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MAJOR ARTICLE

# Cryptococcal and *Histoplasma* Antigen Screening Among People With Human Immunodeficiency Virus in Ghana and Comparative Analysis of OIDx *Histoplasma* Lateral Flow Assay and IMMY *Histoplasma* Enzyme Immunoassay

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***Background.*** Cryptococcal meningitis (CM) and disseminated histoplasmosis (DH) are common in people with human immunodeficiency virus (PWH) and diagnosed by detecting cryptococcal antigen (CrAg) and *Histoplasma* antigen (HistoAg), respectively. In Ghana, CM and DH are rarely suspected by clinicians due to limited epidemiological data.

***Methods.*** This study was conducted among PWH in Ghana who are unwell. Sociodemographic and clinical data were collected by questionnaire. Serum and/or urine were screened for CrAg and HistoAg, using IMMY CrAg lateral flow assay (LFA) and IMMY *Histoplasma* enzyme immunoassay (EIA) kits, respectively, regardless of symptoms. Samples run with IMMY *Histoplasma* EIA were simultaneously run with Optimum Imaging Diagnostics (OIDx) *Histoplasma* LFA. Laboratory investigations were conducted by the research team, and diagnosis incorporating clinical assessment, screening, and confirmatory testing results and treatment decisions were made by the clinical team. Treatment and outcome information on CM and DH patients were evaluated.

***Results.*** Overall, 150 participants were recruited. There were 73% ($n = 109$) females, and the age range was 18–62 years. The prevalence rates of CrAg and HistoAg were 2.7% (4 of 150) and 4.7% (5 of 107), respectively. The OIDx *Histoplasma* LFA showed a high concordance (98.4%) with the IMMY *Histoplasma* EIA. All antigen-positive cases by standard tests were diagnosed with CM and DH. Antifungal treatment was given in 5 patients and follow-up revealed 2 deaths and 3 recoveries.

***Conclusions.*** Histoplasmosis among PWH may be more common than previously anticipated and may be more frequent than cryptococcosis in Ghana. The performance of the OIDx *Histoplasma* LFA should be further explored.

**Keywords.** antigen tests; cryptococcosis; Ghana; histoplasmosis; people with HIV.

Invasive fungal infections (IFIs) are an important cause of ill health and deaths among people with human immunodeficiency virus (PWH). Despite the global rollout of highly active antiretroviral therapy (ART), IFIs continue to affect PWH particularly in sub-Saharan Africa (SSA). This has largely been attributed to delayed human immunodeficiency virus (HIV) diagnosis, interruption of ART care, and high burden of advanced HIV disease (AHD) [1]. Globally, IFIs are collectively estimated to cause approximately 47% of all acquired immune deficiency syndrome (AIDS)-related deaths [2]. The IFIs associated with the highest morbidity and mortality in PWH are cryptococcal meningitis (CM), disseminated histoplasmosis (DH), and *Pneumocystis jirovecii* pneumonia [3].

Annually, over 200 000 CM cases occur globally, with 73% in SSA and responsible for 15% of AIDS-related deaths [4]. At this time, the World Health Organization (WHO) recommends testing for cryptococcal antigen (CrAg) in PWH with a CD4 count less than 100 cells/μL [5]. This recommendation has been evaluated to be cost-effective even at a low CrAg prevalence rate of 1.4% [6]. Earlier studies on CrAg screening and CM had focused on ART-naive patients, but recent studies among ART-experienced patients report similar rates [7–9].

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Among the ART-experienced population, virologic failure or viral nonsuppression has been associated with a high burden of CM [10, 11]. In Ghana, only 2 major studies have been specifically conducted on CM. One was a retrospective study in PWH with CD4 count less than 100 cells/μL and the other a prospective study in PWH presenting with signs of meningitis; both reported a low prevalence of approximately 2% (2 of 92 and 1 of 53) [12, 13]. In related studies, *Cryptococcus neoformans* was the etiological agent in 6% (5 of 84) and 11.7% (19 of 163) of cerebrospinal meningitis [14, 15]. In addition, during the early ART era, a retrospective study reported CM as the cause of death in 3.3% (4 of 123) of hospitalized HIV/AIDS patients in Ghana [16].

Despite histoplasmosis being endemic in Africa, it seems underdiagnosed in many parts of the continent with scarce epidemiological data [17]. In Cameroon, among patients with a clinical suspicion of histoplasmosis, *Histoplasma capsulatum* was detected in 7% [18]. In Latin America, another endemic area, histoplasmosis is estimated to be a predominant HIV coinfection [19]. In view of this, the Pan-American Health Organization (PAHO) in collaboration with the WHO released guidelines for the diagnosis and management of DH among PWH [20]. The burden of DH in Latin America is believed to be similar in SSA [21, 22]. In Ghana, of 12 individual histoplasmosis cases reported in the last 6 decades, 11 occurred in PWH [17].

Several antigen detection assays exist for CM and DH, and they have been exploited in screening programs or studies in some countries in the Americas and Africa [23–29]. These assays have been evaluated in previous studies [22, 23, 26, 28, 29, 30–32]. In Guatemala, an implemented screening for CM, DH, and TB reduced HIV deaths by 7% [24]. For CM, there are many rapid diagnostic tests (RDTs) with an established, high analytical performance and clinical relevance, and more recently semiquantitative forms have been introduced and are being evaluated [31, 33]. Although these tests have revolutionized CM diagnosis, increasing availability and accessibility to these tests have been slow and remain absent in many SSA countries including Ghana [34, 35]. Unlike CM, most available assays for DH are based on EIA, a technique that may not be readily available in many resource-limited laboratories due to required equipment and personnel training. The RDTs have been recently introduced by manufacturers including MiraVista Diagnostics and Optimum Imaging Diagnostics (OIDx) for detecting HistoAg in urine and/or serum, but only the former has been widely evaluated [36–38]. However, internal evaluation studies of OIDx *Histoplasma* lateral flow assay (LFA) reported a sensitivity of 95.1% and specificity of 96.1% [39]. These *Histoplasma* RDTs are anticipated to consolidate the diagnostic efforts in detecting cases of DH in PWH especially in resource-limited settings. However, aside from antigen detection tests, conventional techniques such as direct microscopy, histopathology, and culture may be used as confirmatory tests to prove infections and identify etiological agents.

In Ghana, studies on CM are scarce, and there are no studies on histoplasmosis in any risk group. Cryptococcal meningitis and DH are thus rarely on the diagnostic radar of clinicians due to the limited epidemiological data. Generating epidemiological data is critical to informing and directing practice and policy changes. In this study, we screened for CrAg and HistoAg, and we subsequently established proven cases of cryptococcosis and histoplasmosis among PWH in Ghana. We also compared the performance of the OIDx *Histoplasma* LFA with IMMY *Histoplasma* EIA.

## METHODS

### Settings, Participants, and Samples

In this prospective cross-sectional study, PWH returning to care (currently on ART or lost to follow-up on ART) and newly diagnosed HIV patients aged 18 years and above were recruited irrespective of presenting symptoms, disease stage, CD4 counts, or ART status. Patients who had taken antifungal drugs for at least 2 weeks in the last 3 months, or with previous CM or DH, were excluded. The study was conducted at the Korle-Bu Teaching Hospital (KBTH) and Juaboso Government Hospital, in the Greater Accra and Western North regions of Ghana, respectively. Patients were recruited from September 2020 to November 2021. A well structured questionnaire was used to anonymously collect sociodemographic by interviews and clinical data extracted from medical records. Blood and urine samples were collected from all participants. Additional samples such as cerebrospinal fluid (CSF), biopsies, blood, or sputum were received for further confirmatory tests from patients with either a positive serum CrAg (with titer $>$1:160) or positive urine *Histoplasma* EIA test as part of routine clinical care. To serve as control for the screening tests, healthy PWH with well controlled viral load ($<$20 copies/mL or target not detected), no new complaints, and, so, likely at low risk for CM or DH returning to clinic for antiretroviral (ARV) restock were recruited. Blood and urine samples were collected from these group for screening tests (Figure 1).

### Screening and Confirmatory Testing

Cryptococcal antigen screening was done on sera, using the IMMY CrAg LFA (Immuno-Mycologics Diagnostics, Norman, OK). When CrAg was positive, CrAg semiquantitative test was done to determine the titer. Urine samples were analyzed with both IMMY *Histoplasma* EIA (Immuno-Mycologics Diagnostics, Norman, OK) and OIDx *Histoplasma* LFA (Optimum Imaging Diagnostics, Scarborough, ME). The optical density (OD) of IMMY *Histoplasma* EIA was recorded from a microplate reader, and test line intensity of OIDx *Histoplasma* LFA was determined

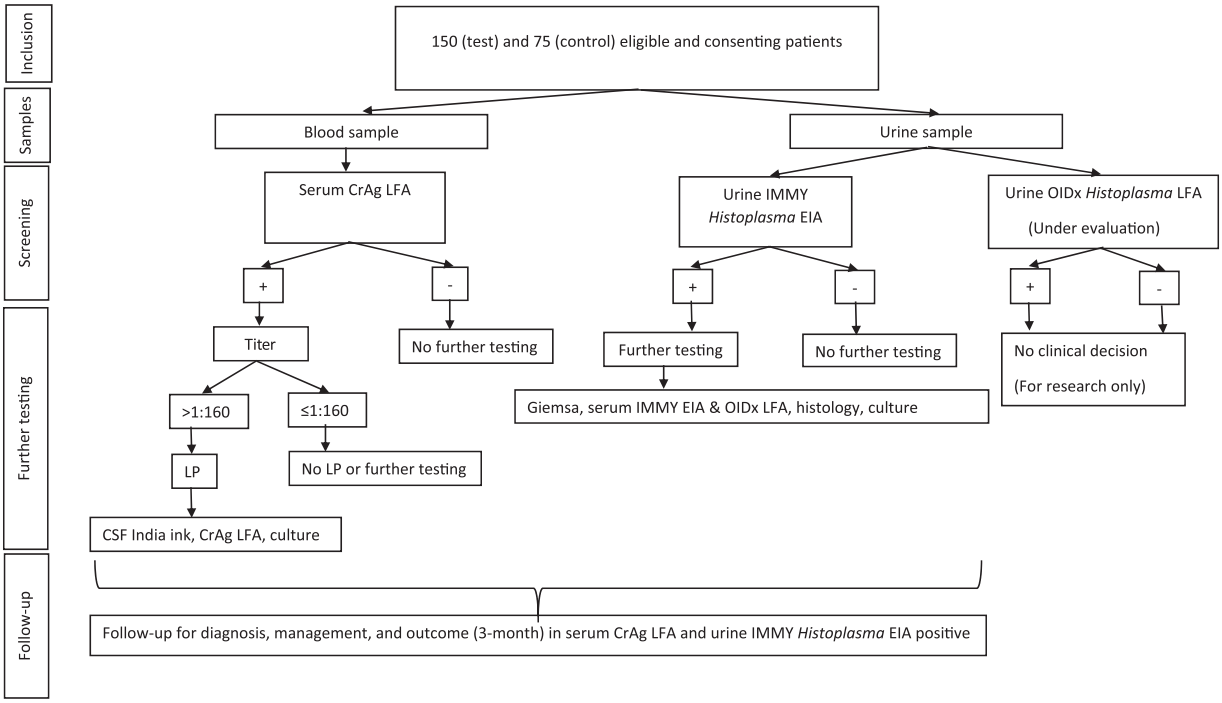


**Figure 1.** Study workflow: inclusion, sample collection, screening, further testing, and follow-up of patients. CrAg, cryptococcal antigen; CSF, cerebrospinal fluid; EIA, enzyme immunoassay; LFA, lateral flow assay; OIDx, Optimum Imaging Diagnostics.

visually. All tests were done strictly following the manufacturer's instructions. In patients with a positive urine IMMY *Histoplasma* EIA and in those unable to provide urine samples, both HistoAgs were tested on their serum. None of the study centers were performing these antigen tests at the time of the study. All antigen tests were run within 48 hours of sample receipt (after only refrigeration without freezing), and usually the same day. All screening test results were shared with the clinical team.

When serum CrAg was positive at a titer $\geq$1:160 or 1:80 with strong correlating neurological manifestations, the clinical team analyzed eligibility for lumbar puncture (LP). The LP was performed as soon as feasible (mostly within 72 hours), and CSF was tested by CrAg LFA, India ink, and fungal culture. When urine *Histoplasma* EIA was positive, sputum, biopsy, or blood was collected, based on patients' clinical manifestation of possible disease. Direct examination with Giemsa, histological examination with periodic-acid Schiff, and fungal culture on Sabouraud dextrose agar were done on received samples. Outpatients were called back for admission appropriately. The results of all investigations were passed on to the clinical team.

#### Diagnosis, Management, and Outcome

Diagnosis, and directed management, of cryptococcosis and histoplasmosis was made by the clinical team incorporating clinical assessment, screening, and confirmatory test results [5, 20, 40]. Treatment and 3-month outcome details of patients who were diagnosed with cryptococcosis and histoplasmosis was obtained from medical records.

#### Data Analysis

Data analysis was done using SPSS, version 25 (IBM Corp., Armonk, NY). Descriptive statistics were used to summarize the patients' sociodemographic and clinical features for positive and negative screening cases, confirmatory results, and performance characteristics for IMMY *Histoplasma* EIA and OIDx *Histoplasma* LFA. Kappa index was used to measure the agreement between the IMMY *Histoplasma* EIA and OIDx *Histoplasma* LFA, with the former as the reference standard with the following interpretation for strength of agreement: poor (<0%), slight (0 to 20%), fair (21 to 40%), moderate (41% to 60%), substantial (61% to 80%), and near perfect (81% to 100%). In addition, point biserial correlation ($r_{pbis}$) was used to determine associations between the OD of IMMY *Histoplasma* EIA and the test line intensity of OIDx *Histoplasma* LFA. The alpha level was set at 0.05.

#### Patient Consent Statement

Ethical clearance for the study was obtained from the Institution Review Board of the Korle-Bu Teaching Hospital (STC/IRB/00058/2020), University Research Ethics Committee (UREC) of the University of Manchester, United Kingdom (UREC Ref. no. 2020-9372-16067), and administrative authorization

was obtained from the Juaboso Government Hospital. Before recruitment, a Participant Information Sheet was shared with patients, any questions or concerns, respectively, were answered and resolved, and written informed consent was subsequently obtained.

## RESULTS

### Patients' Background Details

Overall, 150 patients with 54 (36%) inpatients were recruited into the study. There were 109 (72.7%) females, and the mean age was 42.9 years (range, 18–62). Recent (within the last 3 months) CD4 count and viral loads were available for 73 (48.7%) and 139 (92.7%) patients, respectively. The median and interquartile range for CD4 count and viral load were 1049.1 cells/μL (258.4–1480.6) and 18 367.9 copies/mL (4524.1–44 633.9), respectively. Of the recruited patients, 78 (52.0%) were presently on ART, 41 (27.3%) were lost to follow-up on ART, and 31 (20.7%) were ART naive or newly diagnosed. The main exposure risks identified were farming or agricultural activities (20.7%, $n = 31$) and livestock rearing or animal contact (10.0%, $n = 15$) (Table 1).

**Table 1. Demographics, Clinical Details, and Exposure Factors in Positive Antigen and Negative Antigen Groups**

| Participants' Features | Total (150) Number (%) | Positive CrAg+HistoAg (9) Number (%) | Negative CrAg + HistoAg (141) Number (%) |
|---|---|---|---|
| Sex | | | |
| Male | 41 (27.3%) | 2 (22.2%) | 39 (27.7%) |
| Female | 109 (72.7%) | 7 (77.8%) | 102 (72.3%) |
| Age (Years) | | | |
| Median | 42.9 | 43.0 | 42.6 |
| ART Status | | | |
| Newly diagnosed | 31 (20.7%) | 2 (22.2%) | 29 (30.9%) |
| Defaulter | 41 (27.3%) | 3 (33.3%) | 38 (30.0%) |
| On ART | 78 (52.0%) | 4 (44.5%) | 74 (52.5%) |
| Patient Group | | | |
| Outpatient | 96 (64%) | 3 (33.3%) | 93 (66.0%) |
| Inpatient | 54 (36%) | 6 (66.7%) | 48 (34.0%) |
| CD4 Count (Cells/μL) | | | |
| Number | 73 (48.7%) | 4 (44.4%) | 69 (48.9%) |
| Median | 1409.1 | 204.5 | 1362.0 |
| Viral Load (Copies/mL) | | | |
| Number | 139 | 8 | 131 |
| Median | 18 367.9 | 105 345.3 | 16 890.3 |
| Exposure Factors | | | |
| Farming | 31 (20.7%) | 7 (77.8%) | 24 (17.0%) |
| Animal contact | 15 (10.0%) | 3 (33.3%) | 12 (8.5%) |

Abbreviations: ART, antiretroviral therapy; CrAg, cryptococcal antigen; HistoAg, *Histoplasma* antigen.

### Prevalence of Serum Cryptococcal Antigen and Urine *Histoplasma* Antigen and Confirmatory Tests

Of the 150 patients, 43 did not provide urine samples. Cryptococcal antigen LFA was positive in 2.7% (4 of 150) of patients, whereas IMMY *Histoplasma* EIA was positive in 4.7% (5 of 107) of patients. All 4 serum CrAg-positive samples had titers above 1:160, but CSF sample was received from only 3, and positivity rates were 100% (3 of 3), 66.7% (2 of 3), and 33.3% (1 of 3) for CrAg LFA, India ink, and culture, respectively. Four whole blood and 3 skin biopsies (patients showing skin lesions) were received from the urine IMMY *Histoplasma* EIA-positive patients for confirmatory testing. Recorded positivity was 50% (2 of 4) for whole blood Giemsa staining and 100% (3 of 3) for histology on skin biopsy, but there was no *Histoplasma* growth in any sample. Of the 5 serum samples from positive urine IMMY *Histoplasma* EIA cases, only 3 tested positive. IMMY *Histoplasma* EIA performed on the serum of 43 patients without urine samples were all negative (Table 2). No sample was positive for both CrAg and HistoAg. In addition, serum CrAg LFA and urine IMMY *Histoplasma* EIA was negative among all patients in the control group.

### Comparison of IMMY *Histoplasma* Enzyme Immunoassay and OIDx *Histoplasma* Lateral Flow Assay

IMMY *Histoplasma* EIA and OIDx *Histoplasma* LFA were performed on 230 samples comprising 107 urine samples from the test group, 43 serum samples from patients unable to provide urine samples, serum samples from 5 patients whose urine

**Table 2. Screening Results in Total Participants and Confirmatory Findings in Cryptococcosis and Histoplasmosis Cases**

| | Total | Cryptococcosis | Histoplasmosis |
|---|---|---|---|
| Screening Tests | | | |
| Serum CrAg | | | |
| Number | 150 | 4 | 5 |
| Pos (%) | 4 (2.7) | 4 (100) | 0 (0) |
| Urine Histo Ag EIA | | | |
| Number | 107 | 4 | 5 |
| Pos (%) | 5 (4.7) | 0 (0) | 5 (100) |
| Confirmatory Tests | | | |
| CSF | | | |
| Number | 3 | 3 | – |
| CrAg Pos (%) | 3 (100) | 3 (100) | – |
| India ink Pos (%) | 2 (66.7) | 2 (66.7) | – |
| Culture Pos (%) | 1 (33.3) | 1 (33.3) | – |
| Whole Blood | | | |
| Number | 4 | – | 4 |
| Giemsa Pos (%) | 2 (50) | – | 2 (50) |
| Skin Biopsy | | | |
| Number | 3 | – | 3 |
| Histology Pos (%) | 3 (100) | – | 3 (100) |
| Culture Pos (%) | 0 (0) | – | 0 (0) |

Abbreviations: CrAg, cryptococcal antigen; CSF, cerebrospinal fluid; EIA, enzyme immunoassay; Histo Ag, *Histoplasma* antigen; Pos, positive.

**Table 3. Comparison of EIA and LFA in Urine and Serum Samples and Their Performance Characteristics**

| Specimen | IMMY Histo EIA | OIDx Histo LFA | % Agreement |
|---|---|---|---|
| Urine ($n = 107$, Test Group) | | | |
| Pos (%) | 5 (4.7%) | 6 (5.6)[a] | 99 |
| Serum ($n = 43$, No Urine Samples) | | | |
| Pos (%) | 0 (0) | 3 (7.0) | 93 |
| Serum ($n = 5$, Urine *Histoplasma* GM EIA Positive) | | | |
| Pos (%) | 3 (60) | 3 (60)[a] | 100 |
| Urine ($n = 75$, Control Group) | | | |
| Pos (%) | 0 (0) | 2 (2.7) | 97 |
| Urine ($n = 182$, Test and Control Groups) | | | |
| Pos (%) | 5 (4.7%) | 2 (2.7) | 98 |
| Parameter (Based on Urine Samples) | | | |
| Sensitivity | 100.0% | 100.0% | |
| Specificity | 100.0% | 98.3% | |
| PPV | 100.0% | 62.5% | |
| NPV | 100.0% | 100.0% | |
| Accuracy | 100.0% | 98.4% | |
| Parameter (Based on Serum Samples) | | | |
| Sensitivity | 60.0% | 60.0% | |
| Specificity | 100.0% | 93.0% | |
| PPV | 100.0% | 50.0% | |
| NPV | 95.6% | 95.2% | |
| Accuracy | 95.8% | 89.6% | |

Abbreviations: EIA, enzyme immunoassay; GM, *Histoplasma* galactomannan; LFA, lateral flow assay; NPV, negative predictive value; OIDx, Optimum Imaging Diagnostics; Pos, positive; PPV, positive predictive value.

[a]EIA positives were also LFA positive; Kappa = 0.90; $P < .0001$.

were positive for IMMY *Histoplasma* EIA, and 75 urine samples from the control group (Table 3). Among the 107 urine samples, there were 6 positives with OIDx *Histoplasma* LFA including all 5 that were positive for IMMY *Histoplasma* EIA. The OIDx *Histoplasma* LFA also recorded 3 positives among the 43 serum samples and 2 positives in control urine samples. In addition, for the 3 IMMY *Histoplasma* EIA-positive sera from patients with *Histoplasma* antigenuria, the OIDx *Histoplasma* LFA were also positive. Thus, there were 6 positives for only OIDx *Histoplasma* LFA (where IMMY *Histoplasma* EIA was negative). Evaluation of agreement between the OIDx *Histoplasma* LFA and IMMY *Histoplasma* EIA showed an overall high concordance (98%). The Kappa index indicated a near perfect agreement between the 2 test kits, and this was statistically significant (Kappa value = 0.90; $P < .0001$) (Table 3). The point biserial correlation analysis involving the IMMY *Histoplasma* EIA optical densities and the OIDx *Histoplasma* LFA test line intensities of all the positive cases (for both the test and control groups) revealed a medium but statistically insignificant association between the 2 variables ($r_{pbis} = .38$, $P = .19$).

### Diagnosis, Treatment, and Outcome

Cryptococcal meningitis was diagnosed in all patients with positive serum CrAg LFA, whereas 5 of the 6 patients with positive urine HistoAg (both IMMY *Histoplasma* EIA and OIDx *Histoplasma* LFA) were diagnosed with DH (Table 4). Among the cases with only a positive OIDx *Histoplasma* LFA both in test and control groups, there was no evidence of histoplasmosis infection. Thus, the prevalence of CM was determined to be 2.7% (4 of 150), and that of DH was 4.7% ($n = 5$ of 107). The patients diagnosed with an IFI had a lower CD4 count (median IFI, 204.5 vs median non-IFI, 1362.0) and a higher viral load (median IFI, 105 345.3; vs median non-IFI, 16 890.3) than those not diagnosed with IFI. However, there was no significant difference ($P = .26$) between CD4 and viral load for CM and DH patients.

Two patients diagnosed with DH were lost to follow-up. One patient with CM died within 1 week of diagnosis before antifungal treatment. One DH patient, who was newly diagnosed with HIV, refused any form of treatment. Of the 5 patients who received antifungal therapy, one CM patient was treated with amphotericin B deoxycholate 1 mg/kg per day for 2 weeks. Alternatively, two CM patients were placed on fluconazole 1200 mg/daily for 12 weeks as induction regimens. The choice of regimen generally depended on availability and patients' financial capacity, whereas the 2 DH patients were placed on itraconazole 400 mg/daily for 6 months. A 3-month follow-up showed all CM were doing well but 1 DH patient had died. Overall mortality rate for patients diagnosed with IFIs was 33.3%.

## DISCUSSION

These findings show the potential impact of antigen detection assays for diagnosis of CM and DH in low- and middle-income country (LMIC) settings where WHO-listed essential diagnostics are unavailable [34, 41]. More importantly, the prevalence of histoplasmosis (4.7%) was shown to be higher than that of cryptococcosis (2.7%). This is the first epidemiological study of histoplasmosis among any risk group in Ghana providing important data to guide diagnostic decisions for this largely unrecognized infection. Similar studies in South Africa, Guatemala, and Mexico also reported higher HistoAg positivity than other IFIs [24, 27, 29]. In our study, direct examination and histopathology for DH were helpful and were contributory in establishing proven CM or DH. However, culture positivity was very low. The possible explanation for this observation is early treatment for CM patients because serum CrAg titer was >1:2560 in all patients and not directly inoculating biopsy on media because *H capsulatum* rarely survives in clinical specimen and must be inoculated as soon as possible to increase recovery. Considering the time, cost, equipment, and training required to run an EIA, efforts are now directed at developing

**Table 4. Demographics, Clinical Details, Risk Exposure, Laboratory Findings, Diagnosis, Treatment, and Outcomes of Patients Diagnosed With Cryptococcosis and Histoplasmosis**

| Age | Sex | Patient Group | ART Status | Risk | sCrAg | uHisto Ag | sHisto Ag | cCrAg | cDM | cCul | bDM | tDM | tPath | tCul | Dx | Rx | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 43 | F | In | LA | – | + | – | – | + | + | – | ND | ND | ND | ND | CM | FLC, FLC + AMB | Recovered |
| 30 | F | Out | A | Fa, AC | – | + | + | ND | ND | ND | – | – | + | – | DH | LTFU | – |
| 45 | F | In | LA | Fa | + | – | – | + | + | – | ND | ND | ND | ND | CM | FLC, FLC + AMB | Recovered |
| 43 | F | In | A | Fa | – | + | + | ND | ND | ND | + | + | + | – | DH | ITR | Died |
| 67 | M | Out | A | – | + | ND | – | ND | ND | ND | ND | ND | ND | ND | CM | FLC | Recovered |
| 50 | M | In | A | Fa, AC | – | + | – | ND | ND | ND | ND | – | – | – | CH | LTFU | – |
| 28 | F | Out | N | Fa, AC | | + | + | ND | ND | ND | ND | + | + | – | DH | RT | – |
| 19 | F | In | N | Fa | + | – | – | + | + | + | ND | ND | ND | ND | CM | NT | Died |
| 43 | M | In | LA | Fa | – | + | + | ND | ND | ND | + | – | – | – | DH | ITR | Recovered |

Abbreviations: A, on antiretroviral therapy (ART); AC, animal contact; AMB, amphotericin B (conventional); bDM, blood direct microscopy; CM, cryptococcal meningitis; cCrAg, CSF cryptococcal antigen; cCul, CSF culture; cDM, CSF direct microscopy; DH, disseminated histoplasmosis; Dx, diagnosis; F, female; Fa, farming; FLC, fluconazole; In, inpatient; ITR, itraconazole; LA, lost to follow-up on ART; LTFU, lost to follow-up; M, male; ND, not done; NT, not treated; Out, outpatient; R, recovered; RT, refused treatment; Rx, treatment; sCrAg, serum cryptococcal antigen; sHisto, serum *Histoplasma*; uHisto Ag, urine *Histoplasma* antigen; tCul, tissue culture; tDm, tissue direct microscopy; tPath, tissue histopathology.

LFAs [36–38]. The world's first LFA for *Histoplasma* detection was developed by MiraVista Diagnostics. Evaluation studies have revealed high concordance, Kappa index ranging from 0.66 to 0.90 between the MiraVista *Histoplasma* LFA and EIAs in both urine and serum [36–38]. The recently introduced OIDx *Histoplasma* LFA only has internal evaluations to date [39]. Our study shows that the OIDx *Histoplasma* LFA has excellent sensitivity (100%) and specificity (98.3%) and, in comparison with the IMMY *Histoplasma* EIA, shows a near perfect agreement (98.4%). However, there were 6 false positives. Comparing our findings with a study of the MiraVista *Histoplasma* LFA on urine samples, the OIDx *Histoplasma* LFA had a higher sensitivity (OIDx, 100.0% vs MVista, 93.2%) but lower specificity (OIDx, 98.3% vs MVista, 99.3%) in proven cases [38]. In contrast, on sera, the OIDx *Histoplasma* LFA had a lower sensitivity (OIDx, 60.0% vs MVista, 96%) but higher specificity (OIDx, 93.0% vs MVista, 90%) [36]. However, it is worth noting that our study had a different population and very few positives compared with the MiraVista *Histoplasma* LFA studies, and thus the comparison may be invalid. In another evaluation using frozen urine samples (Nigeria), much lower sensitivity of the OIDx *Histoplasma* LFA was found (R.O., 2021, unpublished data). Our findings also suggest OIDx *Histoplasma* LFA may be superior to the existing OIDx *Histoplasma* EIA in terms of sensitivity, specificity, and accuracy (OIDx LFA 100%, 98%, and 98% vs OIDx EIA 92%, 32%, and 51%) [42]. Our study is the first using freshly collected samples.

Unfortunately, however, the current Ghana HIV guidelines only acknowledge CM as the HIV-associated IFI of concern [43], and this limitation requires critical attention. This shortcoming, in addition to inadequate awareness, has resulted in IFIs being rarely suspected or investigated. In a few circumstances in which these tests may be requested, they are not available in the in-hospital laboratory, and they are expensive at private facilities, because patients need to pay out-of-pocket. This is because the investigations are not captured on the National Health Insurance Scheme in Ghana, despite falling under the HIV/AIDS symptomatic management for opportunistic infections category [44]. However, both CrAg LFA and HistoAg EIA are on the WHO essential diagnostic list, and Ghana is yet to develop its Essential Diagnostic list, as in many other countries [41].

The prevalence of cryptococcosis in the present study is slightly higher than reported in prior studies [12, 13]. Cryptococcal disease studies in Ghana have consistently reported lower rates in comparison to other countries in SSA [7, 9, 31, 45, 46]. One probable reason for this observation is that all the studies in Ghana were done in the 2 largest cities, whereas most of the other studies elsewhere are conducted in small towns or rural areas and mostly in AHD. In this era when CD4 testing is not popular locally, this is challenging.

Clinical suspicion of IFI was only made before screening tests in 3 of 9 patients who were eventually diagnosed. Therefore, we extrapolate that the majority of these IFIs are likely to be missed in Ghana particularly for DH. Among patients diagnosed with IFI, the majority were on admission to hospital (66.7%) and not receiving ART (55.5%). This is a well established phenomenon, not just for IFIs, but for other opportunistic infections [14, 24, 29, 47]. By improving early HIV diagnosis and maintaining those taking ART in care, clinicians should reduce the prevalence of IFIs.

At this time, the treatment process for CM and DH in Ghana is challenging due to inadequate availability and accessibility of antifungal agents, especially amphotericin B and flucytosine [48]. These difficulties were demonstrated in our study. For the CM patients, 2 received fluconazole alone initially due to accessibility and financial constraints but later changed to amphotericin B when they failed to respond. This also comes with the additional challenge of local clinicians' experience with the use and managing the side-effects of amphotericin B [5]. Fortunately, in a recent study, clinicians revealed a single

high-dose of amphotericin B is as efficient as 1 week of daily amphotericin B [49]. The use of fluconazole monotherapy is popular in many LMIC settings, but it is suboptimal in the management of CM [50, 51]. Notwithstanding, 1 CM patient has been on fluconazole monotherapy and is doing well with good adherence to ART. Regarding DH, both patients received itraconazole. However, 1 patient had severe disease and amphotericin B would have been the preferred therapy, if available. Four patients comprising 1 CM and 3 DH did not receive antifungal treatment. The CM patient died while efforts were being made to acquire amphotericin B, which was out of stock at the hospital. This re-emphasizes the importance of prompt initiation of effective antifungal agents although mortality remains high at approximately 44% even with treatment [52]. Two patients with DH were lost to follow-up, and the third person refused any form of treatment including free ARV, possibly because patient had to be admitted longer and the cost of treatment is borne by the patient out-of-pocket.

There were some limitations to our study. The selected study sites, sampling, and number of positives may not reflective of a broader nationwide conclusion. The diagnosis of CM or DH may be missed if there is a false-negative antigen assay, which occasionally happens with low fungal burden or prozone effect. The study population shifted from only high-risk, ART-experienced patients to include newly diagnosed patients due to the decline in clinic attendance during the peak of coronavirus disease 2019. This resulted in convenient sampling and thus a relatively low proportion of patients with AHD. Moreover, CD4 and viral load tests results were not available for many patients. CD4 count has become unpopular in the era of "test and treat", and attention is now focused on viral loads. It is unfortunate that viral load tests were not done, sometimes due to reagent shortages. Furthermore, postmortem investigation was not done on patients who died with IFIs to ascertain whether it was the cause of death. Despite the above limitations, the present study generated novel epidemiological data for DH in Ghana, showing that DH is an important but largely unrecognized opportunistic fungal infection in PWH.

## CONCLUSIONS

This study reveals that histoplasmosis and cryptococcosis may be an unrecognized but relatively common IFIs in Ghana, and diagnostics such as CrAg LFA and *Histoplasma* EIA could facilitate diagnosis. It is notable that histoplasmosis may be at least as common as cryptococcosis. The OIDx *Histoplasma* LFA appears to have good sensitivity and acceptable specificity, but more data are needed to confirm this.


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