Original Article

Molecular epidemiology of Microsporidia among HIV-positive and HIV-negative patients in the Limpopo province, South Africa

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Abstract

Introduction: Human microsporidiosis represents an important and rapidly emerging opportunistic disease. The present study investigated the prevalence of microsporidia among HIV positive and HIV negative patients with or without diarrhoea in Vhembe and Mopani Districts in the Limpopo Province.

Methodology: A total of 170 stool samples were collected from these patients and microsporidia species was detected using a Real-Time PCR targeting a conserved region of the small ribosomal subunit rRNA (SSU-rRNA) gene of *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *Encephalitozoon hellem*, and *Encephalitozoon cuniculi*.

Results: Fifty six (32.9%) were positive for microsporidia. The prevalence was higher in HIV negative patients (36.6%) while 24.1% of patients who were HIV positive had microsporidia. Microsporidia was more common among patients aged between 1 and 10 years (52.6%). However among the HIV positive patients, microsporidia prevalence was higher among those that were not taking antiretrovirals (ARVs) compared to those who were on ARVs, (36.6%) and (24.1%), respectively. Microsporidia was also noted to be significantly associated with diarrheal and stomach pains; $p = 0.02$ and $p = 0.048$, respectively. Furthermore, microsporidia infections was more prevalent among patients who had animals at home ($p = 0.037$).

Conclusions: Study has shown a high prevalence of microsporidia among patients attending primary health centers in the Mopani District for the first time. Prevalence of microsporidia was higher among HIV negative and HIV positive patients who were not on ARV treatment. Keeping animals in the household appeared to be a risk of getting infected with microsporidia. Further studies are needed to determine the genetic characteristics of these organisms in the study population.

Key words: HIV; Microsporidia; diarrheal; Venda.

Introduction

Microsporidiosis is one of the most common emerging infections that have been reported worldwide. Known to infect mainly arthropods and fish [1], these parasites have gained relevance in the past decade because of their increased infections in individuals having an impaired immune system [2].

The most prevalent pathogens of humans include *Enterocytozoon bieneusi* which causes symptoms such as diarrhea which is life threatening among people with HIV/AIDS [2,3]. Infections caused by *E. bieneusi* are increasing among travellers and residents of tropical countries who do not have HIV infection. Other species such as *Encephalitozoon cuniculi* which cause encephalitis and nephritis; *Nosema* spp which affect the cornea and *Encephalitozoon intestinalis* which causes diarrhea and nephritis have also been described [4].

Previous studies conducted in South Africa have indicated that microsporidia were very common in the general population and was even higher among HIV and AIDS patients [5]. However, that study focused mostly on the Vhembe district and samples were not obtained from other districts like Mopani. Furthermore, this study was carried out among HIV patients who were not exposed to Antiretrovirals (ARVs).

Humans acquire microsporidia though ingestion or inhalation of microsporidia spores which are highly resistant to environmental conditions. These spores can be found in surface water and human strains have been identified in municipal water supplies and ground water [6]. Significant contact with infected animals may also transmit the disease but cases are rare [7].

Prevalence rates with microsporidia infections in HIV positive individuals vary worldwide [3,8-10].

However, very few studies have been conducted in these organisms in the African continent and in South Africa in particular. Previously, many studies have been performed focusing on HIV-infected patients or other immunocompromised individuals worldwide. In South Africa, studies have not discussed other risk factors of microsporidia infections such as water sources, animal contact or other concurrent infections or symptoms. Therefore, the current study investigated the prevalence of microsporidia in HIV positive and HIV negative patients with or without diarrheal in both Vhembe and Mopani Districts in the Limpopo Province. Furthermore, different risk factors were also evaluated in relation to the occurrence of microsporidia among the study patients. The main objective of the study was to determine the molecular epidemiology of microsporidia among patients attending different primary health care centres in Giyani and Vhembe District municipalities.

Methodology

Ethics statement

The study was approved by the University of Venda Health and Safety committee and the Department of Health at Polokwane, Limpopo Province, South Africa (Ethical approval number: SMNS/05/MBY/0502). Ethical clearance was also obtained from the different hospitals where the study was conducted as well as the Department of Health in Giyani. The objectives of the study were explained to the patients and their consent to participate in the study obtained. They were however, free to get out of the study at any time.

Study area and sample collection

The study was conducted at clinics (primary health care centers) in Mopani and the main hospitals in the Vhembe district. Samples were collected from patients who agreed to participate in the research and signed a consent form and complete the questionnaires in order to obtain socio-demographic information as well as data about diarrhea history and other related symptoms. Stool samples were collected from the study participants and were labelled with the patient's code, sex, age and date of collection. The samples were further transported to the Microbiology laboratory at the University of Venda in cooler boxes with ice within 4 hours of collection for further analysis.

DNA extraction from stool samples and quantitative real-time PCR amplification for the detection of microsporidia

Genomic DNA was purified from the stool samples using the QIAamp DNA mini kit (Qiagen, Inc,

Valencia, CA, USA) with some modification. Briefly, 250µL of homogenized stool sample was added into a 1.5 mL eppendorf tube containing 0.35 g of autoclaved acid-washed glass beads (425-600µm; Sigma-Aldrich Co, St Louis, USA) and 200 µL of buffer ASL (stool lysis buffer) from the QIAamp DNA mini kit (Qiagen, Hilden, Germany). The mixture was homogenized in the bead beater for 3 sequences of 20 seconds each. After beating, 1 mL of the buffer ASL was added to the mixture and the DNA extraction continued following the procedure recommended for QIAamp DNA mini kit and the modifications by Samie *et al*. [5]. Primers used for PCR targeted a conserved region of the SSU rRNA gene of *Enc. cuniculi, E. bieneusi, Enc. hellem* and *Enc. intestinalis.* The expected amplicon sizes were 250bp for *E. bieneusi*, 268 bp for *Enc. cuniculi,* 270 bp for *Enc. intestinalis,* 268 bp for *Enc. hellem.* The forward primer PMP1, analogous to V1 [11] is complementary to position 1 to 22 of *E. bieneusi, Enc. cuniculi*, *Enc. hellem*, *Enc. Intestinalis* and the reverse primer PMP2 was designed to be complementary to the positions 230 to 250 to the published SSU rRNA sequence of *E. bieneusi* [12]; (GenBank accession no. L07123) and GenBank sequence positions 248 to 268 of *Enc. cuniculi* (GenBank accession no. L17072)*,* 259 to 279 of *Enc. hellem* (GenBank accession no. L19070 and 250 to 270 of *Enc. intestinal* (GenBank accession no. L09929) rRNA genes.

Genomic DNA extracted from samples was used in a real-time PCR protocol using the primers described above as previously described by Samie *et al*. [5] with SYBR-Green-490 (Roche Diagnostics). The reaction was performed in light cycler 480 (Roche Diagnostics). The results were analysed with a user-defined threshold of 200 PCR Baseline Subtracted Curve-fit Relative Fluorescence Units (CF RFU). The level of positivity of the samples was then indicated by the cycle threshold (CT) values, which represent the number of cycles necessary for the samples to cross the threshold: the smaller it is the more DNA is in the samples.

Statistical analysis

Analysis was conducted using the statistical package for social sciences (SPSS) program, version 17.1 with the fisher chi square test and the difference between two variables was considered significant if the *p* value was less than 0.05.

Results

Demographic characteristics of the study population

Table 1 shows the demographic characteristics of the study population based on age, origin and age

groups. Samples were collected from 170 patients from different hospitals and clinics. Nkomo clinic provided the highest number of samples which constituted about 25.9% of the total samples followed by Donald Fraser with 24.7%. The least number of samples were collected from Memorial hospital where only two samples were obtained. From the total sample population, 79.5% were females. In terms of marital status, majority of the patients were married (53.6%) and those who were single constituted about 38.6% of the total study population. The remainders of the study population were either divorced (1.8%) or widowed (6%). The population was aged between 4 and 76 years old. The mean age of the population was 35.96 ± 17.81 years. Older patients in the age group (51-76) contributed most of the samples, which is 24.4% of the total population, followed by those in the age group of (41-50). The least number of samples were collected from patients within the age group 11 to 20 years.

Clinical characteristics of the study population

Some of the major clinical symptoms which were identified from patients include weight loss (72.3%), stomach pains (52.3%), respiratory illness (32.7%) and vision problem (28.1%). The other illnesses which were noted include difficulties in breathing, skin infection, and underweight but these occurred less frequently. Close to 20% of the patients indicated that they had had diarrhea in the last three months before sampling and most of these (56.2%) were suffering from loose diarrhea. Diarrhea was mainly acute (77.4%) in some of

the patients whereas a small percentage of patients suffered from chronic diarrhea (22.6%).

Distribution of microsporidia among patients by origin

Of the 170 stools samples tested for the presence of microsporidia spores 56 (32.9 %) were positive for microsporidia by RT-PCR. Microsporidia were detected in patients from all the hospitals and clinics except from samples that were obtained from the Memorial hospital in Louis Trichardt. Overall, 32.4% of patients sampled from all the hospitals and clinics were positive for microsporidia. The highest prevalence of microsporidia was detected in Ngove with 46% of the samples positive for microsporidia followed by Mhlava and Nkomo with prevalence of 42.4% and 29.5% respectively. Overall microsporidia was significantly more prevalent in Mopani 44 (37%) compared to Vhembe 11 (21.6%) ($p = 0.049$). However heavy infection were more prevalent in Vhembe (72.7%) compared to Mopani district (54.2%) but the difference was no significant ($p = 0.761$).

Distribution and prevalence of microsporidia by gender, marital status, age group, history of diarrhea in patients and patients who kept animals at home

Microsporidia was more common among females. Those who were single and widowed had higher prevalence of microsporidia of about 40% compared to 27% among patients that indicated that they were married. Table 2 shows the occurrence of microsporidia according to gender and marital status. Microsporidia

Table 1. Demographic characteristics of the study population.

Characteristics		Frequency	Percentage
Origin	Donald Fraser	42	24.7%
	Memorial Hospital	2	1.2%
	Mhlava	33	19.4%
	Ngove	35	20.6%
	Nkomo	44	25.9%
	Tshilidzini	7	4.1%
	Thomo	7	4.1%
Sex	Male	34	20.5%
	Female	132	79.5%
Marital status	Divorced	3	1.8%
	Married	89	53.6%
	Single	64	38.6%
	Widowed	10	6%
Age groups	1 - 10	19	11.6%
	11 - 20	9	5.5%
	21 - 30	31	18.9%
	31 - 40	32	19.5%
	41 - 50	33	20.1%
	51 – 76	40	24.4%
Total		170	100%

Table 2. Distribution microsporidia by gender and marital status.

		Positive	Total
Sex	Unknown	1 (25%)	4
	Female	45 (34.1%)	132
	Male	9 (26.5%)	34
	Total	55 (32.4%)	170
Marital status	Unknown	1 (25%)	4
	Divorced	0	3
	Married	24 (27%)	89
	Single	26 (40.6%)	64
	Widowed	4 (40%)	10
	Total	54 (32.4%)	170

were highly prevalent among patients in the age group of 1-10 years old (52.6%). The least number of patients who had microsporidia were in the age group of 41-50 years (Figure 1).

Microsporidia was more prevalent among patients who had had diarrhoea during the last three month with a prevalence of 53.1%. Patients who had relative with diarrhoea living in the same household were to have a higher chance of getting microsporidia. However the difference was not statistically significant ($p = 0.238$). The prevalence was high in patients with acute diarrhoea (58.3%) compared to those with chronic diarrhoea (42.9%). In terms of consistency, all three patients (100%) who provided stools containing blood were positive for microsporidia followed by those with watery stools (75%) and those with loose stools (50%) (Table 3).

Patients who had animals at home appeared to be more infected by Microsporidia with a prevalence of 43.1% compared to those who did not have animals at

Figure 1. Age distribution of microsporidia in Vhembe and Mopani Districts.
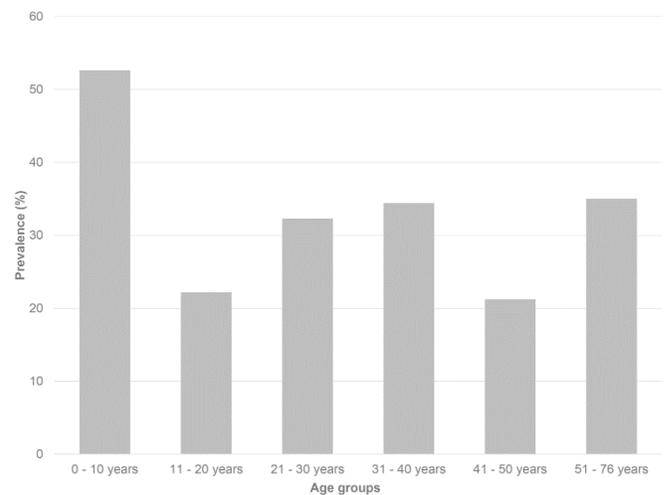

Table 3. Prevalence of microsporidia in relation to history of diarrhoea in patients.

Diarrhoea		Positive n. (%)	Total	Statistics
Diarrhoea for the last three month	YES	17 (53.1%)	134	$\chi^2 = 7.784$, $p = 0.020$
	NO	37 (27.6%)	134	
Has anyone had diarrhoea at home	YES	9 (40.9%)	22	$\chi^2 = 2.873$, $p = 0.238$
	NO	34 (35.1%)	97	
Stool consistency	Unknown	38 (27.5%)	138	
	Bloody/Mucous	8 (57.1%)	14	$\chi^2 = 4.284$, $p = 0.038$
	Loose	9 (50%)	18	$\chi^2 = 2.865$, $p = 0.091$
Type of diarrhoea	Unknown	38 (27.3%)	139	
	Acute	14 (58.3%)	24	$\chi^2 = 8.619$, $p = 0.003$
	Chronic	3 (42.9%)	7	$\chi^2 = 0.368$, $p = 0.544$
Patients who kept animals at home				
Animal at home	YES	31 (43.1%)	72	$\chi^2 = 6.189$, $p = 0.013$
	NO	23 (24.7%)	93	
Types of animals	Chickens	16 (40%)	40	$\chi^2 = 1.268$, $p = 0.260$
	Cattles	13 (43.3%)	30	$\chi^2 = 1.873$, $p = 0.171$
	Dogs	4 (23.5%)	17	$\chi^2 = 0.728$, $p = 0.393$
	Cats	1 (14.3%)	7	$\chi^2 = 1.129$, $p = 0.298$
	Donkeys	1 (25%)	4	$\chi^2 = 0.111$, $p = 0.739$
	Goats	7 (46.7%)	15	$\chi^2 = 1.456$, $p = 0.228$

Table 4. Prevalence of microsporidia among the patients in relation to their water sources and storage.

		Positive n. (%)	Total	
Source of drinking water	Unknown	1 (25%)	4	
	Borehole	23 (35.9%)	64	$\chi^2 = 0.551$, $p = 0.458$
	Communal tap	18 (26.1%)	69	$\chi^2 = 2.234$, $p = 0.134$
	Direct from the river	0	2	
	Still water	0	1	
	Tap in the house	13 (43.3%)	30	$\chi^2 = 1.947$, $p = 0.496$
Do you treat water	YES	1 (33.3%)	3	
	NO	53 (32.7%)	162	
Water storage	1-2 days	17 (35.4%)	48	$\chi^2 = 0.256$, $p = 0.613$
	3-4 days	7 (20%)	35	$\chi^2 = 3.173$, $p = 0.075$
	5 days and above	30 (36.1%)	83	$\chi^2 = 0.988$, $p = 0.320$
Total		54 (32.5%)	166	

home (24.7%) and the difference was statistically significant ($\chi^2 = 6.585$, $p = 0.037$). Microsporidia were detected mostly among patients who had goats with a prevalence of 46.7% followed by those who had cattle with the prevalence of 43.3% (Table 3).

Prevalence of microsporidia among the patients in relation to their water sources and storage

Different water sources were used by the study population. The most commonly used was communal taps; however, microsporidia was more common among patients who had taps inside the house (43.3%) while patients who were using water from boreholes had a prevalence of 35.9% (Table 4). There was no difference between the prevalence of microsporidia

among patients who treated water before use and from those who did not treat their water. This could be due to the little number of patients who indicated that they treat their water before drinking. In terms of water storage, patients who stored their water for 5 days and above after collection were highly infected (36.1%) and was similar to those who indicated that they stored their water for one or two days. The least number of patients positive for microsporidia were those storing water 3 - 4 days (20%).

Relationship between the prevalence of microsporidia and health status of patients

About 34.8% of patients who were taking medication (particularly antibiotic) tested positive for

Table 5. The relationship between the prevalence of microsporidia and health status of patients.

		Positive n (%)	Total	Statistics
Taking medication	YES	16 (34.8%)	46	$\chi^2 = 0.482$, $p = 0.786$
	NO	38 (31.9%)	119	
Are you taking alternative treatment	YES	2 (14.3%)	14	$\chi^2 = 3.130$, $p = 0.209$
	NO	52 (34.7%)	150	
Skin infection	YES	13 (46.4%)	28	$\chi^2 = 3.074$, $p = 0.215$
	NO	41 (29.7%)	138	
Respiratory illnesses	YES	18 (34%)	53	$\chi^2 = 0.890$, $p = 0.828$
	NO	36 (32.4%)	111	
Currently coughing	YES	20 (37.7%)	53	$\chi^2 = 1.254$, $p = 0.534$
	NO	34 (30.4%)	112	
Difficulties in breathing	YES	13 (34.2%)	38	$\chi^2 = 1.353$, $p = 0.717$
	NO	39 (33.3%)	117	
UTI over the past three month	YES	13 (31.7%)	41	$\chi^2 = 0.736$, $p = 0.692$
	NO	41 (33.3%)	123	
Vision Problems	YES	11 (31.4%)	35	$\chi^2 = 0.126$, $p = 0.939$
	NO	43 (32.8%)	131	
Stomach pains	YES	33 (41.8%)	79	$\chi^2 = 6.090$, $p = 0.048$
	NO	18 (25%)	72	
Weight loss	YES	10 (29.4%)	34	$\chi^2 = 1.797$, $p = 0.407$
	NO	40 (35.4%)	113	
WAZ-2	Under weight	7 (31.8%)	22	$\chi^2 = 0.010$, $p = 0.922$
	Normal weight	47 (32.9%)	143	

WAZ-2: Weight for Age z score less than negative 2; UTI: Urinary tract infections.

Microsporidia and from those who were not taking medication, 31.9% had microsporidia. Microsporidia were highly detected in patients not taking alternative treatment (34.7%) compared to those were using alternative treatment. In terms of infections and illnesses, the prevalence was high in patients with skin infection (46.4%), respiratory illness (34%), currently coughing (37.7%), difficulties in breathing (34.2%) and stomach pains (41.8%). It was also found that patients who did not have Urinary tract infection over the past three month before sampling had high prevalence compared to those who had UTI. Patients who did not lose weight were mostly infected. Weight loss did not have any correlation with the occurrence of microsporidia among the patients. The prevalence rate of microsporidia was high in patients who complained about stomach pains in both the HIV positive and HIV negative patients (Table 5).

Prevalence of microsporidia in relation to different income and educational status

There was no specific association between income and microsporidia occurrence. In fact microsporidia were detected in 46.7% patients earning South African Rands (ZAR) 5,000.00 and above, followed by those who were earning ZAR 1,001 - 3,000. The least number of patients infected by microsporidia were obtaining income range of ZAR 1,000.00 and ZAR 3,001 - 4,999, (25%) and (24.2%), respectively.

Prevalence of microsporidia in stool samples of HIV positive and HIV negative patients in clinics and hospitals of Vhembe and Mopani district

Patients who were HIV negative were highly infected by microsporidia while those who were HIV positive were less infected. The prevalence of microsporidia in patients who were not taking ARVs was higher compared to those who were taking ARVs (Table 6). According to CT values, microsporidian infections were classified as heavy infection (CT value more than 15) or mild infection (CT value less than 15). More HIV positive patients had heavy microsporidia infections compared to HIV negative patients (Table 6).

Among the HIV positive patients those who were not on ARV had more heavy infections compared to those who were taking ARV.

Discussion

Microsporidia have been identified as the cause of opportunistic infections in immunocompromised patients such as HIV patients as well as the immune-competent individuals. Most of human microsporidiosis have been limited to studies on HIV positive patients with diarrhoea and different prevalence have been described throughout the world. In this study, the overall prevalence of microsporidia was 32.9% which was determined by real-time PCR. Other studies in the African continent have found similar prevalence. For example, Tumwine *et al.* [13] in a study done to determine the occurrence of cryptosporidiosis and microsporidiosis in Ugandan children with persistent diarrhoea with and without current infection with HIV found similar results with 32.9% of the children infected. Similar results in HIV infected patients were reported in Niamey, Niger and Hanoi, Vietnam [14].

In a Portuguese study by Lobo *et al.* [15], an infection rate of 12% with microsporidia was reported. Moreover, Lobo and colleagues observed a prevalence of 13.9% and 8.5% for HIV+ and HIV- samples, respectively in the same study [15]. In another study conducted by Anane *et al.* [16] focusing on the epidemiological and clinical characteristics of intestinal microsporidiosis, an overall prevalence of 2.4% was found where 3.6% among the HIV infected patients were infected and 1.4% among patients without HIV. In our study, the prevalence of microsporidia was higher in HIV negative patients (36.6%) compared to HIV positive patients.

The prevalence of microsporidia among symptomatic HIV infected individuals ranges between 7 and 50% varying in the location of the study and the diagnostic methods to detect microsporidia [20]. In the present study we found a prevalence of 24.1% among HIV infected patients. We also found that the prevalence of microsporidia among HIV negative

Table 6. Comparative occurrence of heavy and mild Microsporidia infections between HIV negative and HIV positive patients.

HIV status	ARV status	CT range		Total
		Mild infection	Heavy infections	
HIV negative	ARV	20 (45.5%)	24 (54.5%)	44
	Not on ARV	2 (28.6%)	5 (71.4%)	7
HIV positive	Taking ARV	3 (37.5%)	5 (62.5%)	8
	Subtotal	5 (33.3%)	10 (66.7%)	15
Overall	Total	25 (42.4%)	34 (57.6%)	59

CT: Cycle Threshold; ARV: Antiretroviral treatment; HIV: Human immuno- deficiency virus.

patients was 36.6% which was higher compared to those who were HIV positive (24.1%) and were taking ARVs (23.3%). In a previous study conducted in Vhembe District, Limpopo province by Samie *et al.* [5] on PCR detection of microsporidia from stool sample of HIV positive and HIV negative individuals and the school children, *E. bieneusi* was the only detected microsporidian species among the individuals and a high prevalence was found among HIV positive patients compared to HIV negative patients. It should be noted that, unlike the previous study, some of the HIV positive patients in the current report were on ARV. This indicates that it is possible that the use of ARV by the present patients could have contributed to the reduction of the prevalence of microsporidia among these patients. Similar findings have been described by Maggie and colleagues [21]. Therefore continued roll out of ARV could be beneficial for the reduction of microsporidia infections among HIV patients in this part of the country.

Diarrhoea is a commonly known sign associated with the infection caused by microsporidia species [20]. In our study it was found that 53.1% of patients who were infected had diarrhoea. Similar results were reported from a Zimbabwean and a Venezuelan study [22,23]. Previous studies also reported the occurrence of Microsporidiosis with self-limited diarrhoea in immune-competent individuals [24]. Cases of human microsporidiosis were also reported in non-HIV infected immunocompetent patients. These reports include travellers to developing countries as well as residents of various tropical countries. In a study performed by Muller *et al.* [4] to detect microsporidia in 148 stool samples from travellers with diarrhoea, microsporidia were diagnosed in five individuals by light microscope and nine by PCR. Moreover, Wumba *et al.* [25] reported a prevalence of 5.1% and 0.6% of *E. bieneusi* and *Enc. intestinalis*, respectively in AIDS hospitalized patients in Kinshasa, Congo. Microsporidia were also detected in patients who had diarrhoea only with prevalence rates of 4.6% and 1.1%, respectively.

The present study also reported the prevalence of microsporidian infection in patients aged 1 to 10 years (52.6%). The results were similar to the previously reported studies by Nkinin *et al.* [17] who reported that the prevalence of microsporidiosis among teenagers was 81.5% in a study that investigated microsporidia infection in healthy people in Cameroon. The study population was immunocompetent individuals without tuberculosis (TB), HIV positive and HIV negative with TB. A study conducted in Malaysia by Norhayati *et al.*

[19] on the prevalence of intestinal microsporidia in patients with or without gastrointestinal symptoms also described the prevalence of microsporidia in children 6 years of age or younger (26.1%) who had available medical records. An earlier report by Samie *et al.* [5] in the Vhembe district also reported a prevalence of 25% in children aged between 3 to 5 years old.

Microsporidia have been recognized as intracellular microorganisms that cause opportunistic infection in a wide range of humans and animals [25]. In the present study, 72 patients were having animals at home and 43.1% of 72 of patients were infected by microsporidia indicating that microsporidia might be associated with animal transmission in the region. In a previous study conducted by Lores *et al.* [18] *E. bieneusi* was demonstrated in fecal samples from domestic animals from Galicia, Spain and *E. bieneusi* spores were detected in fecal samples of two goats and one dog by PCR. Detection of human microsporidiosis in the environmental water samples has been reported [6]. In this study microsporidia infection were mostly detected in patients who were obtaining water from safe sources. These can be due to the factors that potentially favor contamination of water in the homes which include the way the water is collected from the containers and also the frequency of cleaning the containers. Also, the small size of microsporidia spores will allow them to escape filtration as well as the unknown potency of resistance of microsporidia spores to physical agents and disinfectants [27].

Other factors that may have contributed to contamination of water include pumping of surface water directly from recreational areas that is mainly frequented by swimmers in summer and treated the water with flocculation, ozoflotation and filtration instead of using chlorine [27]. Previous studies reported the links between the contact with water and intestinal microsporidiosis which include swimming in pools, lakes, ponds and rivers [28] and also drinking unfiltered tap and well water [29]. In an earlier report that investigated the prevalence of microsporidia from drinking water, waste water and recreational rivers, microsporidia were detected in 8 (21%) samples out of 38 and *E. intestinalis* was the only microsporidia species detected using PCR [30]. In the present study, microsporidia were detected in patients indicated to have clinical symptoms that might be associated with the presence of these parasites. In our study, we did not differentiate microsporidia species but many studies conducted previously reported on the prevalence of microsporidia species in immunocompetent and immunocompromised individuals. In the study done in

Addis Ababa, Ethiopia by Endeshaw *et al.* [8] on intestinal microsporidiosis in diarrheal patients infected with HIV-1, microsporidial parasites were identified as *E. bieneusi* (in 30 samples), *Enc. intestinalis* (6 samples) and double infection (3 samples) by PCR [8].

Conclusions

This is the first study to determine prevalence of microsporidia in HIV negative and HIV positive patients in the Giyani region. Microsporidia were more common in Mopani district compared to Vhembe district. The prevalence of microsporidia was significantly higher among patients who kept animals compared to those who did not. Microsporidia infections were significantly associated with diarrhoea and stomach pains irrespective of HIV status although the infection load was heavier among HIV positive patients. The use of ARV appears to be beneficial in reducing the prevalence of microsporidia among HIV positive patients. Further investigation should determine the molecular characteristics of these organisms in Vhembe and Mopani district.

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