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# Comparison of the two Diagnostic Methods Used for the Detection of *Cryptosporidium* Infection among HIV Patients in Osogbo, Nigeria

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author SAN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AFK and ASO managed the analyses of the study. Author MAA managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

Cryptosporidiosis is the chief AIDS-defining infection in no more than 2% of HIV reported cases.
**Aim of the Study:** This was carried out to assess the efficacy of two diagnostic methods for the detection of Cryptosporidiosis among HIV individuals in Osogbo.
**Methodology:** A total of 188 HIV seropositive patients attending Institute of Human Virology, Nigeria (IHVN) Clinics, Ladoke Akintola University of Technology Teaching Hospital (LAUTECH), Osogbo, Nigeria and 60 HIV negative individuals were selected for the study from January to December, 2016 by random sampling technique. Stool samples were collected and examined for *oocyst* and antigen of *Cryptosporidium protozoan* using Ziehl-Neelsen (ZN) and Ensyme Link Immunoassay (ELISA) methods respectively. Data were analyzed using SPSS version 16.0 and P value < 0.05 was considered significant.

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**Result:** Prevalence of 28.7% (54/188) and 35.1% (66/188) was obtained by ZN and ELISA respectively in HIV-positive subjects while that of HIV negative was 1.7% (1/60). There was a significant association between *Cryptosporidium* infections and diarrhoea for different age groups (P<0.05), but no significant association between *Cryptosporidium* infection and different sex (P> 0.05).

**Conclusion:** *Cryptosporidium* infection was highly prevalent among HIV seropositive individuals in Osogbo, an indication of active infection that is likely to emerge as major human pathogen in a poor social economic setting. This study re-emphasized the need for inclusion of *Cryptosporidium* screening and treatment in HIV seropositive subjects since it is a major cause of morbidity and mortality.

*Keywords: Diagnostic methods; Cryptosporidium infection; HIV patients.*

## 1. INTRODUCTION

*Cryptosporidium parvum* is an important enteric pathogen that causes diarrhea illness in humans and animals [1]. In immunocompetent individuals, infection is usually self-limiting, but in immunocompromised individuals, persistent infections, which can be life threatening, may be developed [2]. *Cryptosporidium* accounted for 50% of the cases of diarrheal in developing countries. HIV/AIDS remains a global pandemic, Nigeria is one of the highly affected Sub-Saharan countries. HIV/AIDS pandemic has brought about a great change in intestinal parasite fauna. As the spectrum of immunodeficiency progresses, HIV infected individuals become susceptible to a variety of opportunistic parasitic infections that occur with greater frequency and severity[2,3]. Almost 80% of AIDS patients die from AIDS-related infections including intestinal parasitic infections rather than HIV infection itself [4]. *Cryptosporidium,* previously considered non-pathogenic in immunocompetent individuals, may likely cause debilitating illness in HIV/AIDS patients as concomitant infections due to depleted immunity [5].

Diarrhoea is a common clinical manifestation of HIV infection both in the developing and the developed countries [6]. It has been shown that at least 40-80% of AIDS patients report diarrhoea episodes during their illness[7]. HIV enteropathy, a condition characterized chronic diarrhoea in AIDS patients in whom no identifiable etiological agent has been found for the diarrhoea [8]. It is also held that HIV related enteropathy is not only the cause of unexplained diarrhoea, but may also create favorable environment for the invasion of intracellular opportunistic intestinal parasites [9].

The advent of newly improved diagnostic techniques has increased detection and recognition of opportunistic intestinal parasites. Poor performance characteristics of the traditional diagnostic procedure used routinely in many laboratories have resulted in the misdiagnosis or underdiagnosis of Crypto-sporidiosis. This may lead to mistreatment and probably promotes drug resistance. Therefore, there is need for continuing surveillance and re-evaluation of the new methods such as immunological method, which appears to be more sensitive and which may likely improve the accuracy and reliability of the results emanated from the diagnosis. This study aimed to determine the prevalence of *Cryptosporidium* infection and the diagnostic performance of modified Ziehl-Neelsen and Enzyme-Linked Immuno Sorbent Assay (ELISA) techniques among HIV infected individuals on Antiretroviral therapy attending LAUTECH Teaching Hospital Osogbo, South-west, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Study Area and Population

The project was carried out in the Department of Medical Laboratory Science, Mercyland Campus, Osogbo. In IHVN Clinic of LAUTECH Teaching Hospital, Osogbo, Osun State, Nigeria, HIV – infected patients have provided written consent to participate in the study. .

### 2.2 Ethical Consideration and Sample Collection

Ethical approval was obtained from Ethical Committee, LAUTECH Teaching Hospital, Osogbo, Nigeria. Informed consent was given to participants. Structured questionnaire was used to fetch demographic information.188 stool samples were collected between July to December, 2014. The samples were preserved at -20°C prior to the time of processing and analysis.

## 2.3 Microscopy

A wet preparation of each stool samples was made with both saline and iodine mounts on clean grease free slides and examined under the microscope (with 100 and 400 magnification) ova and cysts of parasites. Each sample was concentrated using formal ether method concentration technique [10].

Detection of Cryptosporidium oocysts in the concentrated stool was done using the modified cold Ziehl-Neelsen staining technique as describe by Cheesbrough [11] The slide was then air-dried and observed under the compound light microscope using 40x objective lens for the presence of *Cryptosporidium* oocysts, and confirmed using oil-immersion objectives, as small pink to red spherules on pale green background.

## 2.4 Enzyme Linked Immunoassay Assay Technique

A double antibody (sandwich) ELISA Kit [12] using an anti-Cryptosporidium antibody to capture the antigen from the stool supernatant was used. 0.1 ml of supernatent sample or 0.1 g fecal samples were diluted to prepare 0.3 ml of dilution buffer.

100 µl of negative and positive control each was added to well one and two respectively and 50 ul of dilution buffer was added to the remaining wells. 50 ul of sample was also added to each well containing the dilution buffer, covered and incubated at 25°C for 1 hour. The plate was then washed with buffer (5 times), and 2 drops of Enzyme Conjugate was added to each well and incubated at 25°C for 30 minutes. The plate was washed with buffer (5 times) and it was slap out on a clean absorbent towel to remove excess wash buffer. 2 drops of Chromogen were added to the wells and incubated at 25°C for 10 minutes. 2 drops of stop solution were added and absorbance was read in an ELISA plate reader 450 nm. OD values greater than 0.15 were considered positive and OD values < 0.15 were considered negative [13].

## 2.5 Statistical Analysis

Data were analyzed using SPSS version 16.0; Chicago, USA and p-value of < 0.05 was considered statistically significant.

# 3. RESULTS

Fifty four 54 (28.7%) out of 188 samples were positive for *Cryptosporidium species* as showed in Table 1. Other parasites detected were amoebas such as *Entamoeba histolytica* 16 (18.5%), helminths such as *Ascaris* lumbricoides (10.7%) and *Strongyloides stercorali s*.(1.0%). Table 2 shows the association between the prevalence of *Cryptosporidium parvum* among age and sex using Modified Ziehl-Nielsen techniques.

Using ELISA techniques, 35.1% of the samples were positive for *Cryptosporidium parvum; of them* 36.4% were males and 63.6% females. There was no significant association between *Cryptosporidium* infection and Sex (P-value = 0.46). The prevalence of Cryptosporidum was higher in the age group 31-45 Antigen detection by ELISA showed the highest positivity of 66 (35.1%) while reports on presence of Oocyst by MZN showed the lowest positivity of 54 (28.7%) among the participants.

The correlation values based on sensitivity, specificity, positive predictive value, and negative predictive value of the techniques were assessed to define the best possible criteria of true positive for detecting *Cryptosporidium parvum* as shown in Table 4. Significant difference was observed between the negative predictive value of MZN technique (77.0%) and that of ELISA technique (81.3%) in the detection of *Cryptosporidium* among the participants.

# 4. DISCUSSION

Overall, *Cryptosporidium* species prevalence detected in this study was 28.7% and 35.1% using ZN and ELISA respectively. Our results are similar to those obtained in Ilorin (32.2%) [14] but higher than 18.7% in Lagos [15,16] and 23.6% in Jos [17]. The high prevalence of this study may depend on the level of contamination of water, foodstuff and contacts with animals, which are important factors in dissemination of the parasite.

The prevalence is lower when compared with 54.2% in Osogbo [18] and 52.7% in south-west [19]. Factors contributing to this variation may be due to high level of awareness in the urban setting, and probably sample size [20]. With respect to sex, *Cryptosporidium* was most prevalent in females than males, which is similar

**Table 1. Distribution of intestinal parasites among subjects in the study area by microscopy**

| Parasites | Number | Percentage (%) |
|---|---|---|
| *Cryptosporidium* species | 54 | 28.7 |
| *Entamoeba histolytica* | 16 | 8.5 |
| Helminths | 22 | 11.7 |
| *Cryptosporidium* species and Amoebae | 2 | 1.1 |
| *Cryptosporidium* species and Helminths | 8 | 4.3 |
| Amoebae and Helminths | 4 | 2.1 |
| *Cryptosporidium* species, Amoebae and Helminths | 2 | 1.1 |

**Table 2. Association between detected *Cryptosporidium* species, sex and age groups using ZN techniques**

| | *Cryptosporidium species* | | | | $X^2$ | P- value |
|---|---|---|---|---|---|---|
| | Positive | | Negative | | | |
| **Sex** | **Number** | **%** | **Number** | **%** | **2.065** | **0.151(P>0.05)** |
| Male | 22 | 40.7 | 40 | 29.9 | | |
| Female | 32 | 59.3 | 94 | 70.1 | | |
| **Age** | | | | | **4.324** | **0.229(P>0.05)** |
| 16-30 | 4 | 7.4 | 20 | 14.9 | | |
| 31-45 | 34 | 63.0 | 64 | 47.8 | | |
| 46-60 | 14 | 25.9 | 46 | 34.3 | | |
| 61-75 | 2 | 3.7 | 4 | 3.0 | | |

**Table 3. Association between detected *Cryptosporidium* species and age among participants using ELISA technique**

| | *Cryptosporidium* species | | | | $X^2$ | P- value |
|---|---|---|---|---|---|---|
| | Positive | | Negative | | | |
| **Sex** | **Number** | **%** | **Number** | **%** | **0.527** | **0.46** |
| Male | 24 | 36.4 | 38 | 31.1 | | |
| Female | 42 | 63.6 | 84 | 68.9 | | |
| **Age** | | | | | **9.758** | **0.021** |
| 16-30 | 12 | 18.2 | 12 | 9.8 | | |
| 31-45 | 40 | 66.6 | 58 | 47.5 | | |
| 46-60 | 12 | 18.2 | 48 | 39.3 | | |
| 61-75 | 2 | 3.0 | 4 | 3.3 | | |

**Table 4. Sensitivity, specificity, positive predictive value and negative predictive value of the techniques**

| Techniques | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|
| MZN | 57.4 | 82.7 | 65.9 | 77.0 |
| ELISA | 70.2 | 75.3 | 62.3 | 81.3 |

*Key: PPV- Positive Predictive Value*
*NPV- Negative Predictive Value*

to the work conducted by Kaplan et al. [21] but contrary to Pam et al. [12] with slight different rate among males. Moreover, in rural communities of developing countries, females may be more susceptible to diarrhoea disease as they are the primary caregivers for children, and are therefore frequently in contact with their stools samples, diapers and lack of clean water. This could potentially lead to infection as a result of poor hygiene.

Ziehl-Neelsen conventional technique is laborious, with relatively low sensitivity and specificity when performed by inexperienced

staff [22]. Our findings show a slightly high occurrence rate. The highest positivity was shown by ELISA (antigen detection) (35.1%) and MZN staining (Oocyst detection) (28.7%) in comparison to 12% *Cryptosporidium species* from patients in Chennai with HIV-positive and HIV-negative diarrhoea stool samples, 3.4% from India, 2.7% from Tunisia and 29.6% from Peru [23]. Also, other reports documented from India revealed a prevalence of 0.06% *Cryptosporidium* in adults from Chandigarh and 1.5% from Pondicherry [24]. Reports from France indicated 37.3% *Cryptosporidium* in stool samples of HIV-positive patients with diarrhoea [25].

Studies have reported a sensitivity of 83.7% for microscopy [26]. *Cryptosporidium* oocyst is very small in size and can easily be mistaken in stool debris for artefacts. Also, it is easy to confuse with other oocysts, such as those of *Cyclospora* species and cells, especially yeast cells, which resemble *Cryptosporidium* oocysts in size and morphology [27].

In the present study, the antigen detection method (ELISA) gave the highest prevalence of 35.1% in HIV-positive patients. Our result is lower to the earlier reports of 86.0% from the Mexican/US border and higher than 13% in the Limpopo province both in immuno-compromised and immuno-competent patients [28]. The present study shows higher prevalence than that previously reported in the Limpopo province (13%) [29]. This method showed a high sensitivity of 70.2% in HIV-positive stool samples. Our findings agree with other studies which reported that using fluorescent monoclonal reagents increased the sensitivity and specificity of the detection of *Cryptosporidium* cysts.

## 5. CONCLUSION

Our study showed a high sensitivity of ELISA (71%) compared with ZN (54%) in the study population. Therefore, there is need to include the detection of *Cryptosporidium* in the routine diagnosis using more sensitive diagnostic methods such as ELISA and PCR techniques most especially among Immuno-compressed individuals such as HIV/AIDs patients. This will encourage the need to strengthen and sustain the existing intervention measures so as to further reduce the significance of intestinal parasitic infections in people living with HIV/AIDS.

## CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

## ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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