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Cryptosporidium parvum Co-infection in Respect to CD4+ T-Lymphocyte Count of HIV/AIDS Patients Receiving Antiretroviral Therapy at Umaru Shehu Ultra-Modern Hospital Maiduguri, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Cryptosporidium parvum is a leading cause of diarrhoea among immunocompromised individuals, especially those living with HIV/AIDS. This study determined the Co-infection of *Cryptosporidium parvum* in respect to their CD4+ T-Lymphocyte count of HIV/AIDS Patients Receiving Antiretroviral Therapy at Umaru Shehu Ultra-Modern Hospital Maiduguri, Nigeria. The study also considered the correlation between CD4+ T-Lymphocyte Counts and Cryptosporidiosis among co-infected patients. A total of one hundred and twelve (112) patients were recruited for this study, from which stool and blood samples were collected. Modified Ziehl-Nelsen staining technique was used to stain the fixed smeared stool after processing via formal-ether concentration method. CD4+ T-lymphocyte count was determined by Partec flow cytometry machine. Twenty-seven (27) out of one hundred and twelve (112) patients screened tested positive for *Cryptosporidium parvum*, yielding an infection rate of 24.1%. The prevalence was found to be higher among patients between the ages of 20-39years and least among those >60years old (7.4%). Female patients were most affected (70.1%) than males (29.9%). subject within the occupational group of Housewives revealed the highest frequency of 44.4%. Results have also revealed that, 75.0% of the HIV patients and 70.4% of

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patients with cryptosporidiosis had a CD4+ count of below 500 cells/µl, while 48.1% and 26.0% of *C. parvum* positive patients had a CD4+ count of ≤300 cells/µl and ≤100 cells/µl respectively. *Cryptosporidium parvum* is an opportunistic pathogen among HIV/AIDS patients; as such the importance of routine stool examination for *Cryptosporidium* oocysts is hereby stressed.

Keywords: Cryptosporidium; HIV/AIDS; CD4 T-Lymphocytes; Maiduguri.

1. INTRODUCTION

The Apicomplexan parasite is an intestinal parasite. Cryptosporidium species are a common cause of diarrhoea and gastroenteritis in humans [1,2]. It caused severe, large, watery faeces in immunocompromised patients, such as those infected with the human immunodeficiency virus (HIV) [3]. Clinical symptoms vary in severity among infected people. Patients experience simple symptoms for a long time after an infection, while others recover in 1-2 weeks after a minor attack. Different genotypes or species of Cryptosporidium, as well as their shifting load or host immunity, could be the cause of such variety. Cryptosporidium hominis and Cryptosporidium parvum, which are responsible for the majority of Cryptosporidium outbreaks, are the main causes of human Cryptosporidiosis. C. meleagridis, C. cuniculus, C. viatorum, C. muris, C. canis, C. felis, C. suis, and C. andersoni are some of the less frequent species [4]. *Cryptosporidium hominis* may cause more severe infection than *C. parvum* and other zoonotic species [5]. Patients infected with C. parvum and other zoonotic species, on the other hand, experience fever more frequently than those infected with C. hominis [5]. Thus, this variation in clinical manifestations might be due to the different *Cryptosporidium* species. Therefore, the current study was aimed at investigating *Cryptosporidium parvum* infection in respect to CD4+ T-lymphocyte count of HIV/AIDS patients receiving antiretroviral therapy at Umaru Shehu Ultra-modern Hospital, Maiduguri, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Maiduguri, Borno State capital. The city is situated in the North-Eastern part of Nigeria that lies within latitude 11.15ºN and longitude 30.05ºE in the savannah zone of Sudano-Sahelian with a dense population that are commonly fishermen, farmers, traders and herdsmen. Borno State is situated along River Ngadda which disappears into the Firkin swamplands in the areas around Lake Chad. It borders the Republic of Niger, Lake Chad and Cameroun respectively. It also borders the Nigerian States of Adamawa, Gombe and Yobe. The annual average temperature is 25.8^{o}C and average annual rainfall is 613 mm. The driest month is January, February and November. Rainy season starts May through October and there are two major seasons; wet and dry seasons. The population of the state is 4,171,104 [6].

2.2 Sample size Determination

The Sample size of this study was determined by the following formula [**7**].

$$N = Z^2P\,(1-P)/d^2$$

2.3 Sample Population

The sample population for this study comprised in and out-patients receiving antiretroviral therapy at Umaru Shehu Ultramodern Hospital, Maiduguri.

2.4 Inclusion and Exclusion Criteria

All HIV Seropositive in and out patients receiving antiretroviral therapy in the clinics, during the period of this study were included while all HIV patients that discontented to be part of the study were excluded.

2.5 Study Design

This study was a cross-sectional hospital-based study involving HIV patients in the Hospital within Maiduguri metropolis.

2.6 Specimen Collection

A total of 112 Stool samples were collected in a wide mouth sterile universal transparent container with screw cap lid and 112 blood samples were also collected in an ethylene diamine tetra acetic acid (EDTA) bottle from HIV positive patients, The specimens collected were labeled appropriately and registered with the patient's study number, and then transported to the laboratory, safety measures were observed during the period of specimens analysis [8].

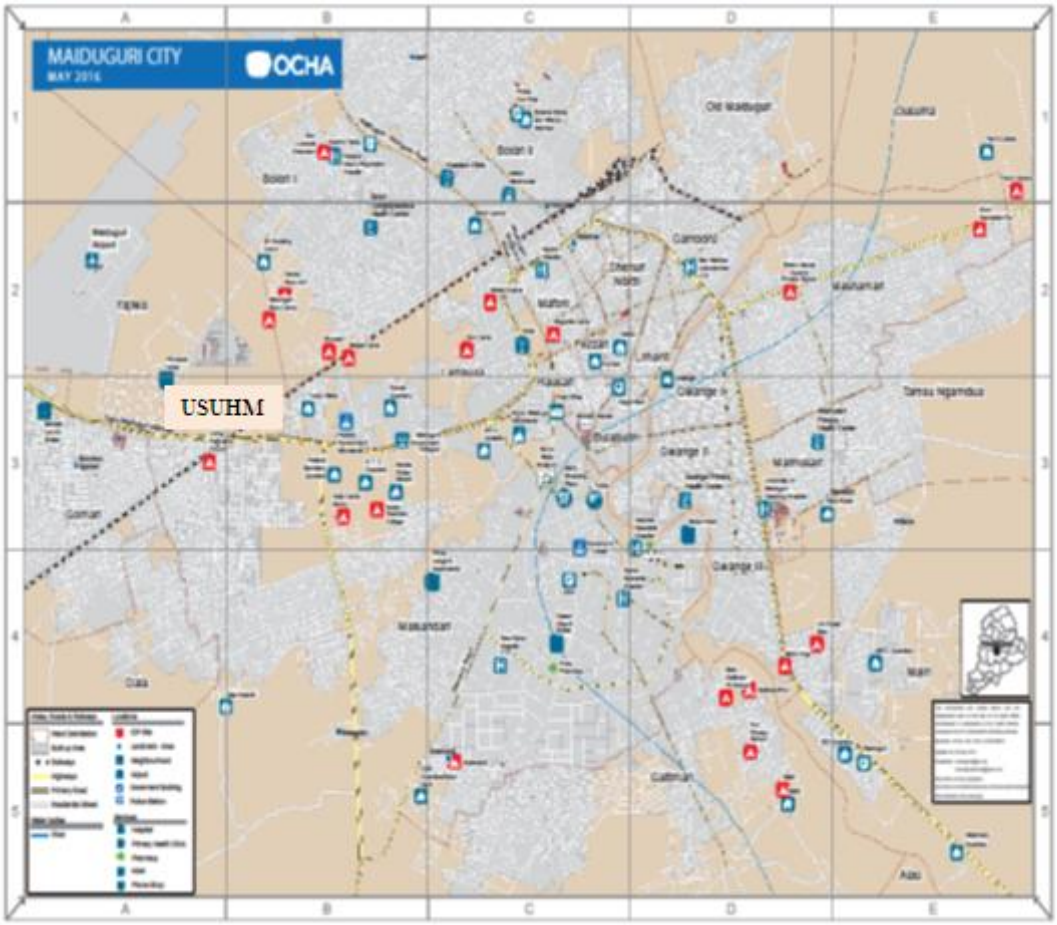

Fig. 1. Map of Maiduguri showing the study location

https://reliefweb.int/sites/reliefweb.int/files/resources/ocha-maiduguri-26052016

KEY= USUH *Umaru Shehu ultramodern hospital Maiduguri*

2.7 Specimen Processing

2.7.1 Macroscopic examination of stool specimens

Stool samples were each studied for colour, consistency and sign of blood, constituent of the stool was also observed [8].

2.7.2 Microscopic examination of stained stool specimens

2.7.2.1 Formalin ether concentration technique

Washington et al. [7] described the formal-ether concentration method by sedimentation technique. With the help of a Pasteur pipette, 7mL of 10% formal saline was put into a screwed centrifuge tube. Approximately 1g (pea-size) of faeces sample was emulsified in 10% formal saline using an applicator stick. To combine, the bottle was capped and vigorously shook. The emulsified material in the screw-capped bottle was filtered into a centrifuge tube using the sieve gauge. The volumes of the centrifuged tubes were flattened by adding 3mls diethyl ether. The tubes were corked with cotton wool and centrifuged at 3000 revolution per minute (rev/min) for 5-minute to concentrate the parasite present in the sample. The centrifuge tubes were brought out after centrifugation and each were carefully inverted to discard the ether, fecal debris and the formal solution. Each tube was tapped to re-suspend the sediment [7,8].

2.7.2.2 Modified Ziehl Nelson Staining

A drop of a deposit from a formal-ether concentrated sample was produced on a clean grease-free slide with the use of a Pasteur

pipette, and the smear was air dried. It was then fixed in 95 percent absolute methanol and stained with the modified Ziehl-Nelsen stain, and the stained smear was air dried. The oocysts were then examined microscopically on the dyed slide. As a quality control slide and for comparison purposes, a proven positive specimen was employed [8,9,10].

2.7.2.3 CD4+ lymphocyte Count

A total of 112 blood samples were collected from patients receiving antiretroviral therapy. 2 mls of blood was aseptically collected via the median cubical vein using 5 ml syringe attached to needles was transferred immediately into a clean and sterile EDTA container to prevent coagulation. A total of 800 µl of CD4 buffer was added to the blood, and was read on the partec Cyflow machine as described by Goldstein *et al*. [11] Based on the CD4+ T- lymphocyte count, patients were grouped into 0-50, 51-100, 101-200, 201-300, 301-400, 401-500,501-600, >600 for the purpose of this study [12].

2.8 Data Analysis

Data generated was analyzed using SPSS 19.0 software was used to establish statistical significance of differences observed between variables. Probability values of $p<0.05$ was considered as significant.

3. RESULTS

According to socio-demography of the patients females were (65.2%). Most of them were between the age of 20-39years and 40-59years (43.8% respectively). Housewives (47.3%) were the most predominant occupational group observed (Table 1). Twenty-seven (27) out of the one hundred and twelve (112) samples processed yielded *C. parvum*, with a parasite prevalence of 24.1%. Female patients (74.1%) recorded the highest infection rate than male patients (23.9%). Patients between the age group of 30-39years were most affected, with an infection rate of 51.9%. The least was observed among those between 0-9years and 10-19years (0.0% respectively) (Fig. 2).

Cryptosporidium parvum was found to be more common among Housewives followed by those engaged in Business ventures. The least was observed among Farmers (Fig. 3).

The highest *C. species* prevalence was recorded among patients with a CD4 count of 0-50cells/µl, 301-400cells/µl and those with >600cells/µl (Fig. 4). Association between CD4 count and *C. species* infection was statistically significant.

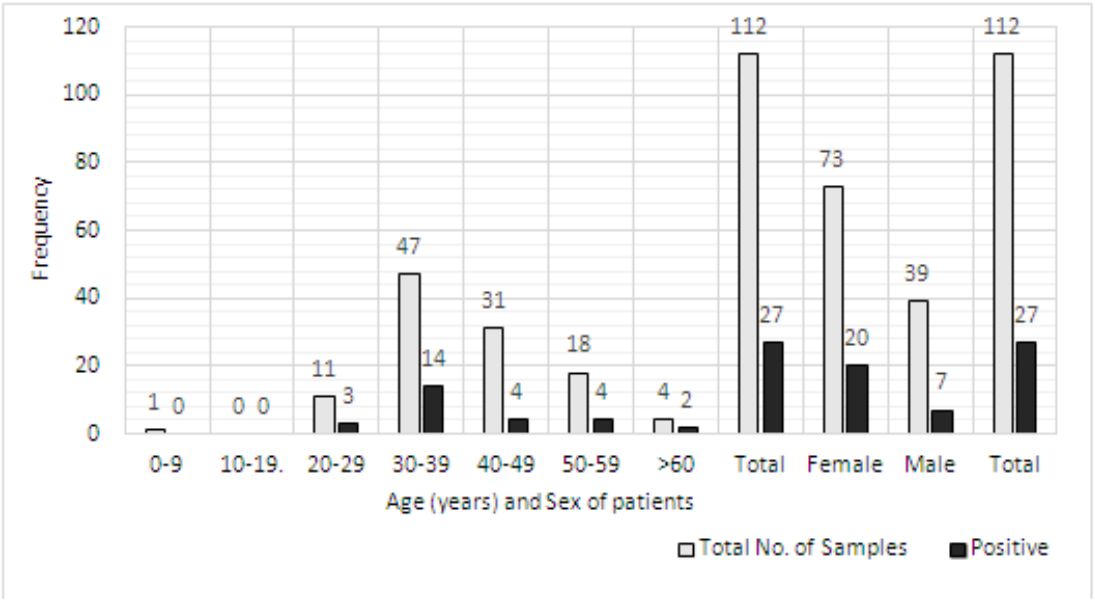

Fig. 2. *Cryptosporidium species* infection in respect to Age and Sex of HIV/AIDS subject receiving antiretroviral therapy USUMH, Maiduguri

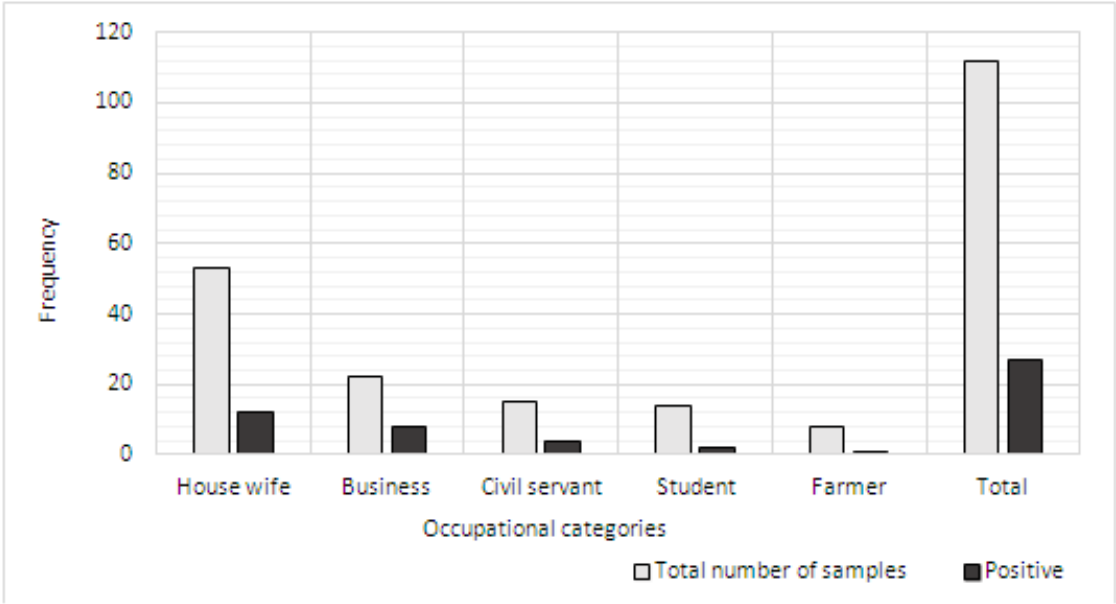

Fig. 3. *Cryptosporidium species* infection in respect to Occupation of HIV/AIDS subjects receiving antiretroviral therapy USUMH, Maiduguri

Table 1. Socio-demography of HIV positive patients attending Umaru Shehu Ultra-modern Hospital Maiduguri, Borno State

Demographic variables	Number of HIV Positive Patients (n =112)	Percentage (%)
Sex		
Female	73	65.2
Male	39	34.8
Age (years)		
0-19	10	8.9
20-39	49	43.8
40-59	49	43.8
≥ 60	04	3.6
Occupation		
Housewife	53	47.3
Business	22	19.6
Civil servant	15	13.4
Student	14	12.5
Farmer	08	7.1

Table 2. Comparison of CD4+ T-Lymphocytes Count in Relation to *Cryptosporidium species* infection among Patients with HIV/AIDS attending USUMH

Variable	Cryptosporidium Infection		p-Value
	Positive	Negative	
	Mean ± SD	Mean ± SD	
CD4+ T-Lymphocytes (Cells/µL)	353.22 ± 280.06	393.84 ± 355.12	0.589*

*Keys: SD = Standard Deviation *p-value ≤ 0.05 was considered statistically significant.*

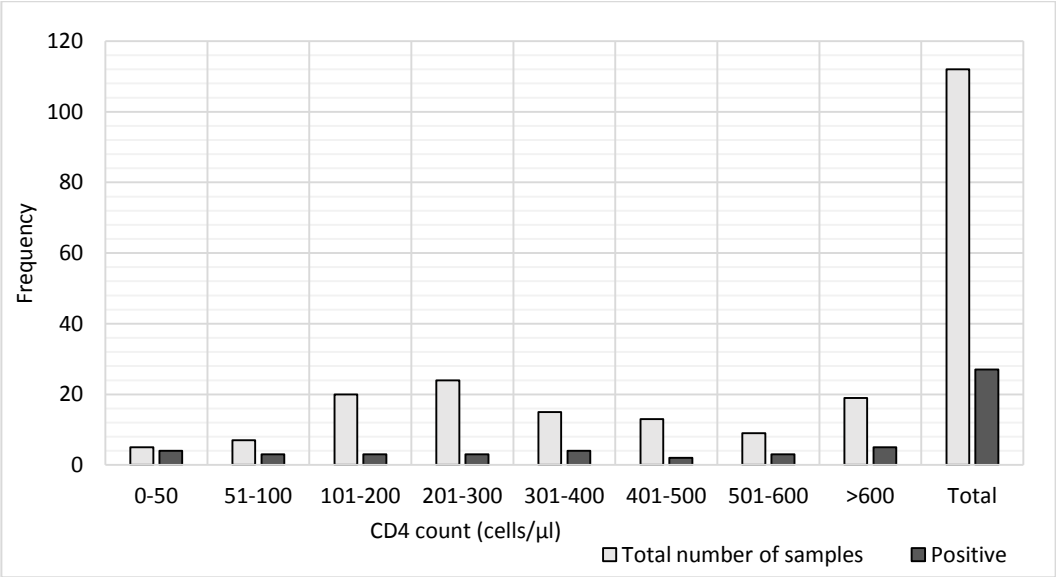

Fig. 4. *Cryptosporidium species* infection in respect to CD4 T-Lymphocytes counts of HIV/AIDS patients attending USUMH, Maiduguri

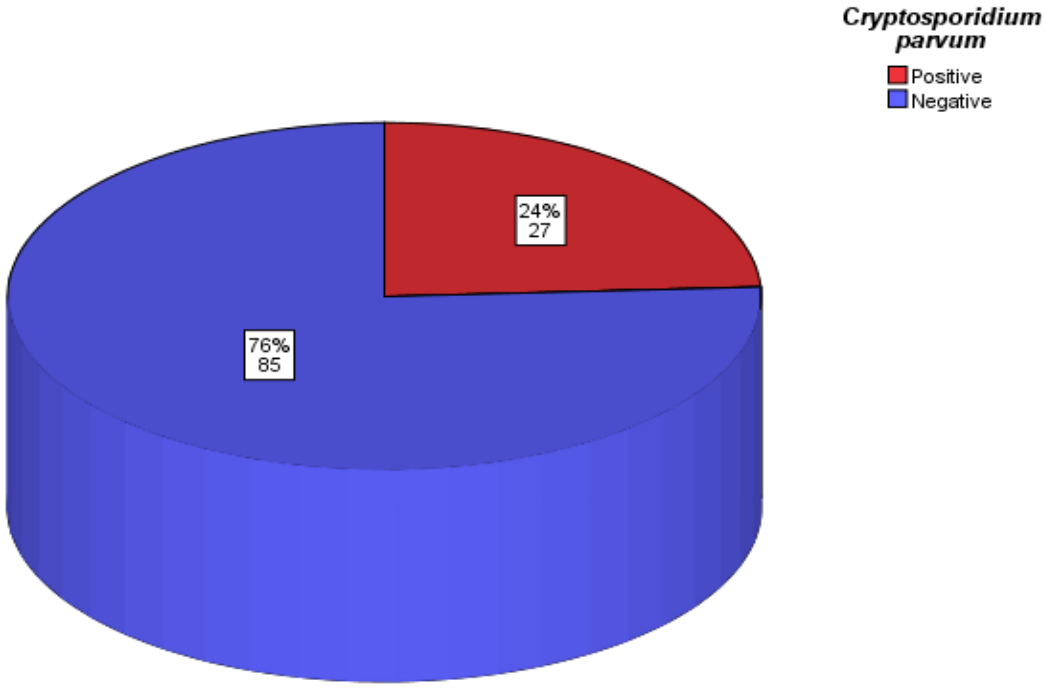

Fig. 5. Prevalence cryptosporidium in patients with HIV/AIDS attending Umaru Shehu Ultra-Modern Hospital Maiduguri, Borno State

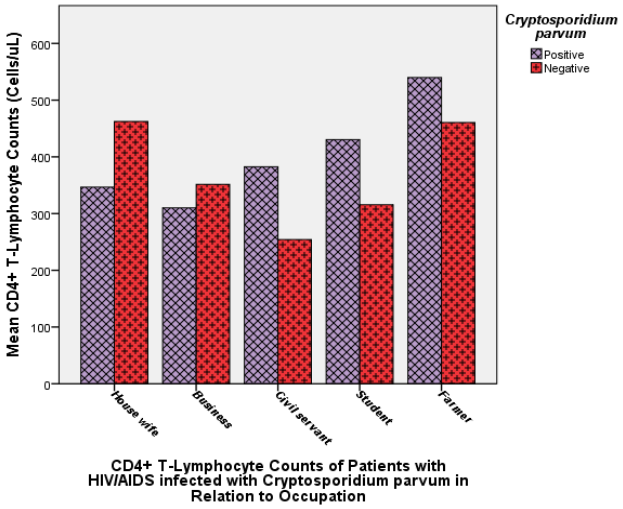

Fig. 6.CD4+ T-Lymphocyte counts of patients with HIV/AIDS infected with *cryptosporidium parvum* in relation to occupation

Table 3. Comparison of CD4+ T-Lymphocytes Count and *Cryptosporidium species* in Respect to Sex and Age of Patients with HIV/AIDS attending USUMH

Variables		Cryptosporidium Infection		p-value
		Positive	Negative	
		Mean ± SD	Mean ± SD	
Sex	Female	355.80 ± 296.81	456.14 ± 407.38	0.316
	Male	345.86 ± 246.63	267.00 ± 152.51	0.290
Age (years)	0-19	288.00 ± 99.00	446.75 ± 271.72	0.493
	20-39	242.79 ± 207.29	408.46 ± 475.11	0.217
	40-59	492.33 ±320.03	379.22 ± 236.85	0.232
	≥ 60	565.50 ± 456.08	218.50 ± 127.99	0.407

*Keys: SD = standard deviation *p-value ≤ 0.05 was considered statistically significant*

4. DISCUSSION

Cryptosporidium species are among the most common protozoans linked to protracted diarrhoea, particularly in developing nations where personal cleanliness and adequate sanitation are not common. The parasites has been reported among HIV/AIDS patients in Nigeria [13] and other parts of the world [14] however, the prevalence rate in this study was said to be 24.1% which is slightly higher than the findings of Pam et al. [15] with prevalence rate of 23.6% and lower than the findings of Yunusa et al. [16] with 31.9% and Adesiji et al. [17] with 52.7%. This study also produces similar report as Vyas et al. [18] with 25.2% of the infections in HIV/AIDS positive patients. This study is higher than studies in Nigeria and other part of the world. As reported in Southeastern Nigeria by Yemisi et al. [19] the prevalence was 0.0% among 161 HIV patients, while a prevalence of 2.9% was reported by Erhabor et al. [10] among 105 HIV-infected adults patients in the south-south, 25.0% prevalence was also reported by Kumurya [13] in Northwestern Nigeria.

The findings of this study contradict those of Kamissky et al. [20] in Honduras, who found no C. parvum among 133 HIV-positive individuals. This could be related to the fact that the prevalence of C. parvum infection varies dramatically between countries and communities, depending on the extent of human and animal excreta pollution of piped and drinking water [21,22].

This study recorded high prevalence rate among female than their male, though was statistically not significant at ($p=0.316$, $p<0.005$). This was consistence with other [23] but in variation with some other reports [20,24,25]. This higher prevalence of females (63.7%) may be attributed to the fact that polygamous family settings is highly encouraged with higher number of females

infected with HIV thereby increasing the chances of more females acquiring opportunistic infection such as *C. parvum* parasite. A higher prevalence was also among patients with age group between 30-39 years old it may not be in connection with their business activity. And come in conformity with similar report of Pam et al. [14] in this study, there was a relationship between HIV seropositivity, CD4 counts and *C. parvum*.

Patients whose CD4 counts were >600 cells/µl and had Cryptosporidiosis were (18.5%) compared to those whose CD4 counts were <51-300 cells/µl (65.6%). This was statistically not significant at ($p=0.589$, $p<0.05$). This was compared favorably with previous reported studies [24,25,26]. In this study, there was a positive correlation between CD4+ T-Lymphocytes count of <51-300cells/µl and infection with *C. parvum.* HIV terminates cell mediated immune system which is provided by the CD4 lymphocytic cell, these lymphocytes when significantly destroyed below 200cells/µl influences the patients to opportunistic infection and regularly more chance of acquisition of *C. parvum* infection [23].

5. CONCLUSION

In this study, it was found that *Cryptosporidium* infection rate was 24.1% mainly in female housewives, age group between 20-39 years. In HIV patients, there is a substantial link between CD4 level and co-infection. Improved health education, the use of clean drinking water, and quick referral to a tertiary health facility can improve the quality of life of HIV/AIDS patients and prevent them from contracting Cryptosporidiosis and other AIDS-Defining illnesses.

ETHICAL APPROVAL

This work has been approved by the research and ethical committee of Umaru Shehu Ultramodern Hospital Maiduguri, Borno State. Number: BO/234/2020.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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