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Full Length Research Paper

Prevalence of malaria and typhoid co-infections in University of Nigeria, Nsukka District of Enugu State, Nigeria

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Mixed infection of malaria caused by *Plasmodium* species and typhoid fever caused by *Salmonella* species is often observed in areas where malaria is endemic, and the infection with *Salmonella* species has been considered by some medical and non-medical personnels to be associated with the malaria parasite infection. Based on this reason, many medical personnel treat both malaria and typhoid simultaneously in every case of suspected *Salmonella* infection and vice versa. In this study, the association between malaria and typhoid infections was investigated. Twenty five malaria patients were screened for antibody against *Salmonella* species using widal test. The stool and blood samples of the patients were also screened for possible isolation of *Salmonella* species. Of the 25 subjects, 23 (92%) had positive antibody titre (≥1/80) for *Salmonella paratyphi* A, 11 (44%) were positive for *S. paratyphi* B, 8 (32%) were positive for *S. paratyphi* C, while 5 (20%) were positive for *Salmonella typhi*. *Salmonella* species were isolated from stool samples of 13 subjects, but only 3 subjects had *Salmonella* species in their blood samples. The results indicated that there is no relationship between malaria and *Salmonella* infection, but there was a significant ($p < 0.01$) association between *S. typhi*, *S. paratyphi* B and *S. paratyphi* C infections. The results also showed a significant ($p < 0.05$) relationship between *Salmonella* in stool and its appearance in blood, suggesting that the presence of *Salmonella* in blood is indicative of its presence in stool.

Key words: *Plasmodium*, *Salmonella*, typhoid fever, malaria, co-infection.

INTRODUCTION

Malaria is a tropical disease of man caused by some species of *plasmodium* and characterised by fever, malaise and weakness. Malaria is the infectious disease that causes incidence estimates of 2 to 3 million deaths and 300 to 500 million clinical cases in the world (Niikura et al., 2008). There are four species of *Plasmodium* that infect humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*. *P. falciparum* is the major human parasite responsible for high morbidity and mortality, and infection with *P. falciparum* is associated with developing fever, a high number of parasites in the blood and pathogenesis, including severe anaemia, body weight loss and cerebral malaria in humans (Niikura et al., 2008).

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Typhoid fever is also an infectious disease. It is caused by species of *Salmonella*. The species and strains of *Salmonella* that commonly cause typhoid fever in humans are *Salmonella paratyphi* A, *Salmonella paratyphi* B, *Salmonella paratyphi* C and *Salmonella typhi* (WHO, 2003; Lerner and Lerner, 2003). The different serotypes of *Salmonella* can coinfect an individual or cause infections differently. Like malaria fever, *Salmonella* infection is characterised by fever, weakness,anaemia, body weight loss, vomiting and sometimes diarrhoea (WHO, 2003; Samal and Sahu, 1991). The detection of high antibody titre for *Salmonella* is not always indicative of current infection(s) (Samal and Sahu, 1991). Therefore, stool and/or blood culture from the patients is/are confirmatory (WHO, 2003; Lerner and Lerner, 2003).

The co-infection of malaria parasite and *Salmonella* species is common, especially in the tropics where malaria is endemic. The common detection of high antibody

titre of these *Salmonella* serotypes in malaria patients has made some people to believe that malaria infection can progress to typhoid or that malaria always co-infect with typhoid/paratyphoid in all patients. Hence, some people treat malaria and typhoid concurrently once they have high antibody titre for *Salmonella* serotypes, even without adequate laboratory diagnoses for malaria and vice versa. This work is therefore aimed at determining the rate of coinfection of malaria and typhoid in an area where both seem to be endemic and have generated a lot of public health concern.

MATERIALS AND METHODS

Sampling

Twenty five adult malaria patients were selected for this study from University of Nigeria, Nsukka Medical Center. An informed consent was obtained from all the subjects used. Blood specimens were collected from each of the subjects with syringe and needle through vein puncture and transferred into commercially prepared sterile EDTA bottles. Sterile specimen containers were given to the subjects and they were instucted to collect their early morning stool specimens.

Serology assay for *Salmonella* spp. antibodies (widal test)

The blood samples were analysed within one hour of collection for antibody against *Salmonella* species. The blood specimens were centrifuged at 5000 rpm for about 20 min. Then, the serum was separated from the plasma using Pasteur pipette. Few drops of the sera were put on a white tile (8 different points), and then few drops each of *Salmonella* serotypes antigens (BioSystem Widal kit) were put onto the sera and mixed. The mixtures were tilted or rocked for about 20 min. Then, the antibody agglutination titre was observed, and recorded. Any serum with antibody titre ≥1/80 for *Salmonella* specie(s) somatic (O) antigen was considered positive for *Salmonella* infection. However, individual serum with titre ≥1/40 but <1/80 was considered to possibly have trace infection.

Isolation and identification of *Salmonella* spp. from stool and blood samples

One millilitre of the blood specimens were inoculated into 5 ml of Trypticase Soy Broth (TSB) supplemented with 0.025% sodium polyanetholesulfonate (SPS) and incubated at 37°C for 24 h. The inoculum for the broth culture was inoculated onto blood agar and *Salmonella-Shigella* agar (SSA) and incubated at 37°C for 48 h but observed at 24 h intervals for growth. The isolates were identified through conventional microbiological procedures.

The stool specimens were also inoculated into the selenit-F broth and incubated at 37°C for 24 h, and then sub-cultured onto SSA and incubated as stated earlier. All the samples were processed within 2 h of collection.

Statistical analysis

The data generated in this study were analysed using statistical package for social sciences (SPSS). The association between malaria and typhoid/parayphoid co-infections was determined by Pearson correlation and Chi-square.

RESULTS

All the malaria patients screened for antibody titre against *Salmonella* species had positive antibody titre (≥1/80) for one, two, three or all of the *Salmonella* species. The statistical analysis of the data using Chi-square showed a non-significant association between malaria and typhoid infections. The results of the infections and/or co-infections between the different *Salmonella* species or strains are described below. Of the 25 subjects, 3 (12%) had positive antibody titres (≥1/80) with *Salmonella* species isolated from their stool and blood specimens; 10 (40%) had antibody titres (≥1/80) to some of the *Salmonella* serotypes with *Salmonella* spp. isolated from their stool but not blood; while 12 (48%) had antibody titres (≥1/80) without *Salmonella* being isolated from either their stool or blood.

Co-infection of malaria and *S. paratyphi* A

Of the 25 subjects, 23 (92%) had positive antibody titre (≥1/80) for *S. paratyphi* A somatic antigens, while 2 (8%) were negative for the antibody (≤1/40) against *S. parayphi* A. Of 23 subjects that had positive antibody titre to *S. paratyphi* A, 4 (16%) had moderate level of infections (titre = 1/80), 11 (44%) were highly infected (titre = 1/160), while 8 (32%) were severely infected (Table 1 and Figure 1). The result in Table 1 indicated high prevalence of *S. paratyphi* A among malaria patients. The correlation analysis indicated that there is no significant ($p > 0.01$ and $p > 0.05$) relationship between malaria infection and *S. paratyphi* A (Table 7).

Co-infection of malaria and *S. paratyphi* B

Of the 25 subjects, 11 (44%) had antibody titre ≥1/80, while 14 (56%) had antibody titre ≤1/40 to *Salmonella paratyphi* B somatic antigens. There were variations in the level of infections as 2 subjects (8%) each had moderate and severe infections respectively, while 7 subjects (28%) were highly infected (Figure 2 and Table 2). There was also no significant ($p > 0.05$ and $p > 0.01$) relationship between malaria and *S. paratyphi* B, but the results indicated a strong significant ($p < 0.01$) association between *S. paratyphi* B and *S. typhi* antibody titres in these malaria patients used for this study.

Co-infection of malaria and *S. paratyphi* C

Of all the subjects, 8 (32%) were positive for antibody against *S. paratyphi* C as compared with the non-antibody positive subjects, 17 (68%). Twelve of the subjects (48%) had antibody titre of 1/20 for *S. paratyphi* C infection, and 5 subjects had antibody tiitre of 1/40. Four (16%) each of the subjects had moderate and high levels

Table 1. Frequency of *Salmonella paratyphi* A co-infection with malaria.

Degree of infection	Frequency	Percent	Valid percent	Cumulative percent
No Infection	1	4.0	4.0	4.0
Trace infection	1	4.0	4.0	8.0
Moderate infection	4	16.0	16.0	24.0
High infection	11	44.0	44.0	68.0
Severe infection	8	32.0	32.0	100.0
Total	25	100.0	100.0	

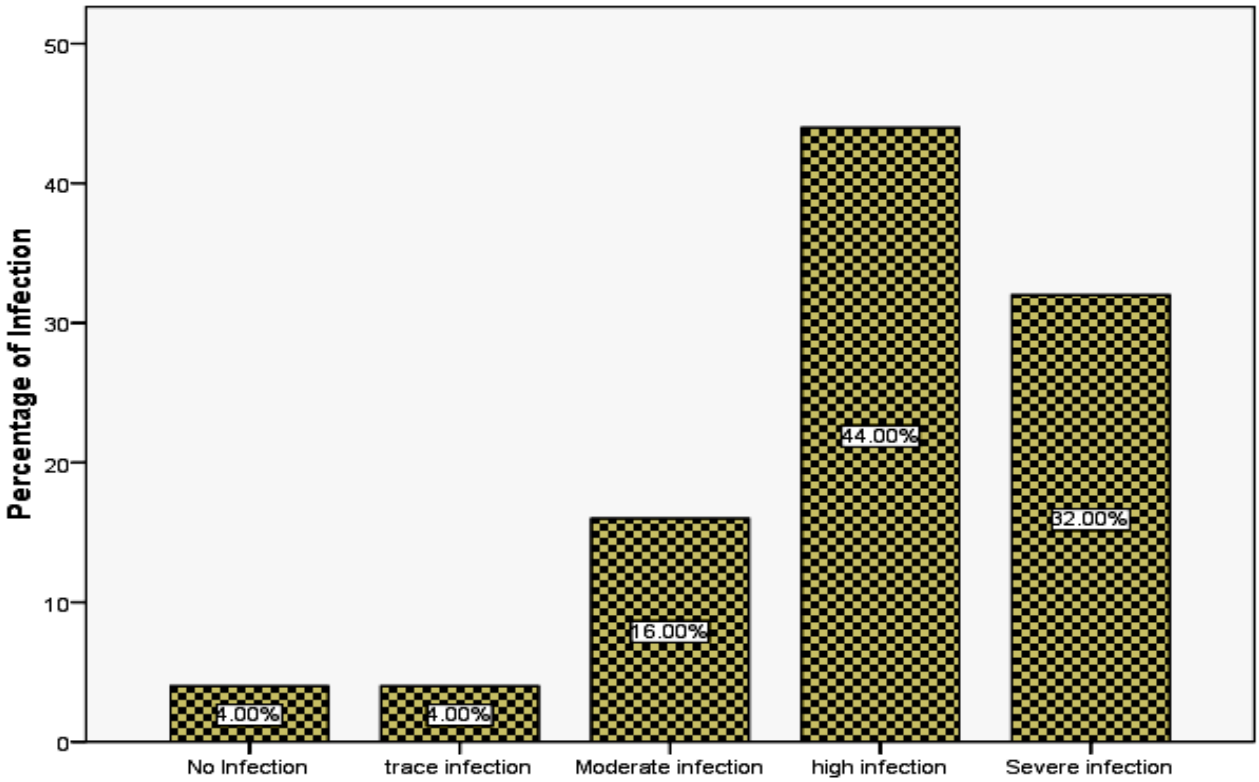

Figure 1. Prevalence of *S. paratyphi* A infection among malaria patients. Antibody titre: 1/20, No infection; 1/40, trace infection; 1/80, moderate infection; 1/160, high infection; 1/320, severe infection.

of infections, respectively (Table 3 and Figure 3). The correlation of malaria with *S. paratyphi* C infections indicated no significant ($p > 0.01$ and $p > 0.05$) association between the co-infection, but there was a significant ($p < 0.01$) association between *S. paratyphi* C and *S. typhi* antibody titres (Table 7).

Co-infection of malaria and *S. typhi*

Of the 25 subjects, only 5 (20%) had positive antibody titre (≥1/80) for *S. typhi*, while 20 (80%) had negative antibody titre (≤1/40) for the *S typhi* antigens (Figure 4 and Table 4). Multiple Pearson's correlation showed that there is significant ($p < 0.01$) association between the presence of *S. typhi* antibody titre and *S. paratyphi* B and C antibody titres (Table 7).

Isolation of *Salmonella* species from stool samples of malaria patients

Of the 25 subjects, *Salmonella* species were isolated from stool samples of 13 (52%) of the subjects but not from 12 (48%) of the subjects (Figure 5 and Table 5). The statistical analysis showed a significant ($p < 0.05$)

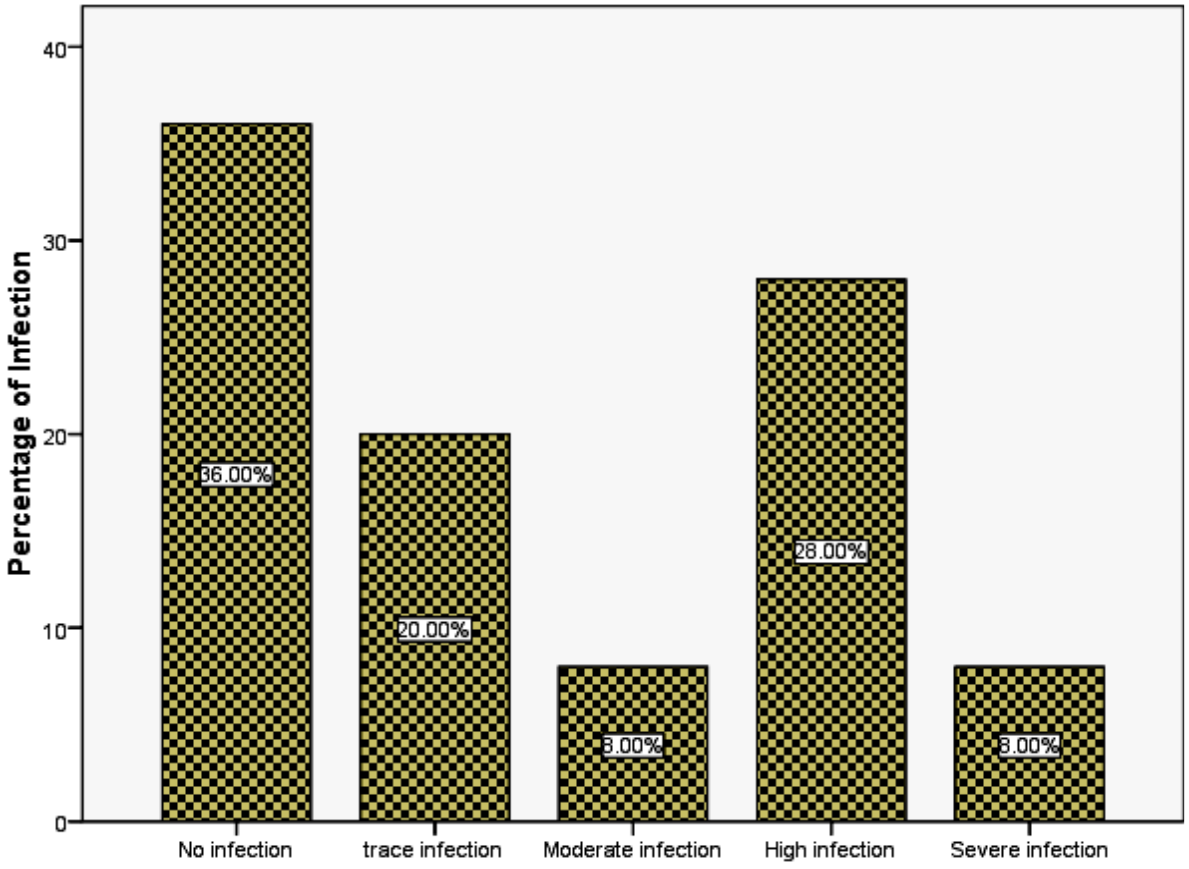

Figure 2. Prevalence of *S. paratyphi* B infection among malaria patients. Antibody titre: 1/20, No infection; 1/40, trace infection; 1/80, moderate infection; 1/160, high infection; 1/320, severe infection.

Table 2. Frequency of *Salmonella paratyphi* B co-infection with malaria.

Degree of infection	Frequency	Percent	Valid percent	Cumulative percent
No Infection	9	36.0	36.0	36.0
Trace infection	5	20.0	20.0	56.0
Moderate infection	2	8.0	8.0	64.0
High infection	7	28.0	28.0	92.0
Severe infection	2	8.0	8.0	100.0
Total	25	100.0	100.0	

Table 3. Fequency of *Salmonella paratyphi* C co-infection with malaria.

Degree of infection	Frequency	Percent	Valid percent	Cumulative percent
No Infection	12	48.0	48.0	48.0
Trace infection	5	20.0	20.0	68.0
Moderate infection	4	16.0	16.0	84.0
High infection	4	16.0	16.0	100.0
Total	25	100.0	100.0	

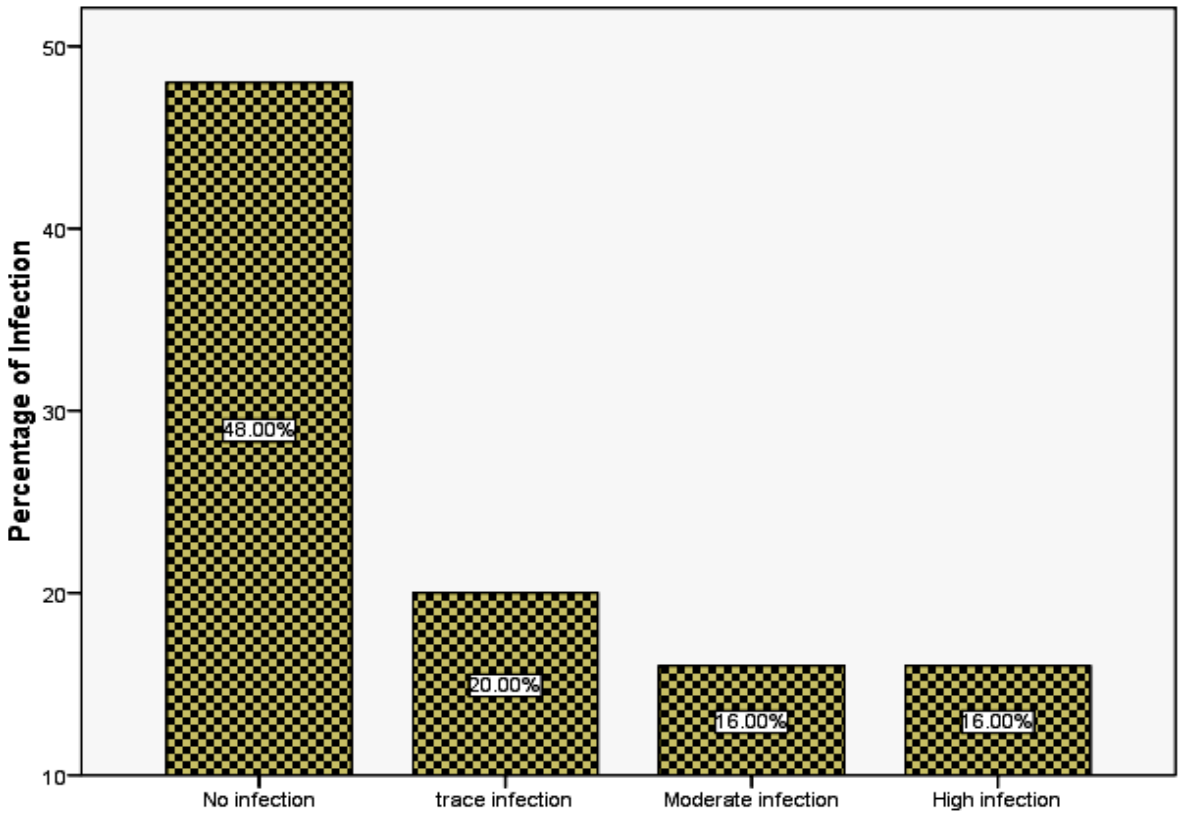

Figure 3. Prevalence of *S. paratyphi* C infection among malaria patients. Antibody titre: 1/20, No infection; 1/40, trace infection; 1/80, moderate infection; 1/160, high infection; 1/320, severe infection.

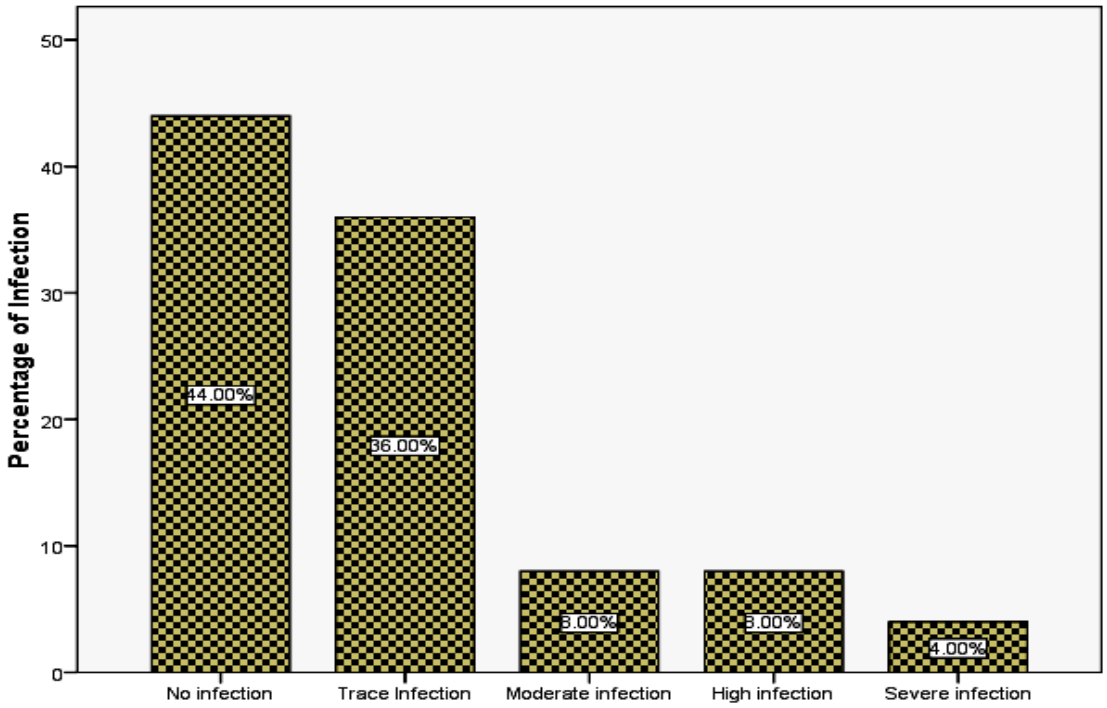

Figure 4. Prevalence of *S. typhi* B infection among malaria patients. Antibody titre: 1/20, No infection; 1/40, trace infection; 1/80, moderate infection; 1/160, high infection; 1/320, severe infection.

Table 4. Frequency of *Salmonella typhi* co-infection with malaria.

Degree of infection	Frequency	Percent	Valid percent	Cumulative percent
No Infection	11	44.0	44.0	44.0
Trace infection	9	36.0	36.0	80.0
Moderate infection	2	8.0	8.0	88.0
High infection	2	8.0	8.0	96.0
Severe infection	1	4.0	4.0	100.0
Total	25	100.0	100.0	

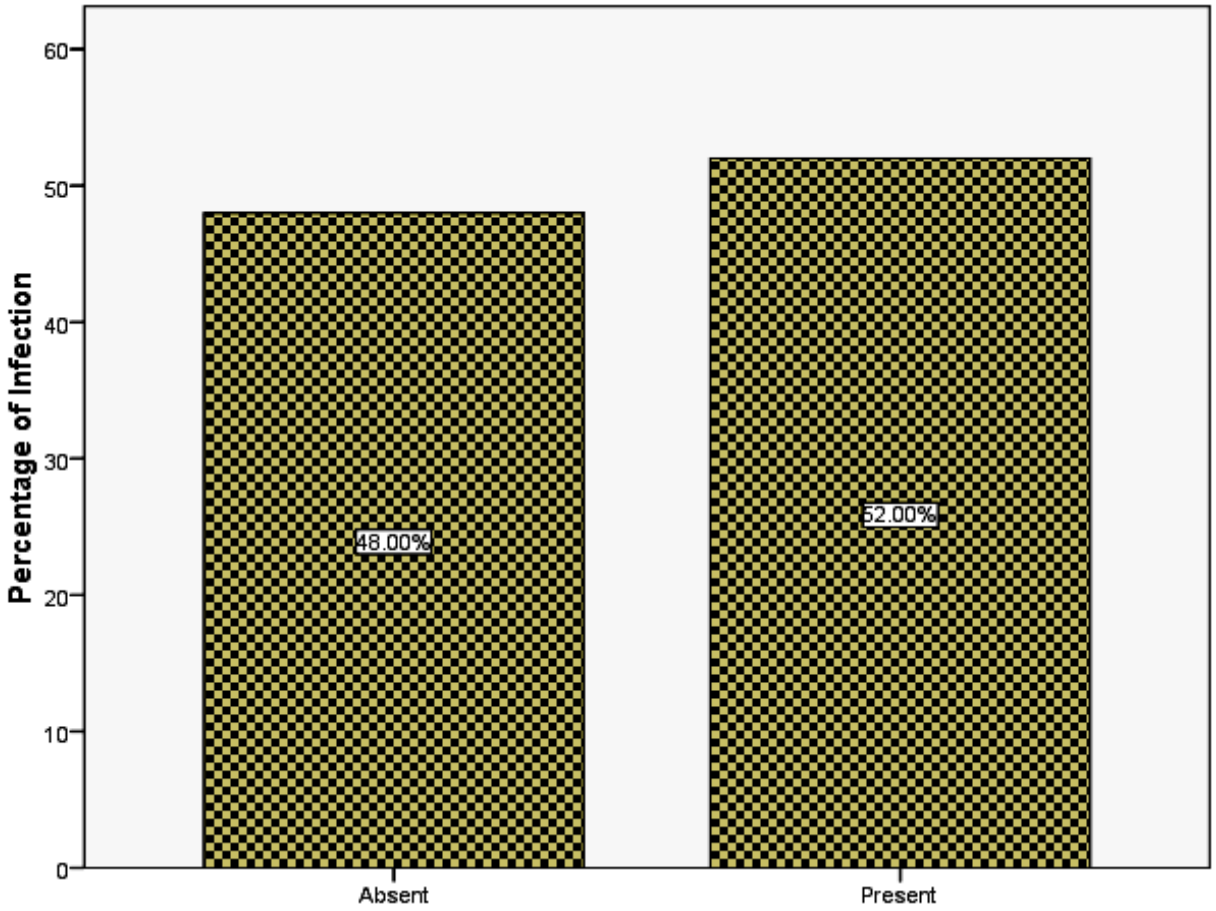

Figure 5. Prevalence of *Salmonella* ssp. in the stool of malaria patients.

relationship between the appearance of *Salmonella* spp. in stool and its presence in blood (Table 7).

Isolation of *Salmonella* species from blood samples of malaria patients

Salmonella species were only isolated from blood samples of 3 (12%) of the subjects, while none was isolated from 22 (88%) of the subjects (Table 6 and Figure 6). The isolation of *Salmonella* species from the blood of these patients is significantly ($p < 0.05$) associated with its presence in stool (Table 7).

DISCUSSION

In this study, the co-infection of *Salmonella* serotypes in malaria patients was evaluated based on their high antibody titres to the *Salmonella* serotypes. *Salmonella*

Table 5. Frequency of isolation of *Salmonella* species from stool specimens of malaria patients.

Availability	Frequency	Percent	Valid percent	Cumulative percent
Absent	12	48.0	48.0	48.0
Present	13	52.0	52.0	100.0
Total	25	100.0	100.0	

Table 6. Frequency of isolations of *Salmonella* species from blood specimens of malaria patients.

Availability	Frequency	Percent	Valid percent	Cumulative percent
Absent	22	88.0	88.0	88.0
Present	3	12.0	12.0	100.0
Total	25	100.0	100.0	

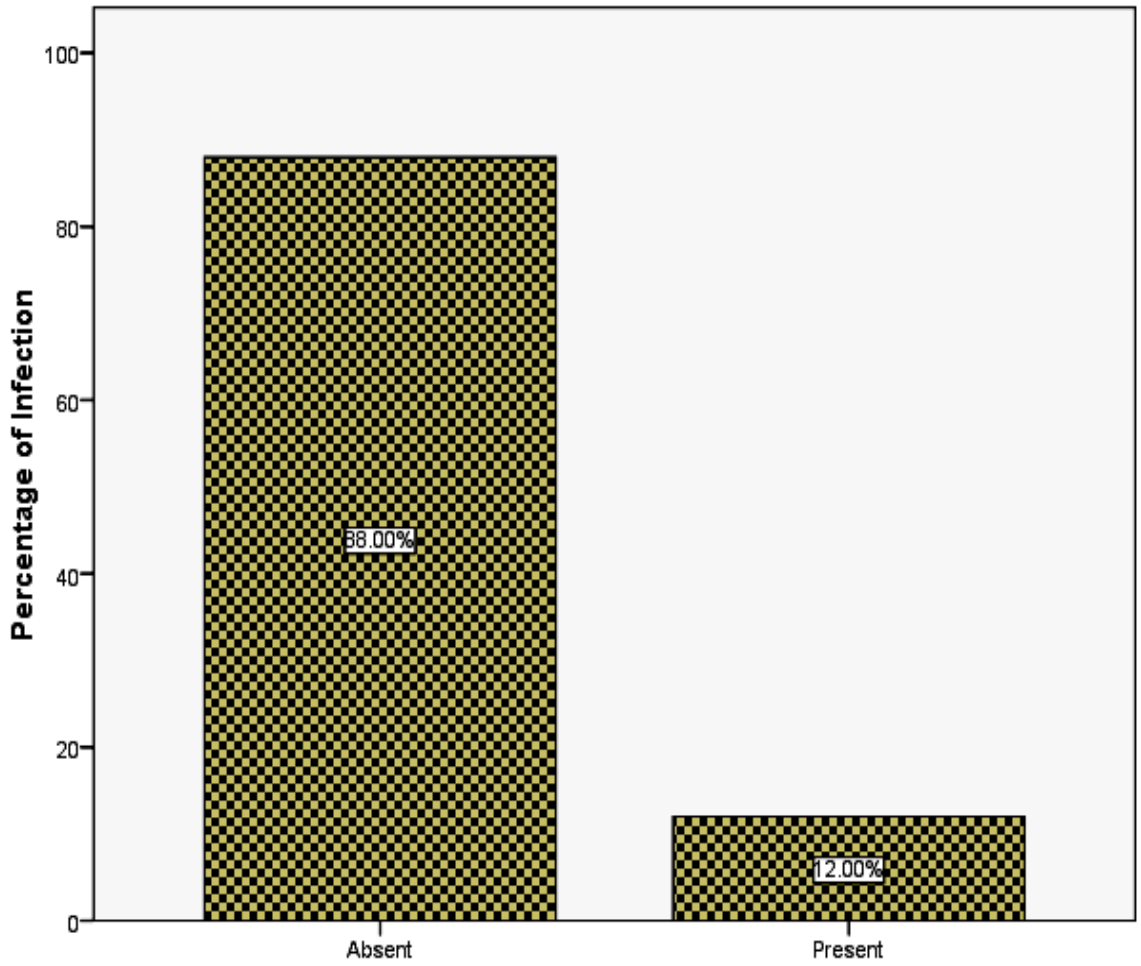

Figure 6. Prevalence of *Salmonella* ssp. in the blood of malaria patients.

serotypes including *S. typhi, S. paratyphi* A, *S. paratyphi* B and *S. paratyphi* C are bacterial causative agents of typhoid fever and its similar paratyphoidal fever (WHO, 2003). Malaria in humans is caused by four species of *Plasmodium* including, *P. falciparum, P. malariae, P. ovale* and *P. vivax* (Lerner and Lerner, 2003; Niikura et

Table 7. Correlations matrix.

		Malaria parasite infection	*S. paratyphi* A	*S. paratyphi* B	*S. paratyphi* C	*S. typhi*	Blood culture for *Salmonella* spp.	Stool culture for *Salmonella* spp.
Malaria parasite infection	Pearson correlation	1						
	Sig. (1-tailed)							
	N	25						
S. paratyphi A	Pearson correlation	0.220	1					
	Sig. (1-tailed)	0.145						
	N	25	25					
S. paratyphi B	Pearson correlation	0.169	0.241	1				
	Sig. (1-tailed)	0.209	0.123					
	N	25	25	25				
S. paratyphi C	Pearson correlation	0.000	-0.212	0.324	1			
	Sig. (1-tailed)	0.500	0.154	0.057				
	N	25	25	25	25			
S. typhi	Pearson correlation	0.220	0.327	0.595**	0.518**	1		
	Sig. (1-tailed)	0.146	0.055	0.001	0.004			
	N	25	25	25	25	25		
Blood culture for *Salmonella* spp	Pearson correlation	-0.185	0.015	0.299	0.218	0.140	1	
	Sig. (1-tailed)	0.188	0.472	0.073	0.148	0.253		
	N	25	25	25	25	25	25	
Stool Culture for *Salmonella* spp.	Pearson correlation	0.080	0.202	0.296	0.212	0.296	0.355*	1
	Sig. (1-tailed)	0.352	0.167	0.075	0.154	0.075	0.041	
	N	25	25	25	25	25	25	25

**Correlation is significant at the 0.01 level (1-tailed); *Correlation is significant at the 0.05 level (1-tailed).

al., 2008). Both diseases have similar symptoms characterized by fever, weakness, body weight loss, anaemia and in some cases gastrointestinal disturbances (Niikura et al., 2008; WHO, 2003; Samal and Sahu, 1991). The similarity in symptoms is one of the causes of difficulties that arises in the preli-minary diagnosis of the diseases. Ensue of poor diagnosis, patients who are suffering from typhoid may be placed on antimalaria therapy or vice versa. However, the two diseases can co-infect an individual causing a more severe illness. The frequent diagnosis of malaria, paratyphoid and typhoid diseases in a particular patient, especially in areas where malaria is endemic (like Nsukka) has made some physcians to always assume the concurrent infections of the diseases. Hence, they always treat the two diseases concurrently without appro-priate diagnosis. Such attitude towards drug adminis-tration can lead to increase in microbial resistance to drugs (antibiotics) and can affect patients rate of recovery.

Findings in this study have shown that all the 25 subjects were positive for antibody titres against *Salmonella* serotypes, but only 3 (12%) of the malaria patients had *Salmonella* species isolated from their stool and blood specimens. The isolation of *Salmonella* species from

blood specimen is confirmatory of typhoid/paratyphoid infections (WHO, 2003). Therefore, only 3 subjects out of the 25 malaria patients were co-infected with typhoid and/or paratyphoid fever. The small percentage of confirmed co-infections found in this study is contraditory to the belief of most of the medical personnels in this study area that malaria and typhoid infections are always concurrent.

Of the remaining 22 subjects, 10 subjects (40% of the total number of subjects studied) had positive antibody titres to *Salmonella* serotypes, but organisms were only isolated from their stool specimens but not from blood specimens. The isolation of *Salmonella* species from stool is suggestive of infections, but not confirmatory, since some individuals habour the organisms as normal flora in the intestine (WHO, 2003). Though these patients can be treated for malaria and typhoid, it would be necessary to repeat the widal test and blood culture after two weeks to confirm *Salmonella* infections (WHO, 2003; Lerner and Lerner, 2003).

Among the other 12 subjects (48%) who had high antibody titres to some or all of the *Salmonella* serotypes, *Salmonella* species were neither isolated from their stool nor their blood specimens. WHO (2003) reported that other infections apart from *Salmonella* infections can elevate antibody responses against H and O antigens which is similar to that of *Salmonella* serotypes. Studies by Romero et al. (1989) showed that oral administration of *Salmonella* vaccine that expresses circumsporozoite protein was protective against murine malaria. Studies by Leitner et al. (1997) have also shown that circumsporozoite protein (CSP) is a target for effector antibody and cell mediated immunity against malaria parasites. This CSP is found in *Salmonella* species (Romero et al., 1989). The circumsporozoite protein has been found to elicit antibody production especially IgG and can induce class switch in IgG isotype from IgG1 to IgG2a (Leitner et al., 1997). The IgG is one of the antibodies commonly detected in widal test (Lerner and Lerner, 2003). Therefore, the high antibody titres observed among the 12 subjects in this study, may be attributable to the antibody produced against the circumsporozoites protein of the malaria parasite, but not that of *Salmonella,* since the Salmonella was neither isolated from their stool nor from blood specimens.

Findings in this study strongly suggest the inappropriateness of the use of widal test only as a diagnostic tool for *Salmonella* infections, since other infections can influence antibody titre against *Salmonella* serotypes. The antibody titre elevation could be as a result of cross reactivity of the antibody with the *Salmonella* antigens. We, therefore, highly recommend appropriate and complete laboratory diagnosis (widal test, stool cultrure and blood culture) for *Salmonella* infections, especially in areas where malaria is endemic like in the tropical Africa, in order to ensure adequate treatment. This study has clarified that malaria infections cannot be associated with typhoid infections, though there could be co-infections.

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