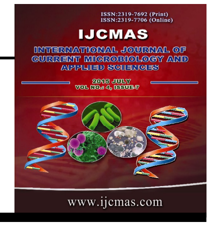

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Original Research Article

Prevalence of Intestinal Parasitic Infection among HIV Infected Patients at SRG Hospital, Jhalawar, India

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A B S T R A C T

Intestinal opportunistic parasitic infections are the major cause of diarrheal disease in Human Immunodeficiency virus (HIV) infected patients in developing countries like India. This study was used to detect enteric parasitic burden causing diarrhea and their association with immune status in HIV- seropositive patients in our area. The present study was conducted in Jhalawar Hospital and Medical College, Jhalawar between October 2013 and March 2014 involving 40 HIV positive patients and 60 HIV negative controls with and without diarrhea. Sero-status from these patients was detected by rapid test method using three tests based on different principle. Stool was examined for enteric parasitic infection by microscopy with saline and iodine mount and by special staining methods. Of the 40 HIV Positive patients, the protozoan parasitic infection was found in 35% (14/40). Out of these, parasitic infection was found in 16 (40%) and 4 (10%) cases who had or not had diarrhea respectively ($P < 0.05$). While in HIV negative Controls 46.7% and 6.7% controls were with diarrhea and without diarrhea respectively. Out of 23 patients whose CD4+ T cells were <200/µl, 17 (73.9%) had opportunistic parasitic infection whereas out of 17 patients whose CD4+ T cells were ≥200/µl, only 3 (17.6%) had opportunistic parasitic infection ($P < 0.05$). Among HIV-seropositive patients, with low CD4 T cell count, enteric opportunistic parasitic infections were very high. Early detection of these help in the timely management of diarrhea as well as improve the quality of life.

Keywords

Diarrhea,
HIV,
CD4,
T cell Count,
Opportunistic parasites

Introduction

Enteric opportunistic parasitic infections in HIV infected patients causing diarrheal disease more prevalent in developing countries. There is worrying projections of 6.5 million deaths in 2030 and in some developing countries by 2020, HIV/AIDS being the main burden of disease. Globally, the number of immuno-suppressed people increases each year, with the HIV pandemic continuing to spread unabated in many parts of the world, with an estimated 14,000 new infections occurring daily (Mathers *et al.*, 2006).

The progressive decline in immune system (CD4 count), ultimately turn into AIDS; usually result in morbidity and mortality due to opportunistic bacterial, viral, parasitic and fungal infections (Durack, 1981). Gastrointestinal infections such as diarrhea or dyscentry are very common in patients with HIV / AIDS, occurs in 30-60 % and 90 per cent of AIDS patients in developed countries and developing countries respectively (Mannheimer *et al*., 1994).

The incidence and prevalence of enteric parasite depend upon the endemicity of parasite in the community (Janoff *et al*., 1988), such as *Cryptosporidium parvum*, *Isospora belli, Microsporidia and Cyclospora cayetanensis*, documented in patients with AIDS (Mukhopadya *et al*., 1999; Cegieslki *et al*., 1994; Sapkota *et al*., 2004; Scharschmidt *et al*., 1999; Ortega *et al*., 2003; Wurtz *et al*., 2006).

Non opportunistic parasites such as *Entamoeba histolytica*, *Giardia lamblia, Trichuris trichiura, Ascaris lumbricoides, Strongyloides stercoralis* and *Ancylostoma duodenale* are commonly encountered in developing countries, not considered opportunistic in AIDS patients (Janoff *et al*., 1988; Ramakrishnan *et al*., 2007).

C. parvum, I. belli and *E. histolytica*, are the most frequent organisms found in HIV infected individuals with diarrhea from India as well as other parts of the world (Wurtz *et al*., 1993; Mohandas *et al*., 2002; Kulkarni *et al*., 2009). Hence the current study was conducted to determine the prevalence of enteric opportunistic parasitic infections among HIV-seropositive patients and controls with and without diarrhea along with the correlation of CD4 count in Jhalawar Hospital and Medical College, Jhalawar, Rajasthan.

Clinico-epidemiology of the opportunistic enteric parasites

Cryptosporidium parvum, a eucoccidian parasite, in 1907 first identified by Tyzzer in the gastric mucosa of laboratory mouse (Parija , 2013), diagnosed first in a homosexual man with AIDS in New York in August 1981 (Ma *et al*., 1983) . *C. parvum* and *C.hominis,* are the species most commonly associated with human cryptosporidiosis, with 97% identical genomes but their host range is strikingly different. *C. parvum* primarily a zoonotic infection, infects humans and animals, whereas *C. hominis,* not detected in animals (Joachim ,2004).

Isospora belli, first described by Virchow, 1860, in villi of intestinal mucosa at autopsy (Cox , 2002), an AIDS defining illness if infection persists for more than 4 weeks (DeHovitz *et al*., 1986).

Cyclospora cayatenensis, noted first by Eimer in 1870 in the intestine of moles (Cox , 2002) but 1st described in 1977 in Papua, New Guinea, found first in patient with AIDS at Haiti in 1983 (Wurtz , 1994). For *C. cayatenensis*, humans are the only known host. With progressive immune suppression (CD4 counts < 200 cells/μl in HIV infected individuals), relapses last for 4 to 7 weeks occur, resulting in severe malnutrition associated with morbidity and mortality (Wurtz , 1994; Santana *et al*., 2000).

Microsporidia, in 1857 reported in silkworms, first case reported in a Japanese boy exposed to farm animal, by Hisakichi Matsubayashi in 1959 (Cox, 2002; Joseph *et al*., 2005). First case in HIV infected patients, reported in 1985 due to *Enterocytozoon bieneusi* (Weber *et al*., 1994), and from India, in 2001, in a patient with diarrhea. (Joseph *et al*., 2005)

The term microsporidia, general nomenclature for the obligate intracellular parasites belonging to the phylum microsporidia, over 1300 species belonging to 160 genera, infecting a wide range of vertebrate and invertebrate hosts. Fourteen species have been identified as human pathogens, include *Anncalia algerae*, *Anncalia connori*, *A.vesicularum*, *Encephalitozoon cuniculi*, *E. hellem*, *E. intestinalis* (syn. *Septata intestinalis*), *E. bieneusi*, *Microsporidium ceylonensis*, *M. africanum*, *Nosema ocularum*, *Pleistophora ronneafiei*, *Trachipleistophora hominis*, *T. anthropophthera*, and *Vittaforma corneae*.

E. bieneusi, most common microsporidian in humans, 2nd most prevalent etiology for diarrhea in immuno-suppressed patients, after *Cryptosporidium* (Weber *et al*., 1994). Human microsporidiosis occurs mainly in severely immunocompromised patients with AIDS, causing dissemination to the hepatobiliary system along with cholangitis, to the maxillary sinus (invasive sinusitis) and to the respiratory system (pulmonary infections) in *E. bieneus*i / HIV co-infection, most common cause of disseminated microsporidiosis. Among *E. cuniculi*, *E. hellem*, and *E. intestinalis* causing human infections, *E. intestinalis* being usually associated with enteric disease (Asmuth, 1994).

The genus *Entamoeba*, amoeboid protozoan parasite, includes six species (*Entamoeba histolytica*, *E. dispar*, *E. moshkovskii*, *E. polecki*, *E. coli*, and *E. hartmanni*). *E. histolytica*, *E. dispar*, and *E. moshkovskii* are capable of infecting the intestinal lumen of humans, are morphologically identical, but genetically different (Parija, 2013). Recent studies have reported *E. moshkovskii* as an enteropathogen, in patients with gastrointestinal symptoms but no adequate studies examining the pathogenic potential of this organism in immuno-suppressed groups and so there is a need to assess the true pathogenicity of this organism (Fotedar *et al*., 2007).

Giardia intestinalis, flagellated protozoan parasite, worldwide distributed, burden of disease remains high in developing countries, in immuno-compromised individuals, especially in children < 10 years of age (Parija , 2013). With progressive reduction in CD4 counts, symptomatic *Giardia* infection is increased (Gazzard, 2009). Complications include steatorrhea leading to malabsorption and weight loss.

Almost 80% of sexually active gay men carry *E. dispar, E. coli, E. hartmanni, Iodamoeba butschlii, Dientamoeba fragilis,* and *G. intestinalis*, as compared to its incidence in the general population of 13%.

Strogyloides stercoralis, the ovoviviparous nematode, first demonstrated in French soldiers with diarrhea in Cochin war (Grove, 1996), causes intractable diarrhea with blood and mucus, mainly in association with HIV-1. Complications include hyperinfection syndrome, respiratory distress, a life threatening condition, if untreated. Overall prevalence of opportunistic enteric parasites in India varies from 7.5% to 73.3%.

Methods

This study was carried out in Jhalawar Hospital and Medical College, Jhalawar between October 2013 and March 2014. Ethical approval was taken from ethical committee of the institute. A total of 40 HIV seropositive patients and 60 HIV negative controls with and without diarrhea participated in the study after giving their consent and provided two consecutive stool samples. Before collection of samples, patient information such as name, age, sex, occupation, family history, literacy, mode of transmission, clinical history as well as history of diarrhea, antibiotic and antiparasitic treatment history was obtained.

An exclusion criterion was patients already on antiparasitic and antibiotic treatment.

Five ml of blood samples was collected in plain vial. Serum samples were used for HIV testing. HIV sero-status of the patients was determined by using commercially available rapid antibody tests (SD Bioline, Signal and Tridot, J Mitra & Co., India).

Stool specimen was collected in clean wide mouth, leak proof, plastic containers from each patient, were examined microscopically for ova, cysts, oocyst, or parasites, by normal saline and iodine mount preparation, preserved in 10% formal saline. Preserved samples were concentrated using formal-ether concentration methods and examined for Oocyst of *Cryptosporidium spp*, *I. belli*, and *C. cayetanensis* finally identified using modified Ziehl-Neelsen technique (Wurtz *et al.*, 1993; WHO, 1991; Sherchand *et al.*, 2001).

The data was analyzed using Chi square (x2) test and appropriate statistical software packages.

Ethical consideration

An informed consent was sought from a cross-section of outpatients (HIV positive, and negative control) attending Jhalawar Hospital and Medical College, Jhalawar before blood and stool samples were collected.

Results and Discussion

Of the 40 HIV positive patients, the protozoan parasitic infection was found in 35% (14/40). Out of 40 patients, parasitic infection was found in 16 (40%) and 4 (10%) cases having diarrhea or not having diarrhea respectively. A significant difference ($p<0.05$) was observed. While in HIV negative Controls 46.7% and 6.7% were with diarrhea and without diarrhea respectively as shown in Table no. 1.

Opportunistic parasitic infection and it's correlation with CD4 count

In the study, Out of 23 patients whose CD4 T cell count was <200/μl, 17 (73.9%) had opportunistic parasitic infection whereas out of 17 patients whose CD4 T cell count was ≥200/μl, only 3 (17.6%) had opportunistic parasitic infection ($P < 0.05$) as shown in Table no. 2.

Enteric opportunistic parasitic infections, remains a problematic task regarding morbidity and mortality, in developing countries like India, in HIV-infected persons presenting with and without diarrhea (WHO, 1981). The World Health Organization 2006 defines diarrhea wasting syndrome in HIV-seropositive patients with etiology could either be due to parasites, bacteria, fungal, virus or HIV itself (WHO, 2006; Guerrant *et al.*, 1990).

The detection of common intestinal parasites in both patients and controls, a reflection of the poor environmental sanitation and personal hygienic practices, emphasize the need for interventional measures to reduce the risk factors of acquiring intestinal parasites in immuno-compromised patients at the community level. Diarrhea, one of the most prevalent manifestations among AIDS patients, associated with presence of intestinal parasites (Feitosa *et al.*, 2001). In tropical countries, chronic diarrhea that begins acutely lasts for > 4 weeks, associated with weight loss, often the first presenting symptoms of HIV infected individuals.

In the present study the enteric parasites were detected in 50% and 26.7% from the samples in HIV + and HIV negative patients respectively. There was significant difference in the infection of opportunistic parasites among HIV- seropositive cases with diarrhea 16/20 (80%) and without diarrhea 4/20 (20%).

There are number of studies reported from India and other countries, with prevalence of intestinal parasites 25 to 50 % which are near to our findings (Prasad *et al.*, 2000; Brink *et al.*, 2000; Gomez *et al.*, 1995). In this study 23 HIV seropositive patients had CD4 count < 200 / µl with gastroenteritis and the infection of opportunistic parasites was 17 (73.9%). Cryptosporidium parvum was the predominant pathogens followed by Isospora belli, Strogyloides stercoralis, Entamoeba histolytica, Giardia lamblia (Dwivedi *et al.*, 2007; Attilli *et al.*, 2006; Wiwanitkit, 2001). Cellular immunity, major defense against intestinal parasitic infections, predisposes to opportunistic infections following reduction in CD4 count of HIV infected patients (Mohandas *et al.*, 2002; Lee *et al.*, 2005). In our study, CD4 count <200 /µl found a significantly higher prevalence of protozoan parasitic infections ($p<0.05$).

C. parvum, predominant opportunistic parasitic infection, found in our studies as compared with other study (Kumar *et al.*, 2002; Call *et al.*, 2000; Goodgame, 1996), in both diarrhea and non-diarrhea cases indicates high risk of infection.

Isospora belli was found to be the major cause of morbidity in symptomatic diarrhea, commonly seen in chronic diarrhoeal patients with HIV-AIDS in developing countries ranges 12- 20%. These finding are similar to other studies, conducted is different part of world in HIV infected patients (Mukhopadya *et al.*, 1999; Cegieslki *et al.*, 1994; Kumar *et al.*, 2002).

Entamoeba histolyitca was also detected in our study so must not be neglected otherwise. (Scharschmidt *et al.*, 1999; Ramakrishnan *et al.*, 2007; Gomez *et al.*, 1995; Dwivedi *et al.*, 2007; Wiwanitkit, 2001)

Difference in the incidence of intestinal protozoal parasitic infection, attributed to the difference in geographical distribution of parasites, sanitary practices, and level of education, economic status, social behavior and different selection cases. (Mohandas *et al.*, 2002; Attilli *et al.*, 2006; Goodgame, 1996; Chhin *et al.*, 2006; Weber *et al.*, 1992)

Although mixed infection is seen in HIV-AIDS patients, but in our study not observed any such findings, reason for the same could not ascertain. Our study was limited by small sample size, number of stool samples analyzed / person, patients referred from ICTC already received antibiotics prior to their visit so number of symptomatic patients was less. The trends observed in this study provide the impetus for future in-depth studies of possible immunologic and epidemiologic associations between HIV infection and intestinal parasites.

Moreover, study report on opportunistic parasitic infection among HIV seropositive patients and CD4 count in our area is very scarce and there is no representative baseline information.

Hence, it is important to investigate further to determine the rate of infection with enteric opportunistic parasite in HIV-AIDS patients in other area of Rajasthan, provide the level of endemicity in the state. Skilled manpower and laboratory support, the most important part, required to investigate the carrier, latent and clinical infection as it is difficult to diagnose by untrained person.

Table.1 Distribution of parasitic infection among HIV sero-positive patients

Parasitic species	HIV Positive			HIV Negative			P Value
	Cases with diarrhea n= 20	Cases without diarrhea n= 20	Total no. of cases n= 40	Cases with diarrhea n= 30	Cases without diarrhea n= 30	Total no. of cases n= 60	
Giardia lamblia	1	1	2	3	1	4	0.06
Entamoeba histolytica	2	0	2	4	1	5	0.07
Cryptosporidium parvum	5	1	6	0	0	0	-
Isospora belli	3	1	4	0	0	0	-
Ascaris lumbricoides	1	0	1	2	0	2	0.03
Hookwoom species	1	1	2	4	0	4	0.06
Strongyloides stercoralis	3	0	3	1	0	1	0.04
Total	**16 (80%)**	**4 (20%)**	**20 (50%)**	**14 (46.7%)**	**2 (6.7%)**	**16 (26.7%)**	

Table.2 Opportunistic parasitic infections and CD4+ T count among HIV sero-positive patients

Characteristics		No. Of tested		No. of infection (%)	
		HIV Positive	HIV Negative	HIV Positive	HIV Negative
Gender	Male	29 (72.5%)	47 (78.3%)	16 (55.2%)	13 (27.7%)
	Female	11 (27.5%)	13 (21.7%)	4 (36.4%)	3 (23.1%)
Clinical symptoms	Diarrhea:	20	30	16 (80%)	14 (46.7%)
	Non-diarrhea	20	30	4 (20%)	2 (6.7%)
CD4 Count (cells/µl) of HIV sero-positive patients	< 200	23 (57.5%)		17 (73.9%)	
	> 200	17 (42.5%)		3 (17.6%)	

Stool sample examination with modified acid fast staining method after concentration method will help to investigate the existence of opportunistic parasitic infection.

In view of AIDS explosion in India, opportunistic enteric parasites are becoming increasingly important, to be identified properly using skilled manpower. Advances in the diagnosis of infectious diseases occur regularly, but the first mode of diagnosis is still by light microscopy of stool by an experienced microscopist. Commercially available fecal immunoassays, widely available for the majority of enteric protozoa, provide a cost effective alternative to traditional diagnosis of protozoan enteric parasites, are rapid, easy to use and more practical for most laboratories than PCR assays. The presence of parasites is normally overlooked in cases of diarrhea, as the provision of appropriate technology in diagnostic laboratories is not yet available. A confirmatory test such as PCR may be run subsequently.

References

Asmuth, D.M. 1994. Microsporidia and diarrhea in AIDS patients, Clin Microbiol Newsl., 16:179‑86.

Attilli, S.V., et al. 2006. Diarrhoea, CD4 counts and enteric infections in a hospital-based cohort of HIV-infected patients around Varanasi, India, *BMC Infect Dis*., 6: 39- 44.

Brink, A.K., et al. 2000. Diarhhoea, CD4 counts and enteric infections in a community-based cohort of HIV-infected adults in Uganda, *J Infect Dis*., 45: 99-106.

Call, S.A., et al. 2000. The changing etiology of chronic diarrhea in HIV patients, with CD4 less than 200/mm3, *Am J Gastroenterology.*, 95: 3142-46.

Cegieslki, J.P., et al. 1994. Cryptosporidium, Enterocytozoon and Cyclospora infection to pediatric and adult patients with diarrhea in Tanzania, *Clin Infect Dis.*, 24: 214-221.

Chhin, S., et al. 2006. Etiology of chronic diarrhea in antiretroviral-naïve patients with HIV infection admitted to Norodom-Sihanouk Hospital, Phnom Penh, Cambodia, Clinical infectious diseases., 43: 925-932.

Cox, F.E. 2002. History of human parasitology, Clin Microbiol Rev.,15:595-612.

DeHovitz, J.A., et al. 1986. Clinical manifestations and therapy of *Isospora belli* infection in patients with the acquired immunodeficiency syndrome, N Engl J Med.,315:87‑90.

Durack, D.I. 1981. Opportunistic infections and Kaposis sarcoma in homosexual men, *New Engl J Med.*, 305: 1465-1467.

Dwivedi, K.K., 2007. Enteric opportunistic parasites among HIV-infected individuals: associated risk factors and immune status, *Jpn J Infect Dis.*, 60: 76-81.

Feitosa, G., et al. 2001. High prevalence of giardiasis and strongyloidiasis among HIVinfected patients in Bahia, Brazil, *BJID*., 5(6): 339-44.

Fotedar, R., et al. 2007. Laboratory diagnostic techniques for Entamoeba species, Clin Microbiol Rev.,20:511‑32.

Gazzard, B. 2009. AIDS and the gastrointestinal tract, Medicine.,37:357‑60.

Gomez, M.A, et al. 1995. Opportunistic and non-opportunistic parasitic in HIV positive and negative patients with diarrhea in Tanzania, *Trop Med Parasitio*l., 46: 109-114.

Goodgame, R.W. 1996. Understanding intestinal spore forming protozoa: Cryptosporidia, Microsporidia, Isospora and Cyclospora, *Ann Intern Med.*, 124: 429-41.

Grove, D.I. 1996. Human strongyloidiasis, Adv Parasitol., 38:251‑309.

Guerrant, R.L., et al. 1990. Diarrhea in developed and developing countries: magnitude, special settings, and etiologies, *Rev Infect Dis.*, 12: S41–S50.

Janoff, E.N., Smith, P.D. 1988. Prospectives on gastrointestinal infections in AIDS, *Gastroenterol Clin North Am.*, 17: 451-463.

Joachim, A. 2004. Human cryptosporidiosis: An update with special emphasis on the situation in Europe, J Vet Med B Infect Dis Vet Public Health.,51:251‑9.

Joseph, J., et al. 2005. Microsporidia: Emerging ocular pathogens, Indian J Med Microbiol., 23:80‑91.

Kulkarni, S.V., et al. 2009. Opportunistic parasitic infections in HIV/ AIDS patients presenting with diarrhea by the level of immunosuppression, *Indian J Med Res.*, 130: 63-66.

Kumar, S.S., et al. 2002. Intestinal parasitic infection in HIV patients with diarrhea in Chennai, *Indian J Med Microbial.*, 20: 88-91.

Lee, J.K., et al. 2005. Prevalence of diarrhea caused by Cryptosporidium parvum in non-HIV patients in Jeollanam-do, Korea, *Korean J Parasitol.*, 43: 111-

114.

Ma, P., Soave, R. 1983. Three‑step stool examination for cryptosporidiosis in 10 homosexual men with protracted watery diarrhea, J Infect Dis., 147:824‑8.

Mannheimer, S.B., Soave, R. 1994. Protozoal infections in patients with AIDS. Cryptosporidiasis, Cyclosporiasis and Microsporiasis, *Infect Dis Clin North Am.,* 8: 483-498.

Mathers, C.D., Loncar, D. 2006. Projections of global mortality and burden of disease from 2002 to 2030, PLoS Med., 3:e442.

Mohandas, K., et al. 2002. Prevalence of intestinal parasitic pathogens in HIV-seropositive individuals in Northern India, *Jpn J Infect Dis*., 55: 83-4.

Mukhopadya, A., et al. 1999. Enteric pathogens in southern Indian HIV infected patients with and without diarrhea, *Indian J Med Res*., 109: 80-89.

Ortega, Y.R., Arrowood, M. 2003. Cyclospora and Isospora, In Murray PR(ED) Manual of Clinical Microbiology, 8th ed., p2008-2016.

Parija, S.C. 2013. In Textbook of Medical Parasitology: Protozoology and Helminthology, 4th ed. New Delhi, All India Publishers and Distributors.

Prasad, K.N., et al. 2000. Identification of enteric pathogens in HIV-positive patients with diarrhea in northern India, *J Health Popul Nutr*., 18: 23-26.

Ramakrishnan, K., et al. 2007. Prevalence of intestinal parasite infestation in HIV/AIDS patients with diarrhea in Madurai South India, *Jpn J Infect Dis.,* 60: 209-10.

Santana Añé, M., et al. 2000. Emergence of a new pathogen: Cyclospora cayetanensis in patients infected with human immunodeficiency virus, Rev Cubana Med Trop., 52:66‑9.

Sapkota, D.A., et al. 2004. Enteric parasitosis in patients with HIV infection and AIDS in Nepal, *J Nep Health Res Council.,* 2: 1-5.

Scharschmidt, Fieldman. 1999. Protozoal infections in patients with AIDS. Cryptosporidiosis, isosporiasis, cyclosporiasis, and microsporidiosis, *Infect Dis Clin North Am.,* 8: 483-98.

Sherchand, J.B., Cross, J.H. 2001. Emerging pathogen Cyclospora cayetanensis infection in Nepal, *Southeast Asian J Trop Med Public Health.,* 32: 143–150.

Weber, R., et al. 1992. Improved light microsporidial detection of Microsporidia spores in stool and duodenal aspirate.The Enteric Opportunistic Infections Working Group, *N Engl J Med.,* 326: 161-166.

Weber, R., et al. 1994. Human microsporidial infections, Clin Microbiol Rev., 7:426‑61.

WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults aged 15 years or older, 2006. SEARO Publications on HIV/AIDS., http://www.searo.who.int

WHO. 1981. Intestinal protozoan and helminthic infections. WHO Technical Report Series 666 Geneva, WHO.

Wiwanitkit, V. 2001. Intestinal parasitic infections in Thai HIV infected patients with different immunity status, *BMC Gastroenterol*., 1: 3-5.

World Health Organization. Basic laboratory methods in Medical Parasitology. 1991. Geneva: World Health Organization., 9-31.

Wurtz, R. 1994. Cyclospora: A newly identified intestinal pathogen of humans, Clin Infect Dis., 18:620‑3.

Wurtz, R.M., et al. 1993. Clinical characteristics of seven cases of diarrhea associated with a novel acid fast organsism in stool, *J Infec Dis.,* 16: 136-138.