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Detection of the Prevalence of Etiological Agents of Enteric Fever through Triple-Antigen Tests in the Northern Part of Bangladesh

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Abstract: Highly virulent *Salmonella enterica* serovars (Typhi and Paratyphi) is the leading cause of typhoid fever and sufferings of enteric fever worldwide and due to this, about 16.6 million infections and 1.6 million deaths are estimated in the world each year. Due to the wide variety of clinical manifestations demonstrated by the pathogen, it can be considered challenging to diagnose it. Several diagnostic techniques are employed throughout South Asia, including Bangladesh, one of which is the Triple Antigen Test.

The purpose of this study was to apply this technique to determine the prevalence of enteric fever in North Bengal, Bangladesh. A total of 176 blood specimens were examined for *Salmonella* Typhi/Paratyphi (Widal test); *Proteus* species, rickettsial infections (Weil Felix test), and brucellosis (*Brucella* antibody test). Overall, 64.04% showed positive results for enteric fever. The results identified *Salmonella enterica* serovars Typhi to be the most prevalent agent, with no Paratyphi or brucellosis cases detected. However, dependence on serology-based testing without antibiogram profiling is inadequate for diagnosis and treatment. Widal test findings can be challenging to interpret due to previous antibiotic treatment and the possibility of false negative results. Improved diagnostic techniques like Typhidot RDT, IgM StripTest, IDL TUBEX Test, and fast stool culture are required for accurate estimations of enteric fever, in addition to following the standards of World Health Organization (WHO) and the Center for Disease Prevention and Control (CDC).

Keywords: Enteric fever, *Salmonella enterica*, Typhi, Paratyphi, Widal test, Weil felix test.

I. INTRODUCTION

Highly virulent *Salmonella enterica* serovars (Typhi and Paratyphi) is the leading cause of typhoid fever and the sufferings of enteric fever worldwide. Enteric fever is a serious bloodstream infection and due to this, about 16.6 million infections and 1.6 million deaths are estimated in the world each year. [1] According to the global burden report 2019, typhoid and paratyphoid fever was the 11^{th} –14^{th} top-leading infection of morbidity and mortality worldwide since 1990, which mostly affects children and young adults [2]. About 12.5 million people of developing countries report typhoid fever cases each year, which could be due to improper sanitation, a lack of safe water, an unhygienic and polluted environment, a lack of consciousness about *S. typhi* vaccination, [3] HIV infections or an imbalanced lifestyle. [4]

The morbidity and mortality rates in Asia, South America and Africa are also significant due their poor sanitation and limited safe drinking water.[5] According to the WHO, almost 90% of morbidity and deaths for enteric fever are reported in South Asia (India, Pakistan, Nepal) and Africa [6, 7]. Bangladesh is also an endemic area for typhoid and paratyphoid fever which account for almost 300 cases per 100,000 populations [8].

Transmission of this pathogen occurs through the fecal-oral pathway after consuming contaminated food or water [9]. There is a short cycle of transmission (7-21 days) from ingestion of the contaminated food to the emergence of symptoms [10]. After attacking the submucosal layer, the pathogen proliferate and cause intestinal bleeding [11, 12].

Signs and symptoms of this systematic infection include abdominal discomfort, fever, headaches, chills, anorexia, constipation, vomiting, diarrhea, enlargement of liver, thrombocytopenia and leucopenia [13, 14].

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Though blood culture is considered as the gold standard for the diagnosis of any disease, it is not suitable for enteric fever [14, 15]. Lack of diagnostic tools in most healthcare settings leads to misdiagnosis and mistreatment of enteric fever in Bangladesh [16]. A triple antigen test is the most appropriate one to investigate the cause of enteric fever [8].

Triple antigen test include the widal test, the weil felix and the brucella antibody test. Widal test is a serological agglutination test that has been used for the diagnosis of typhoid and paratyphoid fever for over a century [6]. Weil-felix test is a non specific agglutination test that occurs between antigens of *Proteus* spp. (OX 19, OX 2, and OXK) with antibodies produced in rickettsial infections [8, 17].

Brucella antibody test is used for the detection of the contagious zoonotic disease brucellosis which is endemic in developing countries of America, Africa and Asia [18]. However, our study aims to detect the current prevalence of enteric fever in the northern part of Bangladesh through this method and suggest constructive methods that can be implemented to prevent the spread of enteric fever in Bangladesh.

II. METHODOLOGY

Study area, design and period: This is a retrospective study carried out from June 2023 to August 2023 at the Department of Microbiology at Stamford University Bangladesh after taking ethical clearance from the institution's ethical clearance committee.

Inclusion and exclusion criteria:
All patients having signs and symptoms of Enteric fever including fever (> 99°F), abdominal pain or discomfort, constipation or diarrhea and giving written informed consent were included. On the other hand, patients with a history of fever and specific infections such as urinary tract infections were excluded from this experiment. Acquired immunizations with various vaccines or antibiotic treatment were also excluded.

Sample size and sampling technique:
A total of 176 participants were included for detecting the prevalence of enteric fever and a convenient sampling method was used to select participants until the required number was achieved.

Data collection and other variables:
The data of this study were collected on demographic variables such as age, sex, food habit, lifestyle and other variables such as drinking water source, toilet availability, hand wash after toilet, food habit and history of enteric fever through a pre-tested structured questionnaire using a face-to-face interview with patients by attending physicians.

Specimen collection, bacterial isolation and identification:
Blood samples from all fever cases were collected aseptically in sterilized vials and transferred to the laboratory. The blood samples were kept to clot and the serum was separated by centrifugation at 2500 rpm for 10 minutes. Then specimens were processed using commercial kits.

The triple antigen test procedure was performed according to manufacturer instructions. The reading for the test was taken for observation overnight and was interpreted according to Yasmin et al. 2020 [8]. Serum agglutinin titres > 1:80 were considered to be significant and the quantitative variables were expressed as frequency and percentage.

III. RESULT

A total of 176 suspects were collected after fulfilling the inclusion and exclusion criteria and among them, 154 (87.5%) cases were positive for any of the triple antigen tests.

Among the study population, the number of *Salmonella typhi* and *Proteus* species were 64 (36.36%) and 90 (51.13%) respectively. There were no cases found for paratyphi and brucella species (Figure 1).

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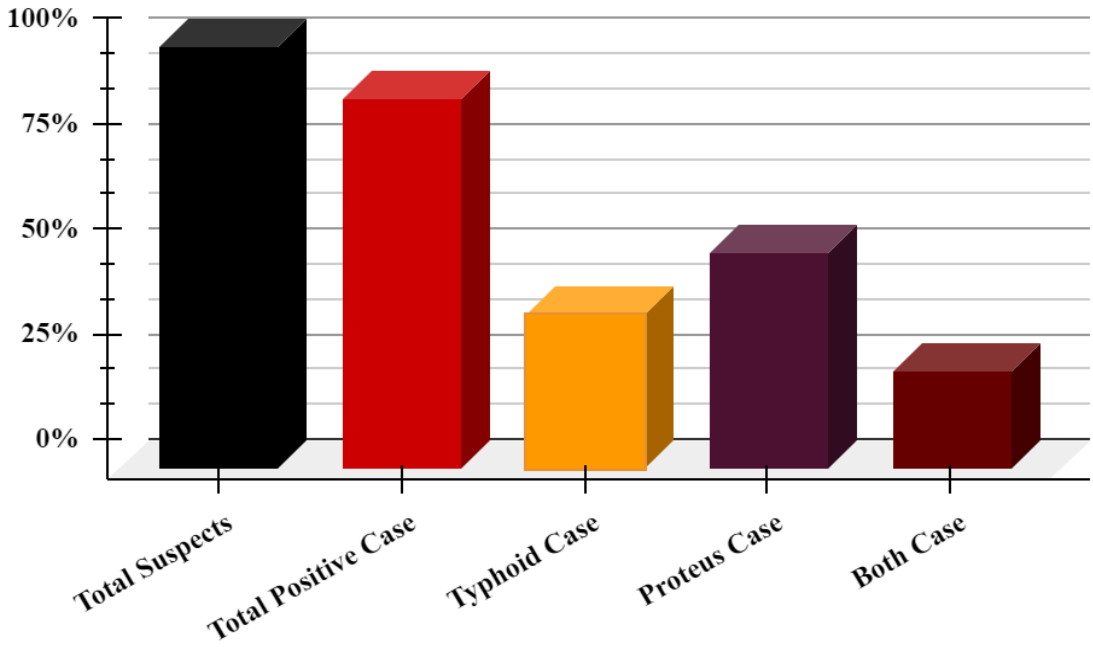

Figure 1: Rate of positive cases among study population.

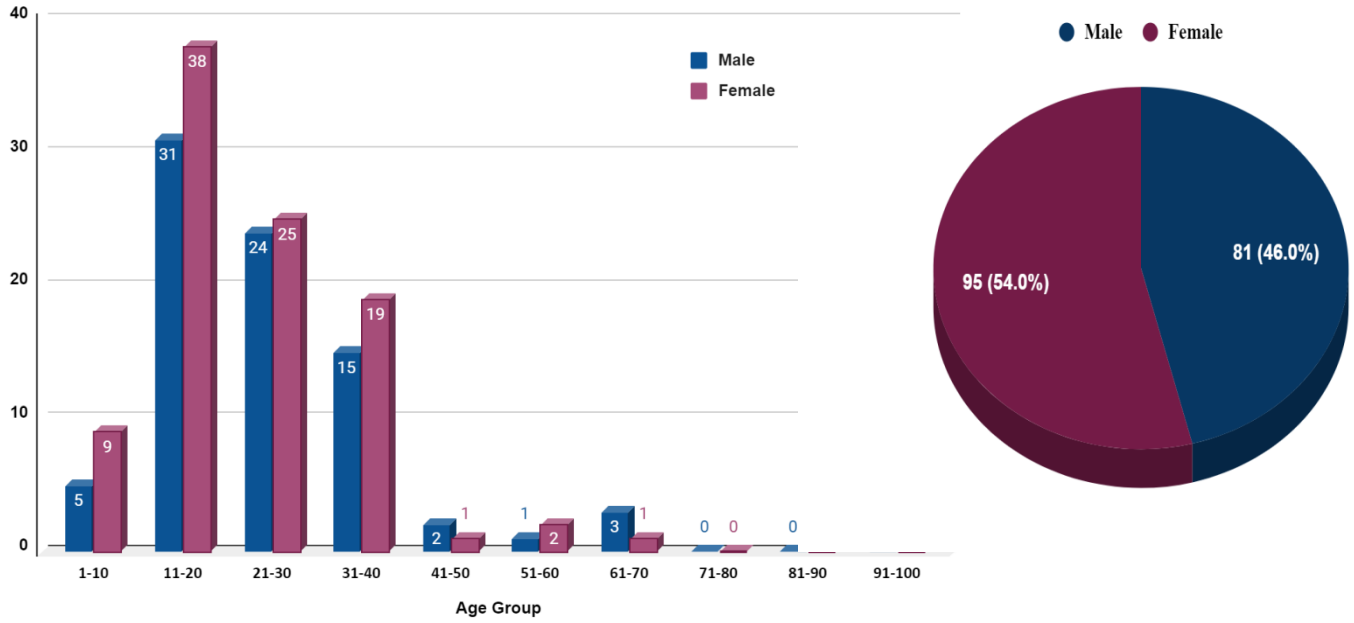

Figure 2: Rate of age and gender group differences among study population.

The infection rate in females is higher than in males. The highest infection rate was found in the 11-20 age group.

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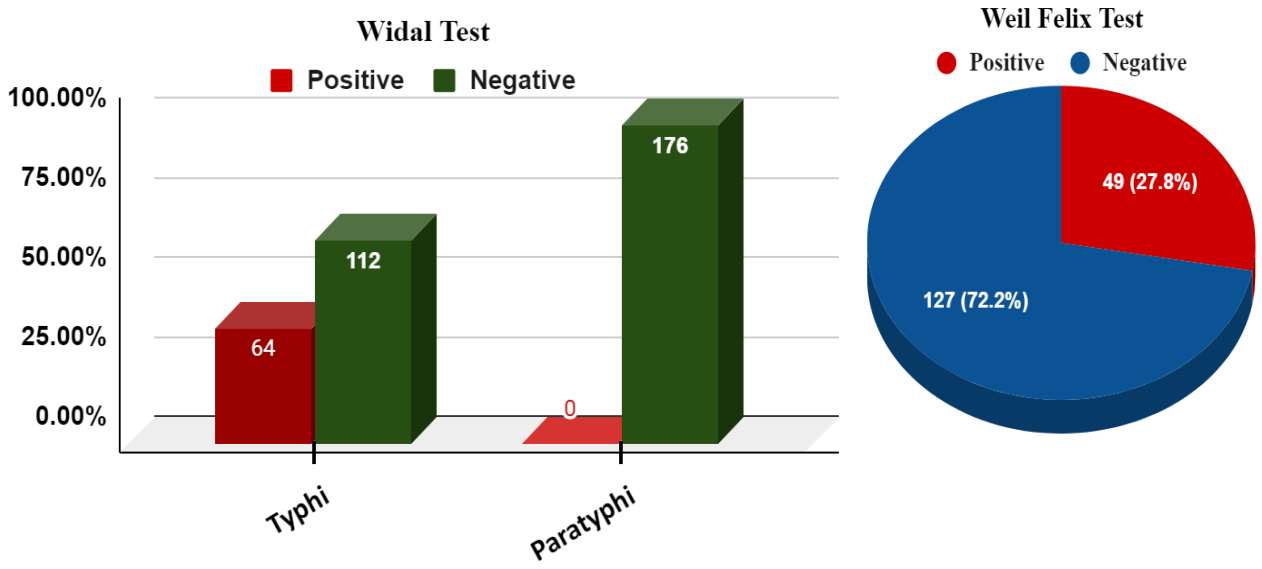

Figure 3: Positive and negative cases of widal & weil felix test.

In the widal test, typhoid cases were found at 36.36% and in the weil-felix test, positive cases were found at 27.8%. No paratyphi or *Brucella* was found.

IV. DISCUSSION

In this study, 154 (87.5%) cases were positive in a total of 176 patients for either any of three antigens. Among them, 64 (36.36%) were positive for *Salmonella* spp., 90 (51.13%) for *Proteus* and *Rickettsia* spp. and 40 (22.73%) were both positive (Figure 1). There was no positive about *Brucella* spp.

A laboratory-confirmed enteric fever case study reported that the rate of enteric fever is 28% in Bangladesh and 22% in Nepal and Pakistan (5). In our study, it was very high in comparison with other studies which may be due to the small sample size and limited time duration.

No *Brucella* species was found in this study, which may be due to the fact that the patients who attended, did not have a history of contact with domestic animals. The prevalence of brucellosis in domestic animals and humans is very uncommon and rare, according to the demographic region of Bangladesh.

The rate of gender group differences among our study population was 95 (54%) female and 81 (46%) male, which is quite common in comparison with related studies.

Females are more vulnerable to enteric fever. This may be due to physiological and immunological differences between males and females.

Among the different age groups, the higher infection rate was observed between 11-20 years and 21-30 years. The age group 31-40 also showed a mentionable infection rate. This finding correlates with the observation made by Yasmin et al. 2020 [8].

V. CONCLUSION

Our study highlights the significance of triple antigen test for the detection of enteric fever, including zoonosis such as rickettsial fever, brucellosis in patients. To reduce the countrywide diagnostic dilemma of enteric diseases, introduction of modern techniques is required.

The best approach for reducing the burden of enteric fever in Bangladesh is to ensure safe drinking water and hygienic toilets with a proper waste disposal system.

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VI. LIMITATIONS

The sample size of our study was very small. There were no control groups in our study.

VII. ACKNOWLEDGMENT

We thank and sincerely appreciate the logistic support provided by the Department of Microbiology, Stamford University, Bangladesh throughout this study.

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