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(RESEARCH ARTICLE)

Prevalence of *Cryptosporidium* oocyts, amongst human immunodeficiency virus patients attending selected hospitals in Nasarawa State, Nigeria

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Abstract

Cryptosporidium a protozoan parasite affects the intestines and is typically an acute short-term infection. The disease is spread through the fecal oral route, often through contaminated water. The study was aimed at determining the prevalence of cryptosporidium oocysts amongst Human Immunodeficiency Virus patients attending selected Hospitals in Nasarawa State, Nigeria. A total of one hundred and ten stool samples were collected from HIV patients attending selected Hospitals in the study area. Faecal samples were analysed using formal diethylacetate the cheesbrough vortex method to detect the presence of cryptosporidium oocysts. The overall prevalence of Cryptosporidium oocyts among HIV patients attending selected Hospitals in Nasarawa state was 29 (26.4%). The prevalence in relation to health care canters was highest in FMCK (26.7%) and lowest in DASH (8.6%). In relation to gender, the prevalence was higher in males with (23.8%) than in females (13.2%). The Prevalence of Cryptosporidium oocyts amongst HIV patients attending selected Hospitals in the study area in relation to occupation showed that patients in other occupation had the highest prevalence (28.6%) with the lowest in farmers (11.1%). The prevalence of Cryptosporidium oocyts in relations to age among HIV patients in the selected health care centres shows that the highest prevalence was amongst range age 0-15 years and 46-60 years (21.4%) with the lowest was for age range 16-30years (7.41%). There was no significant difference between the different age groups ($P > 0.05$). Prevalence of Cryptosporidium oocyts in relation to educational status of patients showed highest prevalence among the uneducated (25.0%) and the least was among primary school (8.70%). The prevalence of Cryptosporidium oocyts amongst HIV patients in relation to livestock farming occupations was higher prevalence among livestock farmers (32.0 %) than non-livestock farmers (9.59%).). this study shows that infection in patients who are livestock farmers was more preponderant indicating that these category of the study population are both highly susceptible to the parasite and could therefore be more capable as agents of its distribution calling to for a more curious concer on these carriers as agents in the epidemiological cycle of cryptosporichosis.

Keywords: Cryptosporidium oocyts; HIV patients; Age; Intestines; Health care canters

1. Introduction

Human cryptosporidiosis is an infection caused by an apicomplexan cellular protozoan of the genus *Cryptosporidium. Cryptosporidium* is transferred, through the faecal oral route in its oocyst form [1], and infection results from the ingestion of their oocysts. *Cryptosporidium* species is a leading waterborne pathogen and the most significant globally, as a result of contaminated water supplies, swimming pools, recreational water, aquatic environment, sachet water, bottle water and raw water. The parasite is resistant to most water purification, especially chlorine, filters, causing an outbreak of gastroenteritis, severe diarrhea and even death [2, 3]. Food borne transmission of *Cryptosporidium* comes from contaminated foods like, milk, fruits, vegetables, and sea foods. Foodborne transmission also comes through direct contamination by infected farm workers, livestock, abattoirs, and food handlers, washing or processed food [4]. Farmyard manure and slurry are used as fertilizers for crop cultivation [5]. Airborne transmission or respiratory cryptosporidiosis, as a result of inhalation of infected droplets of oocyst, has been reported [6]. Other sources of

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transmission include person to person, especially in daycare or child centers, where children, after defecating can transfer oocyst from one child to another orally [7].

Cryptosporidiosis is among one of the possible opportunistic infections in human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) individuals, which presents a very serious public health threat with a severe life threatening diarrhea disease [8]. Human cryptosporidiosis was once believed to be caused by a single species, but molecular studies have demonstrated that it is caused by more than 15 different species. Among the more common species are *Cryptosporidium hominis* for which humans are the only natural host and *Cryptosporidium parvum* [9]. They replicate in the epithelial cell lining of gastrointestinal tract. They complete their life within a single host [10].Genotyping and molecular epidemiology have been used for the assessment of cross species transmission and zoonotic potential of *Cryptosporidium* [11], and these have led to the basic biology of *Cryptosporidium*. This identification has been largely due to the use of molecular biological tools for characterization of the parasite and its interaction with the host. The study was aimed at determining the prevalence of *cryptosporidium* oocysts amongst Human Immunodeficiency Virus patients attending selected Hospitals in Nasarawa State, Nigeria.

2. Material and methods

2.1. Study area

Individuals attending HIV clinics for HIV/AIDS related cases at selected health centers in Nasarawa State namely: Model Hospital Akwanga (MHA) and Our Lady of Apostles Hospital (OLAH), DalhatuAraf Specialist Hospital (DASH), and Federal Medical center, Keffi (FMCK).

2.2. Questionnaire Administration

An interviewer, administered questionnaire to each participant to obtain written demographic and clinical data. The questionnaire contains information on their bio data, educational history, occupation, source of drinking water and involvement in livestock farming

2.3. Research Design

A cross sectional and descriptive study was used for this study.

2.4. Consent Form

A confidential consent form was prepared heightening the purpose of the research, type of specimen required and assurance of their confidentiality, without coercion was given.

2.5. Ethical Approval

Ethical Approval was obtained from the ethics committee of the Nasarawa State Ministry of Health, Lafia; following the submission and defense of the research protocol where patients right to consent and confidentiality was assured

2.6. Sample Size Determination

The sample size formula for this study is: $N= (Z^2pq)/e^2$

The appropriate sample size formula chosen above is that designed for non-comparative single proportion study [11].

The calculation of the sample size

- N=Desired sample size in a population
- Z=Standard deviation set at 1.96, corresponding to the 95% confident interval
- P= Prevalence of the target population estimated to have a particular characteristic. In this case *Cryptosporidium*
- Q= 1-p complimentary probability to P
- e= the degree of precision or absolute error set at 5 % (0. 5)

In this Study P was taken as 7% representing the prevalence rate of *Cryptosporidium* species [12].

2.7. Mathematically, sample size

N= (1.96) ^2 x prevalence x (1-prevalence)/(0 05^2)

0.31462704/0.0025= 100

Minimum sample size=100

10% attrition=10

Sample size=110

2.8. Sample Collection

Fresh stool samples were collected from 3 categories of participants as follows: loose samples, whether they were on anti-retroviral therapy or not; watery stool, whether they were on anti-retroviral or not; any stool texture from immunocompromised participants whose CD4 count of 200 cells/mm'3 and below 100,000 copies/'3 and above .A total of 110 fresh stool samples that met the criteria were collected into a sterile, wide open ,dry, leak proof container with 35 samples from DASH; 35 from MHA ;25 stool samples from OLAH; and 15 from FMCK . Samples collected from these canter were transferred to Omega Int'l Medical Laboratory Ltd Akwanga, Nasarawa State for analysis

2.9. Sample processing

Macroscopic examinations conducted include consistency, presence or absence of mucus and blood were recorded before the microscopy of the samples. Faecal samples were emulsified using sterile rod by estimating 1g of faeces in about 4mls of 10% formal water contained in a screw capped bottle. 3 – 4ml of 10% formol water was added, and the mixture was mixed well by shaking. The emulsified faeces were sieved. The sieved suspension was collected in a sterilized beaker. The suspensions were transferred to a centrifuge tube made up of strong glass, copolymer or polypropylene, and 3-4ml of diethyl ether was added. The content of the centrifuge tube were mixed for 15 seconds with the use of a vortex mixer (Stuart SA7 Vortex mixer Cole parmer LTD). Centrifugation was done immediately at 3000rpm for 1 minute. With the use of sticks or stem of a plastic bulb pipette, the layer of the faecal debris was loosed from the side of the tubes and the tube was inverted to discard the ether. Faecal debris, formol water and the sediment were left [14]. Microscopic Examination of the Slides The microscope (LEICA Microsystems GMBH Wetzlar-Germany) was calibrated using graticule to enable differentiation and confirmation of Cryptosporidium ocysts from other (coccidian oocysts).The prepared slides were examined microscopically for oocysts, using a low power magnification to detect the presence of the oocysts and the oil immersion objective to identify them. Specimens within oocysts that appeared small, round to oval, pink red stained bodies measuring 4–6µm,or a single deeply stained red dot were considered positive [14].

2.10. Staining

A smear from the sediment obtained by formol ether oocyst concentration techniques was prepared and air-dried the smear. The smear was fixed with methanol for 2-3 minutes. Stain was made with unheated carbol fucshsin for 15 minutes and the stain was washed off with water. The stain was decolorized with 1% acid-alcohol for 10-15 seconds and washed off with water. Counter stains were made with 0.05% methylene blue for 30 seconds and washed off with water and the slides transferred to a draining rack for the smear to dry. Each smear was examined microscopically for oocysts using a low power magnification and then by the use of oil emmersion objective for identification.

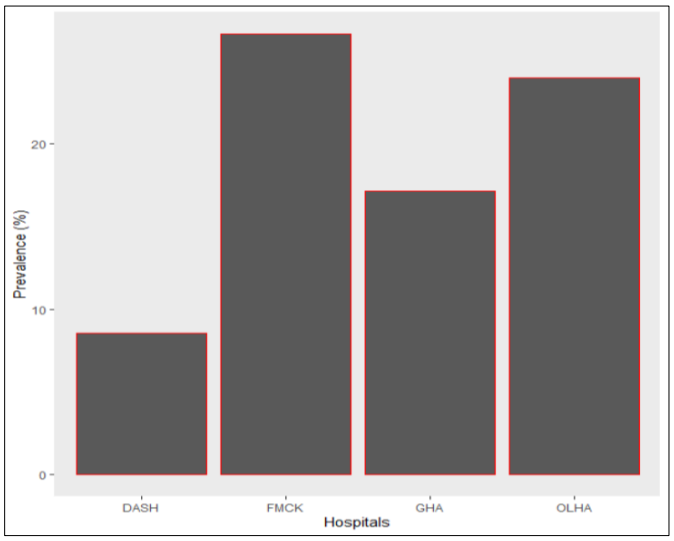
2.11. Data Analysis

Statistical analysis was carried out using R software amplication version 3.6.1. Chi-square was used to test for significance.

3. Results and discussion

For this study, the prevalence of *Cryptosporidium* oocyts among HIV patients attending selected Hospitals in Nasarawa state was 29 (26.4%) and this is higher than study earlier reported by Muhammad and Kumurya [15]. Cryptospodiosis among patients with human immunodeficiency virus who have severely impaired immunity may be a devastating disease. Not only can it cause chronic severe and intractable diarrhoea that greatly reduces the patient's quality of life, but, in many patients, it can also significantly shorten their life expectancy due to low CD4+ cell counts, lack of access to antiretroviral treatment (ART) and poor hygiene [16]. The prevalence in relation to health care canters shows that

FMCK (26.7%) had the highest followed by OLHA (24.8%), GHA (17pp.1%) and the lowest prevalence was observed from DASH (8.6%) as shown in Figure 1. The difference in prevalence observed in different health care facilities might be due to differences in geographical settings, and the sample sizes collected in those health centers.



Key; MHA= Model Hospital Akwanga; OLAH = Our Lady of Apostles Hospital; DASH = DalhatuAraf Specialist Hospital and FMCK = Federal Medical center, Keffi

Figure 1 Prevalence of Cryptosporidium oocyts amongst HIV patients attending selected Hospitals in Nasarawa State

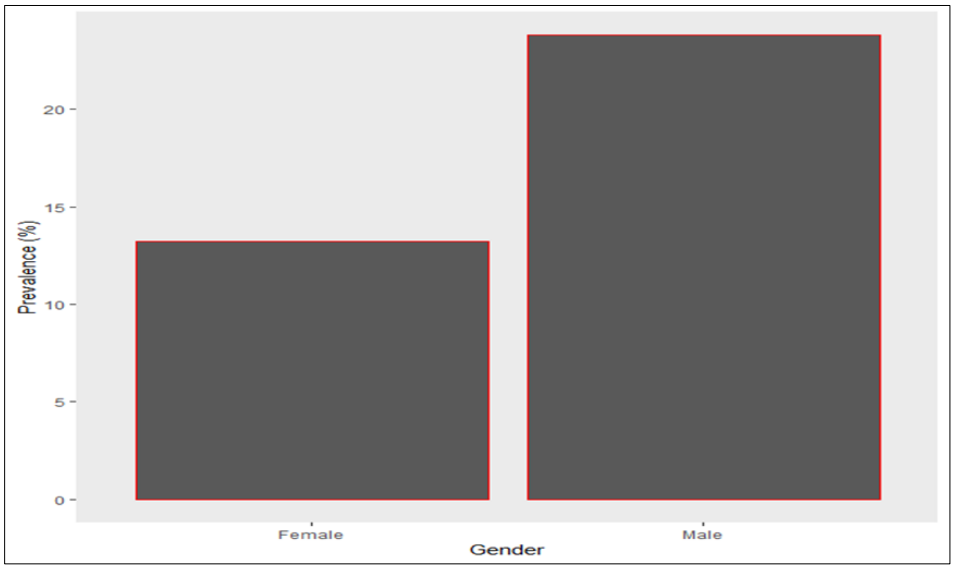


Figure 2 Prevalence of Cryptosporidium oocyts amongst HIV patients attending selected Hospitals in Nasarawa State in relation to gender

Figure 2 shows the prevalence of *Cryptosporidium* oocyts in relation to gender. It was observed that male (23.8%) recorded more prevalent than the female (13.2%) which may be described by the difference in the number of male participants were higher than the female participants but the prevalence were statistically insignificant (p=0.2437). this observation was not in agreement with study reported by Xiao *et al*. [11], that shows a higher prevalence among females than males in HIV-positive patients.

Prevalence of *Cryptosporidium* oocyts amongst HIV patients attending selected Hospitals in Nasarawa State in relation occupation showed that patients with others occupation had the highest prevalence (28.6%) followed by those in business (23.1%), students (15.1%), civil servant (12.6%) and lowest was farmers (11.1%) as given in Figure 3.

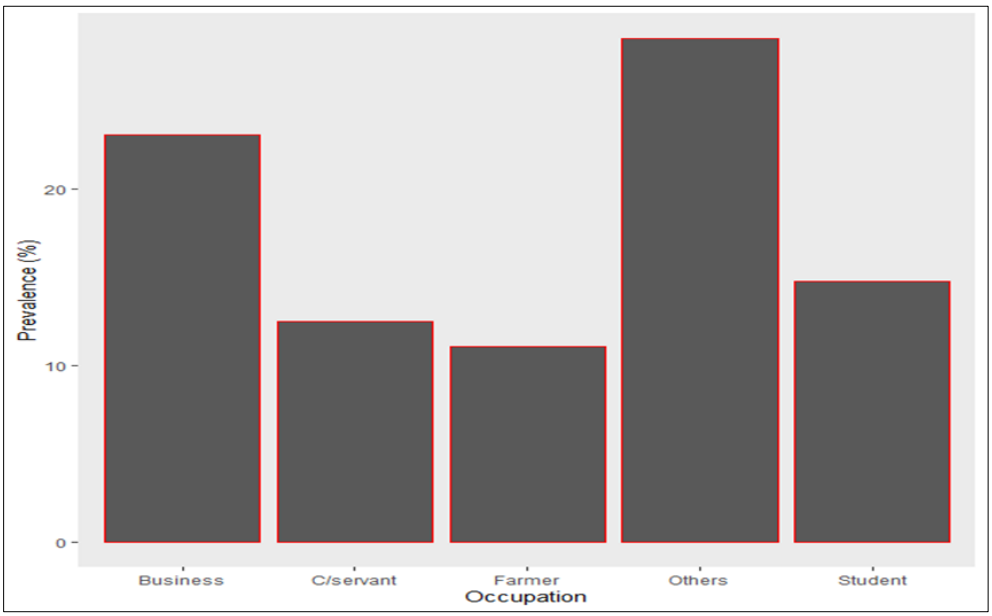


Figure 3 Prevalence of Cryptosporidium oocyts amongst HIV patients attending selected Hospitals in Nasarawa State in relation occupation

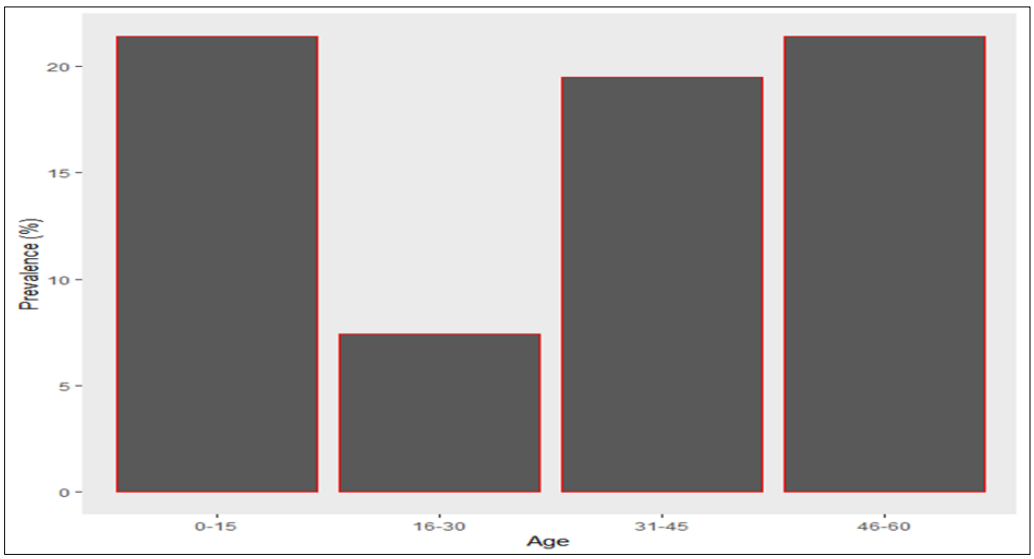


Figure 4 Prevalence of Cryptosporidium oocyts amongst HIV patients attending selected Hospitals in Nasarawa State in relation to age

The prevalence of Cryptosporidium oocyts in relations to age among HIV patients in the selected health care centres shows that the highest prevalence was amomg age 0-15 years and 46-60 years (21.4%) followed by age 31-45 years (19.5%) and the lowest was age 16-30years (7.41%) respectively as shown in Figure 4. But statistically there was no significant difference in Cryptosporidium oocyts among the ages

The prevalence of Cryptosporidium oocyts in relation to education amongst HIV patients shows the highest prevalence was observed among uneducated (25.0%) followed by secondary school (20.0%), tertiary school (12.5%) and the least was among primary school (8.70%) as shown in Figure 4.9. Statistical analysis shows insignificant difference in terms of level of education at p=0.4947

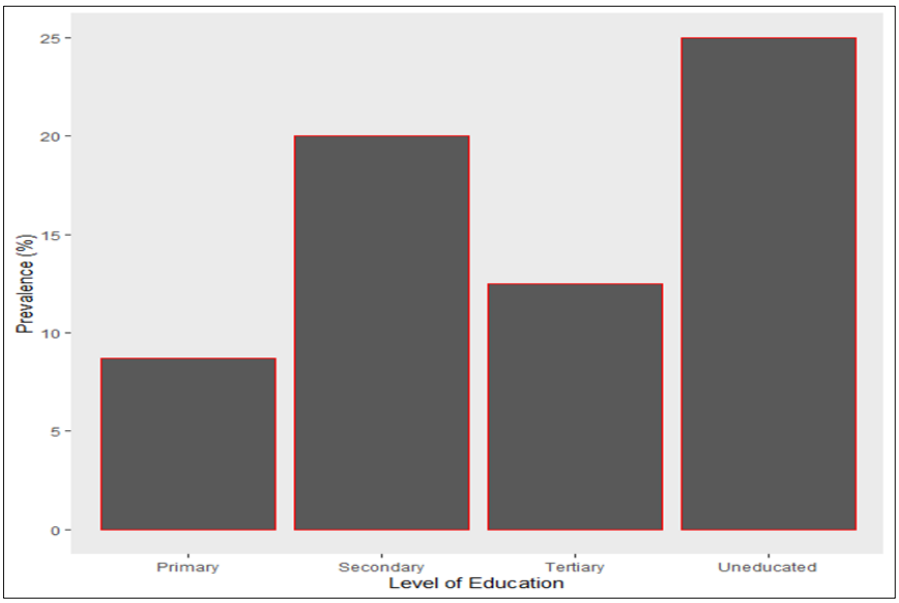


Figure 5 Prevalence of Cryptosporidium oocyts amongst HIV patients attending selected Hospitals in Nasarawa State in relation to level of education

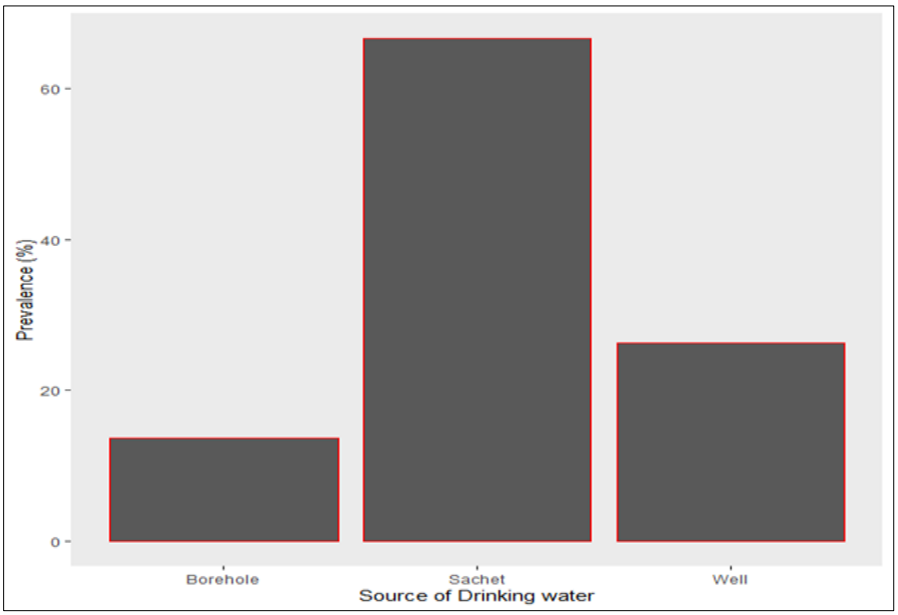


Figure 6 Prevalence of Cryptosporidium oocyts amongst HIV patients attending selected Hospitals in Nasarawa State in relation to source of drinking water

Figure 6: Prevalence of Cryptosporidium oocyts amongst HIV patients attending selected Hospitals in Nasarawa State in relation to drinking water. Showed that the highest prevalence was among those whose source of drinking water was sachet water (66.7%) followed by well water (26.3%) and lowest was borehole (17%) respectively as shown.

The prevalence of Cryptosporidium oocyts amongst HIV patients in relation to livestock farming shows higher prevalence among livestock farmers (32.0 %) than non-livestock farmers (9.59%) as shown in Figure 7. Feacal droppings of animals can contaminate the environment and water bodies [17]. Similar to the reports Shinkafi and Muhammad [18], there was statistically insignificant association between the prevalence of Cryptosporidium oocysts and the presence of among livestock farmers and non-livestock farmers in the present study.

The Cryptosporidium oocysts with high prevalence in the study area call for concern because; the prevalence rate may be on the increase in the study areas since Cryptosporidium lives in the intestine of infected humans or animals. An

infected person or animal sheds Crypto parasites in the stool. Millions of Crypto germs can be released in a bowel movement from an infected human or animal Medinet Com (2010). The prevalence of Cryptosporidium oocyst in this area could also be due to faecal contamination around HIV patients' source of water or from food

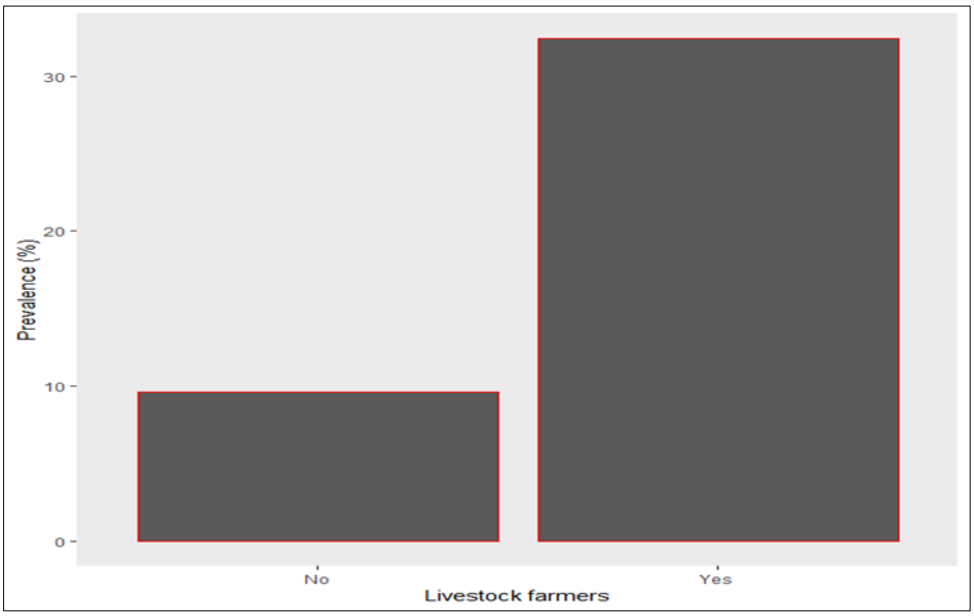


Figure 7 Prevalence of Cryptosporidium oocyts amongst HIV patients attending selected Hospitals in Nasarawa State in relation to livestock farming

4. Conclusion

From the result obtained from this study it was found the prevalence of *Cryptosporidium* oocysts was higher in Federal Medical center, Keffi than any other health care center, also the prevalence was more in male than female. The findings of this study also showed that prevalence of cryptosporidiosis may decreases or increases in HIV patients depend on age and other risk factors associated the infection.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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