

# Distribution Pattern Of Salmonella Typhoidal Serotypes In Benue State Central, Nigeria

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## Abstract

Sera from patients (n=1479; 0-80 years old) seeking medical attention for feverish conditions were screened for significant typhoidal antibody titers (1:160) using Widal test. Salmonella enterica serovar typhi antibodies (57.9%) predominated. Distribution of the typhoidal serotypes was significantly associated with seasons ( $\chi^2$

S. Typhi = 35.8,  $p < .05$ ;  $\chi^2$

S.

Paratyphi = 122.5,  $p < .05$ ), both serotypes occurring highest in wet season. Univariate analyses of variance indicated age-gender, and age-season interaction on distribution pattern: age distribution varied significantly between males and females and between dry and wet seasons ( $p < .05$ ). For instance, paratyphoid antibodies were detected in 30.4% of males aged 41-50 years, but 21.5% in females of same age. Similarly, occurrence of typhoidal antibodies in 11-12 year olds was significantly higher in the wet season (36%) than in dry season (21.0%) ( $p < .05$ ). The findings of this study may be useful in planning infection therapeutic and intervention programs.

## INTRODUCTION

Enteric fevers have continued to pose a serious threat to public health especially in economically poor countries where level of hygiene is below standards and sanitary conditions are poor (Oboegbulam et al., 1995; Zailani et al., 2000, and Okome-Nkoumou et al., 2000). Enteric fever (typhoid or paratyphoid fever) is a systemic infection caused by several Salmonella enterica serotypes including S. Typhi and S. Paratyphi A, B, or C (Kato et al., 2007). Salmonellae are motile, non-lactose fermenting, non-spore, Gram-negative rods which ferment glucose with production of acid and gas. Most species produce hydrogen sulfide ( $H_2S$ ) but not urease. Major symptoms of the infections include persistent high fever with low pulse rate, severe headache, nausea, mental confusion, abdominal tenderness and pain. One of the commonest consequences of the infection is ileal perforation (Ndububa et al., 1992) which even in childhood is associated with high morbidity and mortality (Ekenze et al., 2008). Paratyphoid fevers are usually milder than typhoid fever (Onwubalili, 1989) although not without danger.

The distribution pattern of the infections seems uncertain in Nigeria and appears to show geographical variation. Some

studies found that enteric fevers are more prevalent in males than in females (Kam, 1996; Okome-Nkoumou et al., 2000; Akinyemi et al., 2005), but Zailani et al. (2004) found no influence of age, sex and social class on the distribution pattern of S. typhi/paratyphi in Ile-Ife, south western Nigeria. Nevertheless, a higher prevalence of Salmonella infections and carrier rates was observed in individuals older than 60 years old (Brandis et al., 1980, Bockemuhl, 1976; and Kam, 1996). Typhoid or paratyphoid fevers are usually associated with unstable living conditions and lack of cleanliness (Okome-Nkoumou et al., xxxx). In some parts of the world, infections appear to be associated with seasonal changes (Bockemuhl, 1976), although in others it appears not to be so (Kham, 1996).

In Nigeria, enteric fevers caused by S. Typhi and S. Paratyphi are not only endemic (Oboegbulam et al., 1995; Tanyigna et al., 1999) but constitute a great socio-medical problem (Zailani et al., 2000), being responsible for many cases of pyrexia of unknown origin (Akinyemi et al., 2008), high morbidity and mortality (Ekenze et al., 2008; Nasir et al., 2008; and Effa and Bakirwa, 2008).

In the central area of Benue State, Nigeria, inadequate water supplies is a serious socio-economic problem that has caused

the inhabitants to resort to untreated well water and polluted stream water for domestic water supplies. Polluted and untreated water supplies are responsible for water-borne infections such as enteric fevers. In the study area, diagnostic laboratory investigations frequently report significant typhoid and paratyphoid antibody titres ( $>1$  in 160) in patients seeking medical attention for feverish conditions; yet epidemiological data on the distribution of the disease in the area seems lacking. In the present study the distribution pattern of *Salmonella enterica* serovar typhi and serovar paratyphi agglutinins in patients attending primary and secondary health-care institutions in central region of Benue State, Nigeria was investigated. The information obtained in this study may be useful in rapid diagnosis and treatment of enteric fevers, and in planning the infection intervention programs in the area.

## MATERIALS AND METHODS

### STUDY AREA AND POPULATION

Okpokwu local government area (LGA), Benue State, Nigeria is situated between latitude  $6^{\circ} 52' N$  and  $7^{\circ} 40' E$ , and longitude  $7^{\circ} 40' N$  and  $8^{\circ} 01' E$  (Okpokwu Local Government Area base map, 2007; Ministry of Lands and Survey, Makurdi, 2007). The area is rural and farming is the major occupation of the people with few engaging in petty trading and civil work. Dry season in the study area begins in November and ends in March, and wet season begins in April and ends in October.

### ETHICAL CONSIDERATION

Informed consent was sought in clinically suspected cases and approval for the study was obtained from the ethics committee of the Health Department, Okpokwu Local Government Area, Benue State, Nigeria. Confidentiality was maintained in accordance with standards of medical practice.

### SAMPLE COLLECTION

Five-ml venous blood specimens were collected from 1479 patients who sought medical attention for feverish conditions in health care institutions within the area from November 2006 to July 2007. Defibrinated blood specimens were serologically examined for significant serum agglutinin titer against typhoid/paratyphoid antigens using the Widal test technique.

### SOCIO-DEMOGRAPHIC VARIABLES:

Socio-demographic variables such as age, sex, occupation residential area and source of domestic water supply were collected using questionnaires.

### WIDAL TEST:

The Widal agglutination technique was carried out according to the manufacturer's instruction using plasmatic reagents (Antec Laboratory Products, UK) containing *S. Typhi* O and H antigens, and *S. Paratyphi* A, B, and C antigens. Positive and negative serum controls were included, and a titer of  $> 1/160$  indicated typhoid/paratyphoid fever.

### STATISTICAL ANALYSIS:

SPSS version 10.00 (2003) was used for descriptive and inferential analyses.

## RESULTS

Sera from 1479 patients (569 males and 910 females; age range 0 – 80 years) were examined for typhoidal antibodies using slide agglutination technique. Out of the sera specimens examined, 875 (57.6%) agglutinated *S. Typhi* antigens, and 389 (26.3%) *S. Paratyphi* antigens. Two hundred and thirty (230, 15.6%) showed a mixture of both typhoid and paratyphoid serotypes

(Tables 1). As depicted in Table 1, the percentage frequency of *S. Typhi*

Table 1: Percentage distribution of *S. Typhi* and *S. Paratyphi* serotypes central region of Benue State, Nigeria.

Figure 1

	S. Typhi	S. Paratyphi	Typhoid and Paratyphoid co-infection	Number of sera samples
	856 (57.9%)	389 (26.3%)	230 (15.6%)	N = 1479 (100%)
GENDER				
male	57.6	27.8	16.0	569
female	58.0	25.4	15.3	910
AGE				
$\leq 10$	52.2	18.0	8.1	161
11 - 20	57.4	27.6	16.7	359
21 - 30	57.9	30.7	19.8	349
31 - 40	61.5	24.5	10.4	278
41 - 50	61.8	24.1	17.3	191
51 - 60	54.9	28.3	19.5	113
61 - 70	47.8	30.4	17.4	23
71 and above	40.0	20.0	0	5
SEASON				
Dry	54.0	21.3	12.9	979
Wet	65.4	36.0	20.8	500
OCCUPATION				
Dependant	51.4	18.0	9.8	245
Student	56.7	26.8	16.6	571
Farmer	62.6	26.6	16.7	406
Civil servant	55.9	33.3	16.7	102
Business	61.3	32.3	16.8	155
LOCALITY				
Ugbokolo	57.6	32.3	16.5	462
Edumoga	55.4	27.5	16.5	462
Okpoga	64.2	21.3	16.1	461
Non-indigene	40.4	16.0	4.3	94

serotype was 57.6% in males and 58.0% in females; and that of *S. Paratyphi* was 27.8% in males and 26.4% in females.

The percentage occurrence in both sexes did not differ significantly. The distribution pattern of the various S. Typhi and S. Paratyphi serotypes is presented in Table 2. S. Typhi O had the highest rate of occurrence and was the commonest serotype while S. Paratyphi A was the second commonest. Mixed infection of S. Paratyphi A and C occurred least.

Table 2: Percentage distribution of salmonellae serotypes according to seasons, occupation, gender and age.

**Figure 2**

	Frequency (%)												
	S. Typhi serotype				S. Paratyphi serotype								
	O	H	OH	N	A	B	C	AB	AC	BC	ABC	N	
frequency	570	178	109	875	92	81	50	49	21	29	67	389	
%	38.5	12.0	7.4	100	6.2	5.5	3.4	3.3	1.4	2.0	4.5	100	
Seasons													
dry	44.4	17.5	15.8	530	34.9	22.5	18.7	9.1	2.9	4.8	7.2	209	
wet	44.4	24.0	7.6	327	10.4	18.9	6.1	14.7	8.3	10.4	28.9	180	
Total	44.5	20.8	12.7	857	23.7	20.8	12.9	12.4	5.4	7.5	17.2	389	
*Sig.	.142	.003	.000		.000	.385	.000	.025	.017	.031	.000		
Chi-square	$\chi^2 = 17.650; p > .000$				$\chi^2 = 77.282; p > .000$								
Occupation													
dependant	45.1	19.0	15.9	126	27.3	36.4	11.4	11.4	2.3	4.5	6.8	44	
student	47.4	20.9	11.7	325	20.9	19.0	11.1	12.4	9.2	7.2	20.3	153	
farmer	44.5	20.1	13.4	254	28.7	21.3	11.1	12.0	3.7	7.4	15.7	108	
civil servant	59.6	29.8	10.5	57	23.5	14.7	23.5	14.7	2.9	8.8	11.8	34	
business	49.5	18.9	11.4	95	18.0	14.0	14.0	14.0	2.0	10.0	24.0	50	
Total	44.5	20.8	12.7	857	23.7	20.8	12.9	12.4	5.4	7.5	17.2	389	
Sig.	.772	.505	.756		.499	.082	.322	.989	.126	.893	.146		
Chi-square	$\chi^2 = 4.905; p > .05$				$\chi^2 = 26.975; p > .05$								
Gender													
male	44.8	22.3	11.0	328	24.1	18.4	11.4	15.8	5.7	8.2	14.5	158	
female	44.4	19.8	13.8	529	23.4	22.5	13.9	10.4	5.2	6.9	17.7	231	
Total	44.5	20.8	12.7	857	23.7	20.8	12.9	12.4	5.4	7.5	17.2	389	
Sig.	.900	.399	.228		.878	.323	.478	.113	.830	.432	.741		
Chi-square	$\chi^2 = 1.840; p > .05$				$\chi^2 = 3.785; p > .05$								
Age													
<= 10	73.8	13.1	13.1	84	20.7	27.6	17.2	20.7	3.4	3.4	6.9	29	
11 - 20	71.8	18.4	9.7	206	25.3	19.2	5.1	9.1	10.1	11.1	20.2	99	
21 - 30	60.6	24.1	13.3	203	23.4	17.8	12.1	16.8	6.5	3.7	19.6	107	
31 - 40	44.7	19.3	14.0	171	14.2	19.1	20.6	13.2	1.5	8.8	20.6	48	
41 - 50	63.6	21.2	15.3	118	26.1	19.6	19.6	10.9	2.2	10.9	10.9	46	
51 - 60	64.5	21.0	14.5	62	37.5	31.3	6.3	3.1	3.1	6.3	12.5	32	
61 - 70	54.5	45.5	0	11	14.3	28.6	28.6	14.3	0	0	14.3	7	
>71	100.0	0	0	2	0	100.0	0	0	0	0	0	1	
Total	44.5	20.8	12.7	857	23.7	20.8	12.9	12.4	5.4	7.5	17.2	389	
Sig.	.182	.099	.489		.477	.344	.044	.383	.289	.497	.544		
Chi-square	$\chi^2 = 17.096; p > .05$				$\chi^2 = 49.145; p > .05$								

\*Level of significance for F-statistic

As summarized in the correlation coefficient matrix (Table 3), occurrence of typhoid O, H, and OH antibodies were significantly associated with source of water and seasonal changes, while paratyphoid antibodies were significantly associated with seasonal changes but not with sources of water. There was no significant gender, age, or occupational relationship with the distribution of O, H, and OH antibodies ( $p > .05$ ).

Table 3: Correlation coefficients of typhoidal serotypes and some socio-demographic variables

**Figure 3**

	O	H	OH	A	B	C	AB	AC	BC	ABC	G	AG	OC	W	S
O	1.000														
H	-.293**	1.000													
OH	-.223**	-.104**	1.000												
A	-.008	-.026	-.041	1.000											
B	-.066*	-.094**	-.012	-.062*	1.000										
C	-.033	-.034	-.033	-.048	-.045	1.000									
AB	-.069**	-.024	-.020	-.048	-.045	-.035	1.000								
AC	-.001	-.079**	-.032	-.031	-.029	-.022	-.022	1.000							
BC	.028	-.037	-.016	-.036	-.034	-.026	-.026	-.017	1.000						
ABC	-.052*	-.169**	-.012	-.056*	-.052*	-.041	-.040	-.026	-.031	1.000					
G	.001	-.019	.032	-.015	.013	.010	-.048	-.011	-.018	-.001	1.000				
AG	-.020	.040	.022	.020	.030	.040	-.023	-.046	.002	-.008	.071**	1.000			
OC	.040	.027	-.005	.025	-.011	.061*	.037	-.019	.045	.057	-.001	.339**	1.000		
W	-.078*	-.053*	-.062*	-.028	-.028	-.008	.081**	-.020	.002	.001	.005	-.027	-.009	1.000	
S	.071**	.109**	-.065*	-.072*	.042	-.047	.107**	.095**	.095**	.202**	-.014	-.104**	.099**	-.063*	1.000

N = 1479; \*\* Correlation is significant at the 0.01 level (2-tailed); \* Correlation is significant at the 0.05 level (2-tailed).  
O = serotype O; H = serotype H; OH = serotype OH; A = serotype A; B = serotype B; C = serotype C; AB = serotype AB; AC = serotype AC; BC = serotype BC; ABC = serotype ABC; G = Gender; AG = Age; OC = Occupation; W = Water; S = Seasonal changes.

Regression analytical technique was used to compare the effects of the predictor variables on distribution pattern of the typhoidal serotypes (Table 4).

Table 4: Regression estimates of the predictor variables on the distribution of S. Typhi and S. Paratyphi serotypes

**Figure 4**

Predictor variables	S. Paratyphi	S. Typhi
Source of water	1.159	-4.827**
Seasonal changes	8.841**	2.442**
Occupation of patients	2.454**	.710
Locality of patients	-.044	1.332
Gender of patients	-.926	.462
Age groups of patients	-.047	1.143
R <sup>2</sup>	.071	.020
F-statistic	18.714**	6.044**
Constant	-1.948**	3.940**

For S. Paratyphi, seasonal changes had the greatest impact followed by occupation of the patients, while for S. Typhi, source of drinking and domestic water had the greatest influence followed by seasonal changes.

Univariate analyses of variance shows interactions between some predictor variables. For instance, there were age-gender, gender-occupation, and age-season interactions (Figures 1 – 3). As shown in Figure 1, the age distribution of paratyphoid serotypes differed significantly between males and females ( $F = 2.682; p < .05$ ). Males who were 71 years and above and those between 31 – 40

years had higher rates of paratyphoid infections than females of the same age groups. Also, males between the ages 11 – 20 years had a lower occurrence rate than females of the same age.

Figure 5

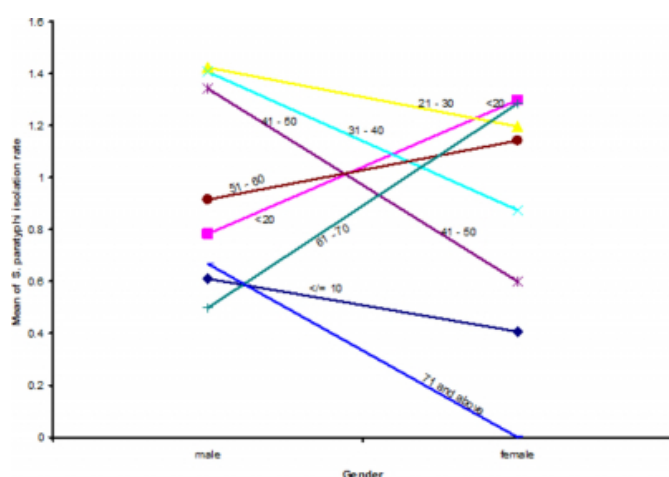


Figure 1: Age - Gender Interactive Effects

In terms of occupation, occurrence rates were higher among male civil servants and farmers than among females in the two occupational categories (Figure 2). Likewise, male students had lower typhoid infection than female students. The seasonal distribution of the enteric fever antibodies varied significantly among the various age groups ( $F = 4.034$ ;  $p < .05$ ). For instance, those that were 70 years and above had a higher rate of infection in the dry season than in the wet season, whereas in some other age groups the infection was higher in the wet season than in the dry (Figure 3).

Figure 6

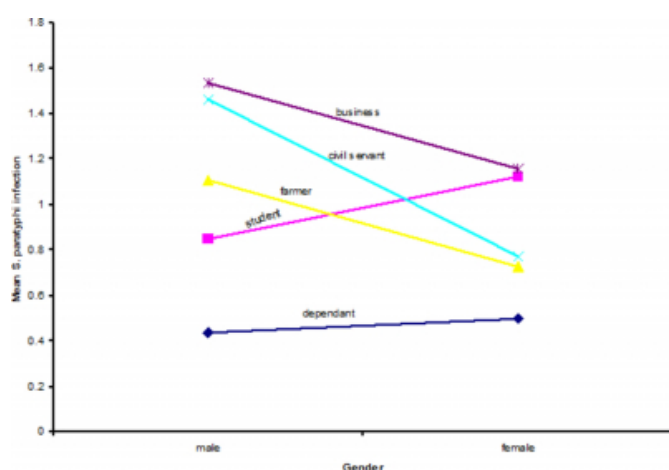


Figure 2: Gender-Occupation interactive effect

Figure 7

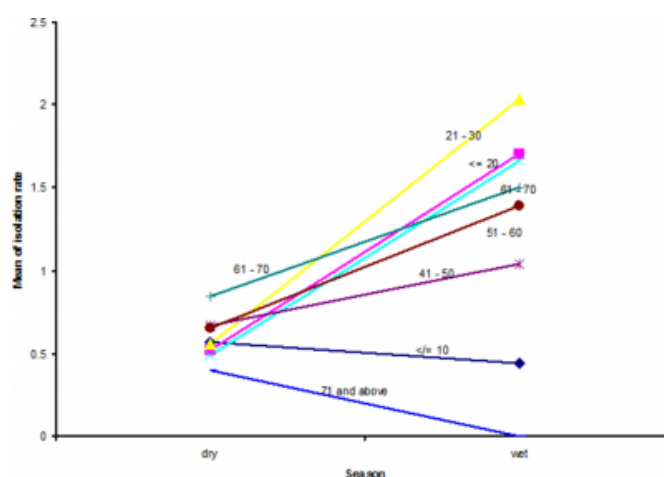


Figure 3: Age-Season Interactive Effect

## DISCUSSION

The number of sera with significant typhoidal antibody titres indicates a high rate of typhoidal infection in the study area, and confirms the endemicity of enteric fever in many rural communities of developing nations (Akinyemi et al., 2005; Oboegbulam et al., 1996; and Akinyemi et al., 2005). *S. enterica* serovar Typhi serotypes predominated with serotype O constituting more than one-half (66.5%) of the enteric fever serological types identified. Paratyphi A was the second commonest serotype. The few cases of co-infection (mixed infection) of both typhoid and paratyphoid serotypes agree with the report of Perera et al. (2006) that mixed infection with multiple *Salmonella* serotypes is unusual.

The predominance of *S. Typhi* agrees with previous reports (Khemiri et al., 1983; Erdem et al., 2004, and French et al., 1977), but differs with those of others (Oboegbulam et al., 1996; Kam et al., 1996; Bharat et al., 2006, Akinyemi et al., 2005; Kumarasinghe et al., 1992; Kam, 1996; and Kam et al., 1996) reported a predominance of different typhoidal and non-typhoidal *Salmonella* serotypes. Variation in geographic distribution is important epidemiologically as it may aid enteric fever diagnosis if some serotypes are known to be more prevalent in certain areas than in others.

Our findings show that, contrary to previous studies (Kam, 1996; Brankis et al. 1980; Bockemühl, 1976; Akinyemi et al., 2005; Okome-Nkounmou, 2000; Ndububa et al., 1992, and Mandeep et al., 2006), the occurrence of the typhoidal antibodies was not correlated with age, sex, and occupation; implying that both sexes, all age groups and occupational groups are equally predisposed to enteric fevers. However, tests of univariate analyses of variance showed that the age



distribution of paratyphoid serotypes varied significantly between males and females, and between dry and wet seasons. For instance, *S. Paratyphi* antibodies occurred more in males aged 31 to 40 years than in females of the same age and in male farmers than female farmers. Males within this age bracket usually work outside their homes and may eat hawked food that are liable to contamination. Also females aged 11 to 20 years had a higher of the typhoidal antibodies than their male counterparts. Young teenage girls who perform most household chores are the ones who fetch water from polluted streams.

With the exception of O agglutinin which had similar occurrence rates in both dry and wet seasons, the distribution of the species showed seasonal disparity, with the *S. Typhi* H, and *S. Paratyphi* AB, AC, BC, and ABC antibodies occurring more in the wet season than in the dry season, and typhi/paratyphi OH, A, B, and C more prevalent in dry season than in wet season. Although seasonal variations of salmonellae infections may not be common in some parts of Africa (Kam, 1996; and Bockemuhi, 1976), increased cases in summer and decreased number of cases in winter have been reported (Hamze and Vincent (2000), and Bockemuhl, 1976). Increased cases of typhoidal antibodies in most age groups occurred more in wet season than in the dry season, and supports the fact that microbial contamination of foods and water are more likely in the warmer seasons when bacterial pathogens multiply very rapidly.

Typhoid and paratyphoid fevers are associated with poor environmental and living conditions especially in economically poor countries (Okome-Nkoumou et al., 2000). In the area studied, treated pipe-borne water is scarce and waste disposal systems are poor. Toilet facilities are usually absent and nearby bushes are used for defecation; while domestic wastes are indiscriminately disposed of in the surroundings. Water is more likely to be polluted in the wet season because the rains may wash debris and littered garbage into wells and streams used as domestic sources of water. Typhoid infections predominated in patients whose sources of drinking and domestic water supplies were the untreated stream water. Constructing covers for wells may prevent pollution of well water. The occurrence of paratyphoid serotype was not associated with domestic source of water probably because, paratyphoid infections are more commonly transmitted through flies and contaminated foods; typhoid fever on the other hand, is transmitted mainly through contaminated water (Huckstep, 1963).

Regression analytical model was used to compare the effects

of the various factors on the distribution pattern of typhoidal organisms. Seasonal changes appeared to have the greatest impact on the distribution of *S. Paratyphi* serotypes, whereas source of drinking and domestic water and then seasonal changes had greater effect on the distribution of typhoid serotypes.

## **CONCLUSION**

These results may be useful to public health agencies in planning infectious disease prevention and control strategies. Treated sources of domestic and drinking water are very important in rural areas where these facilities are lacking. Enlightenment programs on basic rules of hygiene for semi-urban and rural communities of economically poor nations should be encouraged so as to limit the transmission of faecal-orally transmitted infections.

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