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Age Related Co-Infection Of Malaria, Bacteremia And Intestinal Parasites In Primary School Children In Owerri, Imo State, Nigeria.

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Abstract.

Studies on age related co-infections of malaria,bacteraemia and intestinal parasites in primary school children in Owerri metropolis were carried out. Stool and urine specimen were collected with sterile containers, blood specimen were collected with syringe and EDTA bottles from 650 hospitalized and 500 healthy school pupils. Parasitological investigations on the stool specimen included Macroscopic Analysis, Direct Wet Method and Formol Ether concentration technique; On urine specimen, by centrifuging and microscopy, on Blood specimen by Thick and Thin blood smear method. Bacterial inoculation, isolation and identification was done by subjecting the respective specimen to culture. Among the hospitalized pupils, 208 (32%) were infected with both *Plasmodium falciparum* and bacterial organisms. A total of 187 (28.8%) were infected with *Plasmodium falciparum* and intestinal parasites. A total of 125 (19.3%) were infected with *Plasmodium falciparum*, as well as with both intestinal parasites and bacterial organisms. The highest co-infection rates were observed in the 11 – 12 years age group of hospitalized pupils (38.2%) and in the 9 – 10 years age group of school pupils (26.7%) respectively while the least was noted in the 5 – 6 years age groups in both hospitalized (14.2%) and school pupils(12.6%).

Keywords: Co-infection, Malaria, bacteremia, intestinal, infection.

Introduction

Co-infection of malaria, bacteraemia and intestinal parasites, provides some public health challenges to children in tropical countries like Nigeria. This is so because there are favorable climatic, environmental and socio-cultural factors which permit transmission of these diseases for greater part of the year .

For instance, in malaria, an ongoing infection is thought by many not only to induce, but also to be necessary for immunity to a superimposed infection of parasites with the same or different genotype, a phenomenon called premonition (Smith, 1999).

In co-infections, the burden of one or both of the infectious agents may be suppressed or one may be increased and the other suppressed.

Interactions between parasites, bacterial organisms and humans can be synergistic or antagonistic. For example, studies have demonstrated a positive association between intensity and concurrent infection of helminth species, suggesting that individuals harbouring multiple helminth species also harbour the most intense infections (Holland *et al.* 1989; Ferreira, and Nogueira, 1994; Brooker *et al.* 2007). It is conceivable therefore that co-infections may have a greater impact on morbidity than single species infections since morbidity is typically related to infection intensity for most parasite species. Multiple species infections may also increase susceptibility to other infections (Nacher, 2004; Druilhe, *et al*, 2005; Mwangi *et al.* 2006). An examination of age-infection profiles of

different parasite species helps identify those individuals at greatest risk of concomitant infections. For most helminth species, intensity of infection rises dramatically with age, with the age of maximum prevalence varying for each helminth species. Age-profiles of *Plasmodium spp*. suggest that blood stage infection is most prevalent in school-aged children, and it is among this age group that co-infections are most likely therefore to occur (Bundy,1995; Anderson and May,2001)

Understanding the age patterns of infection can provide insight into who is most at risk of the health consequences of polyparasitism, but the underlying age-specific susceptibilities to morbidity are also important.

Materials And Methods

Study Area: This study was conducted in Owerri metropolis, Imo State, South East Nigeria. The geographic position is Latitude 5 ^{o} 45' 0" N and Longitude 7^{o} 7' 0" E, located in the rain forest belt of Nigeria with an ambience temperature of about 27^{0}C. The climatic condition is warm and humid with heavy rain distribution. The rainy season starts from April, reaches its peak in August and diminishes in November. The dry season starts in December and ends in March.

Study Population:

The inclusion criterion was any sick child aged between five and twelve years treated in any of the ten selected hospitals, with infection based on any of the following features; fever ($\geq$38^{o} C), or hypothermia ($\leq$ 35^{o} C), cough, fast breathing, difficulty in breathing, abdominal pain, vomiting, diarrhea or clinical diagnosis of pneumonia, typhoid fever and malaria. 500 apparently healthy pupils were randomly selected from all the classes in selected primary schools and investigated for the infection of bacterial, malarial or intestinal parasites.

Collection of Samples: On reporting to hospital, stool, urine and blood specimen were collected from each sick child. Relevant clinical data such as name, age, sex and weight were also obtained. Similar specimen was also collected from 500 randomly selected seemingly healthy pupils from five selected primary schools.

Relevant permits were obtained from Owerri Municipal Council before samples were collected from primary school pupils. Prior visits were also made to the selected Primary schools to explain to their Head Mistresses the aim of the study and to solicit their approval and co-operation.

Collection of blood samples: A standard clean venepunture technique was used to collect 5mls of blood from both the sick and healthy pupils, into a dipotassium EDTA bottles and samples were analysed within 24 hours of collection.

Thick and thin blood films stained with 3% Giemsa were examined microscopically to establish parasitemia.

Examination of Samples: Each stool specimen was subjected to naked eye examinations for consistency, colour and atypical components such as mucus, blood and parasites. A match-stick head of stool was emulsified in 8.5% saline on a slide. A cover slip (22mm x 22mm) was placed on the suspension and examined with the light microscope, first with x10 objective and again with x40 objective.

The same matchstick head size of stool was emulsified in lugols iodine, covered with a cover slip and examined with a light microscope. The Iodine preparation is particularly suitable for the identification of protozoan cysts. Iodine stains the nuclei and makes them quite visible. The 8.5% saline and iodine wet mount allow for the detection and identification of both protozoan and helminthic human gut parasites. The stool specimen were also examined using formol ether concentration technique (Cheesebrough,1991) and were later subjected to culture in order to look for common enteropathogens. MacConkey, deoxycholate citrate agar(DCA) and thiosulphate citrate bile sucrose(TCBS), Selenite F broth, alkaline peptone water and SS agar were used for the isolation of bacterial pathogens. The bacterial pathogens were identified by standard biochemical tests and by slide agglutination with polyvalent and monovalent sera

The urine samples were concentrated by spinning at 2500rpm for 5 minutes. The supernatant was decanted and the sediments were placed on a slide, covered with a cover slip (22mm x 22mm) and examined with a light microscope using x40 objective. The urine specimen was later cultured to isolate bacterial pathogens.

Blood examination was done by thin and thick films. These were examined with a light microscope, using immersion oil.

Thin Film: Small quantity of the blood sample (a drop) was placed near one end of a clean microscopic slide. A spreader was inclined first at 45^{0} and was then bent up to 30^{0} until the blood touches the spreader. Then the spreader was moved forward such that one layer of evenly distributed blood cells was spread on the slides. The thin film

method allows for the identification of the plasmodium species and filarial worms.

Thick Film: At the other end of the slide, three drops of blood were placed and with the edge of the spreader the blood was evenly spread over an area (2cm in diameter). The slide was labeled accordingly and was allowed to dry by putting it in an oven or by air dry at a dust free environment.

Staining Smear of Blood: Giemsa stain was used for the staining techniques. The stock solution prepared 24 hours prior the staining was diluted in the ratio of 1ml stock solution to 10ml of 7.2 buffer water and was then filtered before use.

The already fixed blood smears were covered with the diluted Giemsa's stain in a tray for about thirty minutes. These were rinsed thoroughly in distilled waters, then each slide was blotted dry at the edges and under surface and placed in a vertical position and allowed to air dry in a dust free environment. The pH 7.2 buffered water was prepared by mixing 1.4gm potassium phosphate plus 2gm di-sodium hydrogen phosphate plus 2 litres of distilled water.

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Blood samples were also cultured in MacConkey, chocolate and blood agar, respectively using oxoid signal system after the manufacturer's instructions. Isolates from distinct colonies from MacConkey, chocolate and blood agar plates were further subjected to bacteriological tests (gram staining) and biochemical tests (coagulase, catalase, oxidase, motility, indole, hydrogen sulphide, citrate utilization and glucose fermentation tests).

RESULTS

Table 1. shows the age related co- infections in hospitalized and school pupils.Four groups of hospitalized pupils were observed. First group, 208(32%) comprised those with co-infection of *Plasmodium falciparum* and bacterial organisms. The second group, 187 (28.8%), comprised those with co-infection of *Plasmodium falciparum* and intestinal parasites. The third group, 125(19.3%), comprised pupils with co-infection of *Plasmodium falciparum* and a combination of intestinal parasites and bacterial organisms. The fourth group, 130 (20.0%) comprised pupils without co- infections. (i.e.those with *Plasmodium falciparum* only).

In the first group, the following bacteria species were identified; *Staphylococcus aureus 40*(6.2%), *Escherichia coli 69*(10.6%), *Salmonella typhi 87*(13.4%), *Klebsiellae Pneumoniae* 10(1.5%) and *Pseudomonas aeruginosa* 2(0.3%).

In the second group, the following intestinal parasites were identified; *Trichuris trichiura* 33(5.1%), *Ascaris lumbricoides* 53(8.1%), *Entamoeba histolytica 43*(6.7%), *Hookworm 34*(5.2%) and *Strongyloides stecoralis* 24(3.6%).

In the third group, the following bacteria species with intestinal parasites were identified; *Ascaris lumbricoides* with *Staphylococcus aureus* 26(4.0%), *Ascaris lumbricoides with Escherichia coli* 16(2.5%), *Entamoeba histolytica with Staphylococcus aureus* 22(3.4%), *Entamoeba histolytica* with *Escherichia coli* 24(3.7%), *Hookworm with Salmonella typhi* 17(2.6%), and, *Hookworm* with *Escherichia coli* 20 (3.1%).

School Pupils: Among the 500 school pupils, The following bacterial organisms were found; *Staphylococcus aureus* 17 (3.4%), *Escherichia coli* 28 (5.6%) and *Salmonella typhi* 9(1.8%). Intestinal parasites isolated were *Hookworm* 22 (4.3%) and *Entamoeba histolytica* 11(2.2%). The malarial parasites identified were only *Plasmodium falciparum* 500 (100%)

Study Group		Hospitalized Pupils					School Pupils				
Age (Years)		5 - 6	7 – 8	9 – 10	11 – 12	Total	5 – 6	7 – 8	9 – 10	11 - 12	Total
Number (%) Infected		119 (18.3)	148 (22.8)	163 (25.1)	220 (33.8)	650	125	125	125	125	500
Co-infection of Malaria with	*Staphylococcus aureus*	8 (6.7)	12 (8.1)	11 (6.7)	9 (4.1)	40 (6.2)	0	4 (3.2)	7 (5.6)	6 (4.8)	17 (3.4)
	Escherichia coli	6 (5.0)	18(12.2)	13 (8.0)	32 (14.5)	69(10.6)	7 (5.6)	8 (6.4)	10(8.0)	3 (2.4)	28(5.6)
	Salmonellae typhi	10 (8.4)	24 (16.2)	16 (9.8)	37 (16.8)	87(13.4)	0	2 (1.6)	4 (3.2)	3 (2.4)	9 (1.8)
	Klebsiellae pneumonia	0	4 (2.7)	3 (1.8)	3 (1.4)	10(1.5)	0	0	0	0	0
	Psedomonas aeruginosa	0	0	1 (0.6)	1 (0.5)	2(0.3)	0	0	0	0	0
	Sub-Total	24 (20.1)	58 (39.2)	44 (27.0)	82 (37.3)	208(32.2)	7 (5.6)	14(11.2)	21(16.8)	12 (9.6)	54(10.8)
Co-infection of Malaria with	*Trichuris trichiura*	4 (3.4)	8 (5.4)	13 (8.0)	8 (3.6)	33 (5.1)	0	0	0	0	0
	Ascaris lumbricoides	9 (7.6)	12 (8.1)	14 (8.6)	18 (8.2)	53 (8.2)	0	0	0	0	0
	Entamoeba histolytica	8 (6.7)	4 (2.7)	11 (6.7)	20 (9.1)	43 (6.6)	1 (0.8)	7 (5.6)	4 (4.2)	10 (8.0)	22(4.4)
	Hookworm	4 (3.4)	5 (3.4)	8 (4.9)	17 (7.7)	34 (5.2)	3 (2.4)	5 (4.0)	2 (1.6)	1 (0.8)	11(2.2)
	Strongyliodes Stercoralis	2 (1.7)	3 (2.0)	6 (3.7)	13 (5.9)	24 (3.7)	0	0	0	0	0
	Sub-Total	27 (22.7)	32 (21.6)	52 (31.9)	76 (34.5)	187(28.8)	4 (3.2)	12 (9.6)	6 (5.8)	11 (8.8)	33 (6.6)
Co-infection of Malaria with	*Ascaris lumbricoides* + *Staphylococcus aureus*	5 (4.2)	6 (4.0)	7 (4.3)	8 (3.6)	26 (4.0)	0	0	0	0	0
	Ascaris lumbricoides + *Escherichia coli,*	3 (2.5)	4 (2.7)	4 (2.5)	5 (2.3)	16 (2.5)	0	0	0	0	0
	Entamoeba histolytica + *Staphylococcus aureus*	4 (3.4)	2 (1.4)	7 (4.3)	9 (4.1)	22 (3.4)	0	0	0	0	0
	Entamoeba histolytica + *Escherichia coli*	2 (1.7)	4 (2.7)	8 (4.9)	10 (4.5)	24 (3.7)	0	0	0	0	0
	Hookworm + *Salmonellae typhi*	4 (3.4)	5 (3.4)	5 (3.1)	3 (1.4)	17 (2.6)	0	0	0	0	0
	Hookworm + *Escherichia coli*	5 (4.2)	3 (2.0)	6 (3.7)	6 (2.7)	20 (3.1)	0	0	0	0	0
	Sub-Total	23(19.3)	24 (16.2)	37 (22.7)	41(18.6)	125 (19.2)	0	0	0	0	0
Sub-Total of all Malaria Co- Infections		74 (62.2)	114 (77)	133 (81.6)	199 (90.5)	520 (80.0)	11 (8.8)	26 (20.8)	27 (21.6)	23 (18.4)	87 (17.4)
Plasmodium falciparum only		45 (37.8)	34 (23.0)	30 (18.4)	21 (9.5)	130 (20.0)	114 (91.2)	99 (79.2)	98 (78.4)	102 (81.6)	413 (82.6)
Grand Total		119 (18.3)	148 (22.8)	163 (25.1)	220 (33.8)	650	125 (25.0)	125 (25.0)	125 (25.0)	125 (25.0)	500

Table 1. Age Related Co- infections in Hospitalized and School Pupils.

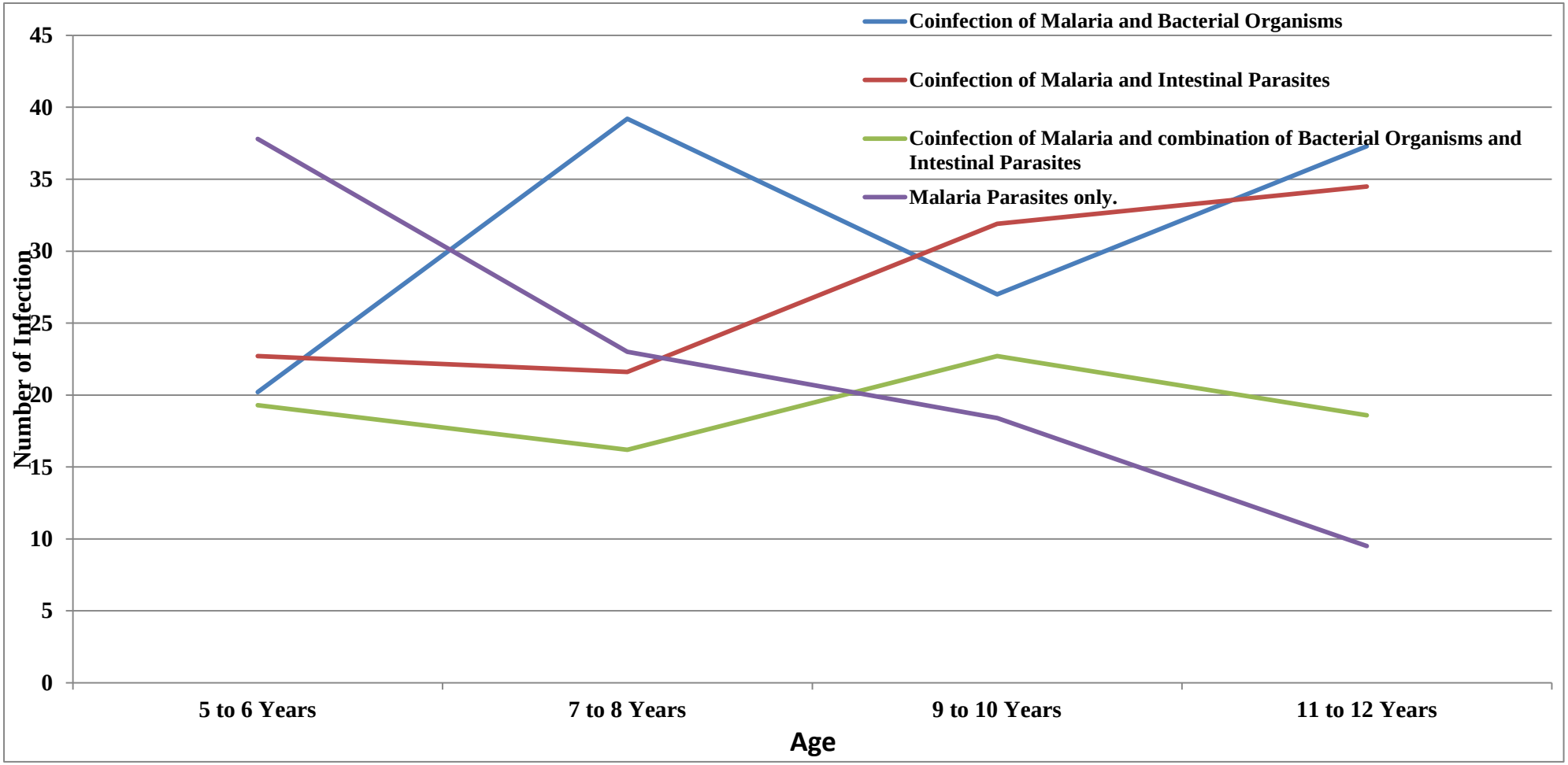

Figure 1 Age distribution of co- infections in hospitalized pupils.

Hospitalized Pupils. Out of 650 hospitalized pupils , 119 were aged from 5 to 6 years out of which 74 (62.2%) had concomitant infections. 148 were aged from 7 to 8 years out of which 114(77.0%) had concomitant infections. 163 were within the age bracket of 9 and 10 years out of which 133(81.6%) had concomitant infections. Finally, 220 were aged from 11 to 12 years out of which 199(90.5%) had concomitant infections. The trend shows a Progressive increase in the number of hospitalized pupils with concomitant infections as their ages increased. Pupils aged from 11 to 12 years had the highest prevalence while pupils within the age bracket of 5 to 6 recorded least prevalence.

In the group that had *Plasmodium falciparum* in concomitance with bacterial organisms, 24(20.1%) were aged between 5 and 6 years while 58(39.2%) were within the age bracket of 7 and 8 years. 44(27.0%) of the pupils in this group were aged 9 to 10 years while 82(37.3%) were aged between 11 and 12 years.

Within the 5 to 6 years age bracket, only three bacterial organisms were isolated namely *Staphylococcus aureus* 8(6.7%), *Escherichia coli* 6(5.0%) and *Salmonellae tyhii* 10(8.4%). In the second age range, 7 to 8 years, four bacterial organisms were isolated namely *Staphylococcus aureus* 12(8.1%), *Escherichia coli* 18(12.2%), *Salmonellae typhi* 24(16.2%) and *Klebsiellae pneumoniae* 4(2.7).

Also in this group, within the age bracket of 9 to 10 years, 5 bacterial organisms were isolated from 44(27.0%) pupils namely *Salmonellae typhi* 16(9.8%), *Escherichia coli* 13(8.0%) and *Staphylococcus aureus* 11(6.7%). Others were *Klebsiellae pneumoniae* 3(1.8%) and a single case of *Psedomonas aeruginosa*.

In the 11 to 12 years age bracket, five bacterial organisms were also isolated, from 82(37.3%) of the pupils, with *Salmonella typhi* 37(16.8%) having the highest prevalence, followed by *Escherichia coli* 32(14.5%) and *Staphylococcus aereus* 9(4.1%). *Klebsiellae pneumoniae* and *Psedomonas aeruginosa* had 3(1.4%) and 1(0.5%) respectively. In this category, number of infected pupils was lowest 24(20.1%) in the 5 to 6 years group while the highest infection rate 82(37.3%) was recorded in the 11 to 12 years age bracket.

In the group that had *Plasmodium falciparum* concomitant with intestinal parasites, a total of 187(28.8%) pupils were infected. 27(22.7%) were aged 5 to 6 years, 32(21.6%) aged 7 to 8 years, 52(31.9%) aged 9 to 10 years while 76(34.5%) were aged from 11 to 12 years. Five intestinal parasites were isolated namely *Trichuris trichiura* 33(5.1%), *Ascaris lumbricoides* 53(8.2%), *Entamoeba histolytica 43*(6.6%), *Hookworm 34*(5.2%) and *Strongyloides stecoralis*24(3.7%

Within the 5 to 6 years age range, *Ascaris lumbricoides* 9(7.6%) had the highest prevalence while *Strongyloides stecoralis* 2(1.7%) had the least prevalence.

In the 7 to 8 years age bracket, *Ascaris lumbricoides* and *Strongyloides stecoralis* had the highest and lowest prevalence of 12(8.1%) and 3(2.0%) respectively. Similarly, in the 9 to 10 years age bracket, *Ascaris lumbricoides* and *Strongyloides stecoralis* recorded the highest and lowest prevalence of 14(8.6%) and 6(3.7%) respectively. Within the 11 to 12 years age bracket, *Entamoeba hystolica* and *Trichuris trichiura* had the highest and lowest prevalence of 20(9.1%) and 8(3.6%) respectively.

In the group that had *Plasmodium falciparum*, intestinal parasites and bacterial organisms, 125 (19.2%) pupils were infected. Highest prevalence of 26(4.0%) was recorded by *Ascaris lumbricoides* and *Staphylococcus aereus*, followed by *Entamoeba histolytica* and *Escherichia coli* 24(3.7%). Others were *Entamoeba histolytica* and *Staphylococcus aereus* 22(3.4%), *Hookworm* and *Escherichia coli* 20 (3.1%), *Hookworm* and *Salmonellae typhi* 17(2.6%) and finally, *Ascaris lumbricoides* and *Escherichia coli* 16(2.5%).

In the first age bracket, 5 to 6 years, *Ascaris lumbricoides* and *Staphylococcus aureus* recorded the highest prevalence of 5(4.2%) *while Entamoeba histolytica* and *Escherichia coli* 2(1.7%) had the least prevalence. Within the 7 to 8 years age bracket, *Ascaris lumbricoides* and *Staphylococcus aureus* 6(4.0%) had the highest prevalence. *Entamoeba histolytica* and *Staphylococcus aureus* 2(1.4%) had the least prevalence. In the third age bracket, 9 to 10 years, *Entamoeba histolytica* and *Escherichia coli* 8(4.9%) had the highest prevalence while *Ascaris lumbricoides* and *Escherichia coli* 4(2.5%) had the least prevalence.

Within the 11 to 12 years age bracket, *Entamoeba histolytica* and *Escherichia coli* 10(4.5%) had the highest prevalence. *Hookworm* and *Salmonellae Typhi* 3(1.4%) had the least prevalence. Similar to other categories, it was observed that the number of hospitalized pupils increased as their ages increased.

Although pupils in the final group did not have concomitant infections (i.e. pupils with only *Plasmodium falciparum* infections), it was observed that out of a total of 130(20.0%) pupils, 45 (37.8%) were aged 5 to 6 years while 34(23.0%)

were between the ages of 7 and 8 years. 30 (18.4%) were aged from 9 to 10 years while 21(9.5%) were between the ages of 11 and 12 years. Unlike the other groups, it was observed that the number of pupils in this category decreased as their ages increased.

Highest number of hospitalized pupils in this category was recorded in the 5 to 6 years age bracket. Figure 1 shows the age distribution of infections in the different age groups among the four categories of hospitalized pupils.

School Pupils: Out of 87 school pupils infected with concomitant infections, 11 or 8.8% were aged 5 to 6 years while 26 (20.8%) were aged 7 to 8 years. A total number of 27 or 22.6% were within the age brackets of 9 and 10 years while 23 (18.4%) were aged from 11 to 12 years. Pupils within the age bracket of 9 to 10 years recorded highest number of infections while those aged from 5 to 6 years had the least infection.

Discussion

A total of 18.3% of the hospitalized pupils were aged from 5 to 6 years, 22.8% were aged from 7 to 8 years, 25.1% were aged from 9 to 10 years while 33.8 % were aged from 11 to 12 years. The number of coinfections increased with the age of the pupils. This could be as a result of increasing level of independence of the pupils from their care givers and increased physical activity as they grew older, which increased their vulnerability to higher risks of infection. This is in agreement with the findings of past researchers, Bundy (1995), Anderson and May (2001).

References.

Anderson, R. M. and May, R. M. (2001). Infectious Diseases of Humans: Dynamics and Control. *Oxford University Press, 374–429.*

Brooker, S., Akhwale, W. S., Pullan, R., Estambale, B., Clarke, S. and Hotez, P. (2007) Epidemiology of Plasmodium-Helminth coinfection in Africa: potential impact on anaemia and prospects for combining control. *American Journal of Tropical Medicine and Hygiene* 77, 88–98.

Bundy, D. A. (1995). *Epidemiology and transmission of helminths.* In *Enteric Infections & Intestinal Helminths*. London: Chapman and Hall Medical 5-24.

Chessbrough, M. (1991). *Medical Laboratory manual for tropical countries. London English Language Book Society Publishers. p189-90.*

Druihe P., Tall, A., Sokhna, C.(2005). Worms can worsen malaria: Towards a new means to roll back malaria. *Trends in Parasitology. 21:359 –62(PubMed).*

Ferreira, C. S., Ferreira, M. U. and Nogueira, M. R. (1994). The prevalence of infection by intestinal parasites in an urban slum in Sao Paulo, Brazil. *Journal of Tropical Medicine and Hygiene 97, 121–127*.

Holland, C. V., Asaolu, S. O., Crompton, D. W., Stoddart, R. C., MacDonald, R.and Torimiro, S. E. (1989). The epidemiology of Ascaris lumbricoides and other soil-transmitted helminths in primary school children from Ile-Ife, Nigeria. *Parasitology 99, 275–285.*

Mwangi, T.W., Bethony, J.M., Brooker, S.(2006). Malaria and Helminth interactions in humans: an epidemiological viewpoint. *Annals of Tropical Medicine and Parasitology; 100: 551- 70.*

Nacher, M. (2004). Interactions between worm infections and malaria. *Clinical Reviews in Allergy and Immunology 26, 85–92.*

Smith D.C.(1999). The rise and fall of typhomalarial fever . *Journal of the History of Medicine and Allied Sciences*; 37: 182-220