



## Research Article

# Influence of Intestinal Helminthiasis on Children Health in Ugbighoko Rural Community of Benin City, Southern Nigeria

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## Abstract

**Background and Objective:** Helminth infections have emerged as a major health concern in African and have been linked to regional ecological and socio-economic status. This study was aimed to ascertain the impact of helminthiasis on the hematological, immunity and antioxidant status of children in the community. **Materials and Methods:** This study was carried out in selected schools of Ugbighoko, Benin, Nigeria. The pupils were grouped; 5-7 years, 8-10 years, 11-13 years, 14-16 years and 17-19 years for control and test. Stool samples were assessed for helminthic parasites (*A. duodenale*, *A. lumbricoides*, *T. trichuira*) and plasma samples were analyzed for CD<sup>4+</sup> level, hemoglobin concentration, packed cell volume (PCV), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), vitamin C and E using standard procedures. The control stools had no observed helminth infection. **Results:** There were significant decrease in PCV and hemoglobin concentration with more severity in age groups 5-7 years and 8-10 years and these correlated negatively with the degree of helminthic parasites. The CD<sup>4+</sup> decreased with helminth infection with more severity at 8-10 years. The SOD activity decreased significantly across the groups, while CAT activity increased significantly across the groups. These antioxidant enzymes correlated significantly with the degree of infection. The non-enzymic antioxidants reduced ( $p < 0.05$ ) with significant correlations to the degree of infection, especially vitamin C. There was significant increase in MDA level. **Conclusion:** Helminthiasis in the school children causes anemia, increased oxidative stress and immune challenges attributable to poor hygienic and low primary health care system. Thus, children of this age groups should be monitored.

**Key words:** Antioxidants, helminthic parasites, helminthiasis, malondialdehyde, hematological helminth infections, immune challenges

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Intestinal parasitic infection is a common affliction of much of the world's population and it has been and is still an important problem in public Health<sup>1</sup>. Parasitic worms or helminths are worm-like organisms that live and feed-off living host, receiving nourishment and protection while disrupting their host nutrient absorption, causing weakness and disease. Those that live mostly within the digestive tract. Approximately 3 billion people globally are infected with helminths<sup>2</sup>. The most common helminthiasis are those caused by infection with intestinal helminths like *Ascaris trichuris* and *Acylostoma*. The inhabitants of rural, impoverished villages throughout the tropics and subtropics are often chronically infected with several different species of parasitic worms<sup>3,4</sup>. The global prevalence of human hookworm infection is greater than all other helminths infection put together and it is now generally accepted that it is among the three most important parasitic infection of man with malaria and schistosomiasis. An estimated 1.05 and 1.3 billion persons harbor the whipworm (*Trichuris trichuria*) and the round worm (*Ascaris lumbricoides*), respectively<sup>5,6</sup>. Among children, an estimated 59 million cases of *A. lumbricoides* infection are associated with significant morbidity and the estimate for acute illness is 12 million cases per year with approximately 10,000 deaths<sup>7</sup>. Both parasites have a widespread distribution in the tropics and subtropics, including both rural areas as well as the slums of large urban areas of the developing world<sup>8,9</sup>. *Ascaris* and *Trichuris* commonly occur both in urban environments, especially urban slums and in rural areas in some instances the prevalence of *Ascaris* infection is actually greater in urban environments in contrast high rates of hookworm infection are typically restricted to areas where rural poverty predominates<sup>10</sup>. The "Five F's" of parasitology, fingers, feces, fomites, flies and food might have originated with *Ascaris* in mind. Transmission through the ingestion of *Ascaris* eggs adhering to vegetables and fruit surfaces is a major route of transmission<sup>11-13</sup>.

This research was aimed at investigating the influence of helminthiasis on the immunity and antioxidant status of young children in a rural area of Benin city, Nigeria. The motivation for this research included the poor health status of growing young children in most neglected poor rural areas of Nigeria and also the lack or scarcity of scientific data to support this claim.

## MATERIALS AND METHODS

**Study area:** The study was carried out, February, 2010 to August, 2011, at Ugbighoko Community, Benin city, Edo State.

Permission to carry out this study was duly granted by the Edo State Primary School Education Board, Ministry of Education Benin city and the parents of the children gave their consents after proper enlightenments. The research study involved primary and secondary school pupils within the ages of 5-19 years. These pupils were 100 in number and were grouped using normal serial sampling and age differences. The research ethics committee guideline principles and consent of the College of Medicine, University of Benin (CMR/REC/2014/57) was obtained, adopted and strictly adhered to.

**Subject:** The research involved the collection of samples and specimen from 100 males and females primary school pupils within the ages of 5-19 years. They were all placed into 5 groups using their age differences; Group 1 (5-7 years), group 2 (8-10 years), group 3 (11-13 years), group 4 (14-16) and group 5 (17-19 years). Each of these groups contained 20 subjects of 10 control and 10 test subjects each.

**Sample collection:** Labeled specimen collection bottles with screw caps were distributed to participating pupils a day before the study. They were instructed to bring freshly passed stool on the following day. Instructions on, how to avoid contamination were also given to each child and the time of stool collection noted. Blood samples of 5 mL of venous blood were carefully drawn into potassium EDTA-containing tubes, the following morning when stool sample bottle was collected and was appropriately labeled.

### Assay methodology

**Fecal analysis:** The fecal samples were examined for parasites using the method described by the World Health Organization<sup>14</sup>. Microscopic examination of stool samples were followed by direct saline and iodine preparation. A portion of the stool sample was emulsified in normal saline on a glass slide, covered with a cover slip and examined microscopically using 10X and 40X objectives for ova of parasites. A drop of iodine solution was added to a homogenous saline preparation of the stool sample, covered with a cover slip and examined microscopically using 10X and 40X objectives for the cysts of parasites. Samples that were negative for parasite ova and cysts were subjected to a concentration method as described by the World Health Organization<sup>14</sup>. The formol ether concentration technique was used. A 1 g sample of the stool was emulsified in 5 mL of formol saline, sieved and the suspension collected in a centrifuge tube, followed by the addition of 3 mL of ether. The well-mixed suspension was centrifuged at 3000 rpm for 1 min. The supernatant was

discarded and the sediment examined microscopically using 10X and 40X objectives for cysts and ova of parasites<sup>14,15</sup>.

**Packed cell volume (PCV):** Anticoagulated blood in a glass capillary of specific length bore size and wall thickness was centrifuged for 3-5 min to obtain constant packing of the red cells. A small amount of plasma remains trapped between the packed red cells. The PCV value was read from the scale of a microhaematocrit reader. Fill about three quarters of a plain capillary tube with well mixed EDTA anti-coagulated blood and seal the unfilled by using a sealant material or a Bunsen burner by rotating the end of a capillary in the flame. Carefully place in the microhaematocrit with the sealed end against the rim gasket (to prevent breakage) and centrifuge for 3-5 min. Immediately after centrifuging, read the PCV.

**CD<sup>4+</sup> T-lymphocyte count and haemoglobin concentration, Hb:** The blood specimens were used for CD<sup>4+</sup> T-lymphocyte count and haemoglobin concentration analysis. The CD<sup>4+</sup> count was determined by flow cytometry (Partec, GmbH, Germany) while haemoglobin concentration was determined using an autoanalyser-sysmex kx-21 (Sysmex Corporation, Kohe, Japan). The Hb concentration was determined by taking the one third of the PCV value<sup>16,17</sup>. Anemia was defined as a hemoglobin concentration <11 g dL<sup>-1</sup> according to WHO criteria<sup>18</sup>.

**Statistical analysis:** Data were entered into the Microsoft excel spread sheet (version 10) prior to descriptive analysis. The data were represented as Mean±SEM. Data were analyzed with the IBM Corp®. Statistical Package for Social Sciences, SPSS®, version 21.0, using the Duncan’s multiple range one-way analysis of variance (ANOVA) at a confidence interval of 95% (p = 0.05); \* was considered significant and \*\* not significant. Correlation analyses were done using the Pearson’s correlation of the IBM Corp®. Statistical Package for Social Sciences, SPSS®, version 21.0. Histograms and line plots were done using Graph Pad software® Prism 5, Version 5.01.

**RESULTS**

*A. duodenale* was the most prevalent helminth across the age brackets especially the children within the 5-7 years age bracket; followed by *A. lumbricoides* shown in Table 1.

The average weights of the test subjects across the different age brackets were lower compared to the control, an indication of weight loss with the infection as described in Fig. 1. The average age of the test subjects across the different

Table 1: Type and degree of helminth parasites present in stool of the children  
Age (years)

Type of helminth	5-7			8-10			11-13			14-16			17-19		
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	
Degree of parasitaemia	--	+++++	++	+++	--	+++	--	+++	--	+++	--	+++	--	+++	
The types of helminth infection (Ad: <i>A. duodenale</i> , Al: <i>A. lumbricoides</i> , Tt: <i>T. trichuria</i> ) and degree of parasite infection (nil, -, 1+, +, 2+, ++, 3+, +++, 4+, +++++) are represented	--	Ad Al Tt	Ad Al Tt	Ad Al Tt	Ad Al Tt	Ad Al Tt	Ad Al Tt	Ad Al Tt	Ad Al Tt	Ad Al Tt	Ad Al Tt	Ad Al Tt	Ad Al Tt	Ad Al Tt	

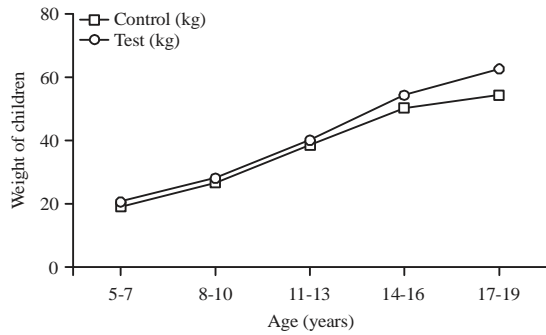


Fig. 1: Average weight of the children

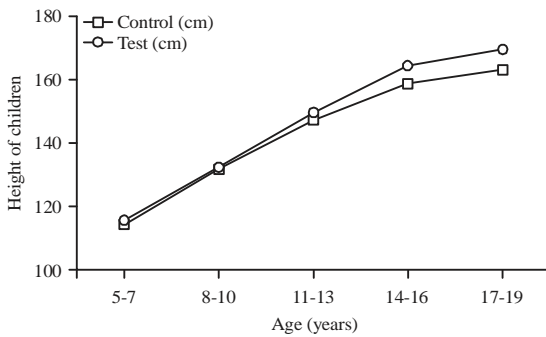


Fig. 2: Average height of the children

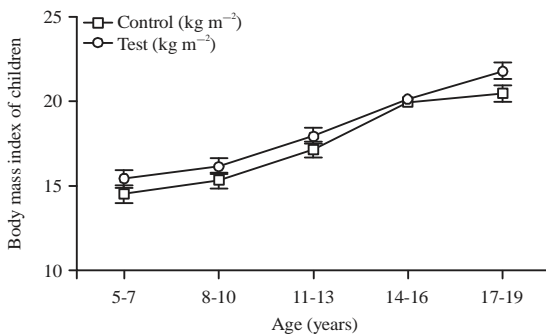


Fig. 3: Average body mass index (BMI) of the children

brackets were lower compared to the control, except the 5-7 age bracket, an indication of stunting of growth of the children with infection as described in Fig. 2 because the height of test group was lowered than control. The average BMI of the test subjects across the different age brackets were lower compared to the control, an indication of malnourishment of the children with the helminths infections (Fig. 3).

The plasma vitamin C concentration of the infected children were significantly decreased across all the age brackets, an indication of poor nutritional state, malnourishment or loss from/or in the gastrointestinal tract (GIT) as described in Fig. 4. The plasma vitamin E concentrations of all the age brackets of the infected children

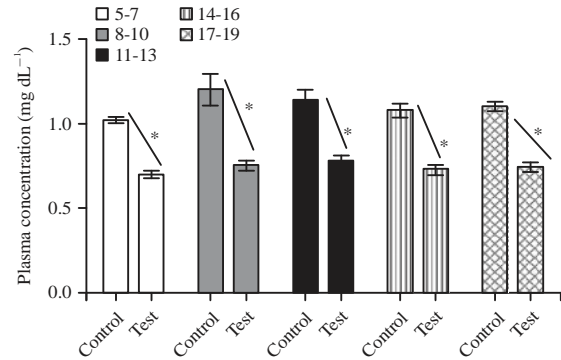


Fig. 4: Plasma concentration of vitamin C of the test compared with the control per age bracket

\*Significant

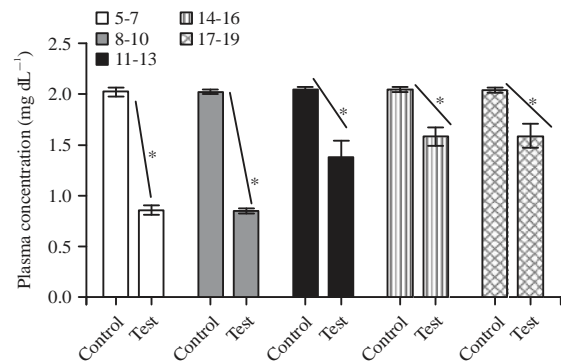


Fig. 5: Plasma concentration of vitamin E of the test compared with the control per age bracket

\*Significant

decreased significantly, with 5-7 and 8-10 years infected age brackets recording the most decreased level, an indication of poor nutritional state, malnourishment or malabsorption from/or in the gastrointestinal tract (GIT) as described in Fig. 5.

The plasma SOD activity of the infected children decreased across all the age brackets ( $p > 0.05$ ), an indication of a challenged health status and oxidative-antioxidant imbalance tended towards oxidative stress as described in Fig. 6. The plasma CAT activity of the infected children demonstrated significant increase across all the age brackets, except the 5-7 years age bracket, an indication of oxidative-antioxidant imbalance tended towards oxidative stress as described in Fig. 7. However, the level of lipid peroxidation as represented by the plasma MDA concentration of the infected children increased significantly across all the age brackets, with 5-7 and 8-10 years age brackets demonstrating steeper increase, an indication of a challenged health status,

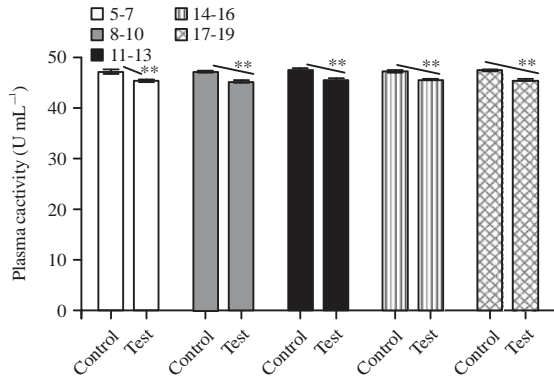


Fig. 6: Plasma superoxide dismutase (SOD) activity of the test compared with the control per age bracket  
\*Significant

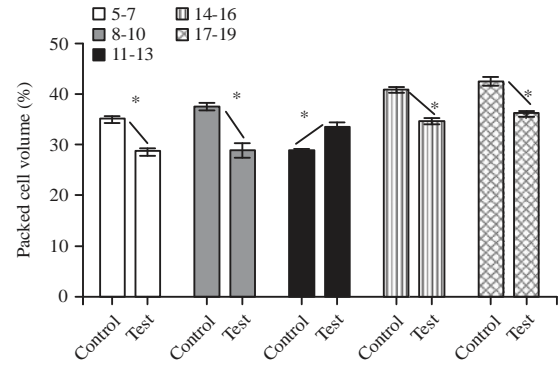


Fig. 9: Packed Cell Volume (PCV) (%) of the test compared with the control per age bracket  
\*Significant

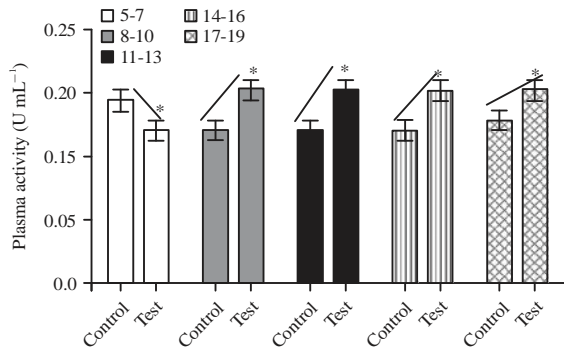


Fig. 7: Plasma catalase (CAT) activity of the test compared with the control per age bracket  
\*Significant

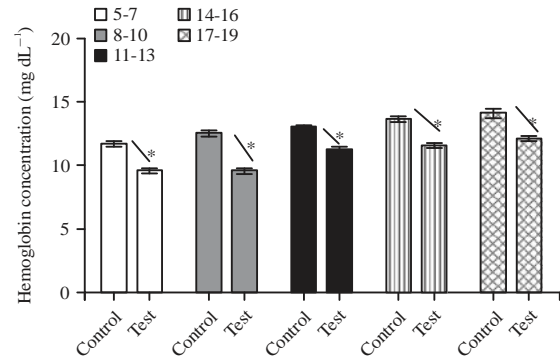


Fig. 10: Hemoglobin (Hg) concentration of the test compared with the control per age bracket  
\*Significant

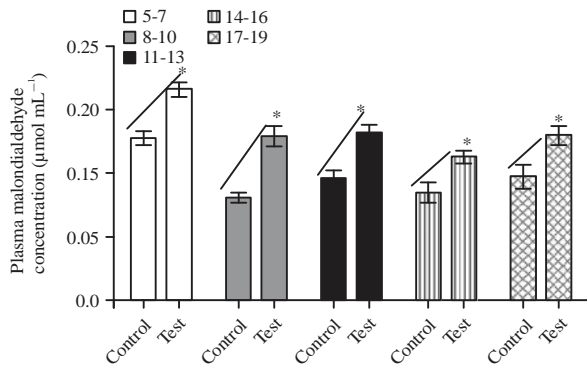


Fig. 8: Plasma concentration of lipid peroxidation marker (MDA) of the test compared with the control per age bracket  
\*Significant

increased lipid peroxidation of membranes (RBC, body tissues, etc.) and oxidative-antioxidant imbalance tended towards oxidative stress as described in Fig. 8.

The PCV levels of the infected children demonstrated significant decrease across the age brackets, except the 11-13 years age bracket, an indication of poor nutritional state, malnourishment, hemolysis and anemia as described by Fig. 9. While the hemoglobin concentration of infected children demonstrated significant decrease across all the age brackets, an indication of poor nutritional state, malnourishment, hemolysis and anemia as shown in Fig. 10.

The percentage CD<sup>4+</sup> of the infected children demonstrated significant decrease across all the age brackets except the 5-7 years age bracket which showed a non-significant decrease. However, the percentage CD<sup>4+</sup> of the infected children of the 8-10 years age bracket decreased markedly compared to the age brackets, an indication of poor health status and challenge from infections as described in Fig. 11.

*A. duodenale* was the most prevalent helminth among the infected children, followed by *A. lumbricoides* and then *T. trichuria* (Fig. 12).

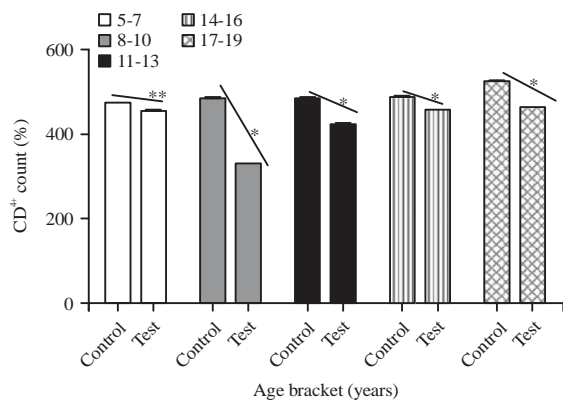


Fig. 11: Percentage CD<sup>4+</sup> of the test compared with the control per age bracket

\*\*Non significant, \*Significant

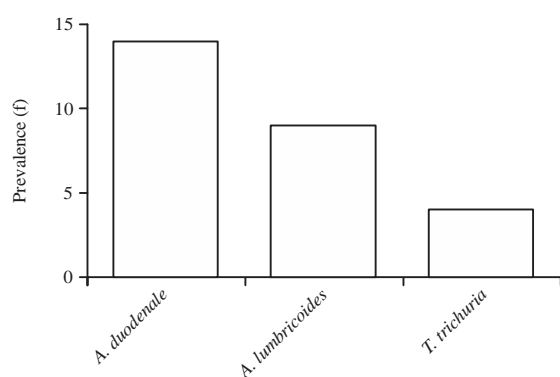


Fig. 12: Helminth prevalence among the school children

## DISCUSSION

Epidemiologic studies conducted throughout the developing world point to school-aged children as the population at greatest risk for acquiring heavy infections<sup>19,20</sup>. These children suffer intestinal obstruction, hepato-biliary ascariasis, *Trichuris* dysentery syndrome or rectal prolapse, physical growth retardation, cognitive and educational impairments, etc<sup>21</sup>.

Figure 4, 5 and 6 demonstrated significant reduction in SOD activity and, vitamin C and E; CAT activity showed a significant increase. These antioxidant enzymes correlated negatively with the degree of helminthic parasites ( $r = -0.817$ ,  $p = 0.000$  and  $r = -0.691$ ,  $p = 0.008$ ). The non-enzymic antioxidants reduced with significant correlations with the degree of parasite. However, a negative correlation between the level of vitamin C and the degree of parasite was observed ( $r = -0.582$ ,  $p = 0.026$ ). Antioxidants are used up in wading off the effect of free radicals generated in the cause of infections especially during respiratory burst involving

white blood cells defense. This explains why reduction in antioxidant level correlates with the severity of the parasitic infection. Redistribution of vitamin A takes place in the extravascular spaces to allow for increase bioavailability to the tissues. Significantly lower vitamin A was observed in severe malaria compared to mild/moderate malaria; vitamin A is utilized during increased parasitemia<sup>2,3,22</sup>.

The negative correlation between the vitamins and degree of parasitaemia may not be unconnected with impaired absorption in gastrointestinal tract due to intestinal obstruction and appetite suppression leading to low nutrient intake. The decrease in vitamin C ( $p = 0.026$ ;  $r = 0.270$ ) was marked in age group (17-19). These deficiencies could lead to serious health problems, including decreased immunity, blindness, lethargy, reduce learning capacity, mental retardation and in some cases, death<sup>23,24</sup>. Impaired nutrition may decrease resistance to intestinal parasite and the parasite themselves may also impair the nutritional status of the individual<sup>25-27</sup>. However the same conditions that lead to intestinal parasite infection that is, poverty and over-crowding, poor environmental sanitation are also characteristics of people at risk of under nutrition<sup>27</sup>. Reduced absorption, increased utilization, increased excretion and reduced food intakes are known to contribute to micronutrient deficiency in the presence of parasite infection<sup>28,29</sup>.

There was significant anaemia in all the age groups (Fig. 9) with more severity in age group 5-7 years (28% PCV) and 8-10 years (29% PCV) compared to controls 35 and 37.5%, respectively; these correlated negatively with the degree of helminthic parasites in their stools ( $r = -0.846$ ,  $p = 0.031$  and  $r = -0.926$ ,  $p = 0.000$ , respectively). This agreed with Hotez *et al.*<sup>4</sup> and Nmorsi *et al.*<sup>20</sup> who claimed that hookworms contributed to anaemia by inducing iron deficiency through chronic intestinal blood loss. The two species of hookworms *Ancylostoma duodenale* and *Necator americanus* cause about 0.2 and 0.15 mL blood loss per day, respectively and release anti-clotting factors like coagulase which ensures continuous blood loss. High *Trichuris* and *Ascaris* infections influence nutritional status negatively affecting protein utilization and amino acid turn-over. Intense whipworm infection in children may result in *Trichuris* dysentery syndrome-growth retardation and anaemia<sup>30</sup>. Heavy burdens of both round worm and whip worm caused protein-energy malnutrition (PEM) and a negative iron balance<sup>31</sup>. A relatively higher prevalence of hookworm in group 1-5 years was observed<sup>32</sup>.

CD<sup>4+</sup> cells of the infected children decreased (Fig. 10). Malondialdehyde (MDA) increased ( $r = 0.837$ ,  $p = 0.005$ ) due to the over production of Free Radicals (FR) from infection<sup>33,34</sup>.

*Ascaris lumbricoides* was the most prevalent helminthic infection among the children (Fig. 11) and possibly among the dwellers of Ugbighoko Community, Benin city, Edo State due to poor sanitation or crowded living conditions<sup>35-44</sup>.

### CONCLUSION

There is no evidence of a country-wide control program against helminthiasis in Nigeria (rural) and school health services are rudimentary as is evident in the findings of this research-decreased antioxidants, anemia and compromised immunity. However, researchers in parasite control had over time advocated for improved sanitation and health care education, especially in schools, to achieve an effective reduction of transmission and intensity. The research made striking observations including, poor health status and malnourishment of the infected children.

### SIGNIFICANCE STATEMENT

This study discovered that young growing school children, especially of rural communities, are at a high risk of health challenges due to helminthiasis and the information from this study can be beneficial for strategic health care planning by stake-holders. The information from this study will furnish researchers in child health with the necessary data to serve as basis for further research.

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