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Exploring Neglected Pathogens In Acute Undifferentiated Fever; A Study Of Microbiological & Immunological Factors At A Tertiary Care Hospital

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ABSTRACT

Background: Acute undifferentiated fever (AUF) is a common clinical presentation in tropical and subtropical regions and often poses diagnostic challenges due to its non-specific symptoms and overlapping clinical features. While malaria and dengue are routinely screened, several neglected tropical infections remain underdiagnosed, resulting in delayed treatment, inappropriate antimicrobial use, and poor clinical outcomes.

Objectives: To determine the prevalence of neglected tropical pathogens among patients presenting with AUF and to evaluate their microbiological and immunological profiles.

Materials and Methods: A prospective cross-sectional study was conducted over six months in a tertiary care hospital. Patients of any age presenting with fever ≥38.5°C for more than five days without identifiable localizing signs were included. Clinical evaluation, baseline laboratory investigations, and serological testing (IgM ELISA) for leptospirosis, scrub typhus, brucellosis, and chikungunya were performed. Data analysis was carried out using SPSS software.

Results: Among 200 AUF patients, neglected tropical pathogens were identified in 6% of cases. Leptospirosis was the most common infection (3%), followed by chikungunya (2%) and scrub typhus (1%). No cases of brucellosis or anti-dsDNA positivity were detected. Patients diagnosed with leptospirosis and scrub typhus demonstrated rapid clinical improvement following empirical doxycycline therapy.

Conclusion: Neglected tropical infections represent an important proportion of AUF etiologies. Incorporating targeted serological assays into routine diagnostic workflows can enable early and accurate pathogen-specific diagnosis, facilitate timely therapeutic interventions, and promote antimicrobial stewardship.

KEYWORDS: Acute undifferentiated febrile illness, scrub typhus, chikungunya, leptospirosis, neglected tropical infections..

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INTRODUCTION

Acute undifferentiated febrile illness (AUFI) remains a major public health concern in tropical and subtropical regions, where patients frequently present with persistent fever without an identifiable clinical focus after initial evaluation [1,2]. The non-specific nature of symptoms such as fever, headache, myalgia, arthralgia, and rash often complicates early diagnosis and leads to empirical antimicrobial therapy. Such practices contribute to inappropriate antibiotic use and accelerate the emergence of antimicrobial resistance.

The etiological spectrum of AUFI is broad and includes infections such as malaria, dengue, scrub typhus, leptospirosis, enteric fever, brucellosis, and chikungunya. Many of these conditions are zoonotic or vector-borne and are classified as neglected tropical diseases due to inadequate surveillance systems, limited diagnostic capacity, and under-recognition within routine healthcare settings [3,4]. In India, AUFI continues to impose a substantial disease burden, particularly among paediatric and young adult populations, resulting in significant morbidity, prolonged hospitalisation, and avoidable healthcare costs [1]. Hospital-based studies from tertiary care centres in North India have further demonstrated that AUFI is caused by multiple pathogens, often with overlapping clinical presentations and occasional co-infections, highlighting the need for improved etiological identification and region-specific diagnostic strategies [23].

Leptospirosis, caused by pathogenic *Leptospira* species, is an environmentally transmitted zoonosis associated with exposure to contaminated water or soil and remains an important cause of febrile illness in endemic regions. Globally, leptospirosis accounts for more than one million infections annually and is associated with considerable mortality [5,6]. Brucellosis, most commonly caused by *Brucella abortus*, typically presents as a prolonged febrile illness with non-specific manifestations and continues to affect hundreds of thousands of individuals worldwide each year [7]. *Chikungunya*, an arboviral infection

transmitted by *Aedes* mosquitoes, is characterised by acute fever, rash, and debilitating arthralgia, frequently mimicking dengue and complicating clinical diagnosis in regions where multiple arboviral infections co-circulate [8,9].

In addition to infectious etiologies, autoimmune conditions such as systemic lupus erythematosus may present with fever and systemic symptoms that closely resemble AUFI. Detection of anti-double stranded DNA (anti-dsDNA) antibodies is essential for identifying autoimmune causes of fever and for differentiating them from infectious conditions. Accurate distinction between infectious and autoimmune etiologies is critical to avoid unnecessary antimicrobial therapy and to ensure timely initiation of appropriate immunomodulatory treatment [10].

Given the overlapping clinical features and limitations of syndromic diagnosis, a comprehensive diagnostic approach incorporating both infectious and non-infectious etiologies is essential for effective management of AUFI. The present study evaluates neglected infectious agents, including leptospirosis, scrub typhus, brucellosis, and chikungunya virus, alongside autoimmune markers such as anti-dsDNA antibodies, in patients presenting with AUFI at a tertiary care hospital. By integrating microbiological and immunological diagnostic strategies, this study aims to improve etiological detection, guide targeted therapy, and promote rational antimicrobial use in endemic healthcare settings. From a global health perspective, this work aligns with the United Nations Sustainable Development Goal 3—Good Health and Well-Being, particularly target 3.3, which focuses on reducing the burden of neglected tropical diseases through early diagnosis and effective management.

MATERIAL & METHODOLOGY

Study Design and Setting

This hospital-based, prospective cross-sectional study was conducted over six months (January–June 2025) in the Department of Microbiology, IIMSR, Lucknow, a tertiary care teaching hospital catering to urban and rural populations.

Inclusion criteria

Patients of any age group presenting with acute undifferentiated fever (temperature ≥38.5 °C) of more than five days' duration, without localising signs of infection on initial clinical evaluation, were eligible for inclusion. Written informed consent was obtained from adult participants or guardians of minors, with assent from children where applicable.

Exclusion Criteria

Patients were excluded if they had chronic or immunosuppressive conditions (e.g., HIV infection, malignancy), were on immunosuppressive therapy, or provided haemolysed, lipaemic, inadequately labelled, or insufficient samples. Those who declined or withdrew consent were also excluded. To prevent diagnostic overlap, individuals who tested positive for dengue (NS1/IgM) or malaria (rapid antigen test or peripheral smear) at initial screening were not included. Only patients with confirmed negative results for both infections were evaluated for alternative AUFI etiologies.

Data Collection and Statistical Analysis

Demographic, clinical, and laboratory data were recorded in a structured proforma. Statistical analysis was performed using Microsoft Excel. Categorical variables were expressed as frequencies and percentages, while continuous variables were presented as means ± standard deviation. Associations between categorical variables were assessed using the Chi-square test; however, when expected cell counts were less than five, Fisher's exact test was applied to ensure validity. Exact 95% confidence intervals (CIs) were calculated for proportions, and p-values were reported only where statistically relevant, with $p < 0.05$ considered significant.

Ethical Approval

This study was approved by the Institutional Ethics Committee of Integral Institute of Medical Sciences and Research, Integral University, Lucknow (Approval No. IEC/IIMSR/2025/21; Date: 11th Feb 2025).

Laboratory Investigations: Serological testing for acute undifferentiated febrile illness (AUFI) was performed using IgM ELISA or rapid test kits for leptospirosis, scrub typhus, and dengue, following the manufacturer's instructions. It is acknowledged that confirmatory testing for scrub typhus and leptospirosis ideally requires paired serum samples or molecular assays such as PCR, indirect immunofluorescence assay (IFA), or microscopic agglutination test (MAT). However, due to logistical and cost constraints in the present study setting, single-sample IgM detection was used for case identification. The diagnostic kits employed had reported sensitivities and specificities exceeding 90%, although cross-reactivity with other febrile illnesses is known. Borderline or equivocal results were considered negative unless supported by consistent clinical features and relevant epidemiological context.

All participants were screened for dengue and malaria as part of the differential diagnosis of AFI. Both positive and negative results were documented. Patients testing positive for dengue or malaria were excluded from further analysis of other febrile illnesses to avoid diagnostic overlap, while negative cases were included for evaluation of alternative etiologies (e.g., *Leptospirosis, Scrub typhus* infections).

Sample Collection and Processing

From each participant, 5 mL of venous blood was collected aseptically. Serum was separated and stored at –20°C until testing. Serum samples from each participant were subjected to the following diagnostic assays:

Leptospira IgM ELISA
Kit: Panbio Leptospira IgM ELISA (Panbio, Australia).
Procedure: Performed strictly according to the manufacturer's instructions. Serum samples, calibrators, and controls were diluted as per kit protocol, added to pre-coated microplates, and incubated under specified conditions. After washing, enzyme conjugate was added, followed by substrate. Optical density (OD) was measured at the recommended wavelength using a microplate reader.
Interpretation: Results were determined by comparing sample OD values to the calibrator/control as per the manufacturer's cut-off criteria.

Anti-Brucella IgM ELISA
Kit: EUROIMMUN Anti-Brucella abortus IgM ELISA (EUROIMMUN, Germany).
Procedure: Conducted according to the kit protocol. Patient sera and controls were diluted appropriately, incubated on Brucella antigen-coated wells, washed, and treated with enzyme-labelled anti-human IgM. Substrate reaction was stopped, and OD was read at the specified wavelength.
Interpretation: Sample-to-calibrator OD ratio was calculated and interpreted according to kit guidelines.

Anti-dsDNA IgG ELISA
Kit: EUROIMMUN Anti-dsDNA ELISA (IgG) (EUROIMMUN, Germany).
Purpose: Screening for autoimmune disease markers.
Procedure: Followed the manufacturer's instructions. Serum samples were diluted and incubated on plates coated with double-stranded DNA. Bound antibodies were detected with enzyme-labelled anti-human IgG, followed by substrate addition. OD was read at the recommended wavelength.
Interpretation: Results expressed in international units per millilitre (IU/mL) and categorised as negative, borderline, or positive according to kit guidelines.

Scrub Typhus IgM/IgG Rapid Test
Kit: Commercial lateral flow immunoassay (J Mitra).
Procedure: Test cassette was equilibrated to room temperature. Using the sample dropper, the specified volume of serum/plasma was added to the sample well, followed by assay buffer. The cassette was incubated at room temperature for 20 minutes.
Interpretation: Presence of a coloured test line along with a control line was considered positive for the respective antibody class.

Chikungunya IgM/IgG Rapid Test
Kit: Commercial lateral flow immunoassay (SD Biosensor).
Procedure: Conducted as per manufacturer's protocol. Serum/plasma was applied to the test cassette sample well, followed by buffer addition. The result was read after 20 minutes.
Interpretation: A visible test line with control line was interpreted as positive for the corresponding antibody.
Quality Control: All ELISA runs included manufacturer-supplied positive and negative controls. Rapid tests were performed with appropriate internal controls (control line validation).

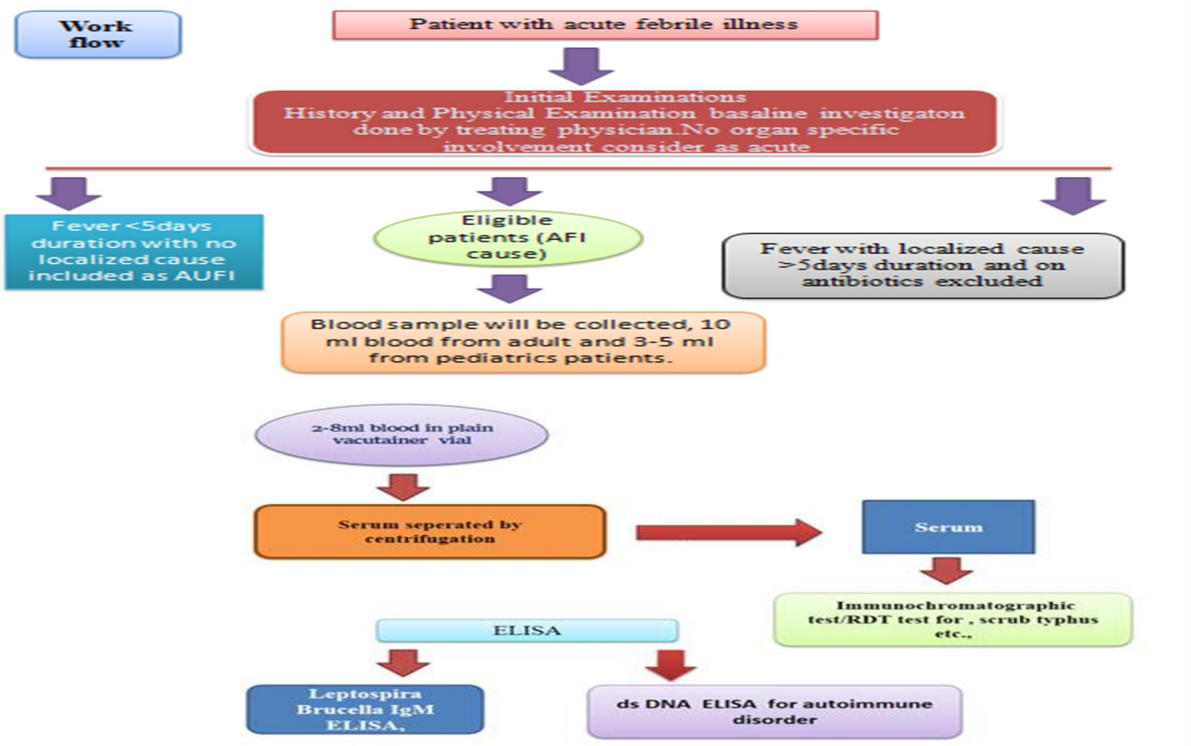

Fig: 1. Work Flow of Acute Febrile illness

RESULT

A total of 200 patients with acute undifferentiated febrile illness (AUFI) of more than five days' duration were enrolled. Males constituted 60% of the cohort, with the highest prevalence observed in the 21–35 years age group (32%), followed by patients aged ≤ 20 years (30%). The most common symptoms were headache (25.5%), arthralgia (25%), myalgia (22%), and rash (16.5%). Hepatomegaly, splenomegaly, and lymphadenopathy were less frequent, occurring in 10.5%, 8%, and 7% of patients, respectively (Table 1).

Table 1: Demographic and Clinical Characteristics of Patients with AUFI (n= 200)

Characteristic	Value
Gender	
Male	120 (60%)
Female	80 (40%)
Age Group (Years)	
<20	60 (30%)
21-35	64 (32%)
36-50	44 (22%)
>51	32(16%)
Clinical features	
Fever (>5 days)	200 (100%)
Headache	51 (25.5 %)
Myalgia	45 (22%)
Arthralgia	50 (25%)
Rash	33 (16.5%)
Hepatomegaly	21 (10.5 %)
Splenomegaly	16 (8%)
Lymphadenopathy	14 (7%)

Diagnostic testing identified *Leptospira* spp. in 6 patients (3.0%) by IgM ELISA, *Scrub Typhus* in 2 patients (1.0%) by IgM/IgG rapid test, and *Chikungunya* virus in 4 patients (2.0%) by IgM/IgG rapid test. No cases of *Brucella* spp. infection or anti-double stranded DNA (anti-dsDNA) positivity were detected (0%) (Table 2).

Table 2: Microbiological Agents and Autoimmune Markers Identified in Patients with AUFI

Pathogen	Positive Cases (N= 12)	Percentage (%)	Diagnostic Method Used
Leptospira spp.	6	3%	ELISA
(Scrub Typhus)	2	1.0%	Immuno-chromatographic assay

			IgM/ IgG
Brucella spp.	0	0%	ELISA
Chikungunya virus	4	2.0%	Immuno-chromatographic Assay IgM/ IgG
Anti-double stranded DNA (anti-dsDNA)	0	0%	ELISA

Dual infections were observed in 2 patients (1.0%), with combinations involving *Leptospira*, *Scrub Typhus*, or *Chikungunya* virus. No patient demonstrated concurrent positivity for autoimmune markers and infectious agents (Table 3).

Table 3: Co-infections and Overlap with Autoimmune Markers in AUFI Patients

Category	Number of Patients (n)	Percentage (%)	Remarks
Patients with dual infections	2	1.0 %	Most common: Leptospira + Scrub Typhus; Chikungunya
Patients with positive autoimmune markers and concurrent infection	0	0 %	Suggests possible infectious trigger or diagnostic overlap

The most frequently reported risk factors included tick or mosquito bites (41.7%), rural residence (33.3%), and recent travel to endemic areas (25%). Additional exposures included contact with stagnant water (25%), animal contact (16.7%), and agricultural work (16.7%). A history of known autoimmune disease was present in 8.3% of cases (Table 4).

Table 4: Risk Factors Among AUFI Patients

Risk Factor	Exposed with Infection (n = 12)
Exposure to stagnant water	3 (25%)
Contact with animals/livestock	2 (16.7%)
Rural residence	4 (33.3%)
Agricultural/field work	2 (16.7%)
Travel to endemic area	3 (25%)
Tick/mosquito bites	5 (41.7%)
Known autoimmune disease	1 (8.3%)

All 12 infected patients were hospitalized for observation and management, while non-infected AUFI cases were managed as outpatients. The mean duration of hospital stay among hospitalized patients was **6 ± 0.58 days**, suggesting a narrow variation owing to the small number of confirmed cases. However, given the limited sample size, the **median duration was 6 days [IQR: 5–7]**, which better represents the central tendency. All patients demonstrated clinical recovery following appropriate antimicrobial or supportive therapy, and **no deaths were reported (0%)** (Table 5).

Table 5: Outcome and Duration of Hospital Stay Among AUFI Patients

Outcome Parameter	Value
Mean duration of hospital stay (days ± SD)	6 days ± 0.58
Median (IQR) duration of stay (days)	6 [5-7]
Type of patient	All 12 infected patients were hospitalized; others were managed as outpatients
Mortality	0
Treatment response	100% (All patients improved with appropriate antimicrobial or supportive therapy)

DISCUSSION

This study provides early but meaningful insight into the etiological profile of acute undifferentiated febrile illness (AUFI) in a tertiary care hospital in Northern India, a region where overlapping clinical presentations often challenge diagnostic accuracy. Despite a modest number of laboratory-confirmed cases, the identification of neglected tropical pathogens—including leptospirosis, scrub typhus, and chikungunya—highlights their ongoing circulation and clinical importance. These findings reinforce the need for strengthened diagnostic vigilance and improved recognition of zoonotic and vector-borne infections that frequently remain underdiagnosed in AUFI.

Leptospira spp. emerged as the most common pathogen (3.0%), aligning with previous Indian studies that report leptospirosis as a persistent public health concern, particularly in settings characterized by heavy rainfall, waterlogging, and inadequate sanitation [11,12]. The association between stagnant water exposure and Leptospira positivity in our cohort supports established environmental and occupational risk factors for transmission [13]. Scrub typhus (1.0%) and chikungunya (2.0%) were detected at lower rates, reflecting their endemic yet sporadic patterns in this region [14]. The limited detection of scrub typhus may be attributable to the known lower sensitivity of rapid antibody tests compared with paired-serum IFA or PCR, which remain the diagnostic gold standards [15,16]. The absence of *Brucella* spp. mirrors earlier Indian data suggesting a limited contribution of brucellosis to AUFI, even among high-risk groups [17].

No cases were positive for anti-dsDNA antibodies, indicating no autoimmune mimicry in this cohort. Although autoimmune disorders such as systemic lupus erythematosus can present as AUFI [18], their absence suggests that infectious etiologies predominated in this setting. Nonetheless, selective autoimmune screening remains important to avoid misdiagnosis and unnecessary antimicrobial use.

The risk factor analysis further supports the multifactorial transmission dynamics of AUFI. Exposures such as tick and mosquito bites, rural habitation, animal contact, and agricultural work were frequently reported—consistent with known epidemiological drivers of rickettsial, leptospiral, and arboviral illnesses [19]. Integration of such epidemiological cues with laboratory testing is critical, particularly in resource-limited healthcare environments where reliance on serology is common.

Clinically, the study underscores the usefulness of an algorithmic diagnostic approach incorporating early serological testing for leptospirosis, *Scrub typhus* and *Chikungunya*, guided by exposure history and clinical presentation. This approach facilitates timely initiation of pathogen-directed therapy, limits reliance on broad-spectrum antimicrobials, and supports antimicrobial stewardship programs [20–22]. The complete recovery of all confirmed cases, with no associated mortality, emphasizes the impact of early diagnosis and appropriate management.

While the number of confirmed infections was small, the findings provide practical, real-world evidence relevant to clinicians managing AUFI in endemic regions. Detecting even a limited number of neglected tropical pathogens offers valuable preliminary data that can inform empirical treatment strategies and reduce the burden of undiagnosed febrile illness. Future studies with larger sample sizes, multi-centre participation, seasonal assessments, and incorporation of molecular diagnostic tools are required to strengthen etiological attribution and better define the epidemiology of AUFI in northern India.

DISCUSSION

The present study contributes valuable preliminary evidence on the etiological landscape of acute undifferentiated febrile illness (AUFI) in a tertiary care centre in Northern India, a region where clinical overlap among febrile illnesses frequently complicates accurate diagnosis. Although the proportion of laboratory-confirmed cases was relatively small, the detection of neglected tropical infections such as leptospirosis, scrub typhus, and chikungunya confirms their persistent circulation and clinical relevance. These findings highlight the continued need for heightened diagnostic awareness and systematic evaluation of zoonotic and vector-borne pathogens that are often overlooked during routine assessment of AUFI.

Leptospira spp. were identified as the most frequently detected pathogen, consistent with earlier Indian studies describing leptospirosis as an enduring public health concern, particularly in areas affected by monsoon rainfall, poor drainage, and suboptimal sanitation infrastructure [11,12]. The observed association with exposure to stagnant water further supports established environmental and occupational risk factors that facilitate transmission [13]. In contrast, scrub typhus and chikungunya were detected at lower frequencies, reflecting their endemic but episodic occurrence in the region [14]. The relatively low detection of scrub typhus may be partially explained by the limited sensitivity of rapid serological assays when compared with confirmatory techniques such as paired-serum indirect immunofluorescence assay or polymerase chain reaction, which remain the reference standards but are often inaccessible in routine clinical settings due to cost and logistical constraints [15,16]. The absence of Brucella species in the study population aligns with previous reports suggesting a comparatively minor role of brucellosis in AUFI presentations in India, even among populations with potential occupational exposure [17].

No evidence of autoimmune disease was identified, as none of the participants demonstrated anti-double stranded DNA antibody positivity. Although autoimmune conditions such as systemic lupus erythematosus are recognised causes of fever with systemic manifestations resembling AUFI [18], their absence in this cohort indicates a predominance of infectious etiologies. Nonetheless, selective screening for autoimmune markers remains clinically relevant to prevent diagnostic errors and avoid unnecessary antimicrobial administration.

Evaluation of exposure history revealed multiple epidemiological risk factors, including insect bites, rural residence, animal contact, and agricultural activities. These findings reflect the complex and multifactorial transmission dynamics associated with rickettsial, leptospiral, and arboviral infections [19]. Incorporating epidemiological context alongside laboratory investigations is particularly important in resource-constrained healthcare environments, where reliance on serological testing is common and diagnostic uncertainty is high.

From a clinical perspective, the findings support the use of a structured diagnostic approach that integrates early serological testing for leptospirosis, scrub typhus, and chikungunya with careful assessment of exposure history and clinical features. Such an approach enables timely initiation of pathogen-specific therapy, reduces dependence on empirical broad-spectrum antibiotics,

and strengthens antimicrobial stewardship efforts [20–22]. The favourable outcomes observed in all confirmed cases, including complete recovery and absence of mortality, underscore the importance of early diagnosis and appropriate management in AUFI.

Despite limitations related to sample size and single-centre design, this study provides practical, real-world insights relevant to clinicians working in endemic regions. Identification of even a small number of neglected tropical infections offers meaningful evidence to inform diagnostic algorithms and guide empirical treatment decisions. Future investigations incorporating larger cohorts, multi-centre participation, seasonal variation analysis, and molecular diagnostic methods are necessary to enhance etiological clarity and better characterise the epidemiology of AUFI in northern India.

From a broader public health perspective, the findings of this study are consistent with the objectives of the United Nations Sustainable Development Goal (SDG) 3—Good Health and Well-Being. Improved etiological identification of neglected tropical pathogens in AUFI enables timely and appropriate therapy, limits unnecessary antimicrobial use, and reduces the risk of complications and prolonged hospitalisation. These outcomes directly support SDG target 3.3, which aims to reduce the burden of neglected tropical diseases, as well as SDG target 3.d, which focuses on strengthening health system capacity for early detection, risk mitigation, and effective management of public health threats in low- and middle-income countries.

CONCLUSION

This study identifies *Leptospira spp*., *Scrub typhus*, and *Chikungunya* as important yet frequently overlooked contributors to acute undifferentiated febrile illness (AUFI) in our region, with *Leptospira* emerging as the leading detectable pathogen. Although detection rates were modest, the consistent presence of vector-borne and environmentally linked infections underscores the critical need for thorough epidemiological assessment in patients presenting with fever without a clear source. The complete absence of *Brucella* spp. and anti-dsDNA positivity further indicates that infectious etiologies are far more prevalent than autoimmune causes in this population. These findings emphasize the importance of early, targeted diagnostic testing to improve clinical decision-making, guide appropriate therapy, and prevent unnecessary antimicrobial use. Collectively, this work strengthens the evidence for incorporating neglected tropical infections into routine AUFI diagnostic algorithms and highlights the need for broader surveillance and advanced diagnostic tools to better define their true burden.

Declarations

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REFERENCES

1. Chrispal, A., Boorugu, H., Gopinath, K. G., Chandy, S., Prakash, J. A. J., Thomas, K., ... & Abraham, O. C. (2010). Acute undifferentiated febrile illness in adult hospitalized patients: The disease spectrum and diagnostic predictors—An experience from a tertiary care hospital in South India. *Tropical Doctor, 40*(4), 230–234. https://doi.org/10.1258/td.2010.100132
2. Indian Council of Medical Research. (2015). *Surveillance of acute febrile illness: Guidance for state public health laboratories*.
3. Susilawati, T. N., & McBride, W. J. H. (2014). Acute undifferentiated fever in Asia: A review of the literature. *Southeast Asian Journal of Tropical Medicine and Public Health, 45*(3), 719–726.
4. Silva-Caso, W., Aguilar-Luis, M. A., Palomares-Reyes, C., Mazulis, F., Zavaleta, M., Del Valle, L. J., ... & del Valle, L. J. (2024). Neglected tropical diseases in acute febrile illness: An under-recognised burden in endemic areas. *The American Journal of Tropical Medicine and Hygiene, 110*(2), 238–247.
5. **Gouveia, A. C., & Freitas, H. (2008).** Intraspecific competition and water use efficiency in *Quercus suber*: Evidence of an optimum tree density? *Trees*, **22**(4), 521–530. https://doi.org/10.1007/s00468-008-0219-5
6. Luce-Fedrow, A., Lehman, M. L., Kelly, D. J., Mullins, K., Maina, A. N., Stewart, R. L., & Richards, A. L. (2018). A review of scrub typhus (*Orientia tsutsugamushi*) and the human immune response. *PLoS Neglected Tropical Diseases, 12*(8), e0006864.
7. Saddique, A., Ali, I., Aslam, M. A., Younus, M., & Irfan, M. (2019). Seroprevalence and associated risk factors of brucellosis in humans and domestic ruminants in Pakistan. *Veterinary Medicine and Science, 5*(4), 556–564.

8. Mohan, A., Kiran, D. H., Manohar, I. C., & Kumar, D. P. (2010). Epidemiology, clinical manifestations, and diagnosis of chikungunya fever: Lessons learned from the re-emerging epidemic. *Indian Journal of Dermatology, 55*(1), 54–63. https://doi.org/10.4103/0019-5154.60355
9. Kumar, N. P., Joseph, R., Kamaraj, T., Sabesan, S., Jambulingam, P., & Murugan, D. (2019). Molecular epidemiology of chikungunya virus isolates from India (2006–2010). *PLoS Neglected Tropical Diseases, 13*(5), e0007365. https://doi.org/10.1371/journal.pntd.0007365
10. Karakeçe, E., Yüceer, E., & Karahan, S. (2015). Anti-nuclear antibody and anti-dsDNA antibody profiles in patients with autoimmune diseases. *Journal of Immunology Research*, 261493. https://doi.org/10.1155/2015/261493
11. **Sethi S, Sharma N, Kakkar N, Taneja J, Chatterjee SS, Banga SS, et al.(2010)** *Increasing Trends of Leptospirosis in Northern India: A Clinico-Epidemiological Study.* PLoS Negl Trop Dis;4(1):e579.
12. **Pappachan MJ, Mathew S, Aravindan KP, Khader A, Bharghavan PV, Abduljaleel F, et al. (2004)** *Risk factors for mortality in patients with leptospirosis during an epidemic in northern Kerala.* Natl Med J India;17(5):240–2.
13. Haake, D. A., & Levett, P. N. (2015). Leptospirosis in humans. *Current Topics in Microbiology and Immunology*, 387, 65–97.
14. **Rathi NB, Rathi AN, Goodman MH, Aghai ZH.** (2011) Rickettsial diseases in central India: proposed clinical scoring system for early detection of spotted fever. *Indian Pediatr.* 2011;48:867–873. Lippincott JournalsPMC
15. **Varghese GM, et al.** (2013) Scrub typhus in South India: clinical and laboratory manifestations… *Int J Infect Dis*;17:e981–e987. Paired-serum IFA testing (2–3 weeks apart) significantly improves sensitivity. PMCFrontiersicjournal.org
16. **Fomda B, Abdullah N, Mir Y, et al.** (2021) Comparative evaluation of ELISA, Weil-Felix with IFA (gold standard) for rickettsial diagnosis in North India; ELISA demonstrated superior performance. *Indian J Med Microbiol.* Dec 2021. ResearchGate
17. **Mantur BG, Amarnath SK.** (2008) Brucellosis in India – a review. *J Biosci.*Nov;33(4):539–547.
18. Zandman-Goddard, G., & Shoenfeld, Y. (2005). Fever and autoimmune diseases. *Autoimmunity Reviews*, 4(1), 33–35.
19. **Kumar NP, Prakash JAJ, Sudharsan TI, Varghese GM, Davis TP.(2018)** *Scrub typhus and other rickettsial infections in India: epidemiology, clinical presentations, and diagnostic challenges.* **Pathog Glob Health**; 112 (1) :3 – 20. https://doi.org/10.1080/20477724.2017.1417755
20. **Ahmed A, Kumar R, Jain P, Sharma P, Singh AK. (2019)** *Diagnostic algorithm for acute undifferentiated fever: a prospective hospital-based study from India.* **PLoS Negl Trop Dis**;13(3):e0007137. https://doi.org/10.1371/journal.pntd.0007137
21. **Kumar A, Verma A, Singh S, Sharma R, Gupta V. (2021)** *Acute undifferentiated febrile illness in India: challenges in diagnosis and management.* **J Assoc Physicians India**; 69(7): 11–15.
22. **Singh R, Basu S, Mehta P, Agrawal M. (2020)** *Role of epidemiological history in the diagnosis of febrile illness in endemic regions.* **Indian J Med Res**; 151 (6) : 516 – 522. https://doi.org/10.4103/ijmr.IJMR_1648_19
23. Siddiqui AH, Dubey S. **Study of acute febrile illness cases in a tertiary care hospital – a cross-sectional study**. In: *Proceedings of the International Scientific and Practical Conference "Status and Development Prospects of Fundamental and Applied Microbiology: The Viewpoint of Young Scientists"*; 25–26 September 2024. Zenodo. https://doi.org/10.5281/zenodo.13828326