

## Pattern of Antibody Titer Against Salmonella Enterica in Apparently Healthy Individuals

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### Abstract

#### BACKGROUND

Enteric fever is a serious health problem in developing countries including Nepal. Widal test is the routinely used for diagnosis of enteric fever. This study aimed to determine the baseline antibody titers for *Salmonella typhi* and *paratyphi* A, B in healthy individuals of Western Region of Nepal.

#### METHODS

A total 150 blood samples were collected from the healthy individuals and pattern of antibody titer was measured using standard quantitative tube method.

#### RESULTS

Among 150 blood samples, 103 had shown significant antibody titers ( $\geq 1:20$ ). The significant proportion (10.7%) of the individuals had anti-O titer  $\geq 1:80$ . Similarly, 86 had anti-H titers of  $\geq 1:20$  to *S. enteric* serotype *typhi*, 23 had a titer of  $\geq 1:80$  and 4 had a titer of  $\geq 1:160$  respectively. We found 10% and 1.3% for *paratyphi* A and B, anti-H titers of  $\geq 1:20$  respectively

#### CONCLUSION

This study concludes that, there should be need to change in the cutoff levels for antibody titer against *S. typhi* to  $> 1:80$  for both anti-O and anti-H titers for Western Development Region of Nepal.

#### KEY WORDS:

Widal test, *S. typhi*, Enteric fever



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## INTRODUCTION

Enteric fever is an important public health problem in many developing countries including Nepal, with a burden of approximately 16 million cases worldwide annually<sup>1</sup>. The *S. typhi* and *S. paratyphiserotype A, B* are the causative agent for typhoid fever worldwide<sup>2,3,4</sup>. The infection is acquired by the ingestion of contaminated food and water containing the *S. typhi* and / or *S. paratyphi*<sup>5</sup>. The poor hygiene and sanitation is main region for the disease outbreak in Nepal<sup>6,7</sup>. Nepal is the endemic for the Typhoid which is caused mostly by, *Salmonellatyphi* and *Salmonella paratyphi A with paratyphi B*<sup>8,9</sup>. The *S. typhi* has three distinct antigens, somatic (O) antigen, surface antigen, and flagellar (H) antigen<sup>10,11,12</sup>. The principle of various Widal agglutination test is based on O and H antigens<sup>13</sup>.

Clinical manifestation varies from person to person and may delay in case of immuno-compromised individuals. Clinical diagnosis and sign and symptoms of typhoid fever is varies from nonspecific symptoms like non-severe dengue fever and malaria to asymptomatic; for that the confirmative diagnosis can be done by using blood and/or bone-marrow culture which is ultimately time consuming, costly and labor-intensive<sup>14,15</sup>.

In Nepal, the laboratory facilities for blood culture for the confirmation of typhoid fever are not available in district laboratories and below district level health care centers, hence the serological test using the Widal test is done only in few hospitals, private clinics and primary health care centers<sup>16,12</sup>. The diagnosis is made on the basis of findings of Widal test<sup>17</sup>.

Only one study has been conducted regarding the determination of base line Widal titer in Kathmandu valley and referred to the pattern of antibody titer against *Salmonella enterica* entire country<sup>19</sup>. Moreover, this is the first study has been conducted outside Kathmandu valley regarding the pattern of Widal reaction among the apparently healthy individuals. The reference cut off value is not taken as standard

and has not been followed by the hospital laboratories and health care centers till date. The aim of our study is to establish the pattern of Widal agglutination reaction in apparently healthy population of western development region of Nepal.

## METHOD

### *Setting and Design*

A prospective cross-sectional study was conducted at department of Microbiology, School of Health and Allied Sciences, Pokhara University, Lekhnath municipality from June to October-2014. Apparently healthy volunteers were selected randomly from the Pokhara valley and its surrounding areas as a part of blood donation program. The survey was done using questionnaires and informed consent was taken from all the participants. The physical examination was done and all the blood donors were apparently healthy and qualified for this study. The participants were of following religions: Hindu, Buddhist and Christian. Total 150 individuals were participated in this study with age range 17-64 years. Widal Antigen set containing ready to use concentrated and smooth antigen suspension of the bacilli: S typhi O, S typhi H, S paratyphi AH, S paratyphi BH, Poly specific positive control by Tulip diagnostics (Pvt) LTD., India and physiological saline by Nirlife, India was used in this study.

### *Sample collection*

The 2.0 ml of whole blood was collected from the tubes of each blood bag that were not diluted by CPDA-1 (Citrate Phosphate Dextrose Adenine) present inside the blood bags. The samples were then transported to the laboratory within 1-2 hours in sterile condition for the further processing. Serum was separated immediately and stored in cryovial labeled with code number and stored at -20°C for further processing.

### *Assay Procedures*

Appropriate number of sets of 8 test tubes with antigen suspension was taken and tube numbers were labeled from 1-8. The 1.9 ml of normal saline was added in 1<sup>st</sup> tube of all sets,

and 1 ml of normal saline was added in remaining tubes. 0.1 ml of sample serum was added in 1<sup>st</sup> tubes and mixed well. Then serial dilution was made by taking 1ml of well mixed serum solution from 1<sup>st</sup> tube to 2<sup>nd</sup> then 2<sup>nd</sup> to 3<sup>rd</sup> and so on till to tube 7. 0.1 ml. of mixed serum solution was discarded from tube 7 from each set to make the dilution as follows: 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, and 1:1280<sup>1</sup>. Tube No 8 in all the sets, were used as a saline control. After addition of TYDAL antigen suspension in respective antigen, the tube was mixed well. All the test tubes were covered and incubated at 37°C for 18 hours and the agglutination was observed<sup>2</sup>.

#### *Interpretation of the observation*

The results were interpreted by observing the test tubes for 'O' antigen 'H' antigen for larger, loose, flocculent agglutination. The test results were scored as following: 0 (no agglutination), 1+ (25% agglutination), 2+ (50% agglutination), 3+ (75% agglutination) and 4+ (100% agglutination)<sup>2</sup>. Visualization and test to be assumed as positive, there should be at least 50% agglutination, then the screening of positive tests were diluted further for the determination of antibodies like 1:40, 1:80, 1:100, 1:160, and 1:320. The end point of serum antibody titer was finally noted by observing 2+ or 50% agglutination<sup>2</sup>.

#### *Quality Control of the Test*

Quality of each test was maintained by using various standard procedures. With each set of sera positive control using the poly specific positive antigen and negative control using a saline was kept. All the test tubes were covered with cotton plugs to prevent dust in the mixture. The temperature of the water bath was daily monitored. All the test tubes were sterilized before use. The micropipettes tubes were reused after leaving overnight in the disinfectant and cleaned. The expiry date, any coloration and clumping of the reagents were checked daily with each lot of reagents. Disinfectants were applied on the working bench and the sample processing was done in sterile conditions. Thus aseptic conditions were maintained while carrying out all the procedures.

#### *Statistical Analysis*

All the statistical analysis were performed using Statistical Package for Social Science (SPSS) version 16.0 (SPSS Inc., Chicago, IL, USA) and Microsoft excel 2007. The SPSS version 16 was used to carry out descriptive statistics. The results were expressed in the forms of pie-charts, bar diagrams, tables etc.

#### **RESULTS**

The majority of individuals were from the age group 21-30 years. As very few females were donated blood; only 24 blood samples were collected from women. The age and sex wise distribution of the subjects are shown in Figure 1 and 2.

#### *Proportion of antibody titer*

The antibody titers against various *Salmonella enterica* serotypes were determined on the separated serum. Out of 150 samples tested, 103 (68.7%) samples showed agglutination in  $\geq$  1:20 titration for the O or H antibodies against *Salmonella enterica* serotypes *Typhi*, *Paratyphi A* or *Paratyphi B*. The rest 47 samples did not show any agglutination and regarded as negative (Figure 3).

#### **Pattern of Antibody titer**

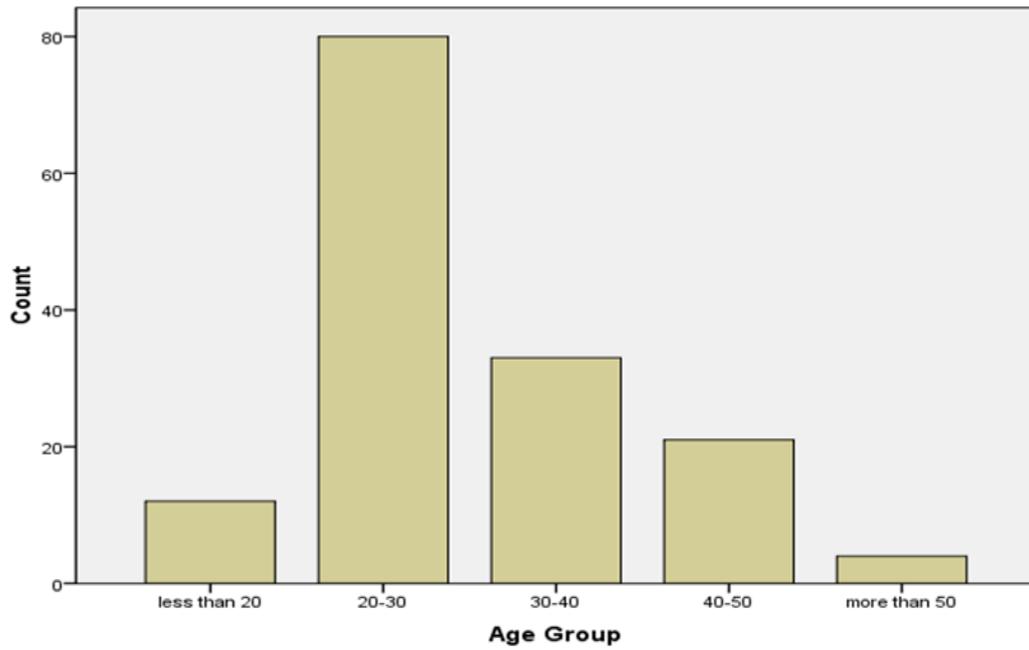
The maximum number of samples were positive for H antibody followed by O antibody and paratyphi AH antibody with only 2 samples positive in case of *S. paratyphi* BH antibody. Higher prevalence of H antibody titer was found in comparison to the O antibody titer among the normal healthy individuals (Table 1).

#### *Pattern of antibody titer against S. typhi O*

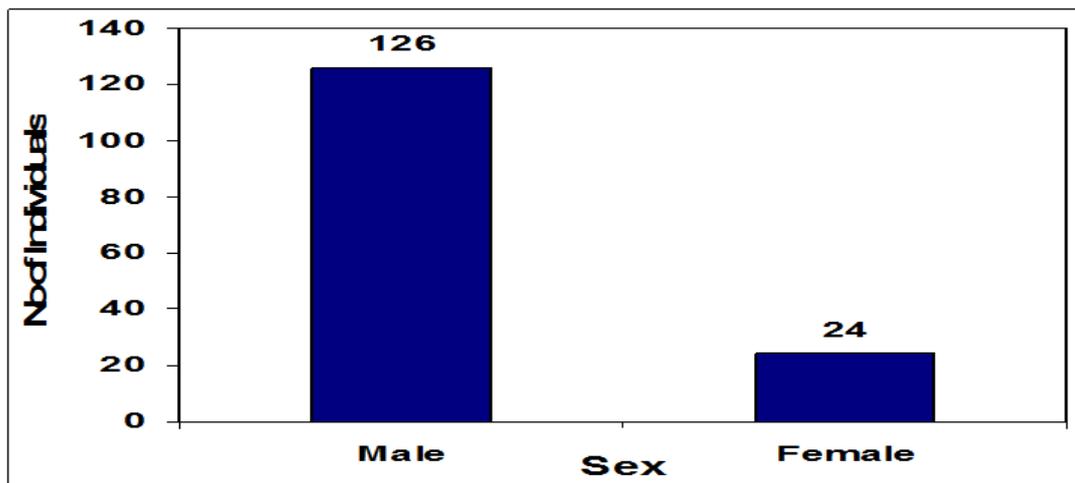
A total 70 samples had shown the end titer of 1:20 and 20 samples (13.3%) had an end titer of 1:40. Moreover, 9 samples (6%) were found to have end titer of 1:80, which can be taken as a significant proportion of population (Figure 4).

#### *Pattern of antibody titer against S. paratyphi AH*

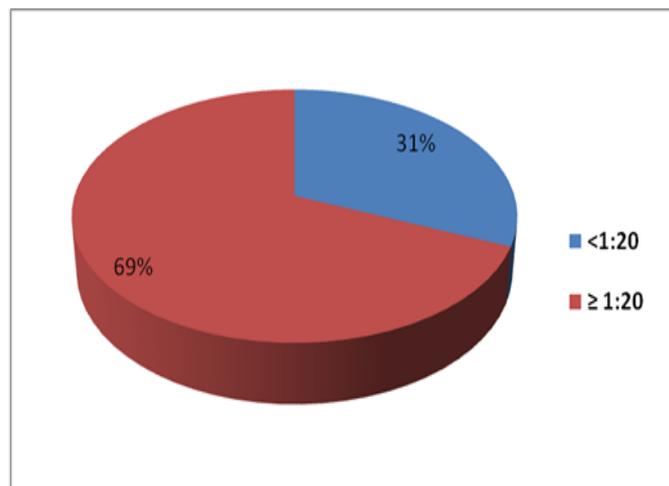
Out of 15 samples positive for AH antibody ( $\geq$ 1:20), a significant population of 10 (6.6%) has shown the end titer of 1:40 and 3 samples (2%) had an end titer of 1:20. Moreover, 2 samples (1.3%) were found to have end titer of 1:80 (Figure 6).



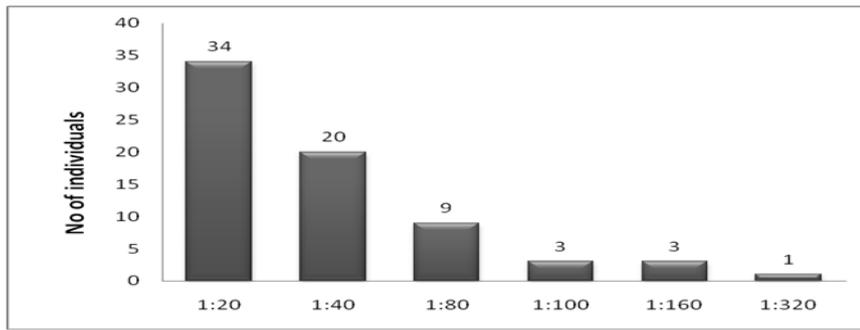
**Figure: 1** Distribution of antibody titer according to Age Group



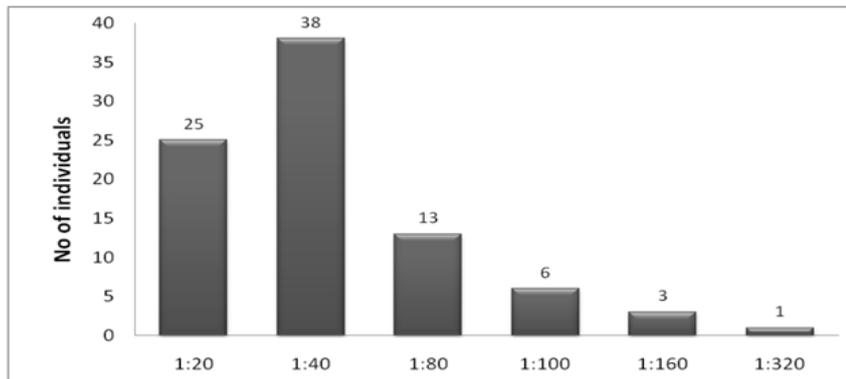
**Figure: 2** Distribution of antibody titer according to sex



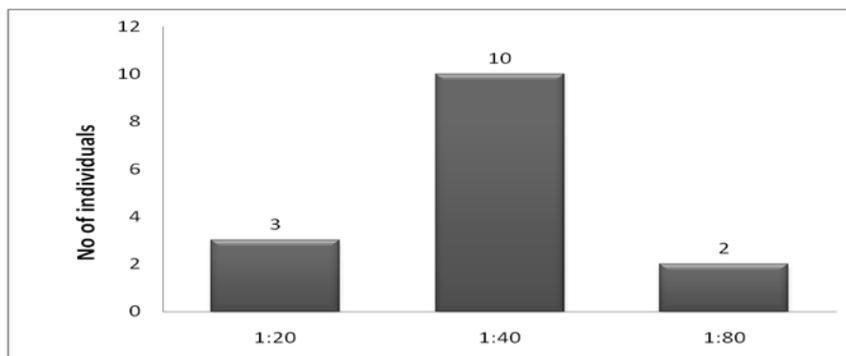
**Figure: 3** Proportion of antibody titres



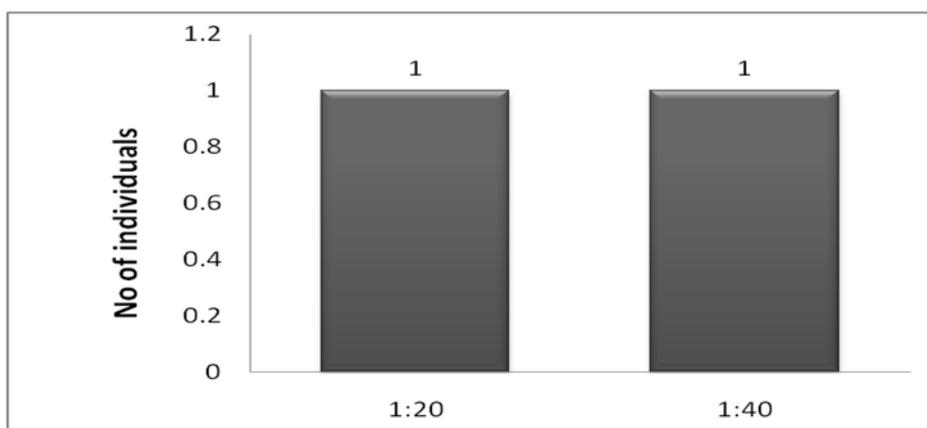
**Figure: 4** Pattern of 70 sera with anti-O end titers  $\geq 1:20$  against *S. enterica* serotype *Typhi*



**Figure: 5** pattern of 86 sera with anti-H and titers  $\geq 1:20$  against *S. enterica* serotype *Typhi*



**Figure: 6** Pattern of 15 sera with Anti 'H' end titers  $\geq 1:20$  against *S. enterica* serotype



**Figure: 7** Pattern of 2 sera with Anti 'H' end titers  $\geq 1:20$  against *S. enterica* serotype *Paratyphi B*

**Table 1:** Distribution of *Salmonella* agglutinin titers of  $\geq 1:20$  in 150 apparently normal individuals.

Salmonellae Antigens	No of positive samples	Percentage
S. typhi O	70	46.7
S. typhi H	86	57.3
S. paratyphi AH	15	10
S. paratyphi BH	2	1.3

## DISCUSSION

Although the entire country of Nepal is listed as endemic region for Typhoid infection, major epidemics have taken place in the Kathmandu. Drinking water pipelines and sewage pipelines run underground close to each other. A breach in sewage pipelines and subsequent fecal contamination of the water supply leads to major epidemics. Such close proximity of drinking water supply and sewage pipelines are not seen in most of the rural areas of Nepal making them less prone to outbreaks of Typhoid. This is the first study in the Western region of Nepal to evaluate the pattern of the Widal agglutination reaction to calculate average baseline antibody titer in healthy individuals against various serotypes of *S. enterica*. We found there is a wide variation of antibody titer against *Salmonella enterica* in healthy individuals of this region. A significant number individuals had high titers of antibodies, which direct to impose the consideration on the current cut-off values for diagnostic titer<sup>9</sup>.

Our study also suggests there is presence of certain level of agglutination titer in significant proportion of healthy individuals. It showed that 10.7% of these samples had anti-O antibody titers of  $\geq 1:80$  and 15.3% had anti-H antibody titers of  $\geq 1:80$  against *Salmonella enterica* serotype *typhi*. Also, the 8.0% of the total samples had anti-AH antibody titers of  $\geq 1:40$  and 1.3% had anti-BH antibody titers. In Nepal, diagnostic baseline titer of Widal agglutination test for typhoid fever is 1:80 for both O and H agglutination<sup>9</sup> In contrast, we found the high level of agglutinins for

*Salmonella typhi* in healthy individuals than those used to diagnosis of typhoid fever in Nepal. So, the result of this study suggests increasing the cut off value base line titer more than 1:80 for anti-O and more than 1:80 for anti-H for a probable diagnosis of typhoid fever.

Similar observations were made in Kathmandu, Nepal and Vietnam<sup>9,20</sup>. However, Pokhrel et al; 2009, found 12% cases were observed as anti-O titer greater than 1:80, and  $\geq 1:160$  for anti H titer, in contrast to the present study where H antibody titer was  $\geq 1:160$  in 2.7% only. This difference might be because of the level of titer in endemic area varies and low standard antigen preparation, technical and manual difference<sup>21</sup>. Cross reactions may also be encountered when non-typhoid antigen specific antibody reacts with typhoid specific antigen. Many other diseases caused by non-Salmonella organisms (miliary tuberculosis, chronic liver disease, endocarditis, brucellosis, malaria, dengue) can also play role in cross reactivity which is a source of error in Widal test results<sup>17</sup>. We found the antibody titer may vary among the endemic areas. Moreover, on the basis of life style and other social facilities like poor access to clean drinking water could possibly explain higher titer of salmonella antibodies titer in this group regardless of their immune status, baseline titer may also vary in healthy population of different regions and it should be updated with time.

## CONCLUSION

The finding of this study emphasized that, *Salmonella enterica* agglutinins are also common among apparently healthy people in different levels as determined by Widal agglutination test. We found the only Widal test for the diagnosis of Typhoid fever has no diagnostic value in the Western Region of Nepal. In addition, it should not be used as a screening test for asymptomatic individuals. Moreover, to minimize the false interpretation of the diagnosis of enteric fever, the results of this study suggests changing the currently used cutoff levels of antibody titer against *S.*

*enterica* serotype *typhito* > 1:80 for both anti-O and anti-H titers for Western Development Region of Nepal. Further studies are necessary to understand the long-term implications of titer against Typhoid antibodies in different region of Nepal.

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## CONFLICT OF INTEREST

None declared

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