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Original Research

Detection of zoonotic opportunistic infections in HIV/AIDS patients in selected residential districts of Tigray Region, Ethiopia

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Abstract

Aim: Zoonotic opportunistic infections (OIs) are of the causes a major life-threatening condition in HIV/AIDS patients. In Ethiopia, OIs are the leading cause of death among people who have HIV.

Method: A cross-sectional study was carried out in Hewane, Korem and Alalmata districts from October 2012 to May 2013 to observe the occurrence of concurrent OIs such as tuberculosis, cryptosporidium, candidiasis and toxoplasmosis in HIV patients; to determine the association between possible risk factors and the human-animal interface responsible for development of OIs. Written informed consent was obtained from all participants and questionnaire was administered.

Results: A total of 651 HIV/AIDS patients and of which 300 (46.1%) were affected by OIs. Pregnancy (χ^2 = 11.7; P = 0.0001), educational status (χ^2 = 20.6; P = 0.0001), occupation (χ^2 = 19.8; P = 0.001), raw milk consumption (χ^2 = 32.14; P = 0.0001), raw meat consumption (χ^2 = 16.82; P = 0.0001), regular contact with animals (χ^2 = 7.83; P = 0.005) and the diabetic mellitus status (χ^2 = 23.1; P =0.0001) were found to have significant statistical association with the detection of OIs in patients.

Conclusion: In conclusion, OIs are of the causes a major life-threatening condition. Unrecognized, unknown and denied risk behaviors for OIs can pose major diagnostic and therapeutic challenges to clinicians in Ethiopia. In views of the above conclusion; recommendations were suggested for possible control and prevention measures of OIs. Awareness about OIs should be created to HIV patients. All HIV-infected persons at risk for infection with OIs must be carefully evaluated and, if indicated, administered therapy to prevent the progression of latent infection to active disease and avoid the complications associated with HIV-related OIs. All HIV-infected patients undergoing treatment for OIs should be evaluated for antiretroviral therapy.

INTRODUCTION

HIV/AIDS is arguably the most important infectious disease of the 20th century and, very likely, of the 21st century as well. The numbers speak to its unwelcome ranking: HIV/AIDS is the fourth-leading cause of death worldwide behind (*a*) ischemic heart disease, (*b*) cerebrovascular disease, and (*c*) lower respiratory infections [1] and next to malaria and Tuberculosis. In Ethiopia, the incidence of AIDS perhaps seems in decline due to the maximum and potential effort of Ministry of Health, and individuals infected with HIV/AIDS are living longer due to the widespread use of antiviral medication that is often out of reach for the majority of the Ethiopian's HIV-infected populace due to its expense. Today, more than a million people in Ethiopia are infected with HIV, with roughly 25% of them unaware of their status [2]. While the risk of acquiring a zoonotic disease from cat, goat, sheep (toxoplasmosis) and from food of animal origin (tuberculosis) is fairly higher for the general population because of their immunocompromised state, individuals with HIV infection that has progressed to the stage of AIDS face significant health risks from opportunistic (candidiasis, cryptosporidiosis) and pathogens associated with animals, insects, food, and water (cryptosporidiosis)

and are more likely to develop serious illnesses from such infections than nonimmunocompromised people [3]. It is, therefore, important that veterinarians have a clear scientific understanding of HIV/AIDS from which to intelligently advise and educate HIV/AIDS victims, co-workers, employees, and community health professionals on zoonotic diseases and other animal-related hazards, with the ultimate goal of protecting human life [4].

Human, in its body, carry many germs - bacteria, protozoa, fungi, and viruses. When the body immune system is working, it controls these germs. But when the immune system is weakened by HIV disease or by some medications, these germs can get out of control and cause health problems. Infections that take advantage of weakness in the immune defenses are called "opportunistic." The phrase "opportunistic infection" is often shortened to "OI." The rates of OIs have fallen dramatically since the introduction of antiretroviral therapies; However, OIs are still a problem, especially for people who have not been tested for HIV. Many people still show up in hospitals and health with a serious OI. People who are not HIV-infected can develop OIs if their immune systems are damaged. For example, many drugs used to treat cancer suppress the immune treatments can develop OIs. HIV weakens the immune system so that opportunistic infections can develop. If people are HIV-infected and develop opportunistic infections, they might have AIDS. In the early years of the AIDS epidemic, OIs caused a lot of sickness and deaths. Once people started taking strong antiretroviral therapy (ART), however, a lot fewer people got OIs. It's not clear how many people with HIV will get a specific OI. In women, health problems in the vaginal area (*Candida albicans*) may be early signs of HIV. The most common OIs are Tuberculosis, Cryptosporidiosis, Toxoplasmosis, Candidiasis, Herpes simplex viruses, Cytomegalovirus and Pneumocystis pneumonia [5].

Tuberculosis (TB) is a bacterial infection that attacks the lungs, and can cause meningitis. TB caused by *M. bovis* is clinically indistinguishable from TB caused by *M. tuberculosis*. In Ethiopia or in countries where bovine TB is uncontrolled, most human cases occur in young persons and result from drinking or handling contaminated milk; cervical lymphadenopathy, intestinal lesions, chronic skin TB (lupus vulgaris), and other nonpulmonary forms are particularly common. HIV-infected or other potentially immunogenically-compromised populations are the population at risk of Tuberculosis. Tuberculosis is more commonly known and extremely serious opportunistic infection for someone who is HIV positive. It can be found in water, food of animal origin (raw dairy products), soil and dust. It gets into the body through the mouth - either transferred from the hands or by breathing in infected soil or dust. Humans become infected, most commonly through consumption of unpasteurized milk products from infected cows [6, 7].

Toxoplasma gondii (T. gondii) is an intracellular protozoan organism with large number of intermediate hosts, including all warm-blooded animals and humans. Toxoplasmosis is an infection that can damage your brain. It is caused by a parasite that can be found in cat feces, raw meat, and soil and it is common and doesn't cause illness in most people with healthy immune systems. It can be serious in people with HIV with depressed immune system. Risk of toxoplasmosis is highest when the CD4 count is under 100. Toxoplasmosis causes headache, confusion, fever, seizures, poor coordination, eye pain, problems seeing, and nausea. When toxoplasmosis infects the brain in someone with HIV, that person has AIDS [8]. The habit of eating raw and/or under cooked meat, unavoidable contact between humans and domestic animals and the high prevalence of HIV/AIDS are the potential factors for the occurrence of toxoplasmosis. Toxoplasmosis can be spread in two ways: by eating undercooked meat and by touching cat feces that have toxoplasmosis germs. Felids, particularly the domestic cat, are its definitive hosts and the only animal species in which oocyst develop [9]. The importance of this parasite in food safety, human health and animal husbandry has been well recognized [10]. *T. gondii* cause Toxoplasmosis which is an important zoonotic disease and responsible for major economic losses in all classes of livestock through abortion, stillbirth and neonatal losses, especially in sheep [11].

Candidiasis is commonly an endogenous opportunistic infection. Occasionally exogenous acquisition has also been proven. Of the causative agents, the most common species is *Candida albicans*. Other non-albicans *Candida* species, especially *C. tropicalis* are increasingly being reported. Candidiasis is the most common fungal infection found in HIV/AIDS patients. Extensive esophageal candidiasis is an AIDS-defining infection. But oral candidiasis, thrush, unless very extensive and causing symptoms, unequivocally is not diagnostic of AIDS. It is of prognostic value only as its presence indicates progression of immunodeficiency. Vulvovaginal candidiasis, though not unequivocally shown to occur more frequently in AIDS patients, nevertheless affects a considerable proportion of HIV-positive women with extensive disease. In advanced AIDS cases, with neutropenia and very low CD4 counts, disseminated candidiasis is certainly a possibility [5].

Cryptosporidiosis is a substantial threat to HIV

infected individuals, who have a lifetime risk of infection of around 10 percent. Cryptosporidiosis remains an important cause of diarrhoea in the immunocompromised due to the lack of effective therapy. Although cryptosporidiosis in HIV infected patients has been widely reported in India including three reports from our centre [12], little is known about circulation and transmission patterns of the infection in this part of the world. Studies from developed countries have found sexual behaviour patterns, immigrant status, pet ownership especially dogs and farm animals, travel outside the country, toileting children and some ethnic populations to be some of the risk factors associated with cryptosporidiosis [13].

Opportunistic infection concerns and justifications

At the point in which the immune system is most weak - the AIDS stage - those with HIV are highly susceptible to opportunistic infections (OIs), including zoonoses (tuberculosis, candidiasis, and toxoplasmosis) [14]. Prior to 1998 globally, approximately 90% of deaths among those with HIV/AIDS were due to OIs, 7% to cancer, and 3% to other reasons [15]. Today, thanks to highly active antiretroviral therapy and prophylactic treatment of OIs, roughly 50% of HIV/AIDS deaths are from OIs, - a remarkable declining due to unreserved efforts from WHO and local health care approaches. In Ethiopia, opportunistic infections are getting more prevalent in parallel to the increment of HIV/AIDS victim population. Available studies on opportunistic infections are absent and there is no any epidemiological information in every regional district of Ethiopia and the prevalence of opportunistic infections has not been well established because of inadequate disease surveillance in immunocompromized individuals and lack of disease specific diagnostic facilities. In general, there is a lack of information on the epidemiology and significance of opportunistic infections in HIV/AIDS patients. The circumstances that promote the occurrence of opportunistic infections (OIs) among HIV/AIDS victim people are still vague to them.

Objectives of the project

This study was conducted to proof the hypothesis that the rates of opportunistic infections in HIV/AIDS patients is dependent up on demographic and living conditions and human-animal interface in daily life. On the ground of the fore mentioned justifications, the objectives of this study were:

- To observe the occurrence of concurrent opportunistic infections such as Tuberculosis, cryptosporidium, candidiasis and Toxoplasmosis in HIV/AIDs exposed individuals,
- To determine the association between possible risk factors and the human-animal interface responsible for the development OIs,
- To determine the knowhow of HIV/AIDS victim individuals on the zoonotic diseases and other animal-related risks, and provide appropriate advice to HIV/AIDS victim individuals.

METHODOLOGY

Description of study districts

Tigray region is the northernmost of the nine ethnic regions of Ethiopia containing the homeland of the Tigray people and there are 36 districts in the Tigray Region. The occurrence of the opportunistic infections in HIV/AIDS patients was assessed in Alamata, Hiwane and Korem districts of Tigray. Korem is located on the eastern edge of the Ethiopian highlands in the Southern Zone of the Tigray Region, this town has a latitude and longitude of 12°30′N 39°31′E with an elevation of 2539 meters above sea level. Based on figures from the Central Statistical Agency in 2005, Korem has an estimated total population of 29,340 of whom 13,842 were males and 15,498 were females [16]. Alamata is a town in northern Ethiopia. Located in the southern lowland zone of the Tigray region, it has a latitude and longitude of 12°25′N 39°33′E and an elevation of 1520 meters above sea level and has an estimated total population of 45,632 of whom 22,712 were males and 22,920 were females [16]. Hiwane is located at 13°14′50"N and 39°-53°E with an elevation of 2100 m.a.s.l. There are a total of 2170 HIV/AIDS patients; 470, 1360 and 340 from Alamata, Korem and Hewane health centers, respectively, registered and receiving Anti-Retroviral Therapy [17].

Study population

The human populations of Hewane, Korem and Alamata districts were the study targets and among which HIV/AIDS patients of both sexes will be included in the study.

Study design

A cross-sectional study was carried out at the medical out-patients and in-patient wards of Hewane and Korem health centers and at Alamata hospital of Tigray region, Ethiopia, from October 2012 to May 2013 to determine the occurrence and distribution of opportunistic infections in HIV/AIDS patients. In this study, 102 from Hewane health center, 408 from Korem health center and 141 from Alamata hospital, which give a total of 651 HIV/AIDS patients, of which 348 were asymptomatic (with no diarrhoea) and 303 symptomatic (with acute or persistent

diarrhoea) HIV/AIDS victims who were presented to the three health facilities were tested for *Cryptosporidium* oocysts by microscopy using modified acid-fast staining [18], Candidiais, Tuberculosis and Toxoplasmosis between October 2012 and May 2013.

Sampling and study methods

Sample size of study participants

The HIV/AIDS victim individuals were sampled from the three health facilities of Hewane, Korem and Alamata district and the number included in the study was calculated proportionally on the basis of the total number of admitted patients in these health centers and 30% of the total number of victims registered and receiving anti-retroviral treatment from the health facilities were included in the study. Thus, 141 (N= 470), 408 (N= 1360) and 102 (N=340) HIV/AIDS patients were obtained proportionally from Alamata hospital, Korem and Hewane health centres, respectively, and a total of 651 patients were sampled.

Sampling and detection of antibody to T. gondii

About 5 ml of whole blood sample was collected by standardized venipuncture from HIV/AIDS patients, and sera were separated and transported to Alamata District Hospital with ice box. Samples to be performed by the next days were stored in deep freezer (-20°c) until serological test. All sera collected were subjected to anti-*T. gondii* antibody detection using commercial ELISA IgG test kits. IgM ELISA Kits was also used to detect acute infection in young women participants. The ELISA kits to be used was the one suitable for several mammalian species that uses the conjugate prepared using the "G protein" that has a high affinity for the conserved fragment (Fc) portions of the various classes of immunoglobulins from numerous species. To undertake ELISA test the procedure described by Villari *et al*. [19] and instructions (standard *ELISA Procedure*) of the manufacture of the kit were followed.

Briefly, 200 micro liters (µl) of the sera (positive and negative controls and samples) at the dilution of 1:20 was dispensed in a micro-titer plate coated with whole Toxoplasma-antigen. Microplates were incubated for 1 hour at 37°C (±3°C.) and washed three times; then, 100 µl anti G-protein peroxidase conjugate (diluted 1:100 in ELISA buffer) was added and plates were incubated again for 30 min. at 37°C (± 3°C). Finally, 100 µl peroxidase substrate was added to each well. After incubation at room temperature for 20 minutes in dark, the reaction was stopped with 100 µl stop solution. The optical density (OD) of each well was measured using a photometer at a wavelength of 450 nano-meters. The test was accepted if the positive control had a minimal mean OD450 value of 0.350 and the ratio between the OD450 value of the positive control and the OD450 value of the negative control was equal or greater than 3.5. The percentage of sero-positivity (S/P%) was calculated:

$$S/P\% = \frac{(\text{OD450 value of the sample}) - (\text{OD450 value of the negative control})}{(\text{Mean OD450 value of the positive control}) - (\text{OD450 value of the negative control})} X100$$

Any sample with a S/P% ≤ 80% was considered negative, > 80% was considered coming from an infected patient. The test was carried out according to manufacturer instructions and the cutoff point was determined from the frequency distribution of the OD values of 651 sera samples. For this study, the IgG result cutoff point was set at > 51 IU as positive. The sera were diluted 1:200.

Clinical history and pathognomonic signs and symptoms of toxoplasmosis [encephalitis, facial paralysis, hemiplegia, convulsions and unilateral uveitis] were also used to diagnose toxoplasmosis.

Sampling and detection of Tuberculosis cases

Study participants were requested to submit sputum samples for acid fast bacilli (AFB) examination and fine needle aspirated (FNA) samples from skin nodules or cervical lymph nodes of patients with suspected TB lymphadenitis. Sputum samples were collected using sterile universal bottles and in ice box (+4^{o}C) to the laboratory and stored in a freezer (-20^{o}C) until processing. In every step of the bacteriological procedure all necessary laboratory safety measures were followed. Examination of Acid-Fast Bacilli from Ziehl-Neelsen stained smears of sputum or fine needle aspirated (FNA) samples was used as a presumptive diagnosis of mycobacterial disease. Hence, sputum and/or cervical lymph node specimens were obtained promptly with a medical research partner for proper technique and testing the specimens for microbiological examination in the microbiology laboratory of Alamata hospital. The FNA procedure was conducted by a medical pathologist at the hospital. The FNA was performed using a 20-22 gauge needle with an attached 10ml syringe which was mounted on an aspiration cameco gun. During each pass, the needle was moved throughout the lesion several times while aspirating. Cervical, axillary, inguinal and occasionally extranodal areas were aspirated. Since other objects can stain acid-fast (i.e. Nocardia, fungal spores, cellular debris, etc) a slide was not be reported out as being positive for acid-fast bacilli unless at least three morphologically correct AFB are seen in the smear. In an instance where less than three AFB were seen per field of vision, it was suggested to re-examine the smear, to make several more smears from the specimen, stain and examine.

Sampling and detection of cases of candidiasis

Oral, vaginal and skin infections are usually diagnosed by appearance and symptoms. Skin and oral lesion samples and vaginal swabs were collected by experienced hospital personnel. A thin smear of skin lesions, samples from the creamy white or yellowish patches in mouth and vaginal exudates on a sterile swab was made on a microscope slide. The smear was air-dried and gram-stained. Yeast cells appeared ovoid and Gram-positive with some of the cells having buds and attached to pseudohyphae. Yeast cells are much larger than staphylococcal cells. Gram stains of smears show gram-positive budding yeasts with or without pseudohyphae.

Sampling and detection of cryptosporidium oocysts

Of the 348 asymptomatic (with no diarrhoea) and 303 symptomatic (with acute or persistent diarrhoea) HIV-infected patients who presented to the outpatient unit or who were admitted to the health centers and Alamata hospital were screened for *Cryptosporidium* oocysts and diagnosis was established by conventional microscopic methods using modified acid-fast (Ziehl–Neelsen) staining. Repeated submissions of fecal samples (2 grams) were taken from symptomatic patients. Diarrhoea was defined as three or more stools per day for at least 72 hours. Acute diarrhoea was defined as diarrhoea of less than 14 days duration. Persistent diarrhoea was defined as diarrhoea for 14 or more days. Oocyst concentration (flotation and sedimentation) method was used to enhance the detection rate and slides with smooth, thick walled, colorless, spherical or slightly ovoid bodies were considered cryptosporidium oocyst positive. Stool samples were not screened for other stool parasites and enteric bacterial pathogens. Identification of different Cryptosporidium species was not the objective of this study except examining *Cryptosporidium* oocysts and recording participants' stool as positive or negative for cryptosporidiosis.

Questionnaire survey

Standardized structured questionnaire was administered to HIV/AIDS sero-positive participants to collect information on variables of interest and to determine the risk factors responsible for the occurrence of concurrent opportunistic infections. The variables of interest were the socio-demographic details such as age, sex, marital status, level of education and occupation, their district, residence (urban or rural), regular contacts with animals and habit of consumption of raw foods of animal origin (meat and/or milk), pregnancy status, signs of HIV/AIDS (symptomatic or asymptomatic), clinical staging of HIV/AIDS (clinical stages 1 to 4), family size (small = 1 to 4; medium = 5 to 7, Large = greater than 7 family members), water source (potable or river), nature of diarrhoea (acute or persistent), knowhow about opportunistic infections, and the diabetic status of each patient were obtained through questionnaire.

Ethical considerations

The study with human subjects was conducted in confirmation with the guidelines of the declaration of National Health Research Ethics Guideline of the Tigray Science and Technology Agency and hence, informed consent was taken from every participant. To ascertain confidentiality, data were collected and recorded in anonymous manner. Participants were asked for their consent to be clinically examined. Special attention was given to vulnerable groups: children, pregnant women, mentally disabled, subordinates and to communities unfamiliar with clinical concepts. HIV/AIDS sero-positive study participants were asked for participation one point in time in this one year study. Written informed consent was obtained from all participants in the study.

Limitations of the study

Since the study subjects are people affected by HIV/AIDS, who are assumed to be the consequence of somebody's sin, it was difficult to get adequate information from HIV affected individuals.

Data analysis

The data were summarized and compiled by summing up the findings of the study subjects. A study participant was said to be positive if it tested positive to the target opportunistic infections. Coded data were stored in Microsoft Excel 2007 spread sheet and transferred to SPSS® Version 20 software for statistical analysis and descriptive and analytic statistics were computed. Multinomial logistic regression and Chi-square test (χ^2) were computed to see the association of risk factors with that of test-positivity to the target concurrent opportunistic infections and the degree of association was calculated using odds ratio (OR) and 95% confidence interval (CI). Odds ratio (OR), a measure risk estimate, was calculated for the variable with a chi-square (χ^2) >3.84 and P-value <0.05 which was considered as statistically significant association between variables. Odd ratio (OR) was used to indicate the degree of association of risk factor with the disease occurrence signified by 95% confidence intervals.

RESULT

Types of concurrent opportunistic infections detected

In the present study a total of 651 HIV/AIDS patients from three districts were examined for the development

of concurrent opportunistic infections (OIs) as a single or mixed infection type. Out of the 651 registered HIV patients; 351 (53.9%) were free of any opportunistic infection and 24 (36.9%) patients were found exposed to mixed infection of tuberculosis, candidiasis and toxoplasmosis. Only 3 (0.46%) patients were having single infection of cryptosporidiosis (Table 1).

Table 1. Type (s) of opportunistic infection (s) detected from the HIV/AIDS patients examined

Type of OIs detected	No found	% out of total (N)
TB	90	13.8%
Candidiasis	117	18%
TB, Candidiasis & Toxoplasmosis	24	36.9%
TB & cryptosporidiosis	6	0.92%
TB & Candidiasis	24	36.9%
TB & Toxoplasmosis	27	4.15%
Cryptosporidiosis	3	0.46%
Cryptosporidiosis & Candidiasis	9	13.8%
Do not develop any OIs	351	53.9%
Total (N)	651	

Episode of opportunistic infections and risk factors

The potential risk factors allied with the occurrence of opportunistic infections (OIs) were investigated. Pregnancy (χ^2 = 11.7; P = 0.0001), educational status (χ^2 = 20.6; P = 0.0001), occupation (χ^2 = 19.8; P = 0.001), raw milk consumption (χ^2 = 32.14; P = 0.0001), raw meat consumption (χ^2 = 16.82; P = 0.0001), regular contact with animals (χ^2 = 7.83; P = 0.005), diabetic mellitus status (χ^2 = 23.1; P =0.0001) and the knowledge of HIV patients about OIs (χ^2 = 4.12; P = 0.04) were found to have significant statistical association with the detection of OIs in patients. Pregnant HIV patient women were 1.83 times (P = 0.0001, OR = 1.83, 95% CI = 1.51-2.40) more at risk of acquiring OIs than those non-pregnant ones. Illiterate patients were found to be 2.62 times (P = 0.0001, OR = 2.62, 95% CI = 1.30-5.40) more at risk to develop OIs than those at tertiary education level (Table 2).

Table 2. Presence opportunistic infections (OIs) in HIV/AIDS patients in different categorical variables

Variable	Category	No examined	Have OIs (%)	χ^2	P-value	OR (95%CI)
Sex	Male	219	108 (49.3)	1.39	0.239	
	Female	432	192 (44.4)			
Pregnancy status	Pregnant	81	33 (40.7)	11.7	0.0001	1.83 (1.51-2.40)
	Non-pregnant	351	159 (45.3)			1
Age in years	<18	24	9 (37.5)	1.7	0.427	
	18-40	447	213 (47.7)			
	>40	180	78 (43.3)			
Educational status	Illiterate	381	165 (43.3)	20.6	0.0001	2.62 (1.30-5.40)
	Primary	156	87 (55.8)			1.59 (1.07-3.40)
	Secondary	78	24 (30.8)			4.5 (1.94-10.50)
	Tertiary	36	24 (66.7)			1
Occupation	Civil servant	69	36 (52.2)	19.8	0.001	1.75 (1.18-3.88)
	Farmer	438	204 (46.6)			1.40 (1.10-2.73)
	Student	66	18 (27.3)			0.60 (0.26-1.39)
	Daily labourer	39	27 (69.2)			3.60 (1.41-9.20)
	Trader	39	15 (38.5)			1
Raw milk consumption	Yes	306	177 (57.8)	32.14	0.0001	2.5 (1.81-3.40)
	No	345	123 (35.7)			1
Raw meat consumption	Yes	321	174 (54.2)	16.82	0.0001	1.92 (1.40-2.62)
	No	330	126 (38.2)			1
Contact with animals	Yes	339	174 (51.3)	7.83	0.005	1.56 (1.14-2.12)
	No	312	126 (40.4)			1
Diabetic mellitus status	Diabetic	66	66 (100)	23.1	0.0001	2.50 (2.26-2.76)
	Non-diabetic	585	234 (40)			1
Knowhow about OIs	Yes	93	45 (48.4)	4.12	0.04	1
	No	558	255 (45.7)			1.20 (1.15-1.40)

OR 95% CI = Odds Ratio at 95% Confidence Interval, χ^2 = chi-squared, 1 within the cells of the OR (95% CI) represents the reference category

Those HIV/AIDS sero-positive patients whose occupation was daily laborer were 3.6 times (P = 0.001, OR = 3.60, 95% CI = 1.41-9.20) more at risk than those involved in trade and those having custom of raw milk consumption were 2.5 times (P = 0.0001; OR = 2.50, 95% CI = 1.81-3.40) more at risk of acquiring OIs than those did not. Likewise, those patients who had habit of eating raw meat were 1.92 time (P = 0.0001, OR = 1.92, 95% CI = 1.40-2.62) more at risk of developing OIs than those did not. All diabetic HIV/AIDS patients were having OIs and they were 2.5 time (P = 0.0001, OR = 2.50, 95% CI = 2.26-2.76) more at risk of being infected with opportunistic infections. Livestock owners were also victim of HIV/AIDS and were having OIs and those who had regular contacts with animals were 1.56 times (P = 0.005, OR = 1.56 , 95% CI = 1.14-2.12) more at risk of acquiring OIs than those who did not and patients did not have knowledge about the presence of opportunistic infections allied with the depression of the immune system and hence, those who did not have knowhow about OIs were 1.2 times (P = 0.04, OR = 1.20, 95% CI = 1.15-1.40) more at risk of OIs than those who had (Table 2).

Tuberculosis as an OI of HIV/AIDS patients

Tuberculosis infection, Acid Fast Bacilli (AFB), was detected from sputum and fine needle aspirate (FNA) samples in HIV sero-positive patients and sex (χ^2 = 24.1; P = 0.0001), age (χ^2 = 8.33; P = 0.013), family size (χ^2 = 11.04; P = 0.004), raw cow milk (χ^2 = 7.80; P = 0.005) and beef (χ^2 = 4.33; P = 0.037) consumption and regular contacts (χ^2 = 5.33; P = 0.021) with different animals were the potential risk factors associated with its occurrence. Female HIV sero-positives were 2.4 times (P = 0.0001, OR = 2.40, 95% CI = 1.72-3.54) more at risk of tuberculosis infection than males and those patients from large family members were 2.8 times (P = 0.004, OR = 2.80, 95% CI = 1.93-8.55) more at risk to acquire tuberculosis (Table 3). Likewise, those patients who had regular contacts with animals were 1.52 times (P = 0.021, OR = 1.52, 95% CI = 1.10-2.20) more at risk of tuberculosis infection than those who neither had livestock nor contacts with animals (Table 3).

Table 3. Occurrence of tuberculosis infection in HIV/AIDS patients and allied risk factors

Variable	Category	No examined	TB cases (%)	χ^2	P-value	OR (95%CI)
Sex	Male	219	84 (38.4)	24.1	0.0001	1
	Female	432	87 20.1)			2.40 (1.72-3.54)
Pregnancy status	Pregnant	81	18 22.2)	0.30	0.604	
	Non-pregnant	351	69 (19.7)			
Age in years	<18	24	6 (25)			1.50 (1.15-4.03)
	18-40	447	132 (29.5)	8.33	0.013	1.87 (1.22-2.87)
	>40	180	33 (18.3)			1
Family size	1-4 (small)	480	111 (23.1)			1
	5-7 (medium)	158	53 (33.5)	11.04	0.004	1.70 (1.54-5.31)
	>7 (large)	13	7 (53.8)			2.80 (1.93-8.55)
Raw milk consumption	Yes	306	96 (31.4)	7.80	0.005	1.65 (1.16-2.34)
	No	345	75 (21.7)			1
Raw meat consumption	Yes	321	96 (29.9)	4.33	0.037	1.45 (1.02-2.10)
	No	330	75 (22.7)			1
Contact with animals	Yes	339	102 (30.1)	5.33	0.021	1.52 (1.10-2.20)
	No	312	69 (22.1)			1

OR 95% CI = Odds Ratio at 95% Confidence Interval, χ^2 = chi-squared, 1 within the cells of the OR (95% CI) represents the reference category

Toxoplasmosis as an OI of HIV/AIDS patients

Toxoplasmosis was one of the OIs found in the HIV patients showing nervous symptoms in some and detected serologically from the rest. Pregnancy status ($\chi^2 = 12.7$; P = 0.0001) and regular contact with animals ($\chi^2 = 4.94$; P = 0.001) particularly with cats, sheep and goats, were significantly associated with the development of *Toxoplasma gondii* infection in HIV patient owners. Pregnant HIV sero-positive women were 5.26 times (P = 0.0001, OR = 5.26, 95% CI = 2.11-13.11) more at risk of acquiring *T. gondii* infection than non-pregnant ones. Likewise, those study HIV sero-positive participants who had animals at home were 1.76 times (P = 0.001, OR = 1.76, 95% CI = 1.19-3.20) more at risk of getting toxoplasma infection than those who did not possess animals at all (Table 4).

Candidiasis as an OI of HIV/AIDS patients

Candidiasis, especially vaginal *Candida albicans* infection, was found in 132 (30.6%) of the 432 HIV patient women involved in the investigation and *C. albicans* was found a common cause of vaginal discharge among HIV-seropositive women. Sex ($\chi^2 = 9.61$; P = 0.002) and diabetic mellitus status ($\chi^2 = 19.6$; P = 0.0001) of patients were statistically associated with the occurrence of candidiasis and females were 1.21 times (P = 0.002, OR = 1.21, 95% CI =1.08-1.34) more at risk of getting candida infection than males. Similarly those diabetic patients were 5.42 times (P = 0.0001, OR = 5.42, 95% CI = 4.57-6.42) more at risk to develop candidiasis infection than those did not have diabetes mellitus, a chronic illness (Table 5).

Table 4. Occurrence of toxoplasma infection in HIV/AIDS patients and allied risk factors

Variable	Category	No examined	Toxo cases (%)	χ^2	P-value	OR (95%CI)
Sex	Male	219	18 (8..2)	1.1	0.795	
	Female	432	33 (7.6)			
Pregnancy status	Pregnant	81	15 (18.5)	12.7	0.0001	5.26 (2.11-13.11)
	Non-pregnant	351	18 (5.1)			1
Age in years	<18	24	0 (0)			
	18-40	447	39 (8.7)	3.38[a]	0.184	
	>40	180	12 (6.7)			
Contact with animals	Yes	339	33 (9.7)	4.94	0.001	1.76 (1.19-3.20)
	No	312	18 (5.8)			1

OR 95% CI = Odds Ratio at 95% Confidence Interval, χ^2 = chi-squared, 1 within the cells of the OR (95% CI) represents the reference category

[a] 1 cells (25.0%) have expected count less than 5. The minimum expected count is 2.05. Fisher's Exact Test

Table 5. Occurrence of candida infection in HIV/AIDS patients and allied risk factors

Variable	Category	No examined	Candi cases (%)	χ^2	P-value	OR (95%CI)
Sex	Male	219	42 (19.2)	9.61	0.002	1
	Female	432	132 (30.6)			1.21 (1.08-1.34)
Pregnancy status	Pregnant	81	30 (37)	1.97	0.160	
	Non-pregnant	351	102 (29.1)			
Age in years	<18	24	3 (12.5)			
	18-40	447	120 (26.8)	2.27[a]	0.257	
	>40	180	51 (28.3)			
Diabetic mellitus status	Diabetic	66	66 (100)	19.6	0.0001	5.42 (4.57-6.42)
	Non-diabetic	585	108 918.5)			1

OR 95% CI = Odds Ratio at 95% Confidence Interval, χ^2 = chi-squared, 1 within the cells of the OR (95% CI) represents the reference category

[a] 1 cells (25.0%) have expected count less than 5. The minimum expected count is 2.05. Fisher's Exact Test

Cryptosporidiosis as an OI of HIV/AIDS patients

Both symptomatic (diarrhoeal) and asymptomatic HIV infected patients were screened for cryptosporidiosis. Study participants were interviewed to record socio-demographic information, water supply and animal contact. Of the 18 cases with cryptosporidial diarrhoea, 6 patients had acute diarrhoea and 12 patients had persistent diarrhoea. The source of water for home use (χ^2 = 33; P = 0.0001) and the presence of family member with diarrhoea (χ^2 = 82.5; P = 0.0001) were statistically associated with the detection of cryptosporidium oocyst in faecal samples of the HIV/AIDS patients. Those study participants who used river water for regular consumption were found to be 2.93 times (P = 0.0001, OR = 2.93, 95% CI = 2.63-3.27) more at risk of acquiring cryptosporidium infection than those who were using potable water and those who had family member with diarrhoea were 11.3 times (P = 0.0001, OR = 11.3, 95% CI = 8.80-14.52) more at risk to develop the infection than those who did not have (Table 6).

DISCUSSION

This study attempted to assess the extent of relationship between HIV and concurrent opportunistic infections (OIs) of different socio-economic groups. Overall, the rates of OIs, and co-infection were high and support the report of Anorlu *et al*. [20] that the prevalence of OIs in HIV patients in Nigeria is on the increase. As for the zoonoses that can infect those with HIV/AIDS, the list of potential pathogens is long; thus, it is important to discriminate between what people with HIV/AIDS *can* get and what they often *do* get. Lewis *et al*. [21] reported that some of the zoonoses of primary concern for people with HIV/AIDS include *Toxoplasma gondii*, *Cryptosporidium* spp., *Salmonella* spp., *Campylobacter* spp., *Bartonella* spp., *Mycobacterium* spp., *Giardia* spp., *Dermatophytes* [22], *Mycobacterium marinum* and *Listeria monocytogenes* which in accord with present investigation in that tuberculosis, toxoplasmosis, candidiasis and cryptosporidiosis were detected in HIV/AIDS patients of the study districts. Likewise, Reo *et al*. [23] reported tuberculosis, toxoplasmosis, candidiasis and cryptosporidiosis as a concurrent opportunistic infections of HIV/AIDS patients in India. The findings of the present study were also similar with the result of Stine [15] that AIDS-defining diseases that are animal-related include Cryptosporidiosis, Mycobacteriosis, C*andida albicans* and Toxoplasmosis. It should be noted that food, water, and the environment all can serve as sources of infection for several of these agents, in addition to animals. One study of individuals with AIDS found no significant difference between animals' owners and those do not have in rates of indicating that animal ownership does not dramatically increase the risk of AIDS-defining zoonotic OIs [24].

Table 6. Occurrence of cryptosporidium infection in HIV/AIDS patients and allied risk factors

Variable	Category	No examined	Crypto cases (%)	χ^2	P-value	OR (95%CI)
Sex	Male	219	6 (2.74)	0.067	0.795	
	Female	432	12 (2.8)			
Age in years	<18	24	0 (0)	1.56 [a]	0.458	
	18-40	447	12 (2.7)			
	>40	180	6 (3.3)			
Water source	River	234	18 (7.7)	33 [a]	0.0001	2.93 (2.63-3.27)
	Potable	417	0 (0)			1
Diarrhoea in family member	Yes	74	18 (24.3)	82.5[a]	0.0001	11.30 (8.80-14.52)
	No	577	0			1
Type of diarrhoea	Acute	24	6 (25)	0.01	0.925	
	Persistent	50	12 (24)			

OR 95% CI = Odds Ratio at 95% Confidence Interval, χ^2 = chi-squared, 1 within the cells of the OR (95% CI) represents the reference category

[a] 1 cells (25.0%) have expected count less than 5. The minimum expected count is 2.05. Fisher's Exact Test

People infected with HIV/AIDS are more likely than uninfected people to get sick with other infections and diseases. Tuberculosis (TB) is one of these diseases. In multibacillary diseases with a high mycobacterial load, a positive Ziehl-Neelsen smear is diagnostic of active tuberculosis [7]. Similarly, examination of acid-fast bacilli (AFB) from Ziehl-Neelsen stained smears of sputum was conducted to diagnose for pulmonary TB and the fine needle aspirate (FNA) sampling from cervical lymph nodes of patients with suspected TB lymphadenitis to diagnose for extra-pulmonary TB. As a result 171 were found positive for tuberculosis infection out of 651 study participants to give an overall prevalence of 26.3% (171/651). People who are infected with HIV are especially susceptible to developing active tuberculosis (TB). TB is the leading cause of death among people living with HIV/AIDS (PLWHA) and one of the most common opportunistic infections they experience. The prevalence of HIV infection among patients in TB clinical settings is high, up to 80 percent in some countries. Previous studies have already identified tuberculosis as key causes of morbidity and mortality in HIV patients [25]. Overlap between the acquired immunodeficiency syndrome (AIDS) and TB epidemics continues to result in increases in TB morbidity. The finding of tuberculosis infection in those patients who had animal contacts might indicate the potential of transmission the agent between cattle and man in the study area.

The seroprevalence of toxoplasmosis among 651 immunodeficiency virus patients was 7.8% (51/651) and the majority of the patients were in the 18-40 age groups, and this finding is similar with that of Veeranoot *et al*. [26] who reported that toxoplasmosis infection was found in majority of age group of 25-37 years. Most infections caused by *T. gondii* are asymptomatic and only a minority of patients with clinical evidence of infection exhibit signs and symptoms that cannot be attributed to the presence of the parasite. Often, the diagnosis of a recently acquired *Toxoplasma* infection is based on the detection of specific IgM antibodies in a single serum sample. The present study is in accord with the findings of Remington *et al*. [27] who reported that pregnant women were more at risk of acquiring toxoplasmosis than males and those women who are co-infected with HIV and *T. gondii* and who have developed AIDS are at risk of reactivating their *T. gondii* infection, developing severe toxoplasmosis and/or transmitting the parasite to their offspring. From this study, the seroprevalence of toxoplasmosis in HIV/AIDS patients was 7.8% (51/651) which is lower as compared with previous studies in Malaysia, for example 21% [28, 29], and 51.2% [30], respectively. The differences in seropositivities among previous studies are based on several factors: the geographical distribution of the study sites, socioeconomic status, risk behaviors of the human population pertaining to the acquisition of *Toxoplasma* infection and the variety of commercial serodiagnostic methods used in each study, each of which demonstrated its own sensitivity and specificity. However, *Toxoplasma gondii* serology should be implemented as part of the routine screening for all HIV-infected patients, and toxoplasmosis chemoprophylaxis should be given to those with positive *Toxoplasma* serology in order to prevent the risk of developing the life-threatening secondary reactivation of cerebral toxoplasmosis in association with AIDS [31].

Prevalence of candida infections is frequently correlated with immunological status of host [32], the infection being the commonest fungal infection associated with HIV-infection in women [33]. Duerr *et al*. [32] reported a higher incidence and greater persistence of the infection in HIV-seropositive women. The present study was therefore, in accord with this finding that the infection of candidiasis was associated with the immunological status of the HIV-infected women and was higher than in males. In Lagos, Nigeria, however, the studies of Anorlu *et al*. [20] implicated *C. albicans* as a common cause of vaginal discharge among HIV-seropositive women and this finding was similar with the present study; while in northern Nigeria, Sagay *et al*. [34] found that candida infection is one of the predictors of HIV infection. The present investigation showed similar finding with that of Umeh and Umeakanne [35] in Nigeria who reported that diabetes and pregnancy had the highest coefficients and therefore were the most important risk factors as regards HIV/yeast coinfection.

Cryptosporidiosis is a leading cause of protracted, life threatening diarrhoea in HIV infected patients. There is no data on prevalence of this infection for Ethiopian patients and no information on risk factors for transmission exists. This study is therefore, undertook to identify risk factors for transmission of cryptosporidiosis in HIV infected patients. There were 303 symptomatic HIV/AIDS patients participated in the study and cryptosporidiosis was detected in 6 (25%) out of 24 patients with acute diarrhoea type and in 12 (24%) out of 50 persistent type. It can be concluded that there are bacterial, viral and parasitic agents that can cause acute and chronic types of diarrhoea in immunocompromized patients. However, bacterial, viral and other parasitic agents were not screened in this study. The present study is different from the findings of Rao *et al*. [23] in India who reported that the association between water source and contact with animals and acquisition of cryptosporidiosis was not statistically significant.

CONCLUSION AND RECOMMENDATIONS

In this study; both symptomatic and asymptomatic HIV/AIDS patients were found to have concurrent OIs which included candidiasis, cryptosporidiosis, tuberculosis and toxoplasmosis. Opportunistic infections are of the causes a major life-threatening condition and public health problem, particularly in patients with AIDS. Together, HIV and concurrent opportunistic infections (OIs) are a deadly combination, each disease making the other disease progress faster. HIV makes the immune system weak, so that someone who is HIV-positive and also infected with OIs becomes much more likely to get sick with OIs than someone infected with OIs who is HIV-negative. In Ethiopia, OIs are the leading cause of death among people who have HIV. HIV is the single major reason why there has been such a large increase in cases of tuberculosis, toxoplasmosis, candidiasis and cryptosporidiosis over the past decade. Unrecognized, unknown and denied risk behaviors for *opportunistic* infections can pose major diagnostic and therapeutic challenges to clinicians in Ethiopia. In views of the above conclusion; recommendations were suggested for possible control and prevention measures of OIs. Awareness should be created not only to HIV patients but also to the people in the large residential community about OIs, and indicate the need of their diagnosis for prior therapy. Early diagnosis and effective treatment of OIs among HIV-infected patients are critical for curing OIs, minimizing the negative effects of OIs on the course of HIV, and interrupting the transmission of the identified infections to other persons in the community. All HIV-infected persons at risk for infection with OIs must be carefully evaluated and, if indicated, administered therapy to prevent the progression of latent infection to active disease and avoid the complications associated with HIV-related OIs. All HIV-infected patients undergoing treatment for OIs should be evaluated for antiretroviral therapy, because most patients with HIV-related TB are candidates for concurrent administration of anti-OIs and antiretroviral drug therapies. There should be species level detection in case of tuberculosis as *Mycobacterium tuberculosis* or *M. Bovis*, and cryptosporidiosis as *Cryptosporidium hominis, C. parvum, C. felis, C. muris, and* others and the snapshoot, cross-sectional, study limits to measure the subsequent CD4 counts of HIV/AIDS patients and hence, further research should be conducted.

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