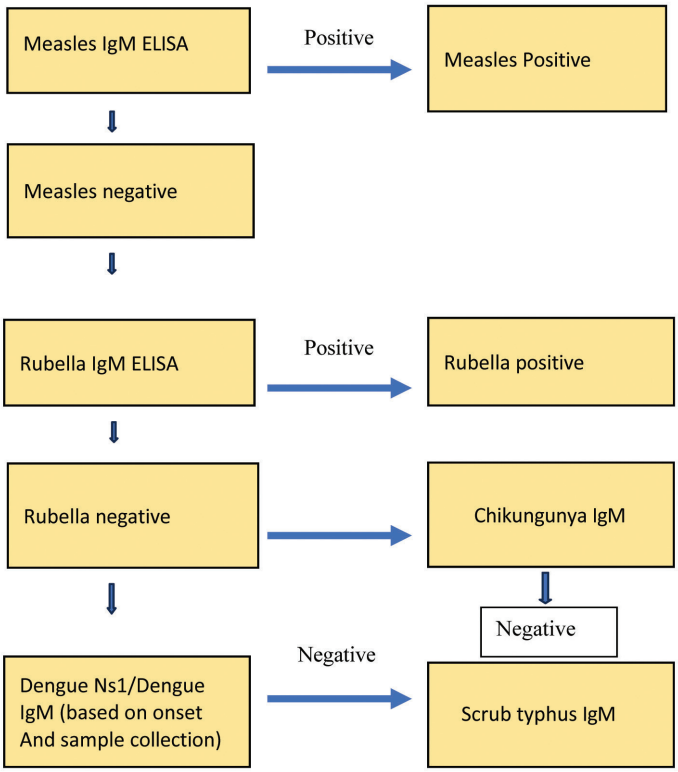
Optical Density (OD) was measured at 450 nm using an ELISA reader (Thermo Fisher Scientific). Based on the onset of the disease,

S. No.	Name of test	Name of the kit	Methodology (as per manufacturer instructions)	Sensitivity, specificity and cut-off criteria/cut-off factor
1.	Measles IgM ELISA	EUROIMMUN (Anti Measles Virus ELISA IgM)	IgM antibody capture (MAC-ELISA)	100%, 98%
2.	Rubella IgM ELISA	EUROIMMUN (Anti-Rubella Virus Glycoprotein ELISA IgM)	IgM antibody capture (MAC-ELISA)	98.6%, 99.5% (Ratio <0.8: Negative Ratio ≥0.8- <1.1: Borderline Ratio ≥ 1.1 : Positive)
3.	Dengue Ns1 ELISA	OSCAR (Dengue Ns1 ELISA)	Sandwich ELISA	100%, 100%, NC+0.200
4.	Dengue IgM ELISA	ICMR-NIV (Dengue IgM capture ELISA)	IgM antibody capture (MAC-ELISA)	98.53%, 98.84% Negative: < NC ODx2 Positive: > NCODx3 Equivocal: NCODx2- NCODx3
5.	Chikungunya IgM ELISA	ICMR-NIV (Chikungunya IgM capture ELISA)	IgM antibody capture (MAC-ELISA)	95% 98% Negative: < NC ODx2 Positive: > NCODx3 Equivocal: NCODx2- NCODx3
6.	Scrub Typhus IgM ELISA	J. MITRA (Scrub Typhus IgM MicroLISA)	Indirect ELISA	100%, 100%, NCx+ 0.350
7.	Varicella zoster IgM ELISA	NOVALISA (Varicella zoster VZV IgM ELISA)	IgM antibody capture (MAC-ELISA)	95%, 98.39%, 0.43
8.	Japanese Encephalitis (JE) IgM ELISA	NIV (JE IgM capture ELISA)	IgM antibody capture (MAC-ELISA)	98%, 98%, Negative: Sample OD ≤ Negative Control ODx3 Positive: Sample OD ≥ Negative Control ODx5 Equivocal : Sample OD <Negative Control ODx5
9.	Hepatitis B Surface Antigen	OSCAR (HBsAg ELISA)	Sandwich ELISA	100%, 100%, 0.1 + Avg NC
10.	Hepatitis A IgM ELISA	Diaprodiagnostic (HAV IgM ELISA)	IgM antibody capture (MAC-ELISA)	98.6%, 99.1%, NC mean+ 0.250
11.	Hepatitis E IgM ELISA	Diaprodiagnostic (HEV IgM ELISA)	IgM antibody capture (MAC-ELISA)	100%, ≥95%, NC mean +0.250
12.	Hepatitis C Anti-HCV	AVANTOR (Microwell ELISAIHF)	Indirect ELISA	100%, 99.3%, NC mean+ 0.2
13.	Leptospira IgM-ELISA	J. MITRA (LeptoIgM MicroLISA)	Indirect ELISA	99.62%, 99.92%, NC mean+ 0.500

[Table/Fig-1]: Details of ELISA kits used in the study.
Abbreviations: NC: Negative control, *OD: Optical density

NS1antigen ELISA test for Dengue and Hepatitis B surface antigen were also performed.

Moreover, Rapid IgM test kits (RDT) were also utilised at a specific time for the detection of Hepatitis A,B,C, and E.(Hepatitis A IgM, Make- Biotest, Specificity- 99.2%, Sensitivity-96%; Hepatitis B Surface Antigen, Make- Avantor, Specificity- 100%, Sensitivity-100%; Hepatitis C virus Antibody, Make- OSCAR, Specificity- 99.8%, Sensitivity-100%; Hepatitis E IgM, Make- Biotest, Specificity- 99.2%, Sensitivity- 98.1%) [17-20]. Laboratory results, like positive IgM for Dengue or JE, etc., were integrated with clinical data to confirm diagnoses. The data was shared with the concerned clinician who correlated serology with symptom timeline (e.g., Dengue NS1 for early fever/rash, IgM for later stages) and physical signs (e.g., encephalitis with JE or jaundice with Hepatitis), and also with the Integrated Disease Surveillance Program (IDSP) of the district to investigate the epidemiology (e.g., mosquito exposure in monsoons for Chikungunya/Dengue) [21].



[Table/Fig-2]: Diagrammatic presentation of the algorithm for testing cases of fever-with-rash syndrome.

For the fever cases with rashes, an algorithm formulated by the Indian Council of Medical Research (ICMR) was considered [Table/Fig-2] [22].

Further, cases of fever accompanied by rash that tested negative for all previously listed parameters in the diagnostic algorithm were subsequently evaluated for VZV IgM to exclude Chickenpox. The other acute febrile cases were tested at the clinician's recommendation.

STATISTICAL ANALYSIS

Statistical analysis was conducted using Statistical Package for the Social Sciences (SPSS) Version 27.0 (Armonk, NY: IBM Corp). Data analysis was performed using both descriptive and inferential methods. Descriptive statistics, such as frequency and percentage, were used to summarise the study population. For inferential analysis, Pearson's Chi-square test and Fisher'sexact test were used to explore associations between clinical features and aetiologic diagnoses. A p-value <0.05 (5% level of significance) was considered statistically significant.

RESULTS

Among the subjects enrolled, fever was prevalent in all 1,544 (100%) cases, with no significant gender difference. Vomiting was significantly more common among females than males (33.79% vs 28.60%; p-value=0.029), while most other symptoms showed no significant gender-wise variation [Table/Fig-3].

No significant difference was observed between male and female patients regarding the outcome of the various diagnostic tests performed. Notably, no case was found positive for Rubella IgM, Hepatitis Anti-HCV ELISA, and Hepatitis HEV IgM (RDT) [Table/Fig-4a,b].

Scrub typhus and leptospirosis were the most frequently detected infections, showing clear seasonal peaks during the monsoon and post-monsoon months. Scrub typhus peaked in October (22 cases), with additional high counts during June-July, while Leptospirosis reached peak positivity in December (25 cases) following a gradual rise from mid-year. Dengue IgM peaked in July (10 cases), whereas

Symptoms	Positive Cases				
	Overall N=1,544	Female N=663	Male N=881	Chi-square	p-value
Fever	1,544 (100%)	663 (100%)	881 (100%)	1.59	0.21
Diarrhoea	52 (3.37%)	21 (3.17%)	31 (3.52%)	0.143	0.70
Dysentery	59 (3.82%)	21 (3.17%)	38 (4.31%)	1.35	0.25
Abdominal pain	347 (22.47%)	162 (24.43%)	185 (21.00%)	2.56	0.11
Vomiting	476 (30.83%)	224 (33.79%)	252 (28.60%)	4.76	0.029
Headache	856 (55.44%)	364 (54.90%)	492 (55.85%)	0.136	0.71
Bodyache	481 (31.15%)	215 (32.43%)	266 (30.19%)	0.881	0.35
Sore throat	4 (0.26%)	1 (0.15%)	3 (0.34%)	--	0.64#
Nausea	56 (3.63%)	31 (4.68%)	25 (2.84%)	3.66	0.056
Cough	636 (41.19%)	263 (39.67%)	373 (42.34%)	1.11	0.29
Rhinorrhoea	280 (18.13%)	107 (16.14%)	173 (19.64%)	3.12	0.077
Neck rigidity	2 (0.13%)	1 (0.15%)	1 (0.11%)	--	>0.99#
Retro-orbital pain	2 (0.13%)	2 (0.30%)	0	--	0.18#
Breathlessness	23 (1.49%)	11 (1.66%)	12 (1.36%)	0.227	0.63
Rashes	21 (1.36%)	10 (1.51%)	11 (1.25%)	0.190	0.66
Chills	52 (3.37%)	26 (3.92%)	26 (2.95%)	1.09	0.30
Unconsciousness	46 (2.98%)	17 (2.56%)	29 (3.29%)	0.693	0.41

[Table/Fig-3]: A comparison of the distribution of symptoms present in patients by gender.
#Fisher's exact test

Test outcomes viral pathogens					
Infections	**Overall[1]**	**Female**	**Male**	**Chi-square**	**p-value**
Measles IgM (n=21)				--	>0.9[#]
Negative	18 (85.71%)	8 (88.89%)	10 (83.33%)		
Positive	3 (14.29%)	1 (11.11%)	2 (16.67%)		
Varicella Zoster IgM (n=5)				--	>0.9[#]
Equivocal	1 (20.00%)	0	1 (33.33%)		
Positive	4 (80.00%)	2 (100.00%)	2 (66.67%)		
Chikungunya IgM (n=24)				--	0.3[#]
Equivocal	1 (4.17%)	1 (11.11%)	0		
Negative	21 (87.50%)	8 (88.89%)	13 (86.67%)		
Positive	2 (8.33%)	0	2 (13.33%)		
Japanese Encephalitis (JE) IgM (n=87)				2.47	0.3
Equivocal	14 (16.09%)	5 (13.51%)	9 (18.00%)		
Negative	53 (60.92%)	26 (70.27%)	27 (54.00%)		
Positive	20 (22.99%)	6 (16.22%)	14 (28.00%)		
Dengue IgM (n=452)				0.987	0.6
Equivocal	38 (8.41%)	14 (7.11%)	24 (9.41%)		
Negative	350 (77.43%)	153 (77.66%)	197 (77.25%)		
Positive	64 (14.16%)	30 (15.23%)	34 (13.33%)		
Dengue NS1 (n=821)				--	0.3[#]
Equivocal	1 (0.12%)	0	1 (0.22%)		
Negative	800 (97.44%)	351 (98.32%)	449 (96.77%)		
Positive	20 (2.44%)	6 (1.68%)	14 (3.02%)		
Hepatitis HBsAg ELISA (n=34)				--	0.2[#]
Negative	30 (88.24%)	6 (75.00%)	24 (92.31%)		
Positive	4 (11.76%)	2 (25.00%)	2 (7.69%)		
Hepatitis HBsAg (Rapid Test) (n=40)				--	>0.9[#]
Negative	35 (87.50%)	12 (85.71%)	23 (88.46%)		
Positive	5 (12.50%)	2 (14.29%)	3 (11.54%)		
Hepatitis HAV IgM ELISA (n=10)				--	0.4[#]
Negative	5 (50.00%)	2 (100.00%)	3 (37.50%)		
Positive	5 (50.00%)	0	5 (62.50%)		
Hepatitis HAV IgM (Rapid Test) (n=19)				--	0.5[#]
Negative	16 (84.21%)	5 (100.00%)	11 (78.57%)		
Positive	3 (15.79%)	0	3 (21.43%)		
Hepatitis Anti-HCV ELISA (n=16)					
Negative	16 (100.00%)	4 (100.00%)	12 (100.00%)		
Hepatitis Anti-HCV (Rapid Test) (n=41)				--	0.5[#]
Negative	39 (95.12%)	14 (100.00%)	25 (92.59%)		
Positive	2 (4.88%)	0	2 (7.41%)		
Hepatitis HEV IgM (Rapid Test) (n=28)					
Negative	28 (100.00%)	10 (100.00%)	18 (100.00%)		
Mumps IgM (n=11)				--	0.5[#]
Equivocal	1 (9.09%)	1 (20.00%)	0		
Positive	10 (90.91%)	4 (80.00%)	6 (100.00%)		
Rubella IgM (n=18)				--	--
Negative	18 (100.00%)	9 (100.00%)	9 (100.00%)		

[Table/Fig-4a]: Test outcome of viral pathogens.
[#]Fisher's-exact test; No positive case found for Rubella IgM so Chi-square test could not be performed

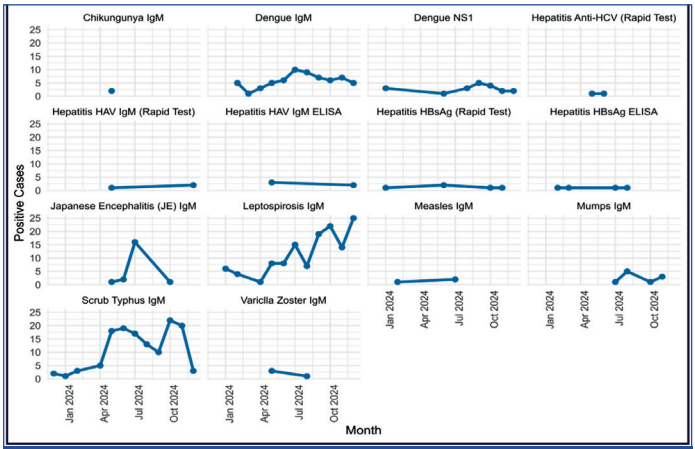
Dengue NS1 showed lower positivity with a peak in September (5 cases). JE demonstrated a distinct outbreak in July (16 cases). Other infections, including Hepatitis A, Hepatitis B, Hepatitis C, Chikungunya, Measles, Varicella-zoster, and Mumps, showed only sporadic or month-specific occurrence during the study period [Table/Fig-5].

A complex multisystem presentation of febrile illness was observed, with headache being the most common associated symptom (55.44%), followed by cough (41.13%) and bodyache (31.15%). Gastrointestinal manifestations such as vomiting (30.57%) and abdominal pain (22.09%) were also prominent, while less frequent symptoms included rhinorrhoea and unconsciousness (2.98%), reflecting variability in clinical severity and presentation [Table/Fig-6].

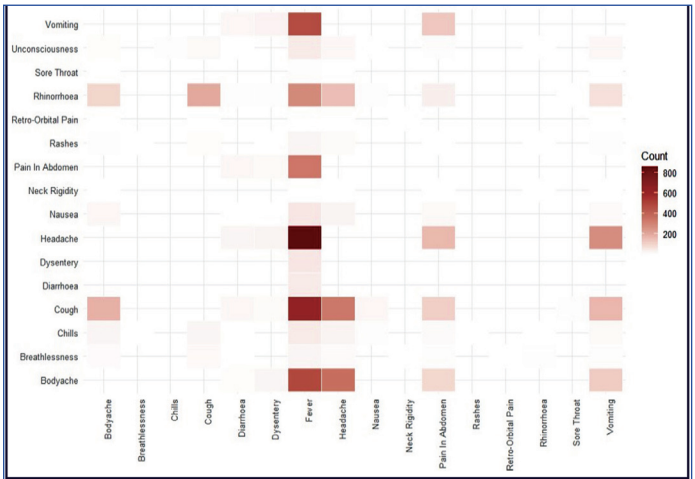
Symptom pairings were analysed instead of isolated symptom frequencies, as the diagnosis of AFI is primarily based on recognising syndromic patterns rather than individual non-specific symptoms [Table/Fig-7].

Test outcome bacterial pathogens					
Infections	**Overall[1]**	**Female[1]**	**Male[1]**	**Chi-square**	**p-value**
Leptospirosis IgM (n=473)				3.92	0.14
Equivocal	43 (9.09%)	19 (8.72%)	24 (9.41%)		
Negative	301 (63.64%)	130 (59.63%)	171 (67.06%)		
Positive	129 (27.27%)	69 (31.65%)	60 (23.53%)		
Scrub Typhus IgM (n=817)				5.97	0.051
Equivocal	13 (1.59%)	5 (1.40%)	8 (1.74%)		
Negative	671 (82.13%)	282 (78.77%)	389 (84.75%)		
Positive	133 (16.28%)	71 (19.83%)	62 (13.51%)		

[Table/Fig-4b]: Test outcome of bacterial pathogens.
n (%).



[Table/Fig-5]: A line diagram presentation of the monthly positive cases.



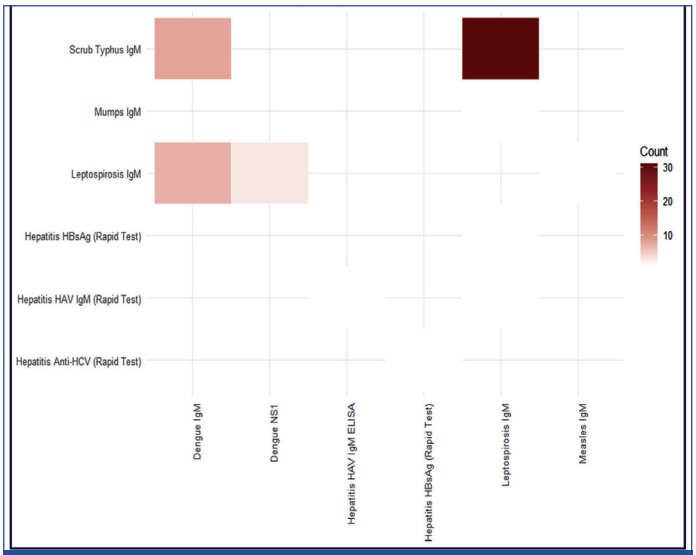
[Table/Fig-6]: A heatmap presentation of the various co-symptoms of the patients.

A complex interplay of pathogens was observed, with Leptospira, confirmed by anti-Leptospira IgM detection by ELISA, emerging as a central and highly promiscuous co-pathogen. Notably, Leptospirosis was present in seven of the ten identified co-infection pairs, frequently co-occurring with other AFI such as Scrub Typhus, Dengue infections (confirmed by either IgM or NS1), and Measles, suggesting significant epidemiological overlap and complicating clinical diagnosis and management. The final pair comparing two Hepatitis A testing methodologies represented a methodological correlation rather than a true biological co-infection [Table/Fig-8].

The combination of Leptospira IgM and Scrub Typhus IgM was the most common co-infection, identified in 31 (10.26%) cases. Additionally, dengue and scrub typhus co-positivity was observed in 8 (3.59%) cases, while co-infections involving dengue IgM and leptospirosis were reported in 7 (5.74%) cases. The occurrence of co-infection between dengue NS1 and leptospirosis was less frequent, identified only in 3 (1.41%) cases. One case each of co-infection pairs was observed in measles with leptospirosis, leptospirosis with

Symptom pair	No. of cases n (%)
Fever and headache	856 (55.44)
Fever and cough	636 (41.19)
Fever and bodyache	481 (31.15)
Fever and vomiting	476 (30.83)
Headache and bodyache	363 (23.51)
Fever and abdominal pain	347 (22.47)
Headache and cough	337 (21.83)
Fever and rhinorrhoea	280 (18.13)
Vomiting and headache	269 (17.42)
Cough and rhinorrhoea	195 (12.63)

[Table/Fig-7]: Top 10 co-symptom pairs seen in the study.



[Table/Fig-8]: A heatmap presentation of the various co-infections present in the patients.

Hepatitis B, and leptospirosis with Hepatitis A. Other unique co-occurrences observed were between Leptospirosis and Mumps, as well as infections involving combinations of hepatitis markers, each noted once. These findings highlight the prevalence and complexity of co-infections and their implications for epidemiological understanding and clinical diagnosis [Table/Fig-9].

Infection pair	No of cases (%)
Leptospirosis IgM and Scrub Typhus IgM	31 (10.26)
Dengue IgM and Scrub Typhus IgM	8 (3.59)
Dengue IgM and Leptospirosis IgM	7 (5.74)
Dengue NS1 and Leptospirosis IgM	3 (1.41)
Measles IgM and Leptospirosis IgM	1 (25.00)
Leptospirosis IgM and Hepatitis HBsAg (Rapid Test)	1 (6.67)
Leptospirosis IgM and Hepatitis HAV IgM (Rapid Test)	1 (8.33)
Leptospirosis IgM and Mumps IgM	1 (100.00)

Hepatitis HBsAg (Rapid Test) and Hepatitis Anti-HCV (Rapid Test)	1 (2.86)
Hepatitis HAV IgM ELISA and Hepatitis HAV IgM (Rapid Test)	1 (100.00)

[Table/Fig-9]: Top 10 infection pairs seen in the sampled population.
Note: The prevalence of the top 10 infection pairs was calculated based on the total number of positive cases found relative to the total number of tested pairs

DISCUSSION

Various infectious and non-infectious conditions can lead to exhibit symptoms of febrile illness. Many of these conditions have distinct combinations of signs and symptoms. Moreover, fever accompanied by a rash in children is a frequent concern among parents during childhood, often caused by benign viral exanthems that require reassurance and supportive care. However, physicians must be vigilant for distinguishing features of serious illnesses that can lead to major complications and significant morbidity and mortality. Febrile cases can also present alongside additional clinical features such as shock, conjunctivitis, abdominal pain, diarrhoea, mental status changes, pulmonary infiltrates, relative bradycardia, adenopathy, and splenomegaly [23].

In this study, the majority of positive cases were attributed to Scrub typhus, followed by leptospirosis and dengue. Scrub typhus is an emerging zoonotic infection caused by *Orientia tsutsugamushi*, an obligate intracellular, Gram-negative bacterium. Humans become infected through the bite of an infected chigger larva that carries *O. tsutsugamushi* [24]. Interestingly, among the 133 positive Scrub Typhus cases identified, eschars were not observed. This contrasts with findings from Jung HC et al., where eschars were present in 56.6% of Scrub typhus cases [25]. However, Sinha P et al., in his study, did not find eschars in any of the 42 scrub typhus patients [23]. Scrub typhus has remarkably increased as a predominant infection across all regions of India. This rise in detection can be attributed to improved availability of serological tests and PCR kits, prompting more routine testing of AFI.

Furthermore, Leptospirosis was identified as the second most common cause of AFI in this study. Out of 473 suspected cases, 129 positive cases were identified. Leptospirosis is a zoonotic infection primarily caused by pathogenic species of the genus *Leptospira*. In most cases, Leptospiral infections are either asymptomatic or present with mild, subclinical features. When symptoms do occur, they are typically non-specific and may include fever, headache, muscle pain- especially in the calves-abdominal discomfort, conjunctival suffusion, and occasionally a skin rash [25].

There were 31 cases among 1544 cases where both Scrub typhus IgM and Leptospira IgM were identified. Additionally, with over 70% of Lakhimpur and adjacent districts' population engaged in agriculture, occupational exposure to these pathogens is significantly increased [26].

Dengue was confirmed in 84 cases through IgM ELISA and early NS1antigen ELISA testing and rank as the third common cause of AFI in this study. Among these, 64 were positive for IgM antibodies, and 20 were NS1 antigen positive. Dengue (DEN) is currently the rapidly spreading viral infection worldwide, with its incidence rising nearly 30-fold over the past five decades [27]. Of the four known dengue virus serotypes (DEN 1-4), the 'Asian' genotypes of DEN-2 and DEN-3 are commonly linked to secondary dengue infections. Factors such as rapid urbanisation, globalisation, inadequate waste and water management, and growing population density have contributed to the creation of new mosquito breeding grounds, leading to a surge in dengue and rise in AFI cases. Moreover, cases of dengue co-infection with both scrub typhus and Leptospirosis were observed in this study. Eight patients were diagnosed with concurrent dengue and scrub typhus, while seven patients had co-infection of dengue and Leptospira. Dengue and Scrub typhus co-infection is uncommon but medically significant due to shared symptoms and the potential for severe consequences. Similar cases are reported from regions where both diseases are highly endemic, including Tamil Nadu, Puducherry, West Bengal, and the Himalayan foothills [28].

In India, pooled prevalence of Dengue and Leptospira co-infection among AFI cases ranges from 2.3% to 4% [29], with higher rates observed in southern regions and areas with heavy rainfall. Such co-infections are more frequently reported in tertiary care settings during the rainy season.

Additionally, 20 positive JE cases were identified in the study population. Annual flooding in Assam because of heavy rainfall, especially in Lakhimpur and Dhemaji districts, creates stagnant water bodies that provide ideal breeding sites for *Culex* mosquitoes, the primary vectors of JE [30]. The district's proximity to the Brahmaputra river intensifies water logging in health blocks like Boginadi and North Lakhimpur, where JE cases are notably concentrated. Additionally, the widespread practice of pig farming- with pigs often reared in loose housing near paddy fields- contributes to the disease's transmission, as pigs serve as amplifying hosts for the JE virus. This is reflected in the high positivity rates observed in both pigs and humans in Lakhimpur district (up to 10.52% in Boginadi) [31].

Eventually, a total of 67% of AFI cases in this study remained undiagnosed, which may be attributed to the absence of testing procedures for rarer infections/inflammatory conditions in this study. Furthermore, none of the undiagnosed cases resulted in any serious complications.

While individual symptoms provide an overview of disease prevalence, analysis of co-occurring symptom pairs offers better insight into the clinical patterns encountered in practice. Frequently observed combinations, such as fever with headache and fever with vomiting, highlight common neuro-constitutional and gastrointestinal presentations. Recognition of these symptom clusters may aid differential diagnosis, improve triaging and early empirical management, and support development of more sensitive syndromic case definitions, particularly in resource-limited settings.

Limitation(s)

The study has ended with a few limitations. First, Polymerase Chain Reaction (PCR) testing could not be performed due to financial and time constraints. Adopting PCR assay might have identified additional causative agents in AFI cases, given its high sensitivity. Secondly, data on prophylactic and post-prophylactic measures, as well as patient outcomes, were unavailable due to limited resources. Additionally, multiple statistical comparisons between sexes raised the potential for inflated Type I error rates. The constraint of the current study is that the diagnosis of Scrub typhus and Leptospira was made only on the basis of ELISA, and the possibility of cross-reactivity cannot be ruled out. To maintain sensitivity in this exploratory analysis, stringent post-hoc adjustments were not applied. As a result, individual significant findings remain preliminary and require validation in larger, prospective studies.

CONCLUSION(S)

The majority of AFI cases can be accurately diagnosed using a combination of detailed medical history, thorough physical examination, and targeted laboratory investigations. In this region, i.e., the north bank of eastern Assam, serological tests like ELISA and Rapid Detection Kits (RDTs) have proven reliable for identifying the aetiology of AFI cases. The primary causes of AFI in this study were scrub typhus, Leptospira, and dengue, which made up most cases. JE followed as a less common but significant contributor. These findings underscore the value of accessible, region-specific diagnostics to guide timely treatment and improve patient outcomes.

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