# Research Article

# Prevalence of *Cryptosporidium* species in human faecal specimens in Minia Governorate, Egypt.

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### Abstract

For evaluation of the prevalence of *Cryptosporidium* spp. infection in diarrheic humans in Egypt, fecal specimens from diarrheic (n = 300) were collected and examined for the presence of *Cryptosporidium* spp. oocysts. The presence of *Cryptosporidium* spp. oocysts was determined by Ziehl- Neelsen acid-fast staining. The results showed that the overall prevalence of infection in all 300 samples was 44.7%. *Cryptosporidium* oocysts were detected either alone (37.3%) or mixed with other parasitic infection (7.3%) with the use of Modified Zeil-Neelsen stain. results indicate that *Cryptosporidium* spp. infection is prevalent in diarrheic humans in Egypt.

Key Words: Cryptosporidium spp, Modified Zeil-Neelsen stain, diarrhia

## Introduction

Cryptosporidium species is one of the infectious zoonotic protozoan parasites that has been recognized as a human pathogen since 1976 (Fayer and Ungar, 1986). Cryptosporidium spp. is the cause diarrhea in a wide range of mammalian spp. (Priest et al., 2006). The disease can affect both immunocompetent and immunocompromised individuals causing a wide spectrum of diseases ranging from asymptomatic carrier state to severe diarrhea. Infection with this parasite results in severe but selflimiting diarrhea in immunocompetent and often lethal diarrhea in immunocompromised individuals (Chen et al., 2002). Cryptosporidium spp. is a primary pathogen causing acute diarrhea and the most evident symptom of cryptosporidiosis is diarrhea. Non-specific signs, such as dehydration, fever, anorexia, weakness, and progressive loss of conditions may be accompanied. There are a variety of methods, including microscopy, immunological and molecular techniques. for the detection of Cryptosporidium oocysts. Microscopic methods include concentration techniques and staining of fecal smears. There are difficulties in distinguishing Cryptosporidium oocysts from other small particles, such as yeasts,

moulds, algae, and plant debris by routine fecal examination techniques in fecal and environmental specimens (Fayer et al., 2000). The modified acid-fast staining technique is useful and the oocysts appear as pink to red, spherical to ovoid, bodies on a blue or purple background. The stained smears are permanent and can be stored for a long time before examination when the samples are high numbers (Sevinc et al., 2003). The purpose of this study was to determine the prevalence of Cryptosporidium spp. infection in different age groups of diarrheic humans in Egypt by the conventional microscopy method using the modified acid-fast staining.

## Materials and methods Subjects and fecal examination

Diarrheic fecal samples (300 in total number) were collected in sterile plastic containers from inpatient and outpatient clinics of University Hospitals and Tropical hospital, Minia District, Egypt, who presented with diarrhea associated with nausea, vomiting, abdominal pain and fever during the period from June 2016 to May 2017. All participants provided verbal approval before participation in the study. Information on potential risk factors for infections was gathered by using structured questionnaires. These factors included source of water, presence of animals (dogs, chicken, ducks, guinea pigs, rabbits, parrots, and sheep).

Samples were subjected to:-

Macroscopic examination to identify color and odor, blood and mucus, round worms, thread worms, or tapeworm proglottids, and consistency. Microscopic by: a- Saline wet mount smear (Melvin and Brooke, 1982): to detect worm eggs or larvae protozoan trophozoites, and cysts, and the presence of RBCs and WBCs, b- Iodine wet mount (Garcia et al, 1983) for glycogen and nuclei of protozoan cysts. c- Water-ether technique (Smith, 2007) and d- stained with Modified Acid Fast (Casemore et *al*, 1985).

## Results

The results are shown in tables (1 & 2).

**Table 1:** Intestinal parasitic infections identified by microscopic examination of stool samples

Parasites	No.	Percentage
Cryptosporidium spp. oocysts	134	44.7%
Blastocystis hominis	60	20%
Entamoeba histolytica cysts	44	14.7%
Giardia lamblia cysts	20	6.7%
Entamoeba coli cysts	16	5.3 %
Hymenolepis nana egg	12	4%
Cyclospora cayetanensis oocysts	12	4%
Isospora belli oocysts	4	1.3%

Table 2: Results of single and mixed parasitic infection

	No	Percentage
Cryptosporidium spp. only	112	37.3%
Cryptosporidium spp.with other parasites	22	7.3%
Parasites other than <i>Cryptosporidium</i> spp.	106	35.3%
No parasite	60	20%
Total	300	100.0%

#### Discussion

The objective of this study was to determine the prevalence of Cryptosporidium spp. infection in diarrheic humans in Minia governorate, Egypt. In study prevalence this the of Cryptosporidium spp. in all specimens was (44.7%). This comes in accordance with Gabr and others who reported a prevalence rate 61% in Minia city (Gabr et al., 2014). where, Abdel-Hafeez et al., (2012) reported a rate of 42.2%, 60.2% in immunocompetent and immunosuppressed individuals respectively (Abdel-Hafeez et al., 2012)

There are discrepancies in the prevalence between different surveys, which were done

on human cryptosporidiosis. In Egypt the prevalence of cryptosporidiosis varied according to the localities. For example, Hassan et al., (2002) reported the incidence of Cryptosporidium spp. was 37.7% and 91% in immunodeficient children and adult patients, respectively. Moreover, Antonios et al., (2010), Shoukry et al., (2009), Helmy et al., (2013) and Mousa et al., (2010) reported 33.3%, 33.3%, 49.1% and 31.1% positive rates of human cryptosporidiosis, respectively. The prevalence rates were 15% in chronic renal failure patients in Zagazig, Egypt (Ali et al., 2000), 23.5% in Abo El-Resh Hospital (Naser et al., 2017) and 19.5% in Benha (Abdel-Maboud et al., 2000).

Countries other than Egypt with high prevalence rate of *Cryptosporidium* spp. infection (34.6%) was Indonesia (Kurniawan et al., 2009a). Furthermore, a study in India stated that the prevalence rate in AIDS was 77.5% (Uppal et al., 2014)

Low prevalence rates were reported in Kenya and Isfahan, Iran as the prevalence rate of cryptosporidiosis in children was 4% (Gatei et al., 2006, Saneian et al., 2010).

The discrepancy in infection rates is expected due to many causes such as immune state of patients, patients' age, environmental habitats or seasonal variation, sample size, virulence of different isolates of the parasite (El-Badry et al., 2015). The intermittent excretion of the parasite oocyst in stool may be another factor (Adamu et al., 2006).

In the present study the *Cryptosporidium* oocysts were detected either alone (37.3%) or mixed with other parasitic infection (7.3%) with the use of Modified Zeil-Neelsen stain. This agreed the detection of mixed infections of 21.7% (5/23) in positive patients (Abd El Kader et al, 2012). No doubt, *Cryptosporidium* is a common pathogen present in cases with mixed infections. This agreed with Philips et al., (1992) in London (Phillips et al., 1992), but in Jakarta and Ethiopia mixed infection occurred in 7% and 2.6% respectively (Kurniawan et al., 2009b; Wegayehu et al., 2011).

Mixed parasitic infection detected by Zeil-Neelsen Modified stain (7.3%)(Cryptosporidium spp. with other parasites) may be explained by the fact that an already established parasite within the host may create an environment suitable for flourishing of other parasites. Furthermore, over crowdedness, poor hygienic conditions, bad habits and contaminated water supply may be other causes (MacKenzie et al., 1995).

# References

 Abd-El Kader, N. M., Blanco, M.-A., Ali-Tammam, M., Abd El Rahman, B., Osman, A., El Sheikh, N., Rubio, J. M.& de Fuentes, I. (2012) Detection of *Cryptosporidium parvum* and *Cryptosporidium hominis* in human patients in Cairo, Egypt. Parasitol Res 110 (1), 161-166.

- Abdel-Hafeez, E. H., Ahmad, A. K., Ali, B. A.& Moslam, F. A. (2012) Opportunistic parasites among immunosuppressed children in Minia District, Egypt. K J P 50(1),57-62.
- Abdel-Maboud, A. I., Rossignol, J. F., El-Kady, M. S., Mostafa, M. S.& Kabil, S. M. (2000) Cryptosporidiosis in Benha, study of some recent modalities in diagnosis and treatment. J Egypt Soc Parasitol 30 (3), 717-725.
- 4. Adamu, H., Endeshaw, T., Teka, T., Kifle, A.& Petros, B. (2006) The prevalence of intestinal parasites in paediatric diarrhoeal and nondiarrhoeal patients in Addis Ababa hospitals, with special emphasis on opportunistic parasitic infections and with insight into the demographic and socio-economic factors. Ethiop J Health Dev 20 (1), 39-46.
- Ali, M. S., Mahmoud, L. A., Abaza, B. E.& Ramadan, M. A. (2000) Intestinal spore-forming protozoa among patients suffering from chronic renal failure. J Egypt Soc Parasitol 30 (1), 93-100.
- Antonios, S. N., Tolba, O. A., Othman, A. A. & Saad, M. A. (2010) A preliminary study on the prevalence of parasitic infections in immunocompromised children. J Egypt Soc Parasitol 40 (3), 617-630.
- Casemore, D. P., Armstrong, M.& Sands, R. L. (1985) Laboratory diagnosis of cryptosporidiosis. J Clin Pathol 38 (12), 1337-41.
- Chen, XM, Keitely, JS, Paya, CV, Larusso, N F, 2002: Cryptosporidiosis. N. Engl. J. Med. 346, 1723-31.
- El-Badry, A. A., Al-Antably, A. S. A., Hassan, M. A., Hanafy, N. A.& Abu-Sarea, E. Y. (2015) Molecular seasonal, age and gender distributions of *Cryptosporidium* in diarrhoeic Egyptians: distinct endemicity. Eur J Clin Microbiol 34 (12), 2447-2453.
- 10. Fayer R, Morgan U, Upton SJ (2000) Epidemiology of *Cryptosporidium*:

Prevalence of *Cryptosporidium* species in human faecal specimens in Minia Governorate, Egypt.

transmission, detection and identification. *Int J Parasitol* 30: 1305-1322.

- 11. Fayer R, Ungar BLP (1986) *Cryptosporidium* spp. and cryptosporidiosis. *Microbiol Rev* 50: 458-483.
- Gabr, N. S., Abdellatif, M. Z. M., El-Hafeez, E. H. A.& Rabou, R. A. M. A. (2014) Comparison between Elisa and Various Stains Techniques in Laboratory Diagnosis of Cryptosporidiosis. J Egypt Soc Parasitol 44 (2), 509-516.
- 13. Garcia, LS, Bruckner, DA, Brewer, TC, Shimizu, RY, 1983: Techniques for the recovery and identification of Cryptosporidium oocysts from stool specimens. J.Clin.Microbiol.18:185-90
- 14. Gatei, W., Wamae, C. N., Mbae, C., Waruru, A., Mulinge, E., Waithera, T., Gatika, S. M., Kamwati, S. K., Revathi, G. & Hart, C. A. (2006) Cryptosporidiosis: prevalence, genotype analysis, and symptoms associated with infections in children in Kenya. Am J Trop Med Hyg 75 (1), 78-82.
- Hassan, S. I., Sabry, H., Amer, N. M., Shalaby, M. A., Mohamed, N. A.& Gaballah, H. (2002) Incidence of cryptosporidiosis in immunodeficient cancer patients in Egypt. J Egyptian Soc Parasitol 32 (1), 33-46.
- Helmy, Y. A., Krucken, J., Nockler, K., von Samson-Himmelstjerna, G.& Zessin, K. H. (2013) Molecular epidemiology of *Cryptosporidium* in livestock animals and humans in the Ismailia province of Egypt. Vet Parasitol 193 (1-3), 15-24.
- Kurniawan, A., Dwintasari, S. W., Soetomenggolo, H. A.& Wanandi, S. I. (2009a) Detection of *Cryptosporidium* sp infection by PCR and modified acid fast staining from potassium dichromate preserved stool. Med J Indonesia 18 (3), 149.
- Kurniawana, A, Karyadib, T, Dwintasaria, SW, Sari, IP, Yunihastutib, E, et al., 2009b: Intestinal parasitic infections in HIV/ AIDS patients presenting with diarrhea in Jakarta, Indonesia. J. Roy. Soc. Trop. Med. Hyg, 103: 892-8.
- 19. MacKenzie, W. R., Schell, W. L., Blair, K. A., Addiss, D. G., Peterson,

D. E., Hoxie, N. J., Kazmierczak, J. J. & Davis, J. P. (1995) Massive outbreak of waterborne *cryptosporidium* infection in Milw-aukee, Wisconsin: recurrence of illness and risk of secondary transmission. Clin Infect Dis 21 (1), 57-62.

- 20. Melvin, D. M.& Brooke, M. M. (1982) Laboratory procedures for the diagnosis of intestinal parasites, US Dept. of Health and Human Services, Public Health Service, Centers for Disease Control, Laboratory Improvement Program Office, Laboratory Training and Consultation Division.
- Mousa, K. M., Abdel-Tawab, A. H., Khalil, H. H.& El-Hussieny, N. A. (2010) Diarrhea due to parasites particularly *Cryptosporidium parvum* in great Cairo, Egypt. J Egypt Soc Parasitol 40 (2), 439-450.
- 22. Naser, M. E., Younis, M. S., Bayoumi, I. R., Eraky, M. A., Aly, N. S.& El Attar, R. E. O. (2017) Evaluation of nanogold-beads-based enzyme-linked immunosorbent assay for detection of *cryptosporidium* antigen in stool samples of diarrheic patients. Benha Med J 34 (2), 88.
- 23. Phillips, AD, Thomas, AG, Walker-Smith, JA, 1992: *Cryptosporidium*, chronic diarrhoea and the proximal small intestinal mucosa.Gut33:1057-61
- 24. Priest JW, Bern C, Xiaol, Roberts JM, et al., 2006: Longitudinal analysis of *Cryptosporidium* species-specific IgG antibody responses in Peruvian children. Clin. Vac. Immunol. 13, 1:123-31.
- Saneian, H., Yaghini, O., Yaghini, A., Modarresi, M. R.& Soroshnia, M. (2010) Infection Rate of *Cryptosporidium parvum* among Diarrheic Children in Isfahan. Iran J Pediatr 20 (3), 343-7.
- 26. Sevinc F, Irmak K, Sevinc M (2003) The prevalence of *Cryptosporidium parvum* infection in the diarrhoiec and non- diarrheic calves. *Revue Méd Vét* 154: 357-361.
- 27. Shoukry, N. M., Dawoud, H. A.& Haridy, F. M. (2009) Studies on zoonotic *cryptosporidiosis parvum* in

Prevalence of *Cryptosporidium* species in human faecal specimens in Minia Governorate, Egypt.

Ismailia Governorate, Egypt. J Egypt Soc Parasitol 39 (2), 479-488.

- Smith, H.V. (2007) Diagnosis of human and livestock cryptosporidiosis. *Cryptosporidium* and cryptosporidiosis 2.
- Uppal, B., Singh, O., Chadha, S.& Jha, A. K. (2014) A comparison of nested PCR assay with conventional techni-

ques for diagnosis of intestinal cryptosporidiosis in AIDS cases from northern India. J Parasitol Res 2014.

 Wegayehu, T, Adamu, H, Petros, B, 2011: Prevalence of *Giardia duodenalis* and *Cryptosporidium species* infections among children and cattle in North Shewa Zone, Ethiopia. BMC Infect. Dis. 13:419-24.