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Original Article

Determination of Local Titre for Interpretation of Widal Test Results of Easthern Bihar

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ABSTRACT

Background: Enteric fever, caused by Salmonella enterica serotypes Typhi and Paratyphi, remains endemic in Eastern Bihar. The Widal test continues to be a widely used serological diagnostic tool; however, its interpretation varies geographically due to differences in background antibody titres. Establishing a local baseline is therefore essential for accurate diagnosis and rational antibiotic use.

Objective: To determine the endemic (baseline) titre of the Widal test by establishing local agglutination titres among healthy individuals and comparing them with titres observed in confirmed cases of enteric fever in and around MGMMC & LSK Hospital, Kishanganj.

Materials and Methods: This cross-sectional study was conducted in the Department of Microbiology, MGMMC & LSK Hospital, Kishanganj, from June 2024 to July 2025. Serum samples from 384 healthy individuals were tested for Salmonella Typhi (O, H) and S. Paratyphi (AH, BH) antigens using the standard tube agglutination method. Titres were analyzed to determine regional baseline and diagnostic cut-off values.

Results: Most participants belonged to the 11–20 year age group (19.5%), with a male-to-female ratio of 1.09:1. O-antigen positivity peaked at 1:40 (79.9%) and H-antigen at 1:40 (77.3%), with no reactivity beyond 1:160. AH and BH antigens showed negligible reactivity (< 3.5%). The 95th-percentile cut-offs were 1:80 for O and 1:160 for H antigens.

Conclusion: The baseline titres in healthy individuals of Eastern Bihar are 1:40 for O and 1:80 for H antigens. Diagnostic cut-offs of ≥ 1:160 for O and ≥ 1:320 for H antigens are proposed to enhance specificity and minimize false-positive diagnosis of enteric fever in this endemic region.

Keywords: *Widal test, Salmonella Typhi, baseline titre, enteric fever, Eastern Bihar, sero-epidemiology.*

INTRODUCTION

The Widal test, developed by Georges Fernand Widal in 1896, remains one of the most widely used serological tests for the diagnosis of enteric fever, particularly in resource-limited settings. [1] Enteric fever, caused by *Salmonella enterica* serotypes Typhi and Paratyphi, continues to be a significant public health concern, especially in developing countries with poor sanitation and limited access to clean water. [2] Eastern Bihar, a region in India, is one such area where enteric fever is endemic due to its socio-economic challenges, including inadequate healthcare infrastructure, poor hygiene practices, and limited access to diagnostic facilities.[3]

The Widal test detects the presence of agglutinating antibodies against the O (somatic) and H (flagellar) antigens of *Salmonella* Typhi and Paratyphi. However, the interpretation of Widal test results is complicated by several factors,

including the variability in baseline antibody titres among populations, cross-reactivity with other infections, and the lack of standardized cut-off values. [4] These challenges underscore the importance of establishing local titre cut-offs for accurate interpretation of Widal test results, as antibody levels can vary significantly between different geographical regions due to differences in endemicity, exposure, and immune responses. [5]

In Eastern Bihar, where enteric fever is highly prevalent, the lack of region-specific titre cut-offs has led to both overdiagnosis and underdiagnosis of the disease.[6] Overdiagnosis can result in unnecessary antibiotic use, contributing to the growing problem of antimicrobial resistance, while underdiagnosis can lead to untreated cases, severe complications, and increased transmission.[7] Therefore, determining local titre cut-offs for the Widal test in Eastern Bihar is crucial for improving diagnostic accuracy, guiding appropriate treatment, and reducing the burden of enteric fever in the region.[8-10] This study aims to establish local titre cut-offs for the interpretation of Widal test results in Eastern Bihar by analyzing the distribution of antibody titres in healthy individuals and comparing them with those in confirmed cases of enteric fever. By doing so, it seeks to provide a more reliable framework for diagnosing enteric fever in this endemic region, ultimately contributing to better patient outcomes and more effective public health interventions.

OBJECTIVE

To determine the endemic (baseline) titre of the Widal test by establishing the local agglutination titre among healthy individuals and comparing it with titres observed in confirmed cases of enteric fever in and around MGMMC & LSK Hospital.

MATERIALS & METHODS

Study design: A Cross-sectional study
Place of study: The study conducted in the department of Microbiology at a tertiary care Hospital, Kishanganj, Bihar
Study period: June-2024 to July-2025.
Sampling technique: Aseptic collection of blood samples for serological testing followed by serum separation. Excess serum samples after recommended testing wasused-up for the study purpose with proper history and informed consent.
Sample size: Using the formula for sample size calculation: n = (Z^2 * p * q) / E^2
Where: n = required sample size Z = Z-score corresponding to the desired confidence level (1.96 for a 95% confidence level) p = estimated proportion of individuals with positive Widal test results (0.5, of the proportion according literature of **John J et al (2023)**[1] q = 1 - p E = margin of error
n = (1.96^2 * 0.5 * 0.5) / (0.05^2) n = 384.16

Inclusion Criteria:

- All serum samples coming for serological testing to Microbiology Deptt of MGMMC & LS Hospital, was taken into consideration irrespective of age and sex.
- Indications for sero-diagnosis for specific disease or routine check-up like pre-operative, antenatal or blood donors.

Exclusion criteria:
Patient with fever

- Samples of apparent immune-compromised individuals,
- Contaminated or haemolysed serum samples,
- Relevant history and informed consent not available.

Widal Test:
Blood samples will be collected from all participants.
The Widal test will be performed following standard laboratory protocols and using specific antigens for Salmonella Typhi and Paratyphi A and B.
The local titre values for the interpretation of Widal test results were determined based on the analysis of the healthy individual.

Ethical Considerations:
The study was adhering to ethical guidelines and obtain necessary ethical approvals from MGM Medical College & Hospital institutional review boards. Informed consent was obtained from all participants prior to their inclusion in the study.

RESULTS & ANALYSIS:

Table 1: Age Distribution of Study Participants (n=384)

Age Group (Years)	Number of Cases	Percentage
1–10	55	14.3
11–20	75	19.5

21–30	50	13.0
31–40	30	7.8
41–50	25	6.5
51–60	35	9.1
>60	44	11.4
Total	384	100.0

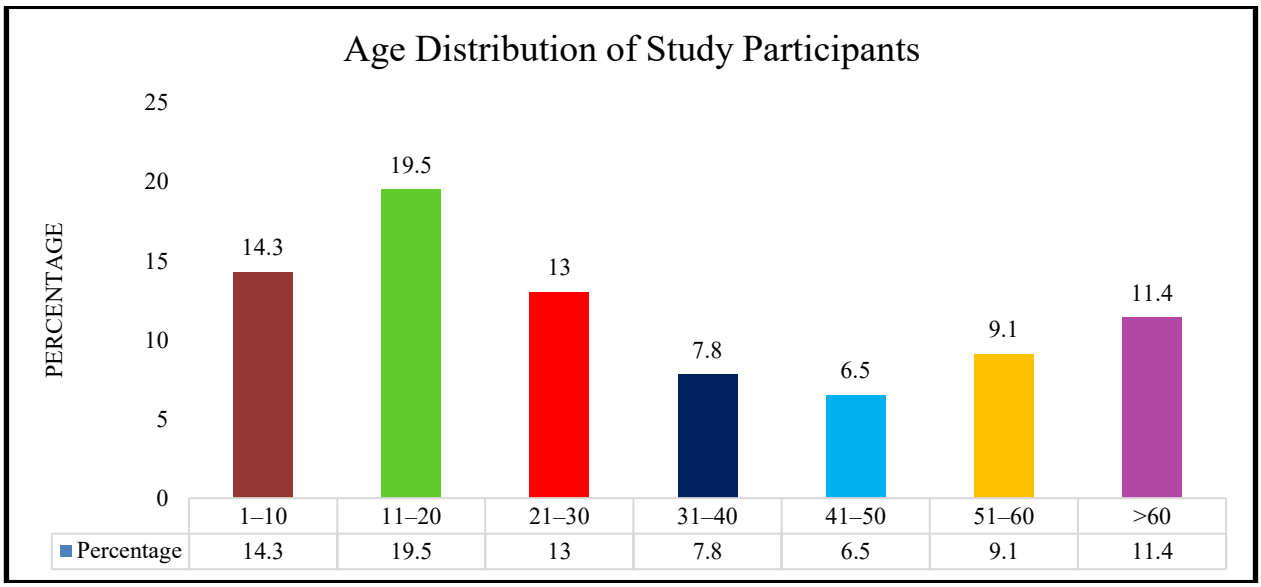

Figure 1: Age Distribution of Study Participants.

The study included 384 participants, with the highest representation in the 11–20 years age group (19.5%, n=75), followed by 1–10 years (14.3%, n=55) and 21–30 years (13.0%, n=50). Older age groups showed declining proportions: 31–40 years (7.8%, n=30), 41–50 years (6.5%, n=25), 51–60 years (9.1%, n=35), and >60 years (11.4%, n=44).

Table 2: Sex Distribution (n=384)

Gender	Healthy Population	Percentage
Male	200	52.1
Female	184	47.9
Total	384	100.0

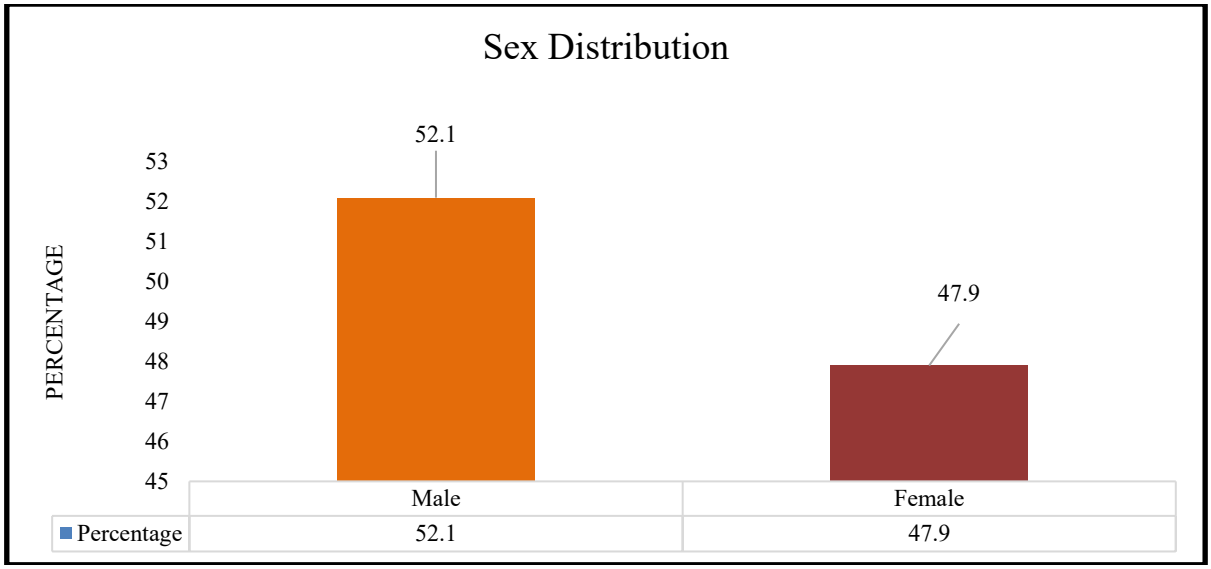

Figure 2: Sex Distribution.

The sex distribution of the study population comprising 384 individuals. Among them, 200 participants (52.1%) were male, while 184 participants (47.9%) were female.

Table 3: Titre Distribution of Healthy Individuals

Antigen	1:20	1:40	1:80	1:160	1:320	Total
O	128 (33.3%)	307 (79.9%)	50 (13.0%)	14 (3.6%)	0 (0.0%)	384 (100%)
H	55 (14.3%)	297 (77.3%)	15 (3.9%)	5 (1.3%)	0 (0.0%)	372(96.9%)
AH	6 (1.6%)	6 (1.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	12 (3.2%)
BH	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

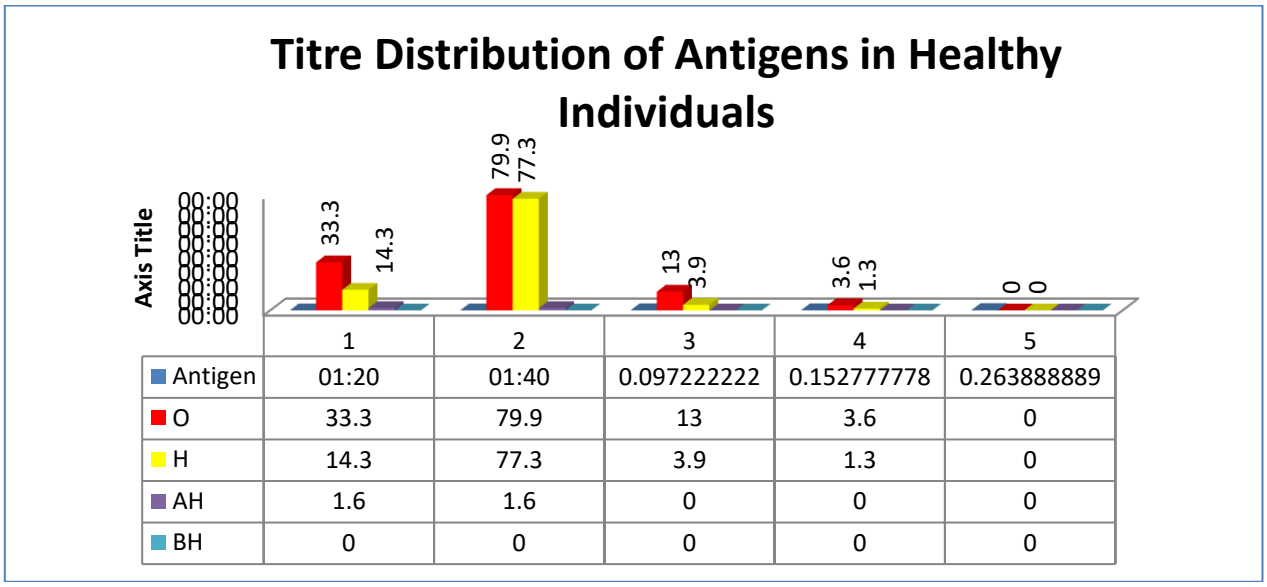

Figure 4: Titre Distribution of Antigens in Healthy Individuals

The Widal titre distribution among 384 healthy individuals revealed distinct antigen-specific patterns. For O antigen, 79.9% (n=307) showed reactivity at 1:40 dilution, decreasing sharply to 13.0% (n=50) at 1:80 and only 3.6% (n=14) at 1:160. H antigen displayed similar attenuation with 77.3% (n=297) positive at 1:40, but just 3.9% (n=15) at 1:80 and 1.3% (n=5) at 1:160. Notably, no samples reacted beyond 1:160 dilution for either antigen. Paratyphoid antigens demonstrated minimal reactivity, with AH antigen showing 1.6% (n=6) positivity exclusively at 1:20-1:40 dilutions and BH antigen showing no reactivity at any dilution. These findings establish critical baseline values, suggesting 1:160 as a potential upper threshold for background antibody levels in this healthy population.

DISCUSSION

In the present study, a total of 384 healthy individuals were evaluated for baseline Widal titres. The age distribution showed the highest representation in the 11–20 years group (19.5%), followed by children aged 1–10 years (14.3%) and young adults aged 21–30 years (13.0%). The representation declined progressively in older age groups, with the lowest percentages observed in individuals aged 41–50 years (6.5%) and 31–40 years (7.8%). These findings suggest that the younger population, particularly adolescents and school-aged children, are more frequently exposed to *Salmonella* antigens, possibly due to poor sanitation, greater outdoor activity, and higher likelihood of asymptomatic carriage in these age groups. A similar trend was noted by **Pal et al. (2013)** [11], where the most reactive sera were obtained from younger individuals, supporting the hypothesis that background exposure to typhoidal organisms is higher in early age.

Peshattiwar et al. (2012) [12] also reported that a substantial proportion of seropositive individuals belonged to the younger age bracket, particularly those under 30 years, which aligns with our findings. This is also consistent with the study by **Pokhrel et al. (2009)** [13], which included healthy volunteers from Nepal, where the majority of the reactive titres were found in younger age groups. These patterns collectively highlight the importance of considering age-related background titres in regions with endemic exposure to enteric pathogens.

With regard to sex distribution, the present study showed a nearly equal representation of both sexes, with males constituting 52.1% (n=200) and females 47.9% (n=184). This slight male predominance is consistent with the findings of **Akhtar SJ (2020)** [14], who also reported a marginally higher male participation among healthy individuals screened for

Widal titres. The minor male preponderance could reflect demographic patterns of healthcare-seeking behavior or blood donation participation, especially in community-based or hospital-attached studies.

Shethwala et al. (2019) [15] and **Gunjal et al. (2013)** [16] also included healthy populations with balanced gender distribution and noted no significant sex-specific differences in agglutinin titres. This suggests that sex may not play a major role in the seroprevalence of *Salmonella* antibodies in endemic settings, although minor variations may be observed based on local population dynamics and study design.

In the present study, O antigen positivity was observed in a significantly high proportion of the healthy population (79.9%), making it the most frequently detected antigen. H antigen followed with a positivity rate of 29.2%, while AH and BH antigens were detected in only 3.4% and 1.3% of individuals, respectively. This distribution indicates that exposure to *Salmonella typhi* O antigen is more prevalent in the community, likely due to repeated subclinical infections or environmental exposure. These findings are in line with those of **Akhtar SJ (2020)** [14], who also reported a predominance of O antigen (45%) and H antigen (53.7%) among Widal-positive healthy individuals in their cohort, although the exact prevalence was lower due to differences in population and diagnostic thresholds.

Gunjal et al. (2013) [16] similarly noted that 31.6% of healthy individuals were positive for O antigen at a titre of 1:40, while H antigen positivity was slightly higher at 33.9%, both observed at the same titre. However, unlike our study, they reported considerably higher frequencies of AH (38.5%) and BH (36.8%) antigen positivity, each at titres of 1:80. This contrast may be attributed to differences in regional endemicity of *S. paratyphi* strains or methodological sensitivity. In our region of Eastern Bihar, the very low positivity of AH and BH antigens (≤3.4%) suggests either a lower circulation of these serotypes or lower antigenic stimulation among the population.

In terms of titre distribution, our findings indicate that the majority of O antigen positivity was confined to a titre of 1:20, with fewer individuals exhibiting titres of 1:40 (33.3%), 1:80 (13.0%), and 1:160 (3.6%). No participant showed an O titre at 1:320. This pattern supports a predominantly low background titre in the community. Similarly, for H antigen, most individuals showed titres at 1:20 (29.2%), with progressively fewer individuals at higher dilutions—1:40 (14.3%), 1:80 (3.9%), and 1:160 (1.3%). Again, titres ≥1:320 were absent.

These patterns are comparable to the findings by **Peshattiwar et al. (2012)** [12], who found that 96% of healthy individuals had O titres ≤1:40, and 91% had H titres ≤1:40, supporting the idea that low titres are common in endemic populations. They also emphasized that any titre ≥1:80 for O or H antigen should be interpreted cautiously and only in the presence of clinical suspicion. Our data also support the notion that titres ≥1:160 are uncommon in asymptomatic individuals, reinforcing their potential utility as diagnostic thresholds.

Furthermore, the study by **Pal et al. (2013)** [11] in Uttarakhand noted similar baseline titres with the majority of reactive sera showing O antigen titres at 1:40 and H titres at 1:80. These titres were considered baseline levels in their setting, and the authors recommended diagnostic cut-offs of ≥1:80 for O and ≥1:160 for H antigens. Our results align with this recommendation, as titres beyond these thresholds were rarely encountered in healthy individuals.

The very low prevalence of elevated AH and BH titres in our study—only at 1:20 with no cases exceeding this level—suggests minimal background immunity or exposure to *S. paratyphi* strains in this region. In contrast, **Pokhrel et al. (2009)** [13] reported AH and BH antibody titres ≥1:20 in 12% and 3% of individuals, respectively, indicating slightly higher exposure in the Nepalese population. Regional variation in circulating strains and environmental exposure could explain these differences.

CONCLUSION

This study establishes region-specific baseline Widal titres for Eastern Bihar. Among healthy individuals, the median titres were **1:40 for O antigen** and **1:80 for H antigen**, with 95th-percentile cut-offs of **1:80 for O** and **1:160 for H**. AH and BH antigens exhibited negligible titres (< 1:20).

These findings indicate that titres ≥ 1:160 for O or ≥ 1:320 for H antigens may serve as diagnostic thresholds for enteric fever in this population, offering high specificity and reducing false positives. Establishing these local benchmarks will enhance diagnostic accuracy and promote rational antibiotic use in endemic regions such as Eastern Bihar.

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