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UNVEILING THE BURDEN OF PARATYPHOID FEVER: A SEROPREVALENCE STUDY FROM NORTH INDIA

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ABSTRACT: Introduction: Paratyphoid fever is an enteric infection caused primarily by *Salmonella enterica* serovars Paratyphi A, Paratyphi B and Paratyphi C. It accounts roughly for 20-30% of total enteric fever cases globally, and this proportion is rising, particularly where typhoid vaccination efforts are underway. The current study was planned to determine the seroprevalence of paratyphoid fever and the epidemiological details of the affected cases in our region. **Material and Methods:** The study was conducted for a period of eighteen months from January 2023 to July 2024 on serum samples from patients suspected of enteric fever were tested with Widal test. The results were interpreted based on regional baseline antibody titers and analyzed. **Results:** Out of 3310 samples, 1102 tested positive by Widal test. Forty five cases showed agglutinins for Paratyphi A or Paratyphi B or both. Twenty six were female (57.8%) and highest burden of infection was observed in age group less than 15 years (46.7%) followed by 16-45 years (42.2%) with a mean age of 18.9 years. **Conclusion:** Epidemiology of paratyphoid fever is still emerging and unclear. There is a need to conduct nationwide studies on paratyphoid fever that will help in making preventive guidelines for control of this disease.

INTRODUCTION: Paratyphoid fever, along with typhoid fever, is collectively referred to as enteric fever. It is caused by *Salmonella enterica* serovars Paratyphi A, B, and C. It is estimated to contribute to about one-fifth of all enteric fever cases, though its relative burden compared to typhoid fever varies widely depending on the geographic region.[1] Its highest burden is observed in South and Southeast Asia, while it remains relatively uncommon in Africa [1, 2]. While once prevalent in Europe and North America, its incidence has markedly declined in these regions due to improved sanitation, clean water, and better food safety practices [3].

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According to the Global Burden of Disease (GBD) Study 2019, there were an estimated 3.8 million cases of paratyphoid fever globally, resulting in approximately 23,300 deaths, with an age-standardized incidence rate of 51.3 per 100,000 population. Emerging evidence indicates a rising trend in paratyphoid incidence, which may be partly associated with the introduction of typhoid vaccination, particularly typhoid conjugate vaccines (TCVs) that do not protect against *Salmonella paratyphi*. Children are the most affected age group, with incidence peaking among those aged 5–9 years [4].

Salmonella paratyphi A is the most common serovar causing paratyphoid fever, followed by *S. paratyphi* B, while *S. paratyphi* C is rare. Like *S. typhi*, *S. paratyphi* A is a human-adapted pathogen that produces a clinical syndrome similar to typhoid fever including fever, chills, and abdominal pain and can be life-threatening in severe cases [5]. Diagnosis relies on clinical presentation but requires laboratory confirmation through bacterial culture or molecular detection of bacterial DNA in blood, stool, or bone marrow. Culture remains the gold standard, with blood culture sensitivity ranging from 40–80%, highest in the first two weeks of illness, while bonemarrow cultures exhibit sensitivity of 80–95% and is less affected by prior antibiotic use [6]. Bone marrow sampling, however, is invasive and seldom performed in many clinical settings [7]. Stool and urine cultures are considerably less sensitive (stool ~30%, urine <1%) and typically reserved for later disease stages or for identifying carriers. Serological tests, such as Widal, are not diagnostic in endemic settings due to low sensitivity and specificity, though they may support diagnosis alongside clinical findings [6]. The Widal test is a classic bacterial agglutination assay that detects antibodies against the O (somatic) and H (flagellar) antigens of *S. enterica* serovars. While it is widely used in the diagnosis of enteric fever in resource-limited settings, its diagnostic accuracy is variable [8]. False positives occur due to baseline antibodies in endemic regions, cross-reactivity with non-typhoidal *Salmonella* or other infections (e.g., malaria), and delayed antibody response post–antimicrobial therapy [6]. Although not definitive, Widal continues in use out of convenience and low cost and functions best as a rule-out tool in association with clinical assessment and culture reports. While several epidemiological studies have been conducted globally on typhoid fever, very few have focused specifically on paratyphoid fever. The current study aims to address this gap by examining the serological prevalence and epidemiological characteristics of paratyphoid fever in the region of North India.

MATERIALS AND METHODS: The present prospective observational study was conducted for a period of eighteen months from January 2023 to June 2024, in the Department of Microbiology, Govt. Medical College, Patiala, Punjab. The serum samplesfrom the patients suspected of enteric fever received in the laboratory during the study period were included in the study. They were tested with 'Widal' test in which somatic 'O'antigen (TO) and flagellar antigens (TH, AH and BH) were used to detect antibodies against *S. typhi* and *S. paratyphi* A & B. All the serum samples were initially tested with slide widal test, and the samples giving positive agglutination were further tested by diluting the serum samples to 1:80, 1:160, 1:320 and 1:640 and final titers were calculated. As per the epidemiological data for our region, the titers of ≥ 1:160 for somatic 'O' antigen and ≥ 1:320 for flageller 'H' antigen was reported as significant [9] and suggestive of enteric fever. The data for all the serum samples testing positive for paratyphoid antigens i.e. TO, AH and BH with or without typhoid antigens (TH) were noted down and analyzed along with the epidemiological details of the patient. The serological prevalence rate for enteric fever and paratyphoid fever shall be calculated based on laboratory test results by dividing the number of samples positive during the study period by the total number of samples received in the laboratory from suspected patients multiplied by 100 [10].

RESULTS: During the eighteen months study period, a total of 3310 serum samples were received in the laboratory from the suspected enteric fever patients. Out of 3310 serum samples, 1102 samples tested positive by slide agglutination test for typhoidal or non typhoidal antigens giving a seroprevalence rate of 33% (n-1102) for enteric fever in our region while for paratyphoid fevers, it was calculated as only 1.3% (n-45) **Table 1**. Among forty five cases, 57.8% (n-26) cases were female while 42.2% (n-19) were male. Twentyone cases (46.7%) belonged to less than 15 years age group, nineteen cases (42.20%) from age group 16-45 years, and fourcases (8.9%) from age group 46-60 years while one case (2.2%) was more than 60 years old. The mean age of patients affected with paratyphoid fever was 18.9 years.

TABLE 1: THE SEROLOGICAL PREVALENCE OF PARATYPHOID FEVER USING WIDAL TEST

Total Number of serum samples examined	Number of samples positive (%)	Number of samples negative (%)	Number of samples positive for Paratyphoid antigens
3310	1102 (33.3%)	2208 (66.7%)	45 (1.3%)

Most of the paratyphoid fever cases showed serological positivity for AH agglutinin for Paratyphi A (53.3%, 24/45) while only 6% of paratyphoid cases (n-3/45) were positive for Paratyphi B. In 28.9% samples (n-13/45), agglutinins for both Typhi and Paratyphi A were detected whereas in 11.11% (n-5/45) samples, agglutinins for both Typhi and Paratyphi B were detected **Table 2**. Amongst all the samples, 0.36% (12/3310) showed agglutinins for all four antigens i.e. TO, TH, AH, BH suggesting previous TAB vaccination.

TABLE 2: EPIDEMIOLOGICAL AND SEROLOGICAL DETAILS OF POSITIVE CASES OF *SALMONELLA* PARATYPHI (N-45)

Gender		Age group	Total number of cases (%)	Widal test result		
				Flagellar antigen	No. of samples	Percentage
Male (% 19 (42.2%)	Female (%) 26 (57.8%)	≤15 years	21 (46.7%)	AH	24	53.5%
		16-45 years	19 (42.2%)	BH	3	6%
		46-60 years	4 (8.9%)	TH + AH	13	28.9%
		>60 years	1 (2.2%)	TH + BH	5	11.1%

DISCUSSION: Enteric fever still causes substantial illness and death in many parts of the world, especially in poorer nations. *S. enterica* serovar Typhi is believed to cause most enteric fever episodes, and a smaller portion are caused by *S. paratyphi* [11]. The incidence of typhoid and paratyphoid fever varies geographically, with south-central and south-east Asia having the highest incidence typically exceeding 100 cases per 100,000 person-years for typhoid and with lower, variable rates for paratyphoid [12].

But this scenario, however, has changed as recent studies have highlighted the increasing occurrence of paratyphoid fever. Yang *et al*. in their study reported a change in the enteric fever situation in south eastern province of China where more number of *S. paratyphi* A cases were isolated than *S. typhi* [13]. In a multicenter surveillance study conducted by Ochiai *et al*., amongst enteric fever cases, a high incidence of paratyphoid fever was observed in many south-east Asian countries, with a reported incidence of over 64% in China, 15% in Pakistan and 14% in Indonesia. In this study, the paratyphoid fever incidence in Kolkata, India was found to be 24% of all enteric cases [11].

Most available data suggest that paratyphoid fever accounts for about 10–30% of enteric fever cases in India, with significant regional variation. A study from CMC Vellore found culture confirmed paratyphoid fever weekly incidence (0-4 cases) to be one sixth of the typhoid fever incidence (0-25 cases).[14]In our study, the overall seropre valence of paratyphoid fever was 1.3% while it constituted around 4% of all enteric cases and more than 50% of the cases were caused by *S. paratyphi* A and few by Paratyphi B (6%). Another study conducted in Punjab, too found very low incidence rate (1.7%; 6/340) of *S. paratyphi* A cases among total number of examined enteric cases [15]. The possible explanation for such an increase in the incidence of paratyphoid fever in some of the studies could be the decrease in *S. typhi*. The reduced *S. typhi* incidence could be attributed to better preventive strategies including increased typhoid vaccine availability and coverage. The use of vaccine containing only *S. typhi* antigen does not give cross protection against *S. paratyphi* resulting in nochange in the incidence of *S. paratyphi* A [11]. The enteric fever has been found to be more prevalent among children typically less than 15 years of age [6]. A multicenter study, found the annual incidence of paratyphoid fever in Pakistan to be highest among children aged 2-16 years with reported incidence of 72 per 100,000 children [11]. A large-scale community study performed in an Indian urban slum showed incidence as high as 2 per 1,000 population per year for children under five, and 5.1 per 1,000 population per year for children under ten [15].

Another study from Northern India, found that the majority of paratyphoid fever cases occurred in children aged 5 to 12 years and 24.8% of cases were found in children up to 5 years of age [16]. *Salmonella* serovars showed an age-related bias, with paratyphoid fever more common in adults. One study from Kolkata showed the incidence of paratyphoid fever was lower (0.8/1000/year), and the mean age of paratyphoid patients was older (17.1 years) compared to typhoid fever (incidence 1.4/1000/year, mean age 14.7 years) [15]. In our study, maximum incidence of paratyphoid fever was seen among <15 years age group followed by 16-45 years with a mean age of 18.9 years. Thus, in our region too, the paratyphoid fever was prevalent among older children and young adults. In enteric fever, the gender variation in incidence is tilted towards males and similar gender distribution was seen in paratyphoid fever. The higher incidence of enteric fever among men could be attributed to variations in *S. typhi* exposure, potentially linked to gender-related differences in dietary practices and hygiene habits [17, 18]. Additionally, the male predominance in typhoid fever might be influenced by differences in the inflammatory response patterns within host Peyer's patches triggered by *S. typhi* or the natural exposure of Peyer's patches to *S. typhi*, considering sexual dimorphism [17]. However, we in our study found the incidence slightly more among females as compared to males. In another study conducted in Nigeria on University students, the female preponderance was observed in the prevalence of enteric fever [19].

In another study done by Varsha *et al* in Akola, Maharashtra found no significant difference in the percentage of male and female affected from enteric fever [20]. Thus, the actual burden of paratyphoid fever in India, its incidence and demographic details are still poorly understood. As humans are the only reservoir for these organisms and the main source of infection is the stool of infected persons; other sources are contaminated water, food, and possibly flies. Thus, keeping environment clean and provision of safe drinking water can largely prevent the occurrence of enteric fever. However, it is seldom possible for the population in poor countries either to boil their drinking water, or to sterilize the water by any other methods, thus the role of vaccination but the choice of vaccination should be based on the regional occurrence of infection [3]. The rising prevalence of paratyphoid A in India may be influenced by TCV rollout, which targets only *S. typhi*, antibiotic resistance patterns and changes in host immunity and environmental exposures. Therefore, future vaccination strategies should include bivalent vaccines that protect against *S. typhi* as well as *S. paratyphi* A, otherwise, the protective effectiveness of typhoid fever vaccines (Vi, Ty21a) against enteric fever may diminish, which could result in a loss of public confidence and reduced public willingness to be vaccinated [12].

The limitation of our study is that we were unable to correlate the serological test results with the blood culture positivity for enteric fever in the same patients. To date, no nationwide population-based study has been conducted on paratyphoid fever, unlike typhoid fever, which has been the focus of multiple large-scale studies. Therefore, there is a pressing need for a multicenter study to assess the exact prevalence of paratyphoid fever across different regions of India. Such a study would provide a clearer, nationwide picture of the disease burden, which could inform targeted public health strategies to control its endemicity.

CONCLUSION: Despite sharing clinical similarities with typhoid fever, paratyphoid fever remains an under-recognized public health concern in India. Current evidence suggests that its true burden is underestimated due to limited surveillance, reliance on serological tests with poor specificity, and the absence of nationwide population-based studies. Strengthening diagnostic capacity, integrating paratyphoid surveillance into existing enteric fever programs, and developing effective prevention strategies, including vaccines, are essential for controlling the disease in endemic regions like India.

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