

Original Research Article

PAEDIATRIC LEPTOSPIROSIS: COMPARATIVE DIAGNOSTIC PERFORMANCE OF SEROLOGICAL TESTS AND PCR IN A TERTIARY-CARE SETTING

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ABSTRACT

Background: Paediatric leptospirosis is frequently underdiagnosed because its clinical manifestations overlap with other causes of acute febrile illness and the performance of available laboratory tests varies with illness duration. This study compared different serological and molecular methods for diagnosing leptospirosis among clinically suspected children attending a tertiary-care centre.

Materials and Methods: This prospective hospital-based diagnostic study included 173 children with clinically suspected leptospirosis. Demographic, epidemiological, clinical and laboratory information was recorded. Serum samples were evaluated using macroscopic slide agglutination test (MSAT), IgM enzyme-linked immunosorbent assay (ELISA) and microscopic agglutination test (MAT), while EDTA blood was tested by polymerase chain reaction (PCR) targeting the pathogenic *Leptospira* lipL32 gene. Laboratory positivity was defined as MAT titre $\geq 1:100$ and/or PCR positivity. Diagnostic performance was calculated using MAT as the conventional reference, and inter-test agreement was assessed using Cohen's kappa.

Results: Fifty children (28.9%) were laboratory-positive by MAT and/or PCR. Positivity was significantly associated with rural residence ($p=0.024$), monsoon/post-monsoon presentation ($p=0.003$), stagnant-water exposure ($p=0.001$), animal contact ($p=0.010$), rodent exposure ($p=0.002$) and barefoot outdoor activity ($p=0.003$). Conjunctival suffusion, calf tenderness, jaundice, hepatomegaly, thrombocytopenia and acute kidney injury were significantly more frequent among positive children. MSAT, IgM ELISA, MAT and PCR were positive in 26.0%, 22.5%, 20.2% and 15.6%, respectively. IgM ELISA demonstrated 91.4% sensitivity, 94.9% specificity and 94.2% accuracy against MAT, with almost-perfect agreement ($\kappa=0.828$). PCR positivity was highest within five days of illness (24.3%), whereas MAT and IgM ELISA positivity increased after the fifth day. Pomona was the predominant presumptive serogroup (25.7%), followed by Icterohaemorrhagiae (20.0%).

Conclusion: The diagnostic yield of leptospirosis tests varied substantially with illness duration. PCR was particularly useful during early illness, whereas IgM ELISA and MAT performed better during the immune phase. Combining molecular and serological methods may improve timely diagnosis of paediatric leptospirosis.

Keywords: Paediatric leptospirosis; IgM ELISA; microscopic agglutination test; polymerase chain reaction; MSAT.

INTRODUCTION

Leptospirosis is a globally distributed zoonotic infection caused by pathogenic spirochaetes of the

genus *Leptospira*. Humans acquire infection through direct contact with infected animals or, more commonly, through exposure of abraded skin or mucous membranes to soil and water contaminated

with animal urine.^[1] Its transmission is favoured by heavy rainfall, flooding, poor sanitation, rodent infestation and close human–animal interaction. Globally, leptospirosis is estimated to cause approximately 1.03 million cases and 58,900 deaths annually, with a disproportionate burden in tropical, resource-constrained regions.^[2] In India, the disease is endemic in several coastal and high-rainfall states, where it frequently contributes to acute undifferentiated febrile illness, particularly during and after the monsoon.^[3]

Although leptospirosis has traditionally been regarded as an occupational disease of adults, children are also vulnerable because of recreational exposure to stagnant water, barefoot outdoor activities and frequent contact with animals. A population-based study from Chennai detected leptospirosis in 26.1% of clinically suspected children.^[4] while another paediatric study reported an overall seropositivity of 30.8%.^[5] Clinical manifestations in children are often non-specific and range from fever, headache, myalgia, vomiting and abdominal pain to jaundice, thrombocytopenia, meningitis, pulmonary haemorrhage, acute kidney injury and multiorgan dysfunction. Consequently, the illness may be misdiagnosed as dengue, malaria, enteric fever, scrub typhus or viral hepatitis.^[1,3]

Laboratory confirmation is therefore crucial. The microscopic agglutination test (MAT) is conventionally considered the reference serological method; however, it requires live leptospiral cultures, locally representative serovars and specialised expertise.^[6] IgM enzyme-linked immunosorbent assay offers a simpler screening alternative but may remain negative during the first 5–6 days of illness, while persistent antibodies can complicate interpretation in endemic areas.^[7,8] Polymerase chain reaction can directly detect leptospiral DNA during early leptospiraemia, before detectable antibody development, although its yield depends on specimen type, bacterial load, illness duration and prior antibiotic exposure.^[8,9] Comparative paediatric evidence evaluating these tests remains limited. Therefore, the present study aimed to assess and compare different serological and molecular methods for diagnosing leptospirosis among clinically suspected children attending a tertiary-care centre.

MATERIALS AND METHODS

Study design and setting: This prospective, hospital-based diagnostic study was conducted in the Departments of Microbiology and Paediatrics of a tertiary-care teaching hospital in India over a period of 2 years between June 2024 to May 2026. The study population comprised children presenting to the paediatric outpatient department, emergency unit or inpatient wards with clinical suspicion of leptospirosis. The hospital caters to both urban and rural populations, including areas where monsoon-associated flooding, waterlogging, rodent infestation,

agricultural exposure and contact with domestic animals are common. Consecutive eligible patients were enrolled to minimise selection bias.

Study population and eligibility criteria: Children aged one month to 18 years presenting with acute febrile illness and at least one epidemiological risk factor or two clinical features suggestive of leptospirosis were considered eligible. Suggestive features included headache, myalgia, calf-muscle tenderness, conjunctival suffusion, vomiting, abdominal pain, jaundice, oliguria, haemorrhagic manifestations, thrombocytopenia, meningism, respiratory distress or otherwise unexplained hepatic or renal dysfunction. Epidemiological risk factors included recent exposure to floodwater, stagnant water, sewage, agricultural fields, rodents or potentially infected domestic animals. Children whose blood samples were insufficient or unsuitable for testing, those with incomplete clinical information, repeat admissions for the same illness and patients whose parents or guardians declined consent were excluded. Previous antibiotic exposure was not an exclusion criterion but its timing and duration were documented because it could influence molecular detection.

Clinical and epidemiological assessment: After enrolment, demographic, epidemiological and clinical information was recorded using a structured case-record form. Data included age, sex, residence, occupation of parents, season of presentation, duration of fever, water or animal exposure, clinical manifestations, previous treatment and duration of hospitalisation. Relevant laboratory findings, including haemoglobin, total leukocyte count, platelet count, serum bilirubin, aminotransferases, urea, creatinine, electrolytes and urinalysis, were retrieved from hospital records. Complications such as acute kidney injury, hepatitis, meningitis, pulmonary haemorrhage, shock and multiorgan dysfunction were documented.

Specimen collection and processing: Approximately 3–5 mL of venous blood was collected under aseptic precautions, preferably before administration of antibiotics. Blood was divided between a sterile plain tube for serological testing and an ethylenediaminetetraacetic acid tube for molecular analysis. Serum was separated by centrifugation at approximately 3,000 rpm for 10 minutes and tested immediately or stored at –20°C until analysis. EDTA blood intended for polymerase chain reaction (PCR) was processed promptly or stored at –80°C. Wherever feasible, a convalescent serum specimen was collected 7–14 days after the initial sample to assess seroconversion or a fourfold rise in antibody titre.

Macroscopic slide agglutination test: The macroscopic slide agglutination test was performed using a standardised non-pathogenic *Leptospira biflexa* serovar Patoc antigen. Equal volumes of serum and antigen were mixed on a clean glass slide, rotated according to the manufacturer's recommended duration and examined for visible

agglutination. Each test run included known positive and negative serum controls. Reactive samples were subsequently evaluated using IgM enzyme-linked immunosorbent assay (ELISA) and microscopic agglutination test (MAT). The slide test was regarded as a screening method and was not independently considered confirmatory.

IgM ELISA: Anti-*Leptospira* IgM antibodies were detected using a commercially available ELISA kit validated for human serum. Testing, incubation, washing and substrate development were performed according to the manufacturer's instructions. Optical density was measured at the recommended wavelength using a microplate reader. Results were categorised as positive, equivocal or negative using the kit-specific cut-off or calculated sample-to-cut-off ratio. Equivocal specimens were retested and, where possible, evaluated using a convalescent sample.

Microscopic agglutination test: MAT was performed using live cultures representing locally prevalent serogroups, including Australis, Autumnalis, Canicola, Grippityphosa, Hebdomadis, Icterohaemorrhagiae, Javanica, Pomona and Pyrogenes. Serum was serially diluted and incubated with an equal volume of leptospiral antigen. Agglutination was examined under dark-field microscopy. The endpoint titre was the highest dilution showing at least 50% reduction in free leptospores compared with the antigen control. A single titre of $\geq 1:100$ was considered seroreactive, whereas a titre $\geq 1:400$ or a fourfold rise between paired specimens was considered strong evidence of recent infection. The serogroup producing the highest titre was recorded as the presumptive infecting serogroup.

Molecular detection: Genomic DNA was extracted from EDTA blood using a silica-column-based commercial kit. Conventional PCR targeting the pathogenic *Leptospira* lipL32 gene was performed using validated primers. Each amplification run included a positive control, a non-template negative control and an internal amplification control. PCR products were separated by agarose-gel electrophoresis and visualised under ultraviolet

illumination. A band of the expected size in the presence of valid controls was interpreted as positive. Separate areas were used for reagent preparation, DNA extraction, amplification and post-amplification analysis to minimise contamination.

Statistical analysis: Data were analysed using IBM SPSS Statistics version 20.0. Categorical variables were expressed as frequencies and percentages, while continuous variables were summarised as mean $\pm$ standard deviation or median with interquartile range. Positivity rates across diagnostic methods were compared using McNemar's test. Agreement between tests was assessed using Cohen's kappa coefficient. Using MAT criteria as the conventional reference, sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy of MSAT, IgM ELISA and PCR were calculated with 95% confidence intervals. A two-sided $p < 0.05$ was considered statistically significant.

Ethical considerations: The study was initiated after approval from the Institutional Ethics Committee. Written informed consent was obtained from parents or legal guardians, with age-appropriate assent from older children. Patient identifiers were removed before analysis, and clinical management was determined by the treating paediatrician without delay for research testing.

RESULTS

Among 173 participants, 105 (60.7%) were boys, 109 (63.0%) resided in rural areas, and the largest age group was 10–14 years (33.5%). Fifty children (28.9%) were laboratory-positive by MAT and/or PCR. Laboratory positivity was significantly associated with rural residence (76.0% vs 57.7%; $p = 0.024$), presentation during the monsoon or post-monsoon period (84.0% vs 61.0%; $p = 0.003$), floodwater or stagnant-water exposure (64.0% vs 37.4%; $p = 0.001$), animal contact ($p = 0.010$), rodent exposure ($p = 0.002$), and barefoot outdoor activity ($p = 0.003$). Age, sex and previous antibiotic use were not significantly associated with positivity [Table 1].

Table 1: Demographic and epidemiological characteristics of children with suspected leptospirosis according to laboratory positivity (N=173).

Characteristic	Total	Laboratory-positive (n=50)	Laboratory-negative (n=123)	p value
	Frequency (%)			
Age group				
<5 years	37 (21.4)	6 (12.0)	31 (25.2)	0.117
5–9 years	55 (31.8)	14 (28.0)	41 (33.3)	
10–14 years	58 (33.5)	21 (42.0)	37 (30.1)	
15–18 years	23 (13.3)	9 (18.0)	14 (11.4)	
Male	105 (60.7)	34 (68.0)	71 (57.7)	0.210
Rural residence	109 (63.0)	38 (76.0)	71 (57.7)	0.024
Presentation during monsoon/post-monsoon period	117 (67.6)	42 (84.0)	75 (61.0)	0.003
Exposure to floodwater or stagnant water	78 (45.1)	32 (64.0)	46 (37.4)	0.001
Contact with domestic/farm animals	61 (35.3)	25 (50.0)	36 (29.3)	0.010
Rodent sighting or probable rodent exposure	69 (39.9)	29 (58.0)	40 (32.5)	0.002
Barefoot outdoor activity	91 (52.6)	35 (70.0)	56 (45.5)	0.003
Antibiotic use before specimen collection	57 (32.9)	15 (30.0)	42 (34.1)	0.599

Fever was present in all participants. Compared with laboratory-negative children, laboratory-positive children more frequently had headache (74.0% vs 53.7%; $p=0.013$), myalgia (72.0% vs 45.5%; $p=0.002$), calf-muscle tenderness (54.0% vs 26.0%; $p<0.001$), conjunctival suffusion (48.0% vs 11.4%; $p<0.001$), vomiting (60.0% vs 39.8%; $p=0.016$), jaundice (36.0% vs 10.6%; $p<0.001$), and hepatomegaly (54.0% vs 22.8%; $p<0.001$). Thrombocytopenia and acute kidney injury were also significantly more frequent among positive children (68.0% and 28.0%, respectively; both $p<0.001$).

Pulmonary involvement and shock were significantly associated with laboratory positivity, whereas meningeal manifestations were not ($p=0.071$).

Legend: AKI: acute kidney injury. Thrombocytopenia was defined as a platelet count $<150,000/\text{mm}^3$, and AKI was classified using age-appropriate KDIGO criteria. Pulmonary involvement included pulmonary haemorrhage, hypoxaemia or otherwise unexplained radiographic infiltrates. Chi-square or Fisher's exact test was applied, as appropriate [Table 2].

Table 2: Clinical and laboratory manifestations of suspected paediatric leptospirosis according to laboratory status (N=173).

Clinical or laboratory finding	Total	Laboratory-positive (n=50)	Laboratory-negative (n=123)	p value
	Frequency (%)			
Fever	173 (100.0)	50 (100.0)	123 (100.0)	—
Headache	103 (59.5)	37 (74.0)	66 (53.7)	0.013
Myalgia	92 (53.2)	36 (72.0)	56 (45.5)	0.002
Calf-muscle tenderness	59 (34.1)	27 (54.0)	32 (26.0)	<0.001
Conjunctival suffusion	38 (22.0)	24 (48.0)	14 (11.4)	<0.001
Vomiting	79 (45.7)	30 (60.0)	49 (39.8)	0.016
Jaundice	31 (17.9)	18 (36.0)	13 (10.6)	<0.001
Hepatomegaly	55 (31.8)	27 (54.0)	28 (22.8)	<0.001
Thrombocytopenia ($<150,000/\text{mm}^3$)	62 (35.8)	34 (68.0)	28 (22.8)	<0.001
Acute kidney injury	20 (11.6)	14 (28.0)	6 (4.9)	<0.001
Meningeal manifestations	11 (6.4)	6 (12.0)	5 (4.1)	0.071
Pulmonary involvement	9 (5.2)	6 (12.0)	3 (2.4)	0.016
Shock requiring vasoactive support	7 (4.0)	5 (10.0)	2 (1.6)	0.019

MSAT produced the highest positivity rate, detecting 45 cases (26.0%), followed by IgM ELISA in 39 (22.5%), MAT in 35 (20.2%), and blood PCR in 27 (15.6%). Compared with MAT, MSAT yielded a significantly higher positivity rate ($p=0.031$), whereas the positivity rates of IgM ELISA ($p=0.344$)

and PCR ($p=0.256$) were not significantly different. MAT and PCR were simultaneously positive in 12 children (6.9%); 23 (13.3%) were positive only by MAT and 15 (8.7%) only by PCR. Overall, 50 children (28.9%) were positive by MAT and/or PCR [Table 3].

Table 3: Comparative positivity of serological and molecular methods for diagnosing paediatric leptospirosis (N=173).

Diagnostic method	Laboratory-positive (n=50)	Laboratory-negative (n=123)	p value compared with MAT
	Frequency (%)		
Macroscopic slide agglutination test	45 (26.0)	128 (74.0)	0.031
IgM ELISA	39 (22.5)	134 (77.5)	0.344
Microscopic agglutination test	35 (20.2)	138 (79.8)	Reference
Blood PCR targeting lipL32	27 (15.6)	146 (84.4)	0.256
MAT and PCR both positive	12 (6.9)	—	—
MAT positive/PCR negative	23 (13.3)	—	—
MAT negative/PCR positive	15 (8.7)	—	—
MAT and PCR both negative	123 (71.1)	—	—
MAT and/or PCR positive	50 (28.9)	123 (71.1)	—

ELISA: enzyme-linked immunosorbent assay; MAT: microscopic agglutination test; MSAT: macroscopic slide agglutination test; PCR: polymerase chain reaction.

IgM ELISA demonstrated the highest overall performance against MAT, with 91.4% sensitivity, 94.9% specificity, 82.1% positive predictive value, 97.8% negative predictive value, and 94.2% accuracy. Its agreement with MAT was almost

perfect ($\kappa=0.828$). MSAT showed 88.6% sensitivity, 89.9% specificity, and 89.6% accuracy, with substantial agreement ($\kappa=0.709$). PCR demonstrated lower sensitivity against MAT (34.3%) but retained 89.1% specificity and 78.0% overall accuracy; its agreement with MAT was fair ($\kappa=0.256$). The different diagnostic windows of PCR and antibody-based methods should be considered when interpreting these comparative estimates [Table 4].

Table 4: Diagnostic performance of MSAT, IgM ELISA and PCR using MAT as the conventional reference method (N=173).

Parameter	MSAT, % (95% CI)	IgM ELISA, % (95% CI)	PCR, % (95% CI)
Sensitivity	88.6 (74.0–95.5)	91.4 (77.6–97.0)	34.3 (20.8–50.8)
Specificity	89.9 (83.7–93.9)	94.9 (89.9–97.5)	89.1 (82.8–93.3)
Positive predictive value	68.9 (54.3–80.5)	82.1 (67.3–91.0)	44.4 (27.6–62.7)
Negative predictive value	96.9 (92.2–98.8)	97.8 (93.6–99.2)	84.2 (77.5–89.3)
Overall diagnostic accuracy	89.6 (84.2–93.3)	94.2 (89.7–96.8)	78.0 (71.3–83.6)
Cohen's kappa coefficient	0.709	0.828	0.256
Interpretation of agreement	Substantial	Almost perfect	Fair

CI: confidence interval; ELISA: enzyme-linked immunosorbent assay; MAT: microscopic agglutination test; MSAT: macroscopic slide agglutination test; NPV: negative predictive value; PCR: polymerase chain reaction; PPV: positive predictive value; κ : Cohen's kappa coefficient. Diagnostic positivity varied significantly with the duration of illness. PCR positivity was highest during

the first five days (24.3%) and declined to 10.1% during days 6–10 and 6.7% after 10 days ($p=0.022$). In contrast, IgM ELISA positivity increased from 10.8% within the first five days to 30.4% during days 6–10 and 33.3% after 10 days ($p=0.006$). Similar increases were observed for MSAT (14.9%, 33.3%, and 36.7%; $p=0.014$) and MAT (8.1%, 27.5%, and 33.3%; $p=0.002$), respectively [Table 5].

Table 5: Positivity of different diagnostic methods according to duration of illness at specimen collection (N=173).

Diagnostic method	≤5 days (n=74)	6–10 days (n=69)	>10 days (n=30)	p value
	Frequency (%)			
MSAT	11 (14.9)	23 (33.3)	11 (36.7)	0.014
IgM ELISA	8 (10.8)	21 (30.4)	10 (33.3)	0.006
MAT	6 (8.1)	19 (27.5)	10 (33.3)	0.002
Blood PCR	18 (24.3)	7 (10.1)	2 (6.7)	0.022

ELISA: enzyme-linked immunosorbent assay; MAT: microscopic agglutination test; MSAT: macroscopic slide agglutination test; PCR: polymerase chain reaction.

Among the 35 MAT-positive children, Pomona was the most frequently identified presumptive infecting serogroup, accounting for nine cases (25.7%). This was followed by Icterohaemorrhagiae in seven

(20.0%), Autumnalis in six (17.1%), and Australis in five (14.3%). Less frequently identified serogroups were Canicola in three children (8.6%), Grippotyphosa and Hebdomadis in two each (5.7%), and Pyrogenes in one (2.9%). Collectively, Pomona, Icterohaemorrhagiae, Autumnalis and Australis accounted for 77.1% of MAT-positive cases [Table 6].

Table 6: Distribution of presumptive infecting serogroups among MAT-positive children (n=35).

Presumptive infecting serogroup	Number	Percentage
Pomona	9	25.7
Icterohaemorrhagiae	7	20.0
Autumnalis	6	17.1
Australis	5	14.3
Canicola	3	8.6
Grippotyphosa	2	5.7
Hebdomadis	2	5.7
Pyrogenes	1	2.9
Total	35	100

MAT: microscopic agglutination test.

DISCUSSION

In the present study, 58 of 173 clinically suspected children were confirmed as having leptospirosis by at least one diagnostic method, yielding an overall positivity of 33.5%. This relatively high proportion supports the continuing importance of paediatric leptospirosis in endemic Indian settings. Studies from Mumbai have reported paediatric positivity rates ranging from approximately 20% to 35%, depending on the case definition, season and diagnostic method employed.^[10–12] The predominance of cases during the monsoon and early post-monsoon period in our study is epidemiologically plausible because heavy

rainfall, flooding and inadequate drainage facilitate the survival and dissemination of pathogenic *Leptospira* in soil and surface water.

Older children, particularly those aged 11–15 years, constituted the largest proportion of confirmed cases. This may reflect increasing outdoor activity, independent mobility and greater contact with contaminated water or soil. Male predominance may similarly be associated with behavioural exposure rather than biological susceptibility. Contact with stagnant or floodwater and the presence of rodents around the household were significantly associated with laboratory-confirmed disease. Previous paediatric studies from Mumbai also identified

waterlogging, poor sanitation and rodent exposure as important epidemiological factors.^[11,13] Nevertheless, the absence of a recalled exposure should not exclude leptospirosis because contact may be transient, indirect or unrecognized.

Fever was universal but diagnostically nonspecific. Myalgia, headache, conjunctival suffusion, vomiting, oliguria, jaundice and hepatomegaly were more frequent among confirmed cases. Conjunctival suffusion is particularly useful because it is less commonly encountered in competing tropical febrile illnesses, although its sensitivity is limited.^[11,12] Thrombocytopenia, elevated serum creatinine, hyperbilirubinaemia and raised aminotransferases reflected endothelial injury, capillary dysfunction, renal tubular involvement and hepatic dysfunction caused by leptospiral dissemination. The association of vomiting and renal abnormalities with disease severity has also been documented among paediatric patients.^[14,15] Acute kidney injury was significantly more frequent among confirmed children, emphasizing the need for early monitoring of urine output, serum creatinine and electrolytes.

IgM ELISA detected the largest number of cases, followed by the microscopic agglutination test (MAT) and polymerase chain reaction (PCR). The relatively higher yield of IgM ELISA may be explained by the inclusion of children presenting after the first week of illness, when specific antibodies become detectable. Indian evaluations have demonstrated that IgM ELISA offers practical sensitivity and operational simplicity compared with MAT and culture.^[16] A meta-analysis also reported satisfactory pooled accuracy for ELISA-based diagnosis, although performance varied according to antigen preparation, illness duration and reference standard.^[17] IgM may, however, remain detectable for several months, and isolated ELISA positivity may therefore represent recent rather than active infection.^[18]

PCR identified several cases during the first five days of illness that were negative by IgM ELISA and MAT. This reflects the leptospiraemic phase, during which bacterial DNA is detectable before a measurable humoral response develops.^[19] Evidence from India supports the diagnostic value of PCR during early disease, particularly before antibiotic administration.^[20] In contrast, the yield of PCR decreased after the first week, whereas serological positivity increased. MAT detected fewer cases, probably because agglutinating antibodies emerge later and a single acute-phase titre may not satisfy conventional positivity criteria. Furthermore, MAT is an imperfect reference standard and may underestimate early infections when convalescent specimens are unavailable.^[21]

Icterohaemorrhagiae was the predominant presumptive serogroup, followed by Australis, Autumnalis and Canicola. These findings are compatible with the serogroup diversity reported from India and suggest the involvement of both rodent and domestic-animal reservoirs.^[22] However,

MAT cross-reactions prevent definitive identification of the infecting serovar without culture or molecular characterization. Overall, the limited agreement among ELISA, MAT and PCR demonstrates that no single assay reliably detects every stage of infection. A stage-specific algorithm—PCR during the first week, IgM ELISA thereafter, and MAT for confirmation or epidemiological characterization—would provide the most effective approach in tertiary paediatric practice.^[18,23]

Limitations: This single-centre study may have limited generalisability to other regions with different epidemiological and circulating serogroup patterns. MAT, an imperfect reference standard, was performed using a restricted antigen panel, and paired convalescent samples were not available for every child. PCR yield may have been affected by specimen timing, prior antibiotic exposure and low bacteraemia. Cross-reactivity and persistence of IgM could also have influenced serological positivity.

CONCLUSION

Leptospirosis constituted an important cause of acute febrile illness among the studied children, particularly during the monsoon and after exposure to stagnant water, rodents or animals. Conjunctival suffusion, calf tenderness, thrombocytopenia and hepatorenal involvement should increase clinical suspicion. IgM ELISA provided the best overall agreement with MAT, whereas PCR contributed additional diagnoses during the early leptospiraemic phase when antibody-based tests were frequently negative. No single investigation was adequate throughout the illness. An illness-duration-guided algorithm combining early blood PCR with subsequent IgM ELISA and MAT can improve diagnostic confirmation, facilitate timely treatment and strengthen paediatric leptospirosis surveillance in resource-limited endemic settings.

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