Original Article

Microbiology

The Prevalence of Coccidian Parasites in and Around Tirunelveli in HIV Positive Individuals and Its Correlation with the CD_4 Count

SUCILATHANGAM G., VELVIZHI G. PALANIAPPAN N., ANNA T.

ABSTRACT

Background and Objectives: Opportunistic infections are the hallmark of the Human Immuno Deficiency Virus (HIV) infection. In recent years, intestinal infections such as *Cryptosporidium, Cyclospora* and *Isospora* are becoming more prevalent in the Acquired Immunodeficiency Syndrome (AIDS) patients. The purpose of this study was to determine the prevalence of coccidian parasitic infections in HIV seropositive patients with or without diarrhoea and to study the association of diarrhoea and the coccidian parasites with the CD_4 counts in our setting.

Materials and Methods: Out of 103 stool samples, 33 stool samples were collected from HIV seropositive patients with diarrhoea and 70 stool samples were collected from HIV seropositive patients without diarrhoea. The samples were examined for intestinal coccidian parasites by microscopy and the modified Kinyoun's acid fast staining methods. The CD_4 counts were estimated by using a PartecCyflow counter (Germany). Statistical analysis was done by using the Chi-square test to evaluate the association between the HIV patients with or without diarrhoea and the coccidian parasitic infections.

Results: Coccidian parasites were identified in 58.2% (60/103) of all the stool samples which were examined. Coccidian parasites were detected in 57.1% patients with non-diarrhoeal complaints and in 60.6% patients with chronic diarrhoea (P>0.05). Among the coccidian parasites, *Cryptosporidium parvum* was the most predominant parasite which was found in the study (42.7%; 44/103), followed by *Microsporidia* (7.8%; 8/103)) *and Cyclospora* (3.9%; 4/103) which were detected alone. Dual infections which comprised of those with *C. cayetanensis* and *C. parvum* (1.9%; 2/103) and *C. parvum* and *Microsporidia* (1.9%; 2/103) occurred in the HIV-positive patients. After being classified by the CD_4 T-cell categories, the opportunistic intestinal parasite infections showed the highest prevalence in patients with a low immune level (CD_4 < 200/µl). Coccidian parasites were seen at a mean CD_4 T cell count of 154.2 cells/µl in our study. The mean CD_4 count in patients who harboured the coccidian infections in the non-diarrhoeal cases (144.7 cells/µl) was lower as compared to those in the diarrhoeal cases (173.2 cells/ µl). *C. parvum* (32) was the most prevalent coccidian parasite, followed by *Microsporidia* (3) and *Cyclospora* (2) in patients with CD_4 counts which were less than 200 cells /µl.

Conclusions: In conclusion, our results make an important contribution to the detection and identification of coccidian parasites in the non-diarrhoeic stool samples of HIV positive patients. The maximum parasitic isolation was in the patients whose CD_4 cell counts were < 200 cells/µl. Our recommendation is that the health practitioners should receive a more intensive education on the emerging diarrhoeal pathogens and the importance of targeting these common coccidial infections while treating HIV-positive patients for opportunistic infections. The routine examination of the stool samples for coccidian parasites must be done even in the non-diarrhoeal HIV-infected individuals.

Key Words: *Intestinal coccidian parasite, Cryptosporidium parvum, Microsporidia, Cyclospora, HIV infection, Diarrhoea,CD_4 count*

KEY MESSAGE

- Opportunistic, intestinal, coccidian parasitic infections should be suspected in any HIV-infected patient with low immunity, who presents with or without diarrhoea.
- The prevalence of these parasites and their potential for compounding the health problems of the HIV-infected patients suggests that the diagnosis and the treatment of the coccidian parasites should be a part of the routine HIV care.
- The routine examination of the stool samples for coccidian parasites must be done even in the non-diarrhoeal HIV-infected individuals and in with CD_4 counts which are < 200 cells/µl.

INTRODUCTION

The Human Immunodeficiency Virus (HIV) and the Acquired Immunodeficiency Syndrome (AIDS) are the leading causes of the morbidity and mortality which are associated with infectious diseases worldwide. India is estimated to have the second largest HIV positive population in the world, with more than 5.7 million persons living with HIV/AIDS [1]. Concomitant infections play a major contributing role in the morbidity which is associated with HIV/AIDS. Effective prevention and the diagnosis and the management of the accompanying infections are critical for improving the health and the well-being of people who are infected with HIV.

Diarrhoea, which is caused by opportunistic protozoa, is one of the commonest complications which is seen in the course of the HIV disease and it is a cause of considerable morbidity and mortality. It occurs in almost 90% of the AIDS patients in the developing countries [2]. In HIV infected patients, the progressive decline in their immunological responses makes them extremely susceptible to a variety of common and opportunistic infections. In recent years, numerous studies have outlined the emergence of important gastrointestinal protozoa like *Cryptosporidium parvum*, *Isospora belli*, *Cyclospora cayetanensis*, *Microsporidia*, *Entamoeba histolytica/Entamoeba dispar* and *Giardia lamblia,* which account for a significant number of cases of diarrhoea in this population [3].

There have been a number of reports regarding the frequency of various pathogens which cause diarrhoea, from different parts of India [4]. However, there is paucity of data on the correlations of the CD_4 levels and the aetiology of diarrhoea among the HIV patients in the southern parts of Tamil Nadu. As most of the protozoan infections are treatable, it is important that an early and accurate diagnosis be made.

Thus, this study was conducted to determine the prevalence of the coccidian parasites in and around Tirunelveli in HIV positive individuals, so as to give an accurate diagnosis to avoid an empirical treatment. An attempt was also made to elucidate the associations between the CD_4 T cell counts and the presence of enteric parasites.

Aims and Objectives

- To study the prevalence of coccidian parasites in and around Tirunelveli in HIV positive individuals with or without diarrhoea.
- To determine the correlation between the CD_4 T cell counts and the presence of enteric parasites.

MATERIALS AND METHODS

Study Cases

After obtaining an approval from the institutional ethical committee and an informed consent from the patients, a total of about 103 stool samples were collected from HIV positive patients who were presented with or without diarrhoea to the Anti Retroviral Therapy (ART) Centre, Inpatients Department and the ICTC (Integrated Counselling and Testing Centre) from March 2009 to February 2010.

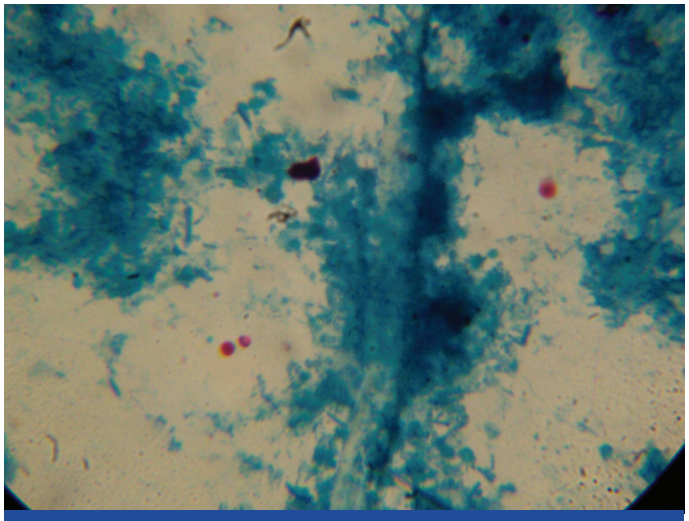
CD_4 Cell Estimation

The HIV serostatus of the patients was determined by using commercially available ELISA/Rapid Tests (J. Mitra and Co, New Delhi). The CD_4 counts were measured by using a PartecCyflow counter (Germany).

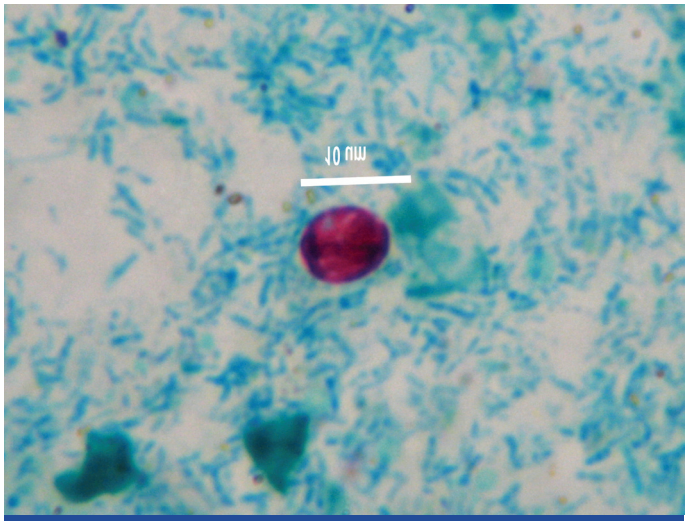
Parasitological Examinations

The stool specimens were collected from the patients at their homes in labeled, leak proof, clean, sterile plastic containers from the ICTC (Integrated Counselling and Testing Centre) and the ART centre and they were transported to the laboratory immediately. The specimens were subjected to saline mounting and modified acid fast staining, without doing the concentration technique.

The methanol fixed, direct smears were examined by modified acid fast staining for coccidian parasites at the 400x magnification by using an Olympus light microscope which was fitted with an eye-piece micrometer that had been calibrated with a stage micrometer. The oocyst sizes which were thus measured under this magnification were identified at the genus level, based on their morphometry and morphology. The coccidian oocysts stained pinkish-red against a uniform green background. The oocysts stained red and were easily recognized against a green background of yeast and faecal debris [5]. *Cryptosporidium parvum* (4–6 µm in diameter) appeared round in shape with 4 naked sporozoites within a thick-walled oocyst [Table/Fig-1] while *Cyclospora cayetanensis* (8–10 µm in diameter) was round to oval in shape [Table/Fig-2]. The microsporidial spores appeared oval in shape (1.5 µm in diameter) and stained a bright pinkish red.



[Table/Fig-1]: Oocysts of *Cryptosporidium parvum* in modified Kinyoun stain (× 1,000)



[Table/Fig-2]: Oocyst of *Cyclospora cayetanensis* in modified Kinyoun stain (× 1,000)

The statistical analysis was done by using the Chi-square test to evaluate any association between the HIV patients with or without diarrhoea and the coccidian parasitic infections. Differences in the p-values which were < 0.05 were considered as significant at 95 % confidence intervals.

RESULTS

A total of 103 stool samples from HIV seropositive patients were examined for the presence of the coccidian parasites. Among these patients, a majority were adults (99; 96%), with a male preponderance (80; 77.7%). The mean age of the HIV infected patients was 35.7 years (range 2-65 years; SD 10.3) and they consisted of 80 males and 23 females. Males had a higher prevalence

(63.8%; 51/80) of the coccidian parasites than the females (39.1%; 9/23) among the HIV-infected persons in our study. This was statistically significant (χ^2=4.452, P<0.05). Chronic diarrhoea was the presenting complaint in 33 patients, while 12 presented with undiagnosed fever, 14 with pulmonary or extra pulmonary or disseminated tuberculosis, 15 with skin lesions and 18 with other non-specific complaints, while 11 were asymptomatic. .

Only 33 (32%) of the 103 HIV seropositive patients presented with chronic diarrhoea and the rest of the 70 (68%) patients had other non-diarrhoeal complaints. Coccidian parasites were seen in 60 patients out of the 103 samples from the HIV infected individuals, thus indicating their overall prevalence of 58.2%. Coccidian parasites were detected in 40 (57.1%) patients with non-diarrhoeal complaints and in 20 (60.6%) patients with chronic diarrhoea. There was no statistically significant association of the coccidian parasites between the diarrhoeal and the non-diarrhoeal patients (P> 0.05) [Table/Fig-3].

Among the coccidian parasites, *Cryptosporidium parvum* was the predominant parasite which was found in the study (42.7%; 44/103), followed by *Microsporidia* [8 (7.8%; 8/103)] and *Cyclospora* [4 (3.9%; 4/103)], which were detected alone. Dual infections which comprised of those with *C. cayetanensis* and *C. parvum* (1.9%; 2/103) and *C. parvum* and *Microsporidia* (1.9%; 2/103) occurred in the HIV-positive patients. *Cryptosporidium spp.* was detected in 32 patients (46%; 32/70) without diarrhoea and in 12 patients (36%; 12/33) with diarrhoea (χ^2 = 0.801, P> 0.05).

Microsporidia was detected in 4 patients (5.7 %; 4/70) without diarrhoea and in 4 patients (12.1 %; 4/33) with diarrhoea (χ^2 = 1.285, P> 0.05). *Cyclospora* was detected in 3 patients without diarrhoea (4.3 %; 3/70) and in 1 patient with diarrhoea (3%; 1/33). Dual infections which comprised of those with *C. cayetanensis* and *C. parvum* were detected in 1 patient without diarrhoea (1.4%; 1/70) and in 1 patient with diarrhoea (3%; 1/33), whereas infections with *C. parvum* and *Microsporidia* were detected in 2 patients with diarrhoea (6%; 2/33) [Table/Fig-4].

In addition, after comparing the coccidian parasites which were found in the diarrhoeal and non–diarrhoeal cases on the basis of the CD_4 counts, the HIV patients were categorized into three groups. The study population consisted of 46 patients with CD_4 counts which were > 500 cells/µl, 16 patients with CD_4 counts which were 200–499 cells/µl and 41 patients with CD_4 counts which were < 200cells/µl. The mean CD_4 cell count in the non-diarrhoeal and the diarrhoeal cases was 319.3 and 313.9 cells/µl respectively. Coccidian parasites were seen at a mean CD_4 T cell count of 154.2 cells/µl. The mean CD_4 count in the patients who harboured the coccidian infections in the non-diarrhoeal cases (144.7 cells/µl) was lower as compared to that in the diarrhoeal cases (173.2 cells/µl).

The coccidian parasites could be detected in all the 41 patients (100 %) with a CD_4 count which was less than 200 cells /µl. *C. parvum* (32) was the most prevalent coccidian parasite which was detected, followed by *Microsporidia* (3) and *Cyclospora* (2) in patients with a CD_4 count which was less than 200 cells /µl. The coccidian parasites could also be detected in all the 16 patients (100 %) with a CD_4 count which was between 200-499 cells /µl. *C. parvum* (10) was the most prevalent coccidian parasite, followed by *Microsporidia* (4) and *Cyclospora* (2) in patients with a CD_4 count which was between 200–499 cells /µl. Among the 46 patients with more than 500 cells /µl, the coccidian parasites could be detected only in 3 patients (6.52 %), among which *C. parvum* was detected in 2 patients and *Microsporidia* in one patient. The proportion of the coccidian parasites in patients with a CD_4 count which was less than 200 and between 200–499 cells /µl was significantly higher than in the patients with a CD_4 count of more than 500 cells/µl. The prevalence of the coccidian parasites in HIV patients with diarrhoea, with a CD_4 count which was less than 200 cells /µl, was 31.7% (13/41) and in HIV patients without diarrhoea, with the same CD_4 counts, it was 68.2% (28/41), whereas in patients with a CD_4 count which was more than 200 cells/µl, it was 11.2 % (7/62) and 19.3% (12/62) respectively (χ^2 = 0.154, P> 0.05) [Table/Fig-5].

Coccidian parasites	HIV patients		Total (n = 103)
	diarrhoeal (n = 33)	non-diarrhoeal (n = 70)	
Positive	20 (60.6 %)	40 (57.1 %)	60 (58.2 %)
Negative	13 (39.4 %)	30 (42.9 %)	43 (41.8 %)

[Table/Fig-3]: Prevalence of the coccidian parasites among HIV patients with diarrhoeal and non-diarrhoeal complaints

Coccidian Species	HIV patients with		Total (n = 103)
	Diarrhoeal (n = 33)	Non-diarrhoeal (n = 70)	
Single infection			
*Cryptosporidium parvum**	12 (36%)	32 (46%)	44 (42.7%)
*Microsporidia spp.***	4 (12.1%)	4 (5.7%)	8 (7.8%)
Cyclospora cayetenensis	1 (3%)	3 (4.3%)	4 (3.9%)
Dual infections			
Cryptosporidium + *Cyclospora*	1 (3 %)	1 (1.4%)	2 (1.9%)
Cryptosporidium + *Microsporidia*	2 (6 %)	0 (0%)	2 (1.9%)

[Table/Fig-4]: Prevalence of coccidian species among HIV patients with diarrhoeal and non-diarrhoeal complaints

**Cryptosporidium parvum*: χ^2 = 0.801, P>0.05.
***Microsporidia spp.* : χ^2 = 1.285, P>0.05.
No significant difference observed in the prevalence rate of *Cryptosporidium and Microsporidia* among HIV patients with diarrhoeal and non-diarrhoeal complaints.

DISCUSSION

Numerous opportunistic infections occur in HIV infected patients due to the deterioration of the immune system. Among these, intestinal parasitic diseases are the commonest and are a major cause of morbidity and mortality in the HIV positive individuals worldwide. The coccidian parasites (*Cryptosporidium* spp., *Isospora belli, Cyclospora* spp. and *Microsporidium* spp.) are the foremost among the enteric parasites in these patients. The line of treatment being different for the diverse parasites, it necessitates a definitive diagnosis and a study of the aetiological agents which cause diarrhoea, especially when it can be fatal in this vulnerable group of individuals.

In the present study, the overall prevalence of the coccidian parasites was 58.2%. It was higher as compared to the findings of other similar studies which were conducted in different regions of India [6,7,8]. Males showed a higher prevalence of infection (63.8%; 51/80) with the coccidian parasites than females (39.1%; 9/23) among the HIV-infected persons in our study. This was statistically significant (P<0.05). The predominance of the male cases may be due to the migration of the males to the metropolitan cities in search of work. This finding was similar to the reports by Mohandas *et al* [3].

Coccidian species isolated	CD_4 cells< 200 cells/µl		CD_4 cells 200–499 cells/µl		CD_4cells 500 & above cells/µl		Total (n = 103)
	Diarrhoeal (n = 13)	Non-diarrhoeal (n = 28)	Diarrhoeal (n = 6)	Non-diarrhoeal (n = 10)	Diarrhoeal (n = 14)	Non-diarrhoeal (n = 32)	
Single infection							
Cryptosporidium parvum	8 (61.5 %)	24 (85.7%)	4 (66.7%)	6 (60%)	0	2 (6.3%)	44
Microsporidia spp.	1 (7.7%)	2 (7.1%)	2 (33.3 %)	2 (20%)	1 (7.1%)	0	8
Cyclospora cayetenensis	1 (7.7%)	1 (3.8 %)	0	2 (20 %)	0	0	4
Dual infections							
Cryptosporidium + *Cyclospora*	1 (7.7 %)	1 (3.8 %)	0	0	0	0	2
Cryptosporidium + *Microsporidia*	2 (15.3%)	0	0	0	0	0	2

[Table/Fig-5]: The association between diarrhoeal and non-diarrhoeal complaints, parasites isolated and CD4 counts of 103 AIDS patients. No significant difference between diarrhoeal and non-diarrhoeal cases and CD_4 counts $\chi^2 = 0.154$, P> 0.05.

There was no significant difference in the prevalence of the infection with the coccidian parasites between the diarrhoeal (60.6%) and the non-diarrhoeal patients (57.1%) in our study. In contrast, other similar studies reported a higher prevalence of the coccidian parasites in the diarrhoeic stools [6,7].

Cryptosporidium parvum was identified as the most prevalent coccidian parasite (42.7%) in the present study. Singh *et al.,* showed similar isolation rates in HIV patients [8] whereas a similar study from southern India revealed a lower *Cryptosporidium parvum* prevalence (9%) [9]. Another study which was conducted in Mumbai showed that the infection rate of the *Microsporidia* spp. in the HIV patients was 17.18% [10]. On the contrary, our study detected 7.8% of the *Microsporidia* spp. In contrary to the findings of Ballal's study, *Isospora belli* was not detected in our study [11]. This discrepancy in the findings may be attributed to the geographical variation. Dual coccidian parasitic infections which comprised those which were caused by *C. parvum* and *C. cayetanensis,* and *C. parvum* and *Microsporidia* which were detected in this study, correlated well with the previous reports [12].

Almost all the patients (57) with a CD_4 count which was less than 500 cells/µl, were found to have opportunistic coccidian parasitic infections (55.3%). The mean CD_4 count in the patients who harboured the coccidian infections in the non-diarrhoeal cases (144.7 cells/µl) was lower as compared to those in the diarrhoeal cases with the coccidian infections (173.2 cells/µl). Moreover, the presence of the coccidian parasites was also associated with a lower CD_{4+} T-cell count (154.2 cells/µl) in these patients. Although these opportunistic parasites can be acquired at any time during the course of the HIV infection, previous reports have suggested that most of the infections are established in patients with CD_{4+} T-cell counts which were less than 200 cells/µl [13].

In our study, *C. parvum* (43%) was the predominant pathogen in patients with CD_{4+} T-cell counts which were less than 200 cells/µl, which was in agreement with the findings of previous studies [6,14]. The reason for this may be that the immunodeficient patients were either more susceptible to acquire particular parasites and/or were unable to get them cleared once the infection was established. However, the mechanism by which immunodeficiency facilitated the selective establishment of certain parasites is not yet clear. Indeed, the magnitude of the impairment on the innate or acquired immunity alters the range of the pathogens to which the host is susceptible [8].

In summary, our results make an important contribution to the detection and identification of coccidian parasites in the non-diarrhoeic stool samples of HIV positive patients. Our study also showed that *Cryptosporidium parvum* was the most prevalent intestinal coccidian parasite in these patients.

Due to the easy availability of HAART in the developed nations, there has been a reduction in the prevalence of the intestinal parasites in the AIDS patients in those nations. At higher CD_{4+} T-cell levels, generally, the spontaneous clearing of the parasite takes place. In resource poor settings like ours, the patients usually go undiagnosed for long periods and present late in the course of the disease. Consequently, the patients usually present with profound, persisting and multiple intestinal infections and low CD_{4+} T-cell counts.

In conclusion, our results make an important contribution to the detection and identification of coccidian parasites in the non-diarrhoeic stool samples of HIV positive patients. Our recommendation is that the health practitioners should receive a more intensive education on the emerging diarrhoeal pathogens and the importance of targeting these common coccidial infections while treating HIV-positive patients for opportunistic infections.

A routine examination of the stool samples for the coccidian parasites even in the non-diarrhoeal HIV-infected individuals will also be of benefit significantly through the reduction of the morbidity and improvement in the quality of life. The need for intervention measures at a community level, with the purpose of reducing the risk factors of acquiring the coccidian diarrhoeal diseases, is emphasised through screening for the presence of coccidian parasites in the HIV patients with or without diarrhoea.

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AUTHOR(S):
1. Dr. Sucilathangam G.
2. Dr. Velvizhi G.
3. Dr. Palaniappan N.
4. Dr. Anna T.

PARTICULARS OF CONTRIBUTORS:
1. M.D., (Micro) Department of Microbiology, Tirunelveli Medical College, Tirunelveli, 627 011, Tamil Nadu, India.
2. M.D., (Micro) Department of Microbiology, Tirunelveli Medical College, Tirunelveli, 627 011, Tamil Nadu, India.
3. M.D., (Micro) Department of Microbiology, Tirunelveli Medical College, Tirunelveli, 627 011, Tamil Nadu, India.
4. M.V.Sc., Ph.D., (Veterinary Parasitology) Department of Veterinary Parasitology, Veterinary College and Research Institute, Namakkal, India.

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Sucilathangam G., M.D. (Micro),
Assistant Professor, Department of Microbiology,
Tirunelveli Medical College,
Tirunelveli District, 627 011, Tamil Nadu, India.
Phone: 94420 63819
E mail: drgsucila@rediffmail.com

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