Journal of the Egyptian Society of Parasitology, Vol. 47, No. 2, August 2017
J. Egypt. Soc. Parasitol. (JESP), 47(2), 2017: 333- 340

CRYPTOSPORIDIUM AMONG ASYMPTOMATIC FOOD HANDLERS

By
AFAF A. TAHA*, FATEN A. MOHAMMED and SABAH M. A. MOHAMED
Department of Medical Parasitology, Faculty of Medicine, Zagazig University, Egypt (*Correspondence: drafaf343@yahoo.com)

Abstract

Cryptosporidiosis in food handlers is an important public health problem. *Cryptosporidium* infection is transmitted by ingestion of *Cryptosporidium* oocysts through consumption of a contaminated food or water. Workers in food sector can play a role in cryptosporidiosis outbreak. This study aimed to detect the existence of *Cryptosporidium* among asymptomatic food handlers using microscopical examination and commercially available antigen capture ELISA. Stool samples collected from asymptomatic 237 food handlers, aged from 17-50 years old and worked at different branches of food sector within Zagazig and its surrounding. Fecal samples were examined microscopically to detect *Cryptosporidium* oocysts by modified Ziehl-Neelsen stain (MZN) and by ELISA for detection of *Cryptosporidium* copro-antigen. Among 237 food handlers asymptomatic cryptosporidiosis has been detected in 10 (4.2%) by MZN, 12 (5%) by ELISA and 13(5.5%) by both techniques. Out of 13 asymptomatic infected food handlers, three of them worked as fruits/vegetables sellers, two at restaurant, three butchers, two as fast food workers, one at Canteen, Café and two at supermarket. These findings indicate that food handlers can be a source of cryptosporidiosis infection. Thus, searching for the existence of asymptomatic cryptosporidiosis food handlers and taking the required measures in case of its determination are helpful in prevention of probable morbidity and protection of consumer health.

Keywords: Asymptomatic; *Cryptosporidium;* Food handlers.

Introduction

Cryptosporidiosis is a gastrointestinal illness caused by protozoon parasite of the genus *Cryptosporidium* (Chen *et al*, 2002). In healthy persons, cryptosporidiosis is manifested by watery diarrhea, which may by associated with abdominal cramps, nausea, vomiting, loss of appetite, low-grade fever, and weight loss but asymptomatic infection occurs frequently (Huang and White, 2006). In the immunocompromised patients *Cryptosporidium* causes an opportunistic infection, which may progress to cholangitis or pancreatitis (Hunter and Nichols, 2002).

Cryptosporidium oocysts are infectious immediately upon being excreted in feces and infection transmitted by ingestion of these oocysts through consumption of contaminated food or water or through direct person-to-person or animal-to-person. Ingestion of as few as 10–30 oocysts can cause infection in healthy persons (DuPont *et al*, 1995 and Okhuysen *et al*, 1999). An infected person can excrete 10^8–10^9 oocysts in the single bowel movement and oocysts can be excreted for up to 50 days after cessation of diarrhea (Chappell *et al*, 1996 and Jokipii and Jokipii, 1986).

Foodborne diseases are large problems in developed and developing countries. The spread of Cryptosporidiosis by food handlers is a common and persistent problem worldwide (Andargie *et al*, 2008; Zain and Naing 2002). Food handlers with poor personal hygiene working in the food service settings can be infected by different enteropathogens (Takalkar *et al*, 2010), causing fecal contamination of foods by their hands during food preparation, and may be implicated in the transmission of many infections to the public in the local community (Kaferstein and Abdussalam 1999).

The prevention of probable morbidity due to cryptosporidiosis and the protection of consumer health depend mainly on proper screening procedure for food handlers. As *Cryptosporidium* can cause extended urgent medical care and even death, cryptosporidiosis outbreak has importance in respect of public health (Quiroz *et al*, 2000). Persistent asymptomatic oocysts shedding can prolong following the clinical infection (Stehr-Green *et al,* 1987).

Cryptosporidium spp. is one of the parasitic protozoa relevant to food production (Dawson, 2005). Moreover, Schlundt *et al.* (2004) indicated *Cryptosporidium spp* among the five most important emerging food-borne zoonotic pathogens. Workers in food sector can play a role in the cryptosporidiosis outbreak (Quiroz *et al,* 2000).

Modified Ziehl-Neelsen staining method is a technique widely used for staining of *Cryptosporidium* oocysts in fecal smears (Casemore, 1991; Insulander *et al,* 2008). ELISA for *Cryptosporidium parvum* antigen in stool samples had been developed in several laboratories and some are commercially available (Rosenblatt and Sloan, 1993; Newman *et al,* 1993). The technical properties of ELISA mean that many specimens can be processed and read by a single technician in a short period of time, thereby maintaining a level standard. Therefore the ELISA proved potentially more suitable than microscopy in epidemiological surveys and in follow up examinations of patients known to be *Cryptosporidium* positive.

This study aimed to detect the existence of *Cryptosporidium* among asymptomatic food handlers by using microscopic examination and commercially available antigen capture ELISA.

Materials and Methods

Study area and period: The study was carried out on 237 asymptomatic food handlers of different occupational categories 213 male and 24 female within Zagazig and its surroundings from May 2012 to May 2015.

Study design: A cross sectional study design was conducted among asymptomatic food handlers.

Study population: A total of 237 food handlers aged from 17-50 years old. They worked at different lines (25 fruits/ vegetables sellers, 13 at bakery, 43 at restaurant, 35 butchers, 40 fast food workers, 23 at Canteen, Café and 58 at supermarket) within Zagazig and its surrounding.

Exclusion criteria: Food handlers who have diarrhea, fever, taking antibiotics, antihelminthics were excluded from the study.

Ethical aspects: An informed written consent was obtained from each one shared in the study. The purpose and procedures were explained to each one according to the Ethics Committee of Faculty of Medicine, Zagazig University.

A structured questionnaire was used to collect data on age, sex, educational level, income and the hygienic status of each study subject.

From all food handlers, three successive stool specimens were collected in suitable clean labeled wide-mouthed plastic containers. A portion of every fresh stool sample was stored immediately at −20◦C and tested later by ELISA. All stool samples were examined macroscopically and microscopically for detection of *Cryptosporidium* oocyst. Each sample was concentrated by formol-ether sedimentation technique (Cheesbrough, 2009), and then stained using Modified Ziehl-Neelsen stain: In this method thin smears were prepared from preserved as well as sediments of concentrated stool samples, air dried, and fixed with absolute methanol for 5 minutes. The smears were stained with carbol-fuchsin for 30 minutes and thereafter, washed with tap water. The slides were decolorized in acid alcohol for 2 minutes and were counter stained with methlyene blue for another 2 minutes. Finally the stained smears were examined using oil immersion objective to detect oocysts of *Cryptosporidium* (Adegbola *et al,* 1994).

Stored samples were tested by a commercially available antigen capture ELISA designed for the detection of *Cryptosporidium* oocyst (Ridascreen *Cryptosporidium:* RBiopharm, Darmstadt, Germany), which was performed according to the manufacturer's instructions.

Statistical analysis: Data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version 16.0. The chi-square test was used to determine the relationship between positive cases, and other parameters such as age, sex, residence, water supply and animal contact. P value <0.05 was considered significant.

Results

Sample was considered positive if oocysts could be detected by MZN staining method or ELISA. The range of patient's ages was 17-50 years, 213 males & 24 females. Of 237 stool samples of food handlers, *Cryptosporidium* oocysts were detected in 10 (4.2%) by MZN and in 12 (5%) by ELISA (Tab. 1). There was 13(5.5%) positive samples by both tests, three of them (12%) worked as fruits/vegetables sellers, two (4.6%) at restaurant, three (8.5%) were worked as butchers, two (5%) as fast food workers, one (4.3%) at Canteen, Café and two (3.4%) at supermarket (Tab. 2). They were eleven male workers and two female workers, eleven were from rural and two from urban areas, seven had history of animal contact and six had no history of animal contact and four had history of using well water while nine had history of using tap water (Tab. 3).

Table 1: MZN and ELISA for Cryptosporidiosis in food handler stool samples

Method used		Modified Ziehl-Neelsen		
		Positive	*Negative*	Total
	Positive	9	3	12
ELISA	Negative	1	224	225
	Total	10	227	237

Table 2: *Cryptosporidium* infection among asymptomatic food handlers

Test / Occupation	No	MZN No +ve	MZN %	ELISA No +ve	ELISA %	Total +ve No +ve	Total +ve %
Fruits/vegetables sellers	25	2	8%	3	12%	3	12%
Bakery	13	0	0%	0	0%	0	0%
Restaurant workers	43	2	4.6%	2	4.6%	2	4.6%
Butchers	35	3	8.5%	2	5.7%	3	8.5%
Fast food workers	40	1	2.5	2	5%	2	5%
Canteen, Café	23	0	0%	1	4.3%	1	4.3%
Supermarket staff	58	2	3.4%	2	3.4%	2	3.4%
Total	237	10	4.2%	12	5%	13	5.5%

Table 3: *Cryptosporidium* in asymptomatic food handlers according to age, sex, residence, water supply and animal contact

Variable		Examined samples	No +Ve	%	χ^2	P
Age	17-25 y	73	4	5.5%	1.098	0.778
	25-35 y	85	5	5.9%		
	35-50 y	79	4	5.1%		
Sex	Male	213	11	5.2%	0.418	0.518
	Female	24	2	8.3%		
Residence	Rural	180	11	6.1%	0.092	0.761
	Urban	57	2	3.5%		
Water Supply	Tap water	225	9	4%	18.9	000
	Well water	12	4	33.3%		
Animal contact	No	178	6	3.4%	6.16	0.013
	Yes	59	7	11.9%		

Discussion

Food contamination may occur at any point during its journey through production, processing, distribution, and preparation. The risk of food getting contaminated depends largely on the health status of the food handlers, their personal hygiene, knowledge and practice of food hygiene (Mudey *et al*, 2010). Infection of asymptomatic persons, especially workers dealing with food (food handlers), could become a potential cause of dissemination of variety of pathogens including intestinal parasites (Jones and Angulo, 2006). *Cryptosporidium* is particularly difficult to control because they are resistant to chlorine disinfection, persist in the environment for a longer period, infect other animal hosts, and are difficult to diagnose and treat (WHO, 1993). As *Cryptosporidium* does not multiply in food, food-borne cryptosporidiosis occur via unhygienic food preparation, storage, preliminary preparation or food processing either through direct contamination by infected individuals during preparation or through fecal contamination of food (such as usage of contaminated water or biosolid, infected employee (Yoder and Beach, 2010).

In this study, from 237 stool samples, *Cryptosporidium* were detected in 10(4.2%) by MZN and in 12 (5%) by ELISA. Also, Radfar *et al.* (2013) demonstrated that capture ELISA can be used as the "golden" test and it is a method capable in detecting *C. parvum* coproantigens with high sensitivity and specificity compared with conventional methods like modified Ziehl-Neelsen stain. The study revealed (5.5%) prevalence of *C. parvum* among asymptomatic food handlers. Also Freites *et al.* (2009) reported that prevalence of *Cryptosporidium* was (11.8%) among food handlers from Zulia State Venezuela. Horman *et al.* (2004) informed that as a result of meta-analysis studies, they estimated the rate of asymptomatic cryptosporidiosis prevalence in Scandinavian countries as 0.99%. The prevalence of intestinal parasites and intensities of helminthic infections were studied in two Amerindian villages in Venezuela. Cryptosporidiosis was identified in (8.8%) in both villages; 60.6% were asymptomatic carriers (Chacín-Bonilla and Sánchez-Chávez, 2000), but the prevalence in asymptomatic Iranian children was 4% (Moghaddam, 2007). *C. parvum* oocysts were detected in 1.5% in western and southern coastal islands of Jeollanam-do Province (Park *et al,* 2006). The difference from the positivity rates obtained by other studies may arise from different geographical (Fayer and Xiao, 2008; Sakarya *et al,* 2012), seasonal (Schlundt *et al,* 2004), or age related differences (Laupland and Church, 2005; Learmonth *et al,* 2003; Krause *et al,* 1995). In Egypt, The prevalence of *Cryptosporidium* was (37.7% & 91%) in children and adult immunodeficient patients (Hassan *et al*, 2002) and 15% in El-Sharkia Governorate (Ali *et al*, 2000).

Faeco-oral transmission of the oocyst stage has resulted in outbreaks through contamination of drinking water, food, and recreational water. In Minnesota, chicken salad was associated with an outbreak among 50 people attending a social event (CDC, 1996). The caterer changed a baby's diaper in her home day-care facility and later prepared chicken salad for that social event. In Spokane, Washington, 54 of 62 persons who attended a catered banquet became ill 3±9 days later (CDC, 1998). The buffet of 18 foods and beverages contained seven uncooked produce items. Stool examination revealed 2 of 14 food preparers were positive for *Cryptosporidium.* Similarly, 88 students and four cafeteria employees were diagnosed with cryptosporidiosis at a university in Washington, DC as a prep cook who cut up vegetables and fruit to be eaten raw was ill for 3 days before the implicated meal and may have acquired infection from a child with diarrhea in his family (Quiroz *et al,* 2000). A recent investigation of food handler contamination of a salad in Denmark identified *C. hominis* (Ethelberg *et al,* 2009). Another food borne outbreak in Japan was caused by eating raw beef and liver contaminated with *C.parvum* (Yoshida *et al,* 2007). Also, Ponka *et al.* (2009) in Finland reported a foodborne outbreak caused by *Cryptosporidium parvum*. They added that outbreak occurred among personnel of the Public Works Department in Helsinki, who had eaten in the same canteen. Four faecal samples obtained from 12 ill persons were positive for *Cryptosporidium* by an antigen identification assay and microscopy. They suspected salad mixture as the source of outbreak and that the workers employed in food sector should be aware of the requirements for the appropriately prepared vegetables as to prevent contamination. These outbreaks highlight important issues. Food handlers should thoroughly wash their hands before handling food items and utensils. Raw fruits and vegetables as well as previously cooked items should not be handled with bare hands. Uncooked produce should be thoroughly washed before being placed on kitchen surfaces. Food preparation surfaces should be washed between preparations. Food workers should not work when experiencing gastrointestinal illness.

In this study, no statistical difference was between sex- and ages (P>0.05) in the cryptosporidiosis infections. Also, in other studies sex and age were not important variables (Hurtado-Guerrero *et al,* 2005; Santos and Merlini, 2010). Some authors mentioned that age did not have any correlation with the presence of *Cryptosporidium* spp., but being an important factor (Chai *et al*, 2001; Park *et al*, 2006; Shakya *et al*, 2006). The elderly were more susceptible to having serious illnesses with age advancement, and this disease can present longer duration.

This study reported eleven *Cryptosporidium* infected cases (6.1%) were from rural areas and two cases (3.5%) were from urban areas without significant difference (P> 0.05). This might be due to direct and indirect contact with animals. Soliman (1992) reported 13.51% of school children infected with *Cryptosporidium* in rural areas in Alexandria Governorate. However, Ranjbar-Bahadori *et al.* (2011) reported that infection rate in children from rural area was similar to urban areas without significant difference.

As regards the history to animal contact our results revealed that (11.9%) had a history of animal contact and (3.4%) gave no history of such contact with significant difference (P<0.05). This finding agreed with Chacin-Bonilla *et al.* (1993) and Chacin-Bonilla *et al.* (1997) and those from developing countries (Mathan *et al,* 1985; Hojlyng *et al,* 1986; Blanco and Samayoa 1988; Janoff *et al,* 1990; Esteban *et al,* 1998). However, there have been a number of studies that did not find an animal association (Khashba *et al*, 1989; Mikhail *et al,* 1989). Given to the fact that most of people in rural areas were farmers and potentially in contact with infected livestock, the initial infection with *C. parvum* would probably cause diarrhea, but in these areas, because of unsanitary and crowded living conditions, people may experience a high frequency of environmental exposure to the parasite and develop acquired immunity that might explain the high percentage of asymptomatic cases.

In the present study there was significant difference (P< 0.001) between nine cases used tap water and four cases not used tap water. In Egypt, Youssef *et al.* (1998) and Antonios *et al.* (2001) reported that *Cryptosporidium* oocysts were detected in water samples from uncovered water tanks, canals, fish ponds and tap water. Also, El-Helaly *et al*. (2012) stated that tap water was the main source of cryptosporidiosis in (96.3%) of patients. Shakya *et al.* (2006) in Turkey reported that the migration of people and the presence of animals as reservoirs, linked to poor sanitary conditions, are factors that contribute to contamination through drinking water which is the main route of transmission of this protozoan. Again in Egypt, Salaby and Salaby (2015) reported a significant relation between cryptosporidiosis infection and low socio-economic level in rural area. Also, a significant relation was obtained between the infection and the presence of animal contact. Watery and loose diarrhea was more significant among infected children. El-Badry *et al.* (2015) highlighted *Cryptosporidium* as a water contaminant distinct en-demicity with a bi-model mostly influenced by population dynamics and an important cause of health problems and recommended further studies of the risk factors. Abouel-Nour *et al*. (2015) reported that cryptosporidiosis *parvum* proved to be a zoonotic protozoan parasite infecting the intestinal epithelial cells causing a major health problem for man and animals. They added that experimentally the immunologic mediated elimination of *C. parvum* required

CD4+T cells and IFN-gamma. But, the innate immune responses also have a significant protective role in both man and animals. The mucosal immune response to *C. parvum* in C57BL/6 neonatal and GKO mice showed a concomitant Thl & Th2 cytokine mRNA expression, with a crucial role for IFN-gamma in the resolution of infection. NK cells and IFN-gamma showed to be important components in immunity in T and B cell-deficient mice, but IFN-gamma-dependent resistance was demonstrated in a-lymphocytic mice. Epithelial cells might play a vital role in immunity as once infected these cells have increased expression of inflammatory chemokines and cytokines and demonstrated anti-infection killing mechanisms.

Conclusion

No doubted, *Cryptosporidium* infection is often missed unless special staining being done by expert technician. Most laboratories do not test for *Cryptosporidium* unless specifically requested. Special staining like MZN is often needed, and therefore many cases will be diagnosed .Also ELISA was needed for survey. Asymptomatic infections and carriers have greater danger to the public because the worker keeps on working unmindful of the infection he is transmitting. So the infected food handlers should have effective treatment and a re-examination of stool. All these examinations should be done before issuing health certificates.

Recommendations

More field studies are advisable; about the role of food handlers on spreading of intestinal parasite. So, there is a need for constant epidemiological surveillance parallel with development of healthcare toward the problem of *Cryptosporidium* infections.

References

Abouel-Nour, MF, El-Shewehy, DM, Hamada SF, Morsy, TA, 2015: The efficacy of three medicinal plants: garlic, ginger and mirazid and a chemical drug metronidazole against *Cryptosporidium parvum*. I- Immunological rtesponse. J. Egypt. Soc. Parasitol. 45, 3:559-70.

Adegbola, RA, Demba, E, Deveer, G, Todd, F, 1994: *Cryptosporidium* infection in Gambian children less than 5 years of age. Am. J. Trop. Med. Hyg. 97:103-7.

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

Ali, MS, Mahmoud, LA, Abaza, BE, Ramad an, MA, 2000: Intestinal spore-forming protozoa among patients suffering from chronic renal failure. J. Egypt. Soc. Parasitol. 30:93-100.

Andargie, G, Kassu, A, Moges, F, Tiruneh, M, Huruy, K, 2008: Prevalence of bacteria and intestinal parasites among food handlers in Gondar town, Northwest Ethiopia. J, Hlth. Popul. Nutr. 26:451-5.

Antonios, SN, Salem, SA, Khalifa, EA, 2001: Water pollution is a risk factor for *Cryptosporidium* infection in Gharbia Governorate. J. Egypt. Soc. Parasitol. 31 (3): 963-64.

Blanco, RA, Samayoa, JC, 1988: Diarrhea y *Cryptosporidium* en Guatemala. Bol. Med. Hosp. Infant. Mex. 45:139-43.

Casemore, DP, 1991: Laboratory methods for diagnosing cryptosporidiosis. J. Clin. Pathol. 44: 445-51.

CDC, 1996: Foodborne outbreak of diarrheal illness associated with *Cryptosporidium parvum* Minnesota, 1995. MMWR Morb. Mortal. Wkly. Rep. 45:783-4.

CDC, 1998: Foodborne outbreak of cryptosporidiosis-Spokane, Washington, 1997. MMWR Morb. Mortal. Wkly. Rep. 47, 27: 565-9.

Chacin-Bonilla, L, Bonilla, MC, Soto-Torres, L, Rios-Ca´ndida, I, Sardina M, *et al*, 1997: *Cryptosporidium parvum* in children with diarrhea in Zulia State, Venezuela. Am J Trop Med Hyg. 56: 365-9.

Chacín-Bonilla, L, Sánchez-Chávez. Y, 2000: Intestinal parasitic infections, with a special emphasis on cryptosporidiosis, in Amerindians from western Venezuela. Am. J. Trop. Med. Hyg. 62, 3:347-52.

Chacin-Bonilla, L, Mejia-Young, M, Cano, G, Guanipa, N, Este´vez, J, *et al*, 1993: *Cryptosporidium* infections in a suburban community in Maracaibo, Venezuela. Am. J. Trop. Med. Hyg. 49:63-7.

Chai, JY, Kim, NY, Guk, SM, Park, YK, Seo M, *et al*, 2001: High prevalence and seasonality of cryptosporidiosis in a small rural village occupied predominantly by aged people in the Republic of Korea. Am. J. Trop. Med. Hyg. 65: 518-22.

Chappell, CL, Okhuysen, PC, Sterling, CR, DuPont, HL, 1996: *Cryptosporidium parvum:* intensity of infection and oocyst excretion patterns in healthy volunteers. J. Infect. Dis. 173: 232-6.

Cheesbrough, M, 2009: District Laboratory Practice in Tropical Countries, 2 vol. 1. New York: Cambridge University Press.

Chen, XM, Keithly, JS, Paya, CV, LaRusso, NF, 2002: Cryptosporidiosis. N. Engl. J. Med. 346:1723-31.

Dawson, D, 2005: Foodborne protozoan parasites. Int. J. Food Microbiol. 103:207-27.

DuPont, HL, Chappell, CL, Sterling, CR, Okhuysen, PC, Rose, JB, Jakubowski, W, 1995: The infectivity of *Cryptosporidium parvum* in healthy volunteers. N. Engl. J. Med. 332:855-9.

El-Helaly NS, Aly MM, Attia SS, 2012: Detection of *Cryptosporidium* infection among children with diarrhea. New York Sci. J. 5, 7:68-76.

Esteban, JG, Aguirre, C, Flores, A, Strauss, W, Angles, R, *et al*, 1998: High *Cryptosporidium* prevalences in healthy Aymara children from the northern Bolivian Altiplano. Am. J. Trop. Med. Hyg. 58:50-5.

Ethelberg, S, Lisby, M, Vestergaard, L, Enemark, HL, Olsen, K, *et al*, 2009: A foodborne outbreak of *Cryptosporidium hominis* infection. Epidemiol. Infect. 137, 3:348-56.

Fayer, R, Xiao, L, 2008: *Cryptosporidium* and Cryptosporidiosis. 2nd Ed., Boca Raton: CRC Press Taylor & Francis Group.

Freites, A, Colmenares, D, Pérez, M, Garcia, M, Díaz, DS, 2009: *Cryptosporidium* sp infections and other intestinal parasites in food handlers from Zulia state, Venezuela. Invest. Clin. 50, 1:13-21.

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. 32:33-46.

Hojlyng, N, Molbak, K, Jepsen S, 1986: *Cryptosporidium* spp., a frequent cause of diarrhea in Liberian children. J. Clin. Microbiol. 23:1109-13.

Horman, A, Korpela, H, Sutinen, J, Wedel, H, Hanninen, ML, 2004: Metaanalysis in assessment of the prevalence and annual incidence of *Giardia* spp. and *Cryptosporidium* spp. infections in humans in the Nordic countries. Int. J. Parasitol. 34:1337-46.

Huang, DB, White, AC, 2006: An updated review on *Cryptosporidium* and *Giardia.* Gastroenterol. Clin. North Am. 35, 291–314.

Hunter, PR, Nichols, G, 2002: Epidemiology and clinical features of *Cryptosporidium* infection in immunocompromised patients. Clin. Microbiol. Rev. 15:145-54.

Hurtado-Guerrero, AF, Alencar, FH, Hurtado-Guerrero, JC, 2005: Occurrence of enteroparasites in the elderly population of Nova Olinda do Norte, Amazonas, Brazil. Acta Amazônica 35, 4:487-90.

Insulander, M, de Jong, B, Svenungsson, B, 2008: A food-borne outbreak of cryptosporidiosis among guests and staff at a hotel restaurant in Stockholm County, Sweden, Euro. Surveill. 13, 51. pii: 19071.

Janoff, EN, Mead, PS, Echeverria, P, Bodhidatta, L, Bhaibulaya, M, *et al*, 1990: Endemic *Cryptosporidium* and *Giardia lamblia* infections in a Thai orphanage. Am. J. Trop. Med. Hyg. 43: 248-56.

Jokipii, L, Jokipii, AMM, 1986: Timing of symptoms and oocyst excretion in human cryptosporidiosis. N. Engl. J. Med. 315:1643-7.

Kaferstein, F, Abdussalam, M, 1999: Food safety in the 21st century. Bull WHO 77:347-51.

Khashba, A, Hilali, M, El-Hennawis, Marei M, 1989: Cryptosporidiosis among children suffering from diarrhea in Banha, Egypt. J. Egypt. Soc. Parasitol. 19, 3:701-5.

Krause, W, Abraham, A, Lehmann, D, 1995: Evidence of *Cryptosporidium* in children with symptomatic enteritis from the Leipzig administrative area 1987-1992. Appl. Parasitol. 36:66-71.

Laupland, KB, Church, DL, 2005: Population-based laboratory surveillance for *Giardia* sp. and *Cryptosporidium* spp. infections in a large Canadian health region. BMC Infect. Dis. 5:72-8.

Learmonth, JJ, Ionas, G, Pita, AB, Cowie, R S, 2003: Identification and genetic characterization of *Giardia* and *Cryptosporidium* strains in humans and dairy cattle in the Waikato Region of New Zealand. Water Sci. Technol. 47:21-6.

Mathan, MM, Venkatesan, S, George, R, Mathew, M, Mathan, VI, 1985: *Cryptosporidium* and diarrhea in southern Indian children. Lancet ii:1172-5.

Mikhail, IA, Hyams, KC, Podgore, JK, Haberberger, RL, *et al*, 1989: Microbiologic and clinical study of acute diarrhea in children in Aswan, Egypt Scand. J. Infect. Dis. 21:59-65.

Moghaddam, A, 2007: Symptomatic and asymptomatic cryptosporidiosis in young children in Iran. Pak. J. Biol. Sci*:* PJBS, 10, 7:1108-12.

Mudey, BA, Kesharwani, N, Mudey, AG, Goyal, RC, Dawale, AK, 2010: Health status and personal hygiene among food handlers working at food establishment around a rural teaching hospital in Wardha District of Maharashtra, India. Global J. Hlth. 2, 2:198-201.

Newman, R, Jaeger, K, Wuhib, T, Lima, A, Guerrant, R, ***et al*****, 1993:** Evaluation of an antigen capture enzyme-linked immunosorbent assay for detection of *Cryptosporidium* oocysts. J. Clin. Microbiol. 31:2080-4.

Okhuysen, PC, Chappell, CL, Crabb, JH, Sterling, CR, DuPont, HL, 1999: Virulence of three distinct *Cryptosporidium parvum* isolates for healthy adults. J. Infect. Dis. 180:1275-81.

Park, JH, Kim, HJ, Guk, SM, Shin, EH, Kim, JL, ***et al*****, 2006:** A survey of cryptosporidiosis among 2,541 residents of 25 coastal islands in Jeollanam-do (Province), Republic of Korea. Korean J. Parasitol. 44, 4:367-72.

Pönka, A, Kotilainen, H, Rimhanen, FR, Hokkanen, P, Hänninen, M, ***et al*****, 2009:** A foodborne outbreak due to *Cryptosporidium parvum* in Helsinki, November 2008. Euro surveillance: bulletin Européen sur les maladies transmissibles. Euro. Commun. Dis. Bull. 14, 28:29-35.

Quiroz, ES, Bern, C, MacArthur, JR, Xiao, L, Fletcher, M, ***et al*****, 2000:** An outbreak of cryptosporidiosis linked to a food-handler. J. Infect. Dis.181:695-700.

Radfar, MH, Gowhari, MA, Khalili, M, 2013: Comparison of capture ELISA and modified Ziehl-Neelsen for detection of *Cryptosporidium parvum* in feces of camel (*Camelus dromedarius*) in Iran. Sci. Parasitol. 14, 3:147-52.

Ranjbar-Bahadori, S, Sangsefidi, H, Shemshadi, B, Kashefinejad, M, 2011: Cryptosporidiosis and its potential risk factors in children and calves in Babol, north of Iran. Trop. Biomed. 28, 1:125-31.

Rosenblatt, J, Sloan, L, 1993: Evaluation of an enzyme-linked immunosorbent assay for detection of *Cryptosporidium spp.* in stool specimens. J. Clin. Microbiol. 31:2944–6.

Shalaby, NM, Shalaby, NM, 2015: *Cryptosporidium parvum* infection among Egyptian school children J. Egypt. Soc Parasitol. 45, 1:125-31.

Sakarya, Y, Kar, S, Tanyuksel, M, Karaer, Z, Babur, C, ***et al*****, 2012:** Detection of *Cryptosporidium spp.* in humans and calves through nested PCR and carbol fuchsin staining methods in Ankara, Turkey. Kafkas Univ. Vet. Fak. Derg. 18: 531-36.

Shakya, B, Rai, SK, Singh, A, Shresta, A, 2006: Intestinal parasitosis among the elderly people in Kathmandu Valley. Nepal Med. Coll. J. 8:243-7.

Santos, SA, Merlini, LS, 2010: Prevalence of enteroparasitosis in the population of Maria Helena, Paraná State. Ciên. Saúde Coletiva 15, 3: 899-905.

Schlundt, J, Toyofuku, H, Jansen, J, Herbst S A, 2004: Emerging food-borne zoonoses. Rev. Sci. Tech. 23:513-33.

Stehr-Green, JK, McCaig, L, Remsen, HM, Rains, CS, Fox, M, ***et al*****, 1987:** Shedding of oocysts in immunocompetent individuals infected with *Cryptosporidium.* Am. J. Trop. Med. Hyg. 36:338-42.

Takalkar, AA, Madhekar, NS, Kumavat, AP, Bhayya, SM, 2010: Prevalence of intestinal parasitic infections amongst food handlers in hotel and restaurants in Solapur City, India. Ind. J Publ. Hlth. 54:47-8.

WHO, 1993: Guidelines for Drinking-water Quality Vol. 1: Recommendations (2nd edn), Geneva.

Yoder, JS, Beach, MJ, 2010: *Cryptosporidium* surveillance and risk factors in the United States. Exp. Parasitol. 124:31-9.

Yoshida, H, Matsuo, M, Miyoshi, T, Uchino, K, Nakaguchi, H, ***et al*****, 2007:** An outbreak of cryptosporidiosis suspected to be related to contaminated food, October 2006, Sakai City, Japan. Jpn. J. Infect. Dis. 60, 6:405-8.

Youssef, MY, Khalifa, AM, El Azzouni, MZ, 1998: Detection of cryptosporidia in different water sources in Alexandria by monoclonal antibody test and modified Ziehl Neelsen stain. J. Egypt. Soc. Parasitol. 28:487-96.

Zain, MM, Naing, NN, 2002: Sociodemographic characteristics of food handlers and their knowledge, attitude and practice towards food sanitation: a preliminary report. Southeast Asian J. Trop. Med. Publ. Hlth. 33:410-7.