Cryptosporidium parvum Co-infection in relation to CD4+ T-Lymphocyte count in patients living with HIV/AIDS attending Umaru Shehu Ultra-modern Hospital Maiduguri, Nigeria

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* among HIV/AIDS patients attending Pepfar Clinic of Umaru Shehu Ultra-modern Hospital (USUH) Maiduguri, Nigeria. The study also ~~analysed~~ 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%. ~~Parasite~~ 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%). Patients within the occupational category of Housewives recorded the highest infection rate of 44.4%. The results also revealed that, 75.0% of the HIV patients and 70.4% of 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,

INTRODUCTION

The coccidian enteric parasite *Cryptosporidium* species is a common cause of gastroenteritis and diarrhea in man [1, 2]. It causes severe, voluminous watery diarrhoea among immunocompromised patients, example of which include those infected with human immunodeficiency virus (HIV) [3].Infected patient's exhibit varying degrees of clinical manifestations. ~~Some~~ Patients do have severe symptoms over long period of infection, while others recover within 1-2 weeks after a mild attack. The reasons for such diversity, ~~which~~ might be attributed to different genotypes or species of *Cryptosporidium* ~~species~~ or their varying load or host immunity~~, have not been adequately investigated so far~~. Human Cryptosporidiosis is mainly caused by *Cryptosporidium hominis* and *C. parvum*, which are responsible for most of the outbreaks of *Cryptosporidium* ~~described~~. Other less common species are *C. meleagridis*, *C. cuniculus*, *C. viatorum*, *C. muris*, *C. canis*, *C. felis*, *C. suis*, and *C. andersoni* [4]. *Cryptosporidium hominis* may cause more severe infection than *C. parvum* and other zoonotic species [5]. In contrast, HIV positive patients infected with *C. parvum* and other zoonotic species tend to have 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 present study was aimed at investigating *Cryptosporidium parvum* infection in relation to CD4+ T-lymphocyte count of HIV/AIDS patients attending Umaru Shehu Ultra-modern Hospital, Maiduguri, Nigeria.

MATERIALS AND METHODS

Study Area

The study was conducted in Maiduguri, the capital of Borno State. The city is located in the North-Eastern part of Nigeria which lies within latitude 11.15ºN and longitude 30.05ºE in the Sudano-Sahelian savannah zone with a dense population that are mostly fishermen, crop farmers, traders and herdsmen. Borno State is situated along the seasonal Ngadda River which disappears into the Firkin swamps in the areas around Lake Chad. It borders the Republic of Niger to the north, Lake Chad to the northeast and Cameroun to the east. On the south and west, it borders the Nigerian States of Adamawa, Gombe and Yobe. The annual average temperature is 25.8^{o}C and average annual rainfall is 613mm. 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].

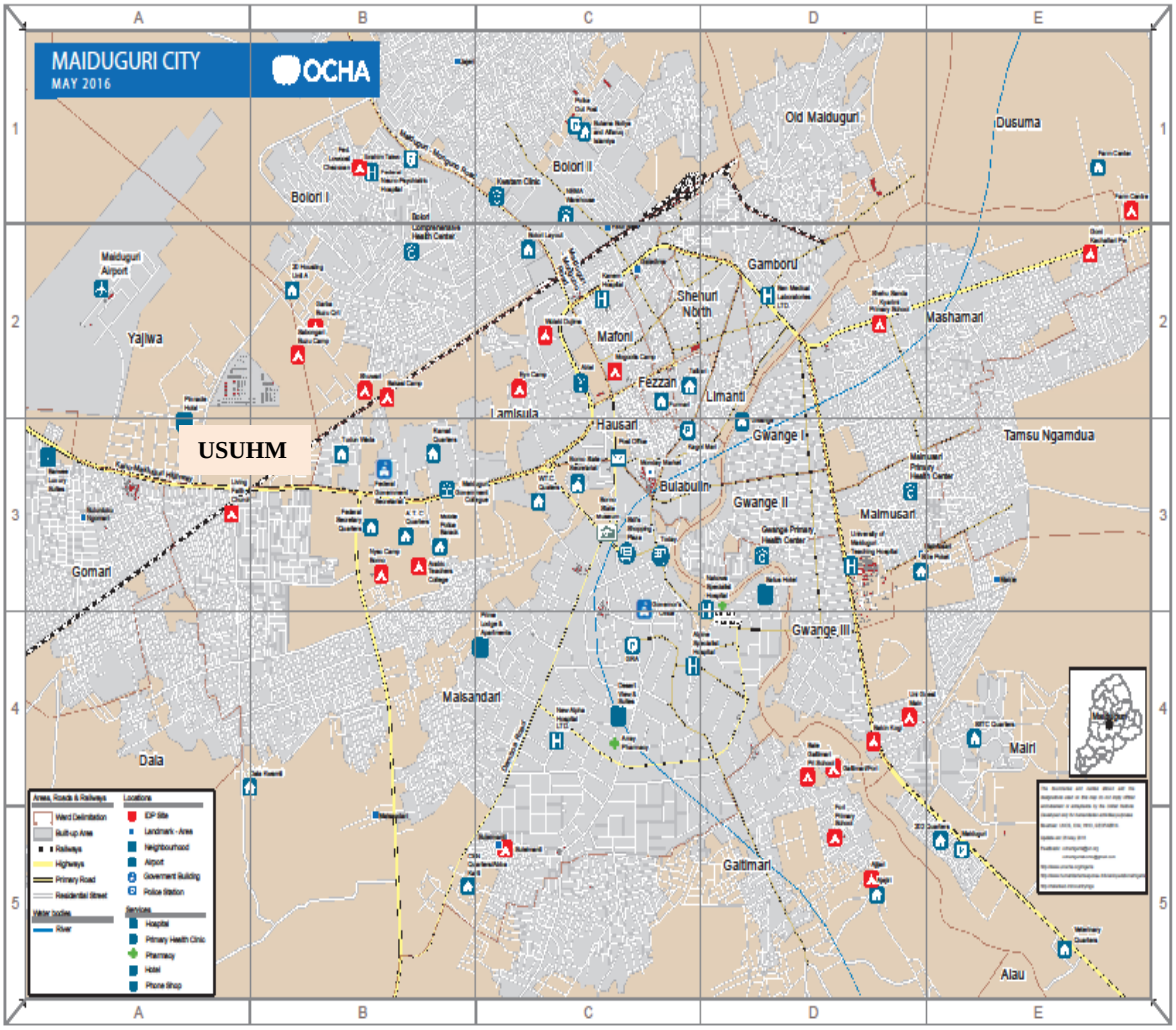

Figure 1: Map of Maiduguri showing the study location

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KEY=

USUHM = Umaru Shehu ultramodern hospital Maiduguri

Ethical Considerations: This work has been approved by the ethical committee of Umaru Shehu Ultramodern Hospital Maiduguri, Borno State.

Sample size Determination

Sample size was determined using the following formula [**7**]

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

Sample Population

The target population for this study include in-patients and out-patients attending Umaru Shehu Ultramodern Hospital, Maiduguri.

Inclusion and exclusion Criteria

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

Study design

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

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 precautions were observed throughout the period of processing the specimens [8].

Specimen Processing

Macroscopic examination of stool specimens

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

Microscopic examination of stained stool specimens

Formalin ether concentration technique

The modified formal-ether concentration method by sedimentation technique was employed as described by Washington *et al.* [7]. 7mls of 10% formal saline was transferred into a screwed centrifuge tube with the aid of a Pasteur pipette. Using an applicator stick, an estimated 1g (pea-size) of stool was emulsified in the formal saline in the bottle. The bottle was capped and shaken vigorously to mix. The emulsified stool in the screw capped bottle was filtered through the sieve gauge into the centrifuge tube. The volumes of the centrifuged tubes were leveled up by adding 3mls diethyl ether. The tubes were corked with cotton wool and taken into the centrifuge machine and was set at 3000 revolution per minute (rev/min) for 5-minute to concentrate the parasite present in the tubes. After centrifugation, the centrifuge tubes were brought out and each was carefully inverted to discard the ether, fecal debris and the formal solution. The bottom of each of the tubes was tapped to re-suspend the sediment [7, 8].

Modified Ziehl Nelson Staining

Using Pasteur pipette, a drop of the sediment from formal-ether concentrate was made on a clean grease free slide and the smear was then allowed to air dry, followed by fixation with 95% absolute methanol and stained using the modified Ziehl-Nelsen stain and air dried. The slide was then observed microscopically for oocysts. A confirmed positive specimen was used as quality control slide and for comparative purposes [8, 9, and 10].

CD4+ lymphocyte Count

A total of 112 blood samples were collected from people living with HIV/AIDS. Samples were collected based on subjects' HIV/AIDS status, CD4+ T- lymphocyte counts, age, sex and occupation of the patients. A total of 2 ml of blood was aseptically collected between 8:00 and 11:00 am via the median cubical vein using 5 ml syringe and 21gauge needles and transfer immediately into a clean and sterile EDTA container to prevent coagulation. A total of 800 µl of CD4 buffer was added to the above volume of blood, and was read on the Cyflow machine ~~within 5 hours of collection at the Umaru Shehu Hospital Laboratory, using the CD4+ easy count~~ 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 [26].

Data Analysis

Data was analyzed using SPSS 19.0 software. The chi square-test and Fischer exact test was used to establish statistical significance of differences observed between variables. Probability values of <0.05 was considered as significant.

RESULTS

Majority of the patients sampled were females (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*, which gives 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) (figure 1).

Table 1: Socio-demographic Characteristics 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

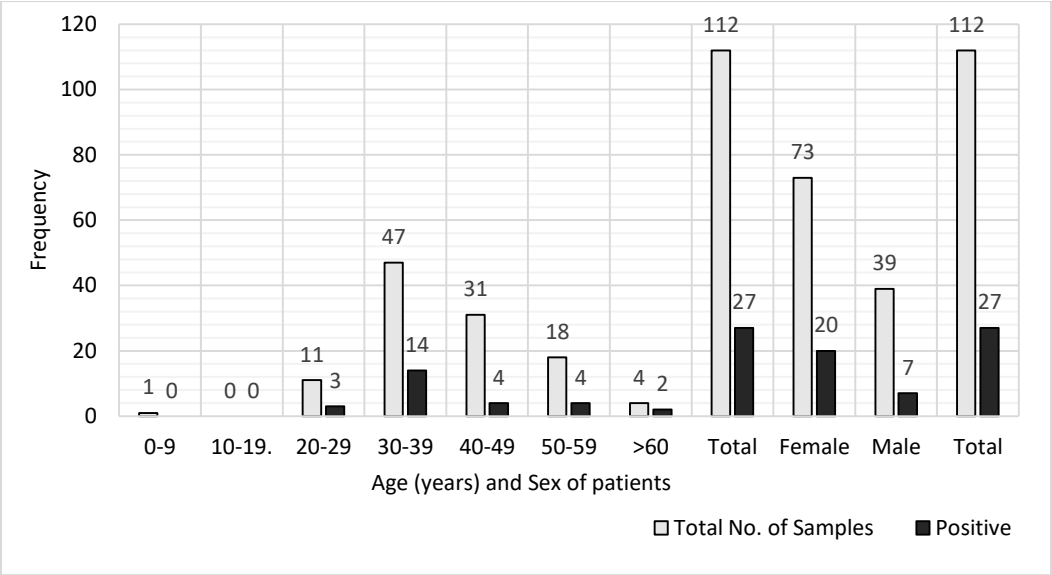


Figure 2: *Cryptosporidium species* infection in relation to Age and Sex of HIV/AIDS patients attending USUH, Maiduguri

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

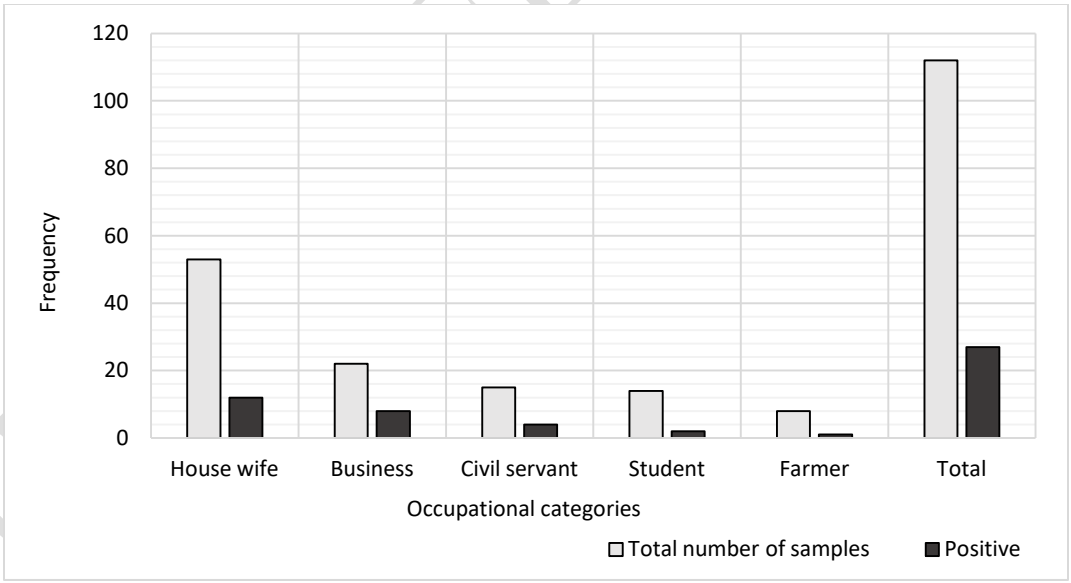


Figure 3: *Cryptosporidium species* infection in relation to Occupation of HIV/AIDS patients attending USUH, Maiduguri

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

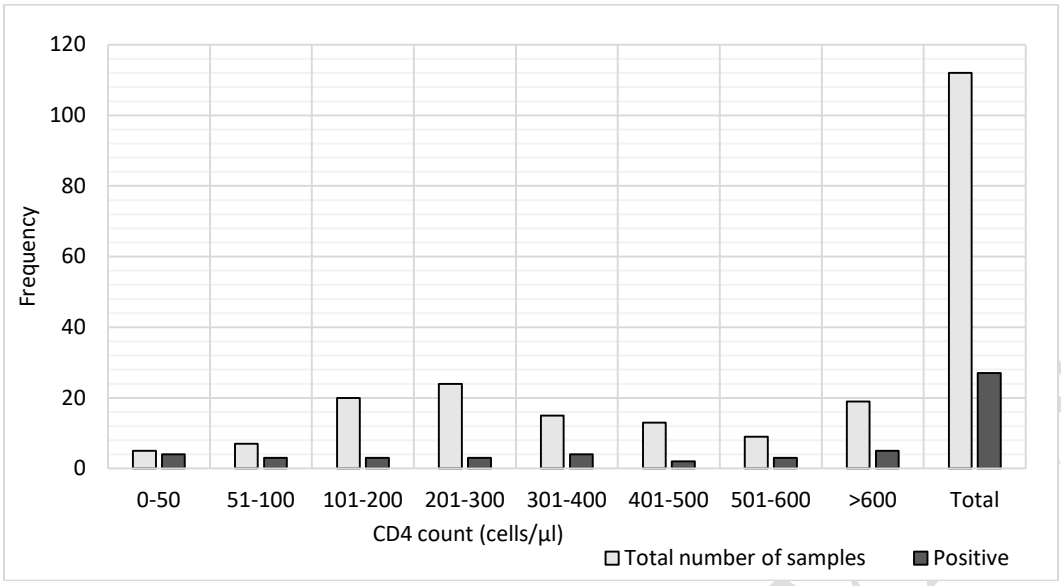


Figure 4: *Cryptosporidium species* infection in relation to CD4 T-Lymphocytes counts of HIV/AIDS patients attending USUH, Maiduguri

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

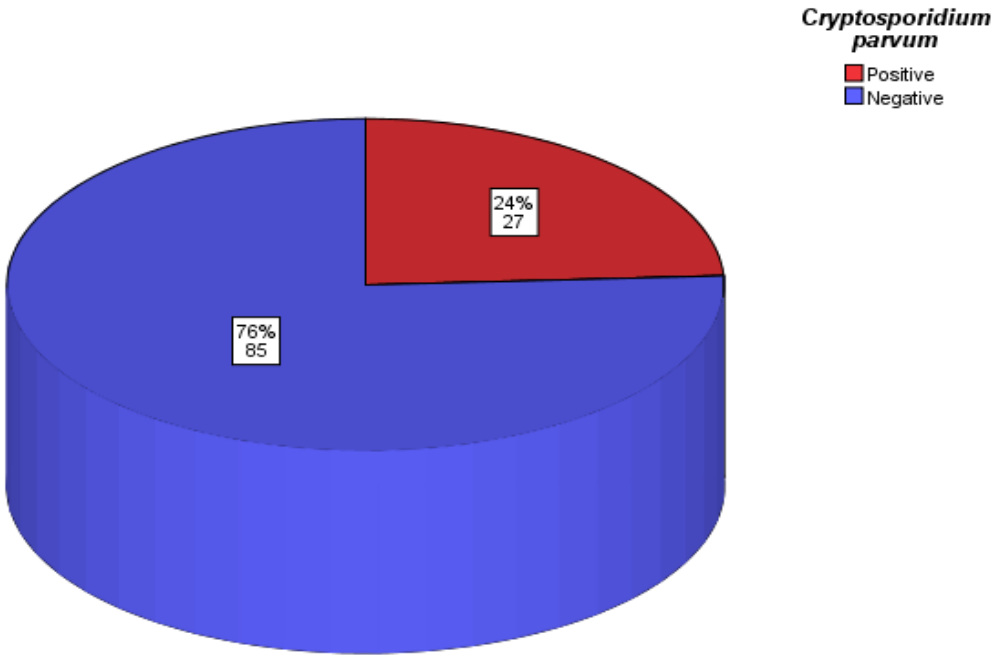
Variable	Cryptosporidium Infection		p-value
	Positive	Negative	
	Mean ± SD	Mean ± SD	
CD4+ T-Lymphocytes (Cells/µL)	353.22 ± 280.06	393.84 ± 355.12	0.589*

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

Tables 3: Comparison of CD4+ T-Lymphocytes Count and *Cryptosporidium species* in Relation 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

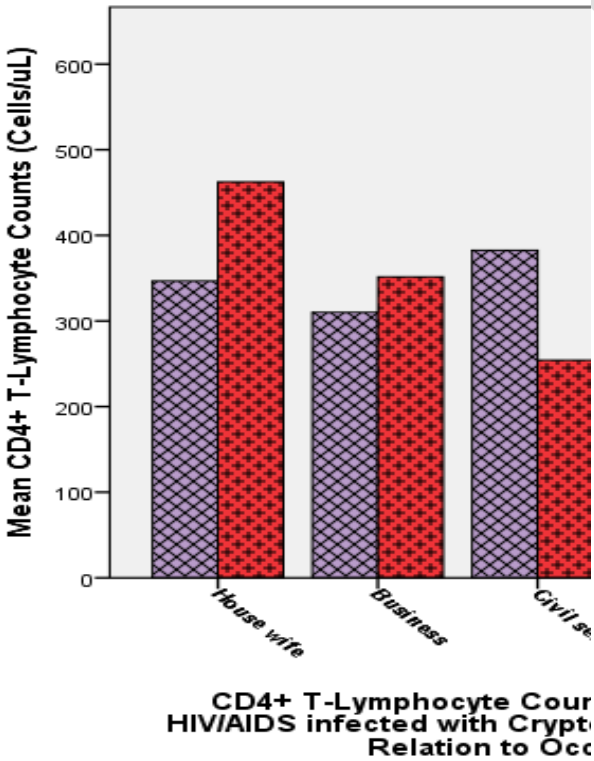
Keys: SD = standard deviation *p-value ≤ 0.05 was considered statistically significant.



Prevalence Cryptosporidium parvum in patients with HIV/AIDS attending Umaru Shehu Ultra-Modern Hospital Maiduguri, Borno State

Figure 5

Figure 6



DISCUSSION

Cryptosporidium species is one of the most important protozoans that are usually implicated in protracted diarrhoea especially in developing countries where personal hygiene and standard sanitation are lacking. *Cryptosporidium* species has been reported among HIV/AIDS patients in Nigeria [12] and other parts of the world [13] however, in this study, the prevalence was recorded to be 24.1% slightly higher than the findings of Pam *et al.* [14] with prevalence rate of 23.6% and lower than the findings of Yunusa *et al.* [15] with 31.9% and Adesiji *et al.* [16] with 52.7%. This study also produces similar report of Vyas *et al.* [17] who reported 25.2% of the infections in HIV/AIDS positive patients. This study is higher than studies in Nigeria and other part of the world. In Nigeria the prevalence was higher than 0.0 % prevalence among 161 HIV patients reported by Yemisi *et al.* [18] in Southeastern Nigeria, 2.9% among 105 HIV-infected adults reported by Erhabor *et al.* [10] in the south-south, 25.0% prevalence reported by Kumurya [12] in Northwestern Nigeria.

The prevalence in this study is higher than that reported by Kamissky *et al.* [19] in Honduras, who did not detect *C. parvum* among the 133 HIV infected patients. The differences may be due to the fact that the prevalence of *C. parvum* infection varies remarkably among regions of the world as well as among communities depending on the level of contamination of piped and drinking water with human and animal excreta. In Nigeria the prevalence obtained in this study is lower than a study done in south-west Nigeria, where the value of 52.7% was obtained [20]. Other lower prevalence reported by Nwokediuko *et al.* [21]. Cryptosporidiasis was up to 50.0% in developing countries. These differences observed may be due to the methods of parasite detection used.

This study recorded high prevalence rate among female than their male counterparts, although this was not statistically significant at ($p=0.316$, $p<0.005$). This was consistence with other [22] but at variance with some other reports [19, 23, and 24]. 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*. [13] in this study, there was a relationship between HIV seropositivity, CD4 counts and *C. parvum*.

HIV 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 [23, 24, and 25]. In this study, there was a positive correlation between CD4+ T-Lymphocytes count of <51-300cells/µl and infection with *C. parvum.* HIV destroys the cell mediated immune system which is provided by the CD4 lymphocytic cell, these lymphocytes when significantly destroyed below 200cells/µl predisposes the patients to opportunistic infection and invariably more chance of acquisition of *C. parvum* infection [22].

CONCLUSION

In this study, it was found that *Cryptosporidium* infection rate was 24.1% mainly in female housewives, age group between 20-39 years. There is a strong association between CD4 count and co-infection among HIV patients. Improved health education, use of clean drinking water and prompt referral to tertiary health institution will improve the quality of life of people living with HIV/AIDS and prevent them from Cryptosporidiosis and other AIDS-Defining diseases.

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