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Research Article

Seroprevalence of *Salmonella typhi* in District Jalalabad, Nangarhar, Afghanistan

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Abstract

The bacterium *Salmonella typhi* is a motile enteric gram negative rod which causes typhoid fever and salmonellosis once reached to small intestines of humans by penetrating into the mucosal epithelium. once the bacterium infects the mucosa it passes 6 to 14 days so that to appear the prodromal symptoms. The most common symptoms of typhoid fever include abrupt fever, nausea, loss of appetite and severe headache. 12 to 30% is the mortality rate if the infection not treated properly; the rate of survival is 99% possible if treated carefully on time. Typhoid Prevalence is very high in South Asia, specifically in India and Pakistan. in the US the annual incidence rate declined from 7.5/100,000 people in 1940 to 0.2/100,000 people in 1990, annual number of cases in the UK declined from 2,500 in 1936 to less than 500 in 1990 - 2008. Nowadays Prevalence in developed countries is in range of 0.1 - 1/100,000 and typhoid cases greater than 80 percent in developed countries are due to the travel of emigrants, for example from India and nearby countries. In Pakistan incidence rate of this disease in children is estimated to be 170 per 100,000 of the population. On serology based which are 710 per 100,000 of the population [1]. The prevalence of typhoid in Egypt is 13/100, 000, in the Belbis District in Nile Delta as well 61/100, 000 personnel in Fayoum Governorate within the south. All the subjects sample were analyzed by rapid test cassettes for typhoid detecting IgG and IgM antibodies. The outcome of the study shows that there were only 4.75% positivity rate for typhoid IgG and IgM antibodies. All subject divided by five areas comprised of 80 population size in which the area one has positivity rate of 5, area two has 2, area three has also 2 the rest of two areas has 5 and 5 positivity rate. Based of sex, the rate of positivity was 4.82% in males while 4.65% in females. Higher positivity rate based on age was found in age group above 50 which was 16.67% while 3.57% in age group 26 - 50. Age group 1 - 10 had 8.33% while 11 - 25 group had 5.88% positive cases. Marital status based passivity rate for typhoid antibodies was found to be 5.28% in married while 4.16% in unmarried subjects of interest. Based on water consumption, 6.07% were positive who were using untreated water while using treated water were 1.67% positive.

Keywords: *Salmonella Incidence; Prevalence; ICT*

Introduction

Salmonella typhi is rod-shaped, Gram-negative, non-spore forming and motile enteric bacteria and 0.7 to 1.5 µm diameter range from 2 to 5 µm and flagella are present that help the bacteria to move in all directions. It is facultative anaerobic and obtain their energy from organic substance by oxidation and reduction reactions. Its produce H_2S by growing on media containing ferrous sulfate [2]. The bacterium caused acute infectious disease. Once the bacterium ingested through food contaminated with the specie it goes to small intestines and penetrate to the epithelium mucosa.

The average incubation period of the bacterium is 6 to 14 days rely on the number of inoculums, if the number of the bacteria is more so the period of incubation will be more as compared to low number of the bacteria present [3]. The general signs and symptoms of the infection include abrupt fever, nausea, loss of appetite and severe headache [2]. 12 to 30% is the mortality rate if the infection not treated properly, the rate of survival is 99% possible if treated carefully on time [2]. Prevalence of typhoid in Asia region is greater than 100 cases/100,000 populations per year. Typhoid Prevalence is very high in South Asia, specifically in India and Pakistan. This disease is more pathogenic to children [1]. Prevalence of *Salmonella typhi* declined in developed countries during 20th century, for example in the US the annual incidence rate declined from 7.5/100,000 people in 1940 to 0.2/100,000 people in 1990, annual number of cases in the UK declined from 2,500 in 1936 to less than 500 in 1990 - 2008. Nowadays Prevalence in developed countries is in range of 0.1 - 1/100,000, and typhoid cases greater than 80 percent in developed countries are due to the travel of emigrants, for example from India and nearby countries. In Pakistan incidence rate of this disease in children is estimated to be 170 per 100,000 of the population. On serology based which are 710 per 100,000 of the population [1]. The prevalence of typhoid in Egypt is 13/100, 000, in the Belbis District in Nile Delta as well 61/100, 000 personnel in Fayoum Governorate within the south [4]. There are many techniques used for the identification of *Salmonella typhi* or other salmonellosis causing agent including Widal test which detects antibodies against the bacterium antigen, it may pose false positive and false negative results [5]. The vital standard used for identifying the base of an infection is the separation and detection of the causative agent of infection [6]. Immunoassay tests are best implemented for the detection of *Salmonella* infections when there is no viable bacterium [6]. Amperometric Immunosensors, Metalloimmunoassays, Enzyme-linked Immunosorbent Assays (ELISA) and dot blot immunoassays are also used to detect specific antibodies against *Salmonella typhi* [7]. PCR multiplex also used to detect the amount of specific DNA particles present in the human genome, it is a very fast, accurate but expensive method for its diagnose [8]. Gold nanoparticle is immune chromatographic strip test used to detect antibodies, proteins, DNA and RNA [7].

Methodology

Sample size

The study was carried out in 5 areas of district Jalalabad. The study was based upon unknown prevalence of *Salmonella typhi* in the study area. For the calculation of samples size, the relevant formula used for a 95% confidence interval is as under:

$$ss = \frac{Z^2 * (p) * (1-p)}{c^2}$$

Where:

Z = Z value (e.g. 1.96 for 95% confidence level)

p = Percentage picking a choice, expressed as decimal (.5 used for sample size needed)

c = Confidence interval, expressed as decimal (e.g. .04 = ± 4)

n = 384.

Blood collection

All the blood collected by myself as conducting a dialogue with the subject of matter. The vein was anchored by holding the person's arm and placed a thumb bellow the venipuncture site. Entered the vein swiftly at a 30 angle and then continued to introduce needle along the vein. After collection of sufficient blood, the tourniquet was released before withdrawing the needle. The needle was withdrawn gentle and gentle pressure was applied to the site with a clean gauze or dry cotton-wool ball.

Materials and equipment

Human blood sample, Vacutainer tubes (cloth activator) BD Vacutainer plus tube clear BD hemogard closure, 3 ml and clear top. Serological pipettes of appropriate volumes, Centrifuge tubes, Cry vials, Bench top centrifuge with swing-out rotor and appropriate carriers.

Procedure

About 5 ml blood was collected in tubes with no anticoagulant treatment. All the samples were kept for about 30 - 45 minutes to allow clotting. Clot-activator tubes were inverted carefully 5 - 6 times to mix clot activator and blood before incubation. Blood samples were centrifuge for 15 minutes at manufacturer's speed (usually 1000 - 2000 rpm). Carefully aspirate the supernatant (Serum) at room temperature and pooled into a centrifuge tube, taken care of not to disturb the cell layer or transfer any cells. A clean pipette for each tube was used. Turbid samples were centrifuge and aspirated again to remove the reaming insoluble matter. Aliquots were made into cryo vials and store at -20°C. It was ensured that the cryovials were adequately labeled with the relevant information, including details of additives present in blood.

Material and equipment

Human blood samples, EDTA Tube, Serological pipettes of appropriate volumes**,** Bench top centrifuge with swing-out rotor and appropriate carrier.

Procedure

About 3 ml of blood was drawn in the EDTA tube which contains anti-coagulant. The tubes were in Vortex mixer to mix the anticoagulant and blood**.** Placed Blood samples were centrifuge for 5 to 10 minutes at manufacture's recommended speed (usually 10000 - 2000 rpm). Carefully aspirated the supernatant (Plasma) at room temperature and pooled into a centrifuge tube, taken care of not to disturb the cell layer or transfer any cells. A clean pipette for each for each tube was used. Aliquots were made into cryovials and store at -20°C. It was ensured that the cryo-vials were adequately labeled with the relevant information, including details of additives present in blood.

Specimen collection and preparation

Separate the serum or plasma from blood as soon as possible to avoid hemolysis. Only clear, non-hemolysed specimens can be used. Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Specimens may be stored at 2 - 8°C for up to 3 days for long term storage specimens should be kept bellow -20°C. Brings specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly. If specimens are shipped, they should be packed in compliance with usual regulations for transportation of etiological agents.

Clinical performance for IgM test

A total of 278 samples from susceptible subjects were tested by the typhoid IgG/IgM rapid test cassette and by a commercial *Salmonella typhi* IgM EIA. There was 97.5% overall agreement between this typhoid IgG/IgM rapid test cassette and *Salmonella typhi* IgM EIA. The overall sensitivity and specificity between these two were respectively 91.8% and 98.5%.

Clinical performance for IgG test

A total 295 samples from susceptible subjects were tested by typhoid IgG/IgM rapid test cassette and by a commercial *Salmonella typhi* IgG EIA. There was 97.6% overall agreement between this typhoid IgG/IgM rapid test cassette and *Salmonella typhi* IgG EIA. The overall sensitivity and specificity between these two were respectively 92.7% and 98.7%.

Results and Discussion

Overall sero-prevalence of *Salmonella typh*i in district Jalalabad by typhoid IgG/IgM rapid test cassette

Out of 400 human blood samples divided to 5 area of district Jalalabad each area having 80 population size in total population size 228 (57%) were male and 172 (43%) female samples these samples were test for *Salmonella typhi* by typhoid IgG/IgM rapid test Cassette showed that 19 (4.75%) in total population size were positive cases.

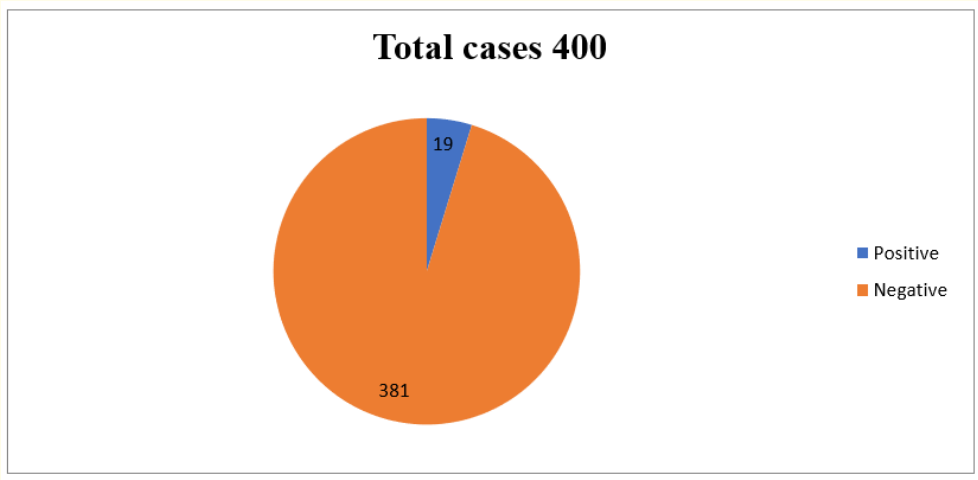


Figure 1

Areas wise sero-prevalence of *Salmonella typhi* by using typhoid IgG/IgM rapid test cassette

Over all areas wise sero-prevalence of *Salmonella typhi* in Jalalabad population was recorded as 6.25% in area 1, 2.5% in area 2, 2.5% in area 3, 6.25% in area 4 and 6.25% in area 5 by using typhoid IgG/IgM rapid test cassette (Figure 2). No significant statistical difference ($P < 0.05$) was observed with respect to different areas by typhoid IgG/IgM rapid test cassette.

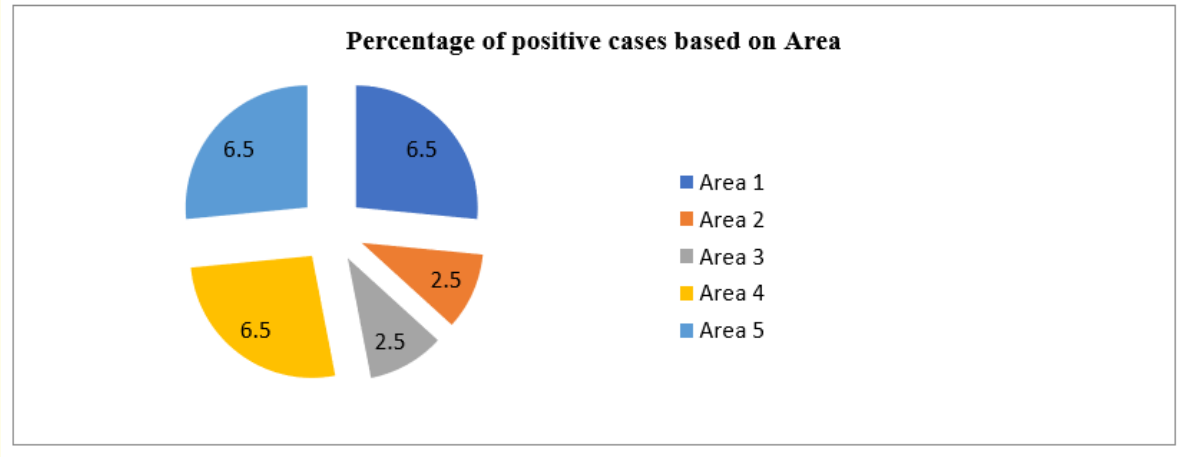


Figure 2

Overall sex based sero-prevalence of *Salmonella typhi* in district Jalalabad by using typhoid IgG/IgM rapid test cassette

By using typhoid IgG/IgM rapid test cassette, it was found that sero-prevalence of *Salmonella typhi* was a bit higher in males (4.82) than females (4.65%) (Figure 3). but statistically, there was no significant difference as ($P < 0.05$) of sero-positivity in relation to sex of the district population.

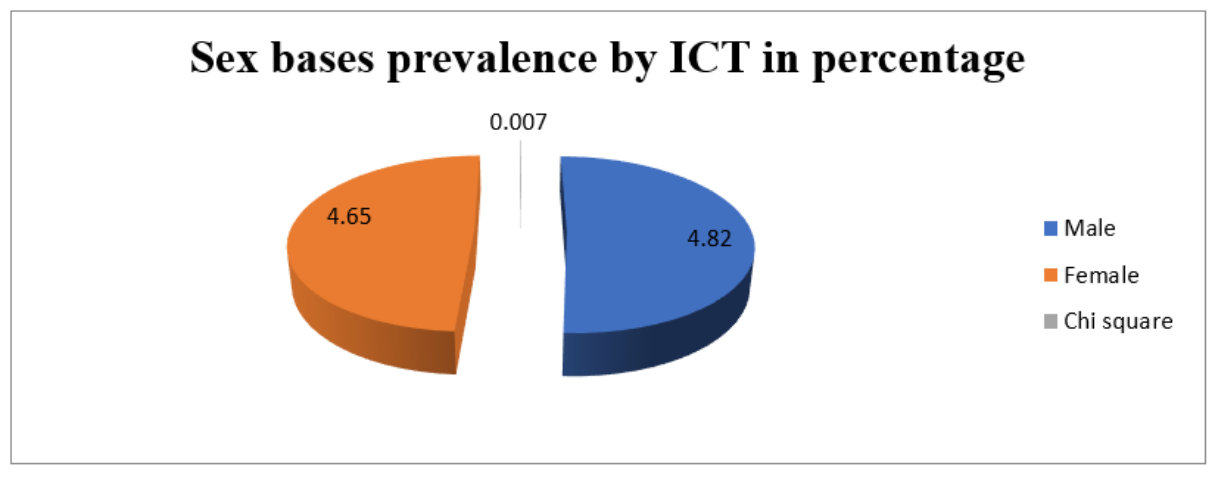


Figure 3

Sex based sero-prevalence of typhoid in different areas of district Jalalabad by using typhoid IgG/IgM rapid test cassette

In area 1, the positivity rate in females depicted as 5.56% while in males it was 6.82%. In area 2, males showed lower positivity rate (2.00%) while females had (3.3%). Non-significant difference statistically observed in both areas, ($P > 0.05$) in relation to sex of the both areas population size. In 3rd area (5.41%) males while females were 0%. In area 4, positivity rate in males was (1.89%) and in females it was (14.81%), area 5, (9.09%) were males positive while (2.78%) were females. There was non-significant difference statistically found in all area between genders where the P-value is ($P < 0.05$).

Areas	Sample Size	Gender	Positive cases percentage	Chi-square	P-value
Area 1	44	Male	6.82	4.017	0.404
Area 2	50	Male	2.00		
Area 3	37	Male	5.41		
Area 4	53	Male	1.89		
Area 5	44	Male	9.09		
Area 1	36	Female	5.56	8.855	0.065
Area 2	30	Female	3.33		
Area 3	43	Female	0.00		
Area 4	27	Female	14.81		
Area 5	36	Female	2.78		

Table 1

Age based sero-prevalence of *Salmonella typhi* in different areas by using typhoid IgG/IgM rapid test cassette

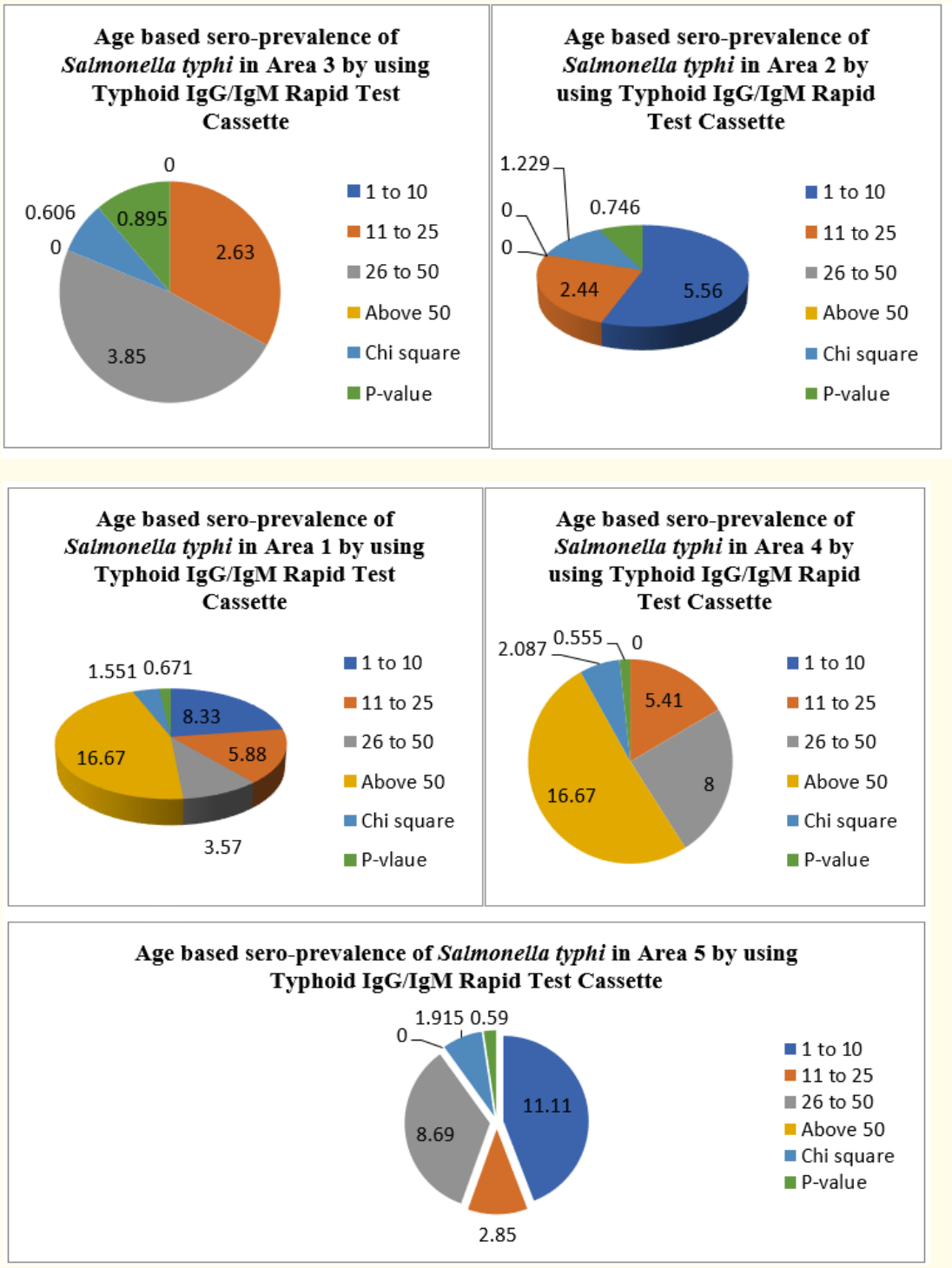


Figure 4

Age based sero-prevalence of *Salmonella typhi* in different sex groups by using typhoid IgG/IgM rapid test cassette

Sero-prevalence of *Salmonella typhi* by typhoid IgG/IgM rapid test was recorded higher in Males age groups of 1 - 10 (6.38%) than males of age groups 11 - 25 (4.90%), 26 - 50 (5.00%), > 50 (0.00%). Statistically non-significant difference was shown as the $p < 0.05$. Similarly, sero-prevalence was recorded higher in Females of age group > 50 (9.09%) then age group of 1 - 10 (0.00%), 11 - 25 (3.70%, 26 - 50 (7.50%). Statistically non-significant difference was shown figure 4.

Age group	Sample Size	Gender	Positive cases percentage	Chi-square	P-value
1 to 10	47	Male	6.38	0.714	0.870
11 to 25	102	Male	4.90		
26 to 50	60	Male	5.00		
Above 50	10	Male	0.00		
1 to 10	25	Male	0.00	2.842	0.417
11 to 25	81	Female	3.70		
26 to 50	53	Female	7.50		
Above 50	11	Female	9.09		

Table 2

Marital status based sero-prevalence of *Salmonella typhi* in different areas of district Jalalabad by using typhoid rapid test cassette

Married showed 9.09% while unmarried showed 2.78. In area 2, unmarried depicted to be positive (2.63%) than married (2.38%). In case of area 3, (2.38%) were unmarried positive while (2.63%) were married positive. In area 4, 3.03% in singles and 8.51% married were positive. In area 5, it was (9.30%) married who were positive while (2.70%) in married. statistically non-significant difference was observed in relation to the marital status which revealed that married are more affected than singles in all the areas where P-value is $p < 0.05$.

Areas	Sample Size	Marital status	Positive cases percentage	Chi-square	P-value
Area 1	44	Single	2.78	3.681	0.451
Area 2	50	Single	2.63		
Area 3	37	Single	2.38		
Area 4	53	Single	3.03		
Area 5	44	Single	9.30		
Area 1	36	Married	9.09	3.983	0.408
Area 2	30	Married	2.38		
Area 3	43	Married	2.63		
Area 4	27	Married	8.51		
Area 5	36	Married	2.70		

Table 3

Marital based sero-prevalence of *Salmonella typhi* in different age groups by using typhoid IgG/IgM rapid test cassette

Marital status based sero-prevalence of *Salmonella typhi* in 1 - 10, years age group was recorded higher in singles (5.5%) than males (0%). In age group 11 - 25, was recorded to be lower in singles (2.38%) then married (4.21%). In age group 26 - 50, was recorded to be higher in married (5.66%) then singles (5.26%), similarly in age group of > 50, it was recorded 0.00% in singles and 10.00% in married. Statistically it was seen to be non-significant in all age groups as $p < 0.05$.

Age group	Marital status	Sample size	Positive cases percentage	Chi-square	P-value
1 to 10	Single	75	5.33	1.060	0.787
11 to 25	Single	84	2.38		
26 to 50	Single	19	5.26		
Above 50	Single	1	0.00		
1 to 10	Married	0	0.00	1.100	0.577
11 to 25	Married	95	4.21		
26 to 50	Married	106	5.66		
Above 50	Married	20	10.00		

Table 4

Marital status based sero-prevalence of *Salmonella typhi* in different sex by using typhoid IgG/IgM rapid cassette

Sero-prevalence of *Salmonella typhi* of marital status by Typhoid IgG/IgM rapid test cassette in single-male was higher (5.93%) then female-single (1.35%). Statistically non-significant difference was observed, similarly the sero-prevalence of *Salmonella typhi* in male-married was observed to be lower (3.70%) then female-married peoples (7.00%). Statistically the relationship between the marital status and sex was non-significant.

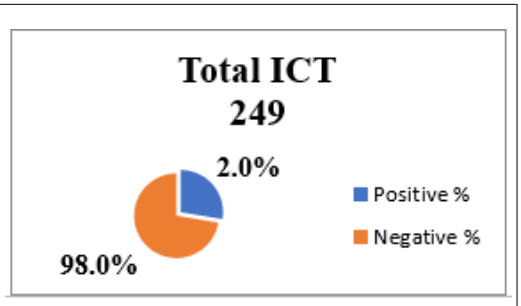
Sex	Marital status	Positive cases	Positive %	
Male	Single	7	5.93	
Female	Single	1	1.35	
Chi square	2.390			
P-value	0.122			

Table 5

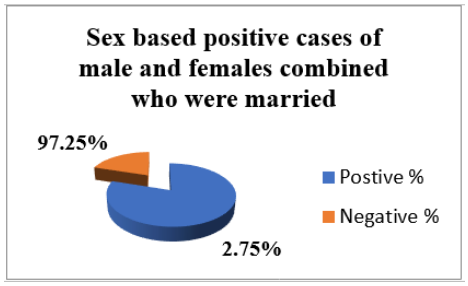
Sex	Marital status	Positive cases	Positive %	
Male	Married	4	3.70	
Female	Married	7	7.00	
Chi square	1.126			
P-value	0.289			

Table 6

Overall water intake based sero-prevalence of *Salmonella typhi* by using typhoid IgG/IgM rapid test cassette

Overall sero-prevalence of *Salmonella typhi* in under study population was recorded as 1.67% among peoples used treated water and 6.07% using untreated water by use of typhoid IgG/IgM rapid test cassette. Statistically, the difference was significant as $p < 0.05$. It's because of poor water supply system in the city and public unawareness.

Water intake	Positive cases	Positive %	Chi-square	P-value
Treated water	120	1.67	3.620	0.052
Untreated water	280	6.07		

Table 7

Overall sewage exposure based sero-prevalence of *Salmonella typhi* by typhoid IgG/IgM rapid test cassette

Sero-prevalence of *Salmonella typhi* by using typhoid IgG/IgM rapid test was higher in peoples who were exposed to sewage (6.67%) then sewage non exposure (5.93%). Statistically non-significant difference in sero-positivity was shown with relation to sewage exposure of the under studied population.

Sewage exposure	Total cases	Positive %	Chi-square	P-value
Yes	30	6.67	0.026	0.781
No	270	5.93		

Table 8

Overall economic status based sero-prevalence of *Salmonella typhi* by using typhoid IgG/IgM rapid test cassette

Sero-prevalence of *Salmonella typhi* by using typhoid IgG/IgM rapid test cassette was a bit higher in poor (7.14%) than moderate (3.64%) and rich (2.50%). Statistically, there was non-significant difference in sero-positivity in relation to economic status of the peoples.

Economic status	Total cases	Positive cases	Positive %	Chi-square	P-value
Poor	140	10	7.14		
Moderate	220	8	3.64		
Rich	40	1	2.50	2.822	0.244
Total	400	19			
No					

Table 9

Conclusion

Prevalence of anti-*Salmonella typhi* antibody is 4.75% in the studied of remote areas population. This bacterium is possibly candidates for typhoid disease and potential source of the disease spread both horizontally and vertically. It is imperative to create mass awareness program on different aspects of these deadly infection in the rural community. Mass screening program should be undertaken to define the infected population properly so that they may be managed as per guidelines and further spread of the disease may be prevented. It's important to aware public and government official about better hygiene conditions and distribution of drinking water, safe drinking water and sanitary disposal of excreta.

Bibliography

1. Imran ZP., *et al*. "Molecular and epidemiological analysis of multidrug-resistant *Salmonella paratyphi* an obtained from different regions of Pakistan". *Pakistan Journal of Botany* 42.4 (2010): 2941-2947.

2. Osuntokun OT., *et al*. "Antibacterial and phytochemical properties of some Nigerian medicinal plants on Salmonella typhi and *Salmonella paratyphi* isolated from human stool in Owo local government, Ondo State, Nigeria". *Journal of Scientific Research and Reports* 4.5 (2015): 5-12.

3. WHO. Background document: "The diagnosis, treatment and prevention of typhoid fever". World Health organization, Dept Vaccines and Biological Geneva Switzerland (2003): 1-38.

4. Rahman BA., *et al*. "Multi-drug resistance and reduced susceptibility to ciprofloxacin among *Salmonella enterica serovar typhi* isolates from the Middle East and Central Asia". *New Microbes and New Infections* 2.4 (2014): 88-92.

5. Elgayar KE., *et al*. "Application of *Salmonella typhi* outer membrane (OMP) in diagnosis of typhoid". *International Journal of Current Research* 5 (2013): 2037-2042.

6. Chart HT., *et al*. "Serodiagnosis of *Salmonella enterica serovar typhi* and *Salmonella enterica serovars paratyphi* A, B and C human infections". *Journal of Medical Microbiology* 56.9 (2008): 1161-1166.

7. Preechakasedkit PK., *et al*. "Development of a one-step immunochromatographic strip test using gold nanoparticles for the rapid detection of *Salmonella typhi* in human serum". *Biosensors Bioelectronics* 31.1 (2011): 562-566.

8. Mandal JM., *et al*. "Development of a multiple polymerase chain reaction protocol for the simultaneous detection of *Salmonella enterica serovar typhi* and Class 1 integron". *Asian Pacific Journal of Tropical Disease* 4.2 (2014): 808-812.

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