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SEROLOGICAL DIAGNOSIS OF INTESTINAL PROTOZOAN PARASITES AMONG CHILDREN WITH DIARRHOEA IN SOME SELECTED HOSPITALS IN ADAMAWA STATE, NIGERIA

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ABSTRACT

Intestinal protozoan parasites, notably *Entamoeba histolytica* and *Giardia lamblia*, are significant etiological agents of diarrhoea in children in low-resource settings. Accurate sero-epidemiological data are essential for understanding the burden and informing control strategies in north-eastern Nigeria. A hospital-based cross-sectional study was conducted at three health facilities in Adamawa State, Nigeria. A total of 500 diarrhoeic children were enrolled. Blood samples were collected and analysed using serological methods, Enzyme Linked Immunosorbent Assay (ELISA) to detect *E. histolytica* and *G. lamblia* antigens. Data were analysed using chi-square with statistical significance set at $p<0.05$. The overall seroprevalence of intestinal protozoan infections was 181(36.2%). *Entamoeba histolytica* was more prevalent, 112(22.4%), than *Giardia lamblia*, 69(13.8%). Prevalence varied significantly by health facility ($p<0.0001$), being highest at General Hospital Numan (50.0%). Higher seroprevalence positivity was recorded among females (38.5%) than males (34.0%). Also, in relation to age, children aged 5-12 years had a higher prevalence (37.0%), while the least was among 13 -17 years (29.2). Additionally, rural subjects had a higher prevalence (31.4%) than urban subjects (26.0). This study reveals a high seroprevalence of intestinal protozoan parasites (*Entamoeba histolytica*) among children with diarrhoea in the State. Public health interventions should prioritize improving water, sanitation, and hygiene (WaSH) infrastructure and integrating enhanced diagnostic capacity into routine surveillance to reduce the high burden of these infections.

Keywords: *Entamoeba histolytica*, *Giardia lamblia*, Seroprevalence, Diarrhoea, Children, Nigeria

INTRODUCTION

Diarrhoeal disease is the world's second leading cause of death among young children (United Nations International Children Emergency Fund, UNICEF, 2024). The primary cause of diarrhoea is the ingestion of primary agents: bacterial, viral, or protozoan, from human or animal faeces. Transmission occurs through zoonotic, food-borne, and water-borne routes (Moore *et al*., 2016). It consists of ingestion of contaminated food or water, direct contact with infected faeces, person-to-person contact, and poor personal hygiene. The World Health Organization (WHO) and UNICEF define diarrhoea as more than three loose or watery stools during 24 hours. A duration of 14 days is proposed as the criterion for acute diarrhoea or persistent diarrhoea (Walker *et al.,* 2019).

Intestinal parasitic infections, such as soil-transmitted helminths (STHs) and parasitic intestinal protozoa, have been described as the major worldwide causes of illnesses and disease in tropical and sub-tropical regions in the world (WHO, 2023). Besides causing mortality and morbidity, intestinal parasitic infections have been associated with impairment of physical and intellectual development as well as worsening of nutritional status during infancy (Berkman *et al*., 2022). Indeed, the intestinal parasitic infections cause under-nutrition, abdominal pain, diarrhoea, intestinal obstruction, anaemia, ulcers, and other health problems that can lead to delayed cognitive development and impaired learning (WHO, 2017).

Eating uncooked foods that may have been grown, washed, or prepared with contaminated water should be avoided. Breastfeeding appears to protect infants from *Giardia lamblia* infection. Breast milk contains detectable titres of secretory IgA, which is protective for infants, especially in developing countries. Furthermore, infected infants who were exclusively breast-fed had fewer clinical manifestations than those who were not exclusively breast-fed (Tyoalumun *et al.,* 2021).

Many research works indicated different factors had an association with the prevalence of intestinal protozoan infections among children; some of these factors are related to poverty, socio-demographic characteristics, and some others might be related to individuals' practices, such as age, gender, residence, and occupation. The protozoan parasitic infections are among the most prevalent infections in people living in the study area, which is a result of poor environmental sanitation and very poor personal hygiene and unclean habits practices by endemic community dwellers, compounded by public ignorance and illiteracy of the population. Generally, intestinal parasitic infections are dangerous disease-causing agents among school children, ultimately resulting in malnutrition. Mohammed *et al.* (2019) stated that in Nigeria, parasitic infection is endemic and commonly reported among individuals in various communities. Conventional methods, such as microscopic examination of stool samples, have been the mainstay for the diagnosis of protozoan intestinal parasites. This method has its limitations, such as low specificity and sensitivity. This is related to the instability and rapid deterioration of some protozoan parasites once outside the host (Nino *et al*., 2017). Microscopic examination cannot distinguish between different species of *Cryptosporidium*, assemblages of *Giardia*, subtypes of *Blastocystis*, and pathogenic and non-pathogenic species of *Entamoeba* (Haque *et al*., 2019). It is therefore imperative for the use of Serological techniques to diagnose intestinal protozoan parasites in vulnerable populations to accurately measure parasite prevalence in

endemic settings. The unsanitary environment in Yola communities and environs, where children are commonly vulnerable, and the consumption of contaminated foods and water, result in many cases of diarrhoea being rampant. Against this background, children presenting with diarrhoea attending the Specialist Hospital, Yola, and General Hospitals in Mubi and Numan, Adamawa State, Nigeria, were chosen to study the Serological prevalence of intestinal protozoan parasitic infection among children in Adamawa State.

MATERIALS AND METHODS

Study Area

This research study was conducted at Specialist Hospital, Yola, and General Hospitals, Mubi and Numan, located in Yola-North, Mubi-South, and Numan Local Government Areas of Adamawa State, North-eastern Nigeria (Figure 1). The hospitals were chosen to represent the three senatorial zones in the state: Specialist Hospital, Yola, represented the central senatorial zone and served patients residing in that area; General Hospital, Mubi, represented the northern senatorial zone; while General Hospital, Numan, represented the southern senatorial zone and served patients attending from that region.

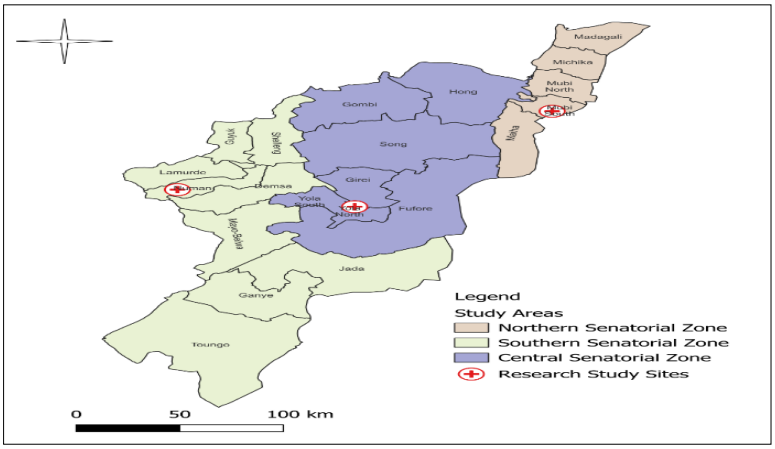

Figure 1: Map of Adamawa State Showing Locations and Study Areas

Adamawa state lies between Latitude 9.3265° N and Longitude 12.3984° E. It is one of the largest states in Nigeria and occupies an area of about 36,917 square kilometres and a projected population of 5,150,630 (National Population Commission [NPC], 2023). Of the total projected population, 1,395,821 are children between the age ranges of 9 months to 14 years old, where 875,607 are below 5 years of age. The State has a tropical climate, marked by dry and rainy seasons. The rainy season commences around May and ends in the middle or late October. The rainfall is characterized by a single maximum with a mean total rainfall of 1,113.3mm. The dry season starts in late October and ends in late April (Adebayo and Tukur, 2020). Maximum temperature in Yola can reach 44^0C around April, while minimum temperature could be as low as 18.3^0C between December and early January. The harmattan season occurs between the end of November and the middle of March. It is characterized by the dry and dusty North-Easterly trade wind, which blows from the Sahara Desert over West Africa into the Gulf of Guinea. The temperature is cold in most places, but can also be hot in certain places, depending on local circumstances (Minka and Ayo, 2013). Relative humidity in the area is about 26% in January, while February is the lowest; with high relative humidity values of 58, 69, 79, 79, 77, and 66, respectively, could be recorded during May to October, particularly during July and August as the peak, with about 80% relative humidity (Adebayo and Tukur, 2020). Most inhabitants are civil servants, traders, fishermen, farmers, and cattle rearers. The study area is positioned within the Sudan savannah vegetation zone, which is characterized by short grasses interspersed with short trees (Yohanna *et al*., 2016). The state is well drained by many rivers, most of which are seasonal. The main river is the River Benue, with major tributaries that have flat sandy beds and steep rocky incised valley sides with an undulating terrain that provides for suitable fishing, irrigational farming, and cattle rearing (Yohanna *et al*., 2016). Flooding is common and occurs seasonally (during the rainy season), which affects most of the LGAs in the state (especially Central and Southern senatorial zones).

Ethical Issues/Approval

Approval and permission for this study were granted by the Adamawa State Ministry of Health Ethical Committee on Health Research with approval number 25/03/2023/016. An introductory letter was obtained from the Department of Zoology, Modibbo Adama University, Yola. The management of the respective hospitals was informed about the study, and their permission was sought and obtained before commencement. Written and verbal informed consent was obtained from patients' relatives or caregivers to ensure voluntary participation.

Study Design

A descriptive cross-sectional hospital-based study was carried out in the Specialist Hospital, Yola, the General Hospital, Mubi, and the General Hospital, Numan. These three hospitals served the population of the study area and its environs and provided both inpatient and outpatient services. The healthcare institutions were selected because of their strategic functions: (a) acting as referral centres for patients from other hospitals and (b) providing specialized medical services to communities in their respective geopolitical zones and to the state at large.

Patients attending the Specialist Hospital, Yola, and General Hospitals, Mubi and Numan, Adamawa State, were selected for this study from November 2024 to December 2025. The sample size was determined using the formula proposed by Smith (2013) for studies involving large or unknown population sizes.

Five hundred (500) Patients were selected for this study using a formula (n= [(Zscore) ^{2xSD} (1-SD)]/e^2) proposed for studies

that can only be used for large populations or unknown population sizes (Smith, 2013).

Blood Sample Collection

A total of 500 blood samples of diarrhoeal patients (outpatients and inpatients) between the ages of 0 – 17 years over a period of 12 months (November 2024 – December 2025). Collected blood samples were transported to Biorcursor Laboratory, Yola, for analysis. The study was conducted in accordance with the recommendations of the Adamawa State Ministry of Health Ethical Committee on Health Research. As the participants were children, a written assent was obtained from caregivers in line with the Declaration of Helsinki (World Medical Association [WMA], 2024). Each blood EDTA container was labelled with a unique identification number capturing age, gender, and location.

Serological Testing

Serological testing for intestinal protozoan infections was performed using enzyme-linked immunosorbent assay (ELISA) kits (e.g., from Diagnostic Automation, Inc., or equivalent), following the manufacturer's instructions. Approximately 3000xg of serum samples were collected using EDTA and centrifuged for 30 minutes at 3000g at 2-8°C within 30 minutes of collection, and processed to detect protozoan antigens. Briefly, serum samples were homogenized in the provided dilution buffer to release soluble antigens, followed by centrifugation to remove particulate matter. The clarified supernatant was then transferred into antigen-coated microplate wells.

During incubation at room temperature (20–25°C), protozoan antigens present in the samples bound specifically to capture antibodies immobilized on the plate surface. After washing to remove unbound material, enzyme-conjugated secondary antibodies were added and allowed to bind to the antigen–antibody complexes. Subsequent washing steps ensured the removal of residual unbound conjugate.

A chromogenic substrate was then added, and colour development occurred in proportion to the quantity of antigen present. The reaction was stopped with a terminating solution, and optical densities (OD) were measured at 450 nm using a microplate reader. Positive and negative controls were included in each assay to ensure validity. Results were interpreted according to the manufacturer's cutoff values, with samples above the cutoff considered positive for infection.

Data Analysis

Data were analysed using both descriptive and inferential statistical methods in R statistical software (R version 4.3.2; R Core Team, 2023). The Chi-square test (χ^2) assessed associations between infections and categorical variables (e.g., location, age, gender). A p-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

A total of 500 children presenting with watery stool or diarrhoea at the selected health facilities were considered for the study. Of this population, 200 children were considered from the Specialist Hospital, Yola, while General Hospital, Mubi, and General Hospital, Numan had 150 children each from the facilities for the study. Serological procedure was conducted for the study population, and the health facility, age, and gender distributions were studied and analysed.

Serological Prevalence of Intestinal Protozoan Infections in relation to Health Facility (Table 1). Serological analysis for *E. histolytica* and *G. lamblia* ELISA revealed that 181 of 500 participants (36.2%) were positive for either parasite, with *E. histolytica* accounting for 112 (22.4%) and *G. lamblia* for 69 (13.8%). The study revealed that infections were most prevalent in General Hospital (GH) Numan (50.0%), followed by General Hospital Mubi (36.0%), and the least was State Specialist Hospital (SH) Yola (26.0%). Also, the coinfection with intestinal protozoan parasites had a similar sequence of infection, where the GH Numan was the most prevalent, while the least was recorded in the Specialist hospital, and this variation was statistically significant.

Table 1: Serological Prevalence of Intestinal Protozoan Parasites with Co-Infections in Relation to Health Facilities

Health Facility	No. Examined	*E. histolytica* (%)	*G. lamblia* (%)	Total Positives (%)	Coinfection	Prevalence	χ^2 (p-value)
GENERAL HOSPITAL Mubi	150	33 (22.0)	21 (14.0)	44 (29.3)	10 (6.7)	54 (36.0)	
GH Numan	150	45 (30.0)	30 (20.0)	59 (39.3)	16 (10.7)	75 (50.0)	21.38 (<0.0001)*
SH Yola	200	34 (17.0)	18 (9.0)	45 (22.5)	7 (3.5)	52 (26.0)	
Total	500	112 (22.4)	69 (13.8)	148 (29.6)	33 (6.6)	181 (36.2)	

Note: GH = General Hospital Numan; GENERAL HOSPITAL = General Hospital Mubi; SH = Specialist Hospital Yola. Only the health facility showed a statistically significant association with protozoan infections ($p < 0.05$).

By Gender, infections were slightly higher among females than males, but the difference was not significant (Table 2). Females had the highest prevalence (38.5%) compared to their males counterpart (34.0%). However, in relation to coinfection, the male subjects had a higher prevalence (6.7%) than the females (6.5%).

Table 2: Serological Prevalence of Intestinal Protozoan Parasites with Co-Infections in Relation to Gender

Gender	No. Examined	*E. histolytica* (%)	*G. lamblia* (%)	Total Positives (%)	Coinfection	Prevalence	χ^2 (p-value)
Female	247	53 (21.5)	42 (17.0)	79 (32.0)	16 (6.5)	95 (38.5)	
Male	253	59 (23.3)	27 (10.7)	69 (27.3)	17 (6.7)	86 (34.0)	0.90 (0.344)
Total	500	112 (22.4)	69 (13.8)	148 (29.6)	112 (22.4)	181 (36.2)	

Note: X^2 = was statistically not significant ($p > 0.05$) in relation to gender.

Table 3 shows the serological prevalence of intestinal protozoan parasites with co-infections in relation to age groups. The highest seroprevalence (37.0%) occurred among children aged 5–12 years, followed by 13–17 years, while children under 5 years accounted for only 2 (100.0%). Coinfection was recorded among only 5-12 years (6.7%). Although not statistically significant, infections were concentrated among the subjects.

Table 3: Serological Prevalence of Intestinal Protozoan Parasites with Co-Infections in Relation to Age-group

Age (Yrs)	No. Examined	*E. histolytica* (%)	*G. lamblia* (%)	Total Positives (%)	Coinfection	Prevalence	χ^2 (p-value)
0–4	2	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)	
5–12	433	100 (23.1)	62 (14.3)	127 (28.3)	33 (6.7)	160 (37.0)	5.00 (0.082)
13–17	65	12 (18.5)	7 (10.7)	19 (10.5)	0 (0.0)	19 (29.2)	
Total	500	112 (22.4)	69 (13.8)	148 (29.6)	33 (6.6)	181 (36.2)	

Note: Yrs=Years. X^2 = was statistically not significant ($p > 0.05$) in relation to age

Serological Prevalence of Intestinal Protozoan Parasites in Relation to Location (Table 4). The study revealed that infections were most prevalent among subjects in the rural settings (31.4%), as compared to their urban counterparts (26.0%), with a statistically significant difference.

Table 4: Serological Prevalence of Intestinal Protozoan Parasites in Relation to Location

Location	No. Examined	Total Positives	Prevalence (%)	χ^2 (p-value)
Urban	169	44	44 (26.0)	
Rural	331	104	104 (31.4)	(0.002)
Total	500	148	148 (29.6)	

Note: X^2 = was statistically not significant ($p < 0.05$) in relation to location

Discussion

Intestinal protozoan parasites are major contributors to diarrhoeal diseases in children, particularly in developing regions where poor sanitation and unsafe water supply facilitate transmission. Serological diagnostic methods provide greater sensitivity than conventional microscopy, enabling more accurate estimation of prevalence and identification of risk factors. This study assessed the serological prevalence of intestinal protozoan parasites among children in some selected Hospitals in Yola, Adamawa State, Nigeria.

Serological prevalence of intestinal protozoan infections by health facility revealed that the highest prevalence was observed in General Hospital Numan (50.0%) in the southern senatorial district of Adamawa State, significantly exceeding rates at General Hospital Mubi (36.0%) and Specialist Hospital Yola (26.0%). This geographic heterogeneity suggests localized environmental or infrastructural factors, such as differences in water supply, sanitation, or food hygiene practices, that facilitate protozoan transmission. Similar intra-regional variations have been reported in Ethiopia, Sudan, and northern Nigeria, where hospital catchment areas with poor WASH conditions consistently record higher protozoan burdens (Hajissa *et al*., 2022; Oyeyemi *et al*., 2022). The significantly elevated rates in Numan may therefore reflect site-specific vulnerabilities that warrant targeted interventions. Also, the predominance of *E. histolytica* aligns with earlier studies in Nigeria and other African settings where amoebiasis remains a leading cause of protozoan-associated diarrhoea (Roro *et al*., 2022; Pukuma *et al*., 2023). Globally, *Giardia* often emerges as the most prevalent intestinal protozoan, especially in community-based surveys of children in Asia and Latin America (Fletcher *et al*., 2020; Certad *et al*., 2017). The lower *Giardia* prevalence in our study compared to global estimates may reflect local transmission ecology and differences in water source contamination patterns.

Although infection was slightly more common among females (38.5%), the difference by Gender was not statistically significant. This pattern is in agreement with most African and Nigerian studies reporting no consistent Gender-associated differences in protozoan prevalence (Sitotaw *et al*., 2020; Tyoalumun *et al*., 2021). Where Gender disparities are detected, they are typically modest and linked to behavioural factors, such as domestic water-related chores for girls or outdoor play for boys, rather than inherent susceptibility. Interestingly, our data suggested that *E. histolytica* was more frequent in males, whereas *G. lamblia* was more common in females, though neither reached significance.

Age-specific distribution showed that infections were overwhelmingly concentrated among children aged 5–12 years (37.0%). This age profile is consistent with global and African evidence identifying school-age children as the group most at risk for protozoan infections due to increased exposure through contaminated water, outdoor play, and inadequate hygiene (Hakizimana *et al*., 2023; Fletcher *et al*., 2022). The near absence of infection in children under five years mirrors findings from Rwanda and Nigeria, where younger children are relatively protected by closer parental supervision and reduced independent exposure to contaminated environments (Oyeyemi *et al*., 2022; Hakizimana *et al*., 2023). Also, this result does not align with research conducted in Southern India by Kattula *et al*. (2015), which indicates a high prevalence of diarrhoeal diseases among children under 5 years.

The prevalence of diarrhoeal diseases was recorded high among the rural (31.4%) than the urban (26.0%) population. The higher prevalence of diarrhoea in rural settings might be attributed to inadequate sanitation, limited access to clean drinking water, poor hygiene practices, and lower socioeconomic status, as well as lower maternal/caregiver education and environmental factors. This result is consistent with research by Fagbamigbe *et al*. (2021), which stated that the prevalence of diarrhoea is higher in those who live in rural areas than in urban settings.

CONCLUSION

This study has shown that Intestinal protozoan infections remain an important contributor to childhood morbidity in Adamawa State. The study reveals critical associations with

age, gender, and location, underscoring both epidemiological and clinical implications. Serological diagnosis uncovered wider exposure patterns, suggesting that the true burden of disease is underestimated when relying on microscopy alone. Collectively, these findings emphasize the need for strengthened WASH interventions, enhanced diagnostic capacity, and integrated policy frameworks to reduce transmission, improve case management, and safeguard child health.

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