Journal of the Egyptian Society of Parasitology, Vol.52, No.1, April 2022
J. Egypt. Soc. Parasitol. (JESP), 52(1), 2022: 117 - 122
(Online: 2090-2549)

CRYPTOSPORIDIOSIS: MOLECULAR ANALYSIS, RISK FACTORS AND SEASONAL ABUNDANCE IN IMMUNOCOMPETENT AND IMMUNO-COMPROMISED PATIENTS, KAFRELSHEIKH UNIVERSITY HOSPITALS

By
MARWA M.I. GHALLAB[1], MOUSA A. M. ISMAIL[2], TAMER HAYDARA[3], And SALWA M. MORSY[4*]
Departments of Medical Parasitology, Faculty of Medicine, Kafrelsheikh University[1], Departments of Medical Parasitology, Faculty of Medicine, Cairo University[2], Departments of Internal Medicine[3], Faculty of Medicine, Kafrelsheikh University, and Departments of Medical Parasitology[4], Faculty of Medicine, Cairo University, and Modern University for Technology and Information.
(*Correspondence: smmorsy@kasralainy.edu.eg)

Abstract

This study detected of the molecular *Cryptosporidium* species in immunocompetent and immunocompromised adults with diarrhea attended Kafrelsheikh University Hospitals and to assess risk factors. Stool samples from 200 immunocompetent & 200 immunocompromised were examined using acid fast (AF) stain and PCR/restriction fragment length polymorphism (RFLP) targeting small subunit (SSU) rRNA gene. *Cryptosporidium* PCR amplified was 15(7.5%) in immunocompetent and 23 (11.5%) immunocompromised, with significance difference as to age, presence of mucous, abdominal pain ($P<0.05$). *Cryptosporidium* was more common in rural areas in autumn and winter seasons. Two genotypes were *C. hominis* and *C. parvum*, but *C. hominis* was 60% in immunocompetent and 82.6% in immunocompromised.

Key words: Patients, immunocompetent, immunocompromized, Cryptosporidiosis, SSU gene, RFLP.

Introduction

Zoonotic species and genotypes of *Cryptosporidium* are transmitted from animal hosts to humans, and non-zoonotic species & genotypes are host-adapted without evidence of transmission from animals to man. *Cryptosporidium parvum* (formerly known as *C. parvum* genotype II) and *C. homin is* (formerly known as *C. parvum* genotype I) are leading causes of human cryptosporidiosis. *C. meleagridis, C. felis, C. canis, C. ubiquitum, C. cuniculus, C. viatorum*, Chipmunk genotype I, *Cryptosporidium* mink genotype, & *C. muris* also infect humans (CDC, 2019). Cryptosporidiosis manifests with abdominal pain and diarrhea similar to that of choleric infection. In the immunocompromised hosts, the parasite causes prolonged infections that can also be fatal, and thus considered one of riskiest opportunistic infections for patients with acquired immunodeficiency syndrome (Gerace *et al,* 2019). However, it can likely be underestimated, since the diarrhea usually resolves without any treatment (Bouzid *et al,* 2013). Anyhow zoonotic cryptosporidiosis was reported in Egypt by many authors (El-Dessouky and El-Masry, 2005; El-Sherbini and Mohammad, 2006: Youssef *et al*, 2008; El-Khodery and Osman, 2008; Mahfouz *et al,* 2014; Abouel-Nour *et al,* 2016, El Bahnasawy *et al,* 2018; El-Missiry *et al,* 2019; Hamza *et al,* 2020 and others).

Thirty-nine species of *Cryptosporidium* were identified, but not all cause human disease, and the majority of human infection is caused by anthroponotic *Cryptosporidium hominis* or zoonotic *C. parvum*. Cryptosporidiosis was included among the neglected diseases initiative, that served to raise awareness for both international and national measures in disease prevention and control (Morris *et al*, 2019).

Diagnosis of cryptosporidiosis is usually made by microscopically identification of oocysts of 4-6µm in diameter in the stool of infected subjects (Khurana and Chaudhary, 2018) However, since the detection of *Cryptosporidium* oocysts can be difficult, three fecal samples collected on separate days should be microscopically examined to det-

ect the oocysts prior to exclude the *Cryptosporidium* infection in subjects with severe diarrhea (Mohammad *et al,* 2021). Routine laboratory diagnosis of cryptosporidial infection by coproscopy and/or copro-immunoassay had many limitations, thus molecular techniques were used for species and genotypic differentiation as the PCR/restriction fragment length polymorphism (RFLP) analysis or sequence analysis (Ghallab *et al*, 2016).

Seasonality of many infectious diseases, including cryptosporidiosis, as socio-demographic distribution, behavioral characters and environmental factors were the main drivers of disease seasonality in transmission (El-Badry *et al*, 2015).

The present study aimed to determine the *Cryptosporidium* species among a cohort of immunocompetent and immunocompromised patients with diarrhea using PCR/RFLP analysis of small subunit (SSU) rRNA gene and to evaluate seasonal pattern and socio-demographic data as risk factors.

Material and Methods

Four hundreds diarrheic patients (200 immunocompetent & 200 immunocompromised) with diabetic, renal, hepatic failure, and/or on immuunosuppressive drugs attended Internal Medicine Outpatient Clinic, Kafrelsheikh University Hospitals were selected from January to December 2019. Medical sheets were filled out on each patient with a designed questionnaire.

Morning fecal samples were collected. A part was fixed in formalin saline for microscopic examination as wet mount or modified Ritchie's biphasic stain & permanent AF staining (Garcia, 2007). Second one was stored at -20^{o}C for copro-molecular assays.

Extraction of the genomic DNA was done by using QIAamp Stool Mini Kit (Qiagen, Germany) according to the manufacturer's instructions after 5 freeze/throw cycles; the obtained DNA yield was measured using spectrophotometer.

Cryptosporidium was genotyped by nested PCR amplification of an approximately 830 bp fragment of SSU rRNA gene using primers for primary PCR; 18s (f1): TTCTAG AGCTAATACATGCG, 18s (R1): CCCAT TTCCTTCGAA & 18s(f2): GGA AGGGTT GTATTTATTAGATAAAG, 18s(R2): AAG GAGTAAGGAACAACCTCCA for second reaction. Standard nested PCR targeting the 18S rDNA was done (Jiang *et al*, 2005) with mild modifications as the annealing temperature for the primary reaction was 55^{o}C & 60^{o}C for secondary one. *C. parvum* and *C. hominis,* RFLP analysis of amplified PCR products by VspI was carried out after the manufacturer's instructions. Amplified PCR and digested products were electrophoresed on 2% ethidium bromide-stained Agarose gel and visualized by the ultra-violet trans-illuminator.

Statistical analysis: Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data was recorded using frequencies (numbers) and relative frequencies (%). Chi square ($\chi 2$) test was performed for comparing categorical data, P-values less than 0.05 were considered as statistically significant.

Ethical statement: The study protocol was approved by the Ethics Committee Board, Kafrelsheikh University, according the rules of Helsinki 2000.

Results

PCR amplified *Cryptosporidium* was 15 (7.5%) & 23(11.5%) in immunocompetent & immunocompromised respectively. AF stain detected 11(5.5%) in immunocompetent & 18(9%) in immuno-compromised. Infection was common in rural areas in autumn and winter, with significance difference in age, mucous, abdominal pain, residence, & seasonal variation, with flatulence difference in immunocompromised ($P < 0.05$).

PCR/RFLP showed 2 genotypes: 9/15 (60%) with genotype I (*C. hominis*), 6/15 (40%) genotype II (*C. parvum*) in immunocompetent &19/23 (82.6%) with *C. hominis*, 4/23(17.4) *C. parvum* in immunocompromised. Details are in tables (1, 2 & 3).

Table 1: Socio demographic, clinical and seasonal data among groups

Variations		Immunocompetent (N= 200)		Immunocompromized (N= 200)	
		Count	%	Count	%
Age	12-20	46	23.0%	68	34.0%
	21-40	125	62.5%	94	47.0%
	41-60	29	14.5%	38	19.0%
Sex	Male	99	49.5%	99	49.5%
	Female	101	50.5%	101	50.5%
Blood	Yes	12	6.0%	21	10.5%
	No	188	94.0%	179	89.5%
Mucus	Yes	26	13.0%	32	16.0%
	No	174	87.0%	168	84.0%
Abdominal pain	Yes	136	68.0%	137	68.5%
	No	64	32.0%	63	31.5%
Flatulence	Yes	63	31.5%	75	37.5%
	No	137	68.5%	125	62.5%
Vomiting	Yes	60	30.0%	64	32.0%
	No	140	70.0%	136	68.0%
Rural/Urban	Rural	67	33.5%	79	39.5%
	Urban	133	66.5%	121	60.5%
Season	Spring	53	26.5%	52	26.0%
	Summer	83	41.5%	79	39.5%
	Autumn	20	10.0%	24	12.0%
	Winter	44	22.0%	45	22.5%

Table 2: Microscopy, AF stain and copro-PCR results:

Variations		Immunocompetent (N= 200)		Immunocompromized (N=200)	
		Count	%	Count	%
Microscopy	*Blastocystis hominis*	9	4.5%	9	4.5%
	Entamoeba coli	1	0.5%	0	0.0%
	Entamoeba histolytica	7	3.5%	5	2.5%
	Giardia lamblia	11	5.5%	7	3.5%
	Cystoisospora belli	1	0.5%	8	4.0%
	No	171	85.5%	171	85.5%
AF stain	Yes	11	5.5%	18	9.0%
	No	189	94.5%	182	91.0%
PCR	Yes	15	7.5%	23	11.5%
	No	185	92.5%	177	88.5%

Table 3: Relation between *Cryptosporidium* and symptoms, urban/rural and seasonal variability in both groups:

PCR positive cases		Immunocompetent (N= 15)		P value	Immunocompromised (N= 23)		P value
		Count	%		Count	%	
Age	12-20	0	0.0%	0.022	9	13.2%	0.840
	21-40	14	11.2%		10	10.6%	
	41-60	1	3.4%		4	10.5%	
Sex	male	6	6.1%	0.444	12	12.1%	0.785
	female	9	8.9%		11	10.9%	
Blood	yes	2	16.7%	0.224	5	23.8%	0.074
	no	13	6.9%		18	10.1%	
Mucus	yes	6	23.1%	0.006	9	28.1%	0.004
	no	9	5.2%		14	8.3%	
Abdominal pain	yes	15	11.0%	0.003	20	14.6%	0.043
	no	0	0.0%		3	4.8%	
Flatulence	yes	5	7.9%	1	14	18.7%	0.014
	no	10	7.3%		9	7.2%	
vomiting	yes	5	8.3%	0.774	9	14.1%	0.436
	no	10	7.1%		14	10.3%	
Residence	rural	10	14.9%	0.005	17	21.5%	< 0.001
	urban	5	3.8%		6	5.0%	
Season	spring	2	3.8%	< 0.001	2	3.8%	< 0.001
	summer	0	0.0%		0	0.0%	
	autumn	9	45.0%		15	62.5%	
	winter	4	9.1%		6	13.3%	

Discussion

In the present study, the PCR analysis showed a rate of 7.5% & 11.5% in immunocompetent and immunocompromised respectively, but AF stain detected 5.5% in immunocompetent and 9% in immunocompromised patients. The reported prevalence of cryptosporidiosis varied between 0.0% and 47% among studies carried out in immunocompetent patients of various ages in several Egyptian areas (Helmy *et al,* 2013). Distribution of *C. hominis* and *C. parvum* in man varied by geographic region, as *C. hominis* tends to predominate in most parts of the world, especially in developing countries, but *C. parvum* was more frequent in the Middle East and both species were common in the Europe (Xiao, 2010)

In immunocompetent adult, Sanders *et al*. (2005) and Abd El Kader *et al*. (2012) detected cryptosporidiosis in 5.7% & 4.6% respectively, slightly higher results (8.3%) was detected by. Rezk *et al*. (2001).

Much lower results was detected in another study in Egypt where 2.5 and 3.3% detection rate in patient with liver diseases (Ramadan *et al*, 2015; Abo-Mandil *et al*, 2020). But, higher results were 22%, 30%, 32% & 36% Shrestha *et al*, 1993), and Hassan *et al*. (2002) detected 91 % in cancer patients.

The increased susceptibility to cryptosporidiosis in chronic diseases may be due to the influence of impaired cellular immune response in these patients making them more susceptible to several opportunistic infections, including *Cryptosporidium* spp. (Mousa *et al*, 2014).

In current study, there were significance difference between cryptosporidiosis and ages with high prevalence among group 21-40 years old. This may be related to sociodemographic differences, contact with contaminated resources and/or occupational risks. Also, there was correlation between the presence of mucous, abdominal pain and flatulence in cryptosporidial infected patients. This agreed with Chauret *et al*. (1999) who reported that gastrointestinal symptoms are a well-known risk factor for cryptosporidiosis.

In the present study, when comparing rates between urban and rural cases, a clear difference is documented with significantly higher rates within the rural population than the urban one. This agreed cryptosporidiosis infection in England, Paris and Switzerland, which showed high prevalent in rural areas (Lake *et al*, 2009; Mons *et al*, 2009; Fuchslin *et al*, 2012) this may be due to contaminated water resources with domestic animals and/or directly contact with farm animals and agriculture wastes, socioeconomic status, occupational risk, sanitary conditions, and higher population densities with a risk of person-to-person transmission as well (Castro-Hermida *et al*, 2008).

In the present study, *Cryptosporidium* transmission occurred via autumn and winter seasons only. This agreed with Sulaiman *et al*. (2005), Natividad *et al*. 2008) and Adamu *et al*. (2010) who reported that cryptosporidiosis was common in rainy and/ or cool seasons but, in Egypt *Cryptosporidium* was detected in Spring (El-Shazly *et al*, 2007; El-Badry *et al*, 2015), as well in USA (Yoder and Beach, 2007)

Difference in *Cryptosporidium* genotypes was related to the differences of infection sources as, human strain predominance was attributed to water resources contamination from different human activities with person-to-person transmission, while the bovine strain was related to the agricultural practices related to calving and contamination of the water supply from the main reservoir; young livestock (Lal *et al*, 2012).

In this study, human strain was predominating in immunocompetent and immunocompromised. This agreed with Tumwine *et al*. (2003), Gatei *et al*. (2003), Abd El Kader *et al*. (2012); Helmy *et al*. (2013) who found that *C. hominis* was more prevalent than *C. parvum*. But, Sulaiman *et al*. (2005), Youssef *et al*. (2008); Eida *et al*. (2009) reported predominance of the *C. parvum.*

Conclusion

Molecular prevalence is the gold standard to diagnose cryptosporidiosis. The commonest *Cryptosporidium* among the immunocompetent and immunocompromised patients was *C. hominis* especially in rural areas during rainy and cool seasons.

Efforts to develop vaccine are limited by insufficient understanding of the immune responses mediating protection. Consequently, early diagnosis is the best to treat infection, molecular techniques data clarify crypotosporidiosis epidemiology to suggest feasible control programs.

References

Abd El-Kader, NM, Blanco, MA, Ali-Tammam, M, Abd El Ghaffar, AERB, Osman, A, *et al,* 2012: Detection of *Cryptosporidium parvum* and *Cryptosporidium hominis* in human patients in Cairo, Egypt. Parasitol Res. 110, 1:161-6.

Abouel-Nour, MF, El-Shewehy, DMM, Hamada, SF, Morsy, TA, 2016: The efficacy of three medicinal plants; garlic, ginger and mirazid and a chemical drug metronidazole against *Cryptosporidium parvum*: ii- Histological changes. J. Egypt. Soc. Parasitol. 46, 1:185-200

Adamu, H, Petros, B, Hailu, A, Petry, F, 2010: Molecular characterization of *Cryptosporidium* isolates from humans in Ethiopia. Acta Trop. 115, 1/2:77-83

Bouzid, M, Hunter, PR, Chalmers, RM, Tyler, KM, 2013: Cryptosporidium pathogenicity and virulence. Clin. Microbiol. Rev. 26, 1:115-34

Castro-Hermida, JA, Garcia, I, Almeida, A, González, M, Da Costa, JM, *et al,* 2008: Presence of *Cryptosporidium* spp. and *Giardia duodenalis* through drinking water. Sci. Total Environ. 405:45-53.

CDC, 2019: Cryptosporidiosis**.** www.cdc.gov/parasites/.

Chauret, C, Springthorpe, S, Sattar, S, 1999: Fate of *Cryptosporidium* oocysts, *Giardia* cysts, and microbial indicators during wastewater treatment and anaerobic sludge digestion. Can. J. Microbiol. 45, 3:257-62**.**

Eida, AM, Eida, MM, El-Desoky, A, 2009: Pathological studies of different genotypes of human *Cryptosporidium* Egyptian isolates in experimentally mice. J. Egypt. Soc. Parasitol. 39, 3: 975-90.

El-Badry, AA, Abdel Aziz, IZ, Shoeib, EY, Ghallab, MMI, 2017: *Cryptosporidium* genotypes and associated risk factors in a cohort of Egyptian children. Comparat. Clin. Pathol. 2*6*, 5: 1017-21.

El-Badry, AA, Al-Antably, ASA, Hassan, M A, Hanafy, NA, Abu-Sarea, EY, 2015: Molecular seasonal, age and gender distributions of *Cryptosporidium* in diarrhoeic Egyptians: distinct endemicity. Europ J Clin Microbiol Infect Dis. 34, 12:2447-53.

El-Bahnasawy, MMM, Morsy, ATA, Morsy, TA, 2018: A mini-overview on zoonotic cryptosporidiosis. J. Egypt. Soc. Parasitol. 48, 1:35-44.

El-Dessouky, SA, El-Masry, NM, 2005: Effect of *Cryptosporidium parvum* infection on the haematology and blood chemistry of buffalo calves with special reference to the prevalence of infection in adult buffaloes. Assiut Vet. Med. J. 51: 105-23.

El-Khodery, S, Osman S, 2008: Cryptosporidiosis in buffalo calves (*Bubalus bubalis*): Prevalence & potential risk factors. Trop. Anim. Hlth. Prod. 40:419-26.

El-Missiry, AG, Abd El-Hameed, L, Saad, G, El-Badry, AA, **Helmy**, **YA, 2019:** Molecular genetic characterization of human *Cryptosporidium* isolates and their respective demographic, environmental and clinical manifestations in Egyptian diarrheic patients PUJ 12, 3:187-96.

El-Shazly, AM, Elsheikha, HM, Soltan, DM, Mohammad, KA, Morsy, TA, 2007: Protozoal pollution of surface water sources in Dakahlia Governorate, Egypt. J. Egypt. Soc. Parasitol. 37, 1:51-64.

El-Sherbini, G, Mohammad, K, 2006: Zoonotic cryptosporidiosis in man and animal in farms, Giza Governorate, Egypt. J. Egypt. Soc. Parasitol. 36, 1:49-58.

Fuchslin, HP, Kotzch, S, Egli, T, 2012: *Cryptosporidium* spp. in drinking water: samples from rural sites in Switzerland. Swiss Med. Wkly. 142:13683.

Garcia, LS, 2007: Diagnostic Medical Parasitology, 5th ed. ASM Press, Washington DC.

Gatei, W, Greensill, J, Ashford, RW, Cuevas, LE, Parry, CM, *et al,* 2003: Molecular analysis of the 18S rRNA gene of *Cryptosporidium* parasites from patients with or without human immunodeficiency virus infections living in Kenya, Malawi, Brazil, the United Kingdom, and Vietnam. J. Clin. Microbiol. 41:1458-62

Gerace, E, Lo Presti, VDM, Biondo, C, 2019: *Cryptosporidium* Infection: Epidemiology, pathogenesis, and differential diagnosis. Eur. J. Microbiol. Immunol (Bp). 9, 4:119-23.

Ghallab, MI, Aziz, IZA, Shoeib, EY, El-Badry, AA, 2016: Laboratory utility of coproscopy, copro immunoassays and copro nPCR assay targeting Hsp90 gene for detection of *Cryptosporidium* in children, Cairo, Egypt. JOPD 40, 3: 901-5.

Hamza, DA, Soliman, SM, Salem, NY, Nader, SM, 2020: Bovine Cryptosporidiosis and the Egyptian diarrheic buffalo calves. Int. J. Vet. Sci. 9, 2:448-52

Hassan, SI, Sabry, H, Amer, NM, Shalaby, M A, Mohamed, NA, *et al,* 2002: Incidence of cryptosporidiosis in immunodeficient cancer patients in Egypt. J. Egypt. Soc. Parasitol. 32, 1:33-46.

Helmy, YA, Krücken, J, Nöckler, K, von Samson-Himmelst, G, Zessin, KH, 2013: Molecular epidemiology of *Cryptosporidium* in livestock animals and humans in the Ismailia province of Egypt. Vet. Parasitol. 193, 1/3:1524

Jiang, J, Alderisio, KA, Xiao, L, 2005: Distribution of *Cryptosporidium* genotypes in storm event water samples from three watersheds in N. Y. Appl. Environ. Microbiol. 71, 8: 4446–54.

Khurana S, Chaudhary P, 2018: Laboratory diagnosis of cryptosporidiosis. Trop. Parasitol. 8, 1:2-7.

Lake, IR, Nichols, G, Harrison, FD, Bentham, G, Sari, R, *et al,* 2009: Using infectious intestinal disease surveillance data to explore illness etiology: A cryptosporidiosis case study. Hlth. Place 15: 333-9.

Lal, A, Hales, S, French, N, Baker, MG, 2012:

Mahfouz, ME, Mira, N, Amer, S, 2014: Prevalence and Genotyping of *Cryptosporidium* spp. in farm animals in Egypt. J. Vet. Med. Sci. 76, 12:1569-75.

Mohammad, SM, Ali, MS, Abdel-Rahman, S A, Moustafa, RA, Sarhan, MH, 2021: Genotyping of *Cryptosporidium* species in children suffering from diarrhea in Sharkyia Governorate, Egypt. J Infect Develop Countr. 15, 10:1539-46.

Mons, C, Dumetre, A, Gosselin, S, Galliot, C, Moulin, L, 2009: Monitoring of *Cryptosporidium* and *Giardia* river contamination in Paris area. Water Res. 43:211-7.

Morris, A, Robinson, G, Swain, MT, Chalmers, RM, 2019: Direct Sequencing of *Cryptosporidium* in stool samples for public health. Front

Mousa, N, Abdel-Razik, A, El-Nahas, H, *et al,* 2014: Cryptosporidiosis in patients with diarrhea and chronic liver diseases. J. Infect. Dev. Ctries. 8, 12:1584-90.

Natividad, FF, Buerano, CC, Lago, CB, Mapua, CA, De Guzman, BB, *et al*, 2008: Prevalence rates of *Giardia* and *Cryptosporidium* among diarrheic patients in the Philippines. Southeast Asian J. Trop. Med. Publ. Hlth. 39, 6:991-9. Publ. Hlth. 7, 11:360-4.

Ramadan, M, Ramadan, M, Yousef, M, 2015: *Cryptosporidium* infection in patients with acute on chronic liver failure (ACLF). Pluralidade 2, 4:102-15.

Rezk, H, El-Shazly, AM, Soliman, M, El-Nemr, HI, Nagaty, IM, *et al,* 2001: Coccidiosis among immuno-competent and -compromised adults. J. Egypt. Soc. Parasitol. 31, 3:823-34.

Sanders, JW, Putnam, SD, Gould, P, Kolisnyk, J, Merced, N, *et al,* 2005: Diarrheal illness among deployed U.S. military personnel during Operation Bright Star 2001-Egypt. Diagn. Microbiol. Infect. Dis. 52, 2:85-90.

Seasonality in human zoonotic enteric diseases: a systematic review. PLoS One 7, 4:31883

Shrestha, S, Larsson, S, Searched, J, *et al,* 1993: Bacterial and cryptosporidial infection as the cause of chronic diarrhea in patients with liver disease in Nepal. Trop. Gastroenterol. 14:55-8.

Sulaiman, IM, Hira, PR, Zhou, L, Al-Ali, FM, Al-Shelahi, FA, *et al,* 2005: Unique endemicity of cryptosporidiosis in children in Kuwait. J. Clin. Microbiol. 43, 6:2805-9.

Tumwine, JK, Kekitiinwa, A, Nabukeera, N, Akiyoshi, DE, Rich, SM, *et al,* 2003: *Cryptosporidium parvum* in children with diarrhea in Mulago Hospital, Kampala, Uganda. Am. J. Trop. Med. Hyg. 68:710-5.

Xiao, L, 2010: Molecular epidemiology of cryptosporidiosis: an update. Exp. Parasitol. 124, 1: 80-9.

Yoder, JS, Beach, MJ, 2007: Cryptosporidiosis surveillance-United States, 2003–2005. MMWR Surveill Summ. 56, 7:1-10

Youssef FG, Adib I, Riddle MS, Schlett CD, 2008: A review of cryptosporidiosis in Egypt. J. Egypt. Soc. Parasitol. 38, 1:9-28

Youssef, FG, Adib, I, Riddle, MS, Schlett, C D, 2008: A review of cryptosporidiosis in Egypt. J. Egypt. Soc. Parasitol. 38, 1:9-28.