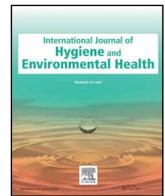




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Giardia and *Cryptosporidium* in children with diarrhea, Kufra, Libya, a North African migration route city

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ABSTRACT

Background: *Giardia* and *Cryptosporidium* are common parasitic diarrhea agents of children contributing to childhood morbidity and mortality in developing countries. Major risk factors, based on the international literature, are expected to include domestic animals, fresh vegetables and drinking water and foodstuffs purchased from street vendors. These factors and sub Saharan migrants are common in the study area. Reports elsewhere indicate that person-to-person transmission is also important.

Objective: To assess the prevalence *Cryptosporidium* and *Giardia* in children with diarrhea in the Kufra City hospital and to assess risk factors for cryptosporidiosis and giardiasis.

Methods: A 10-month study, September 2013–June 2014 provided a fecal sample from 505 patients with diarrhea aged from 2 to 17 attending the outpatient clinics of local Kufra hospital, plus 100 non symptomatic controls. Specimens were assayed for parasitic infection and for bacterial pathogens. Demographic information was obtained by questionnaire.

Results: *Giardia* was found in nearly 1/3 of the symptomatic population but few had *Cryptosporidium*. Mixed parasite infections were found in 1/7th of samples including: *Shigella*, *Salmonella*, *Ascaris* ova, *E. coli*, and *E. histolytica*. Infection frequency was age related, and risk factors included: domestic animals, foreign workers from Africa, contaminated fresh vegetables and drinking water.

Conclusion: *Cryptosporidium* and *Giardia* were frequently associated with diarrhea in children in a remote desert agricultural community with many opportunities for infection. Contact with animals, foreign workers from Africa, fresh vegetables and drinking water sources contaminated with sewage materials are the likely modes of transmission of both organisms.

1. Introduction

In developing countries, diarrhea is common and associated with high rates of mortality among young children, killing an estimated 2.2 million people annually, 1.9 million being children (Gascon et al., 2000; Nkrumah and Nguah, 2011). *Cryptosporidium parvum* and *Giardia lamblia* are important protozoan causes of diarrhea in developing countries (Snelling et al., 2007). Children may be most susceptible, and those less than two years old may have the greatest prevalence (Lima and Guerrant, 1992; Iqbal et al., 2001). The clinical spectrum of the associated diseases is wide, ranging from asymptomatic carriers and self-limited diarrhea in healthy persons (Addiss et al., 1991; Current and Garcia, 1991; Miron et al., 1991) to potentially life-threatening protracted diarrheas in immunocompromised or immunosuppressed individuals (e.g. those with the acquired immunodeficiency syndrome

[AIDS], malnourished children, the elderly) (Weinstein et al., 1981; Current et al., 1983; Forgacs et al., 1983; Meyer, 1988; Greenwood et al., 2007; Rathnayake et al., 2015). Transmission of *Cryptosporidium* and *Giardia* via contaminated drinking water, zoonotic, person-to-person contact, and by food contamination is well documented (Mackenzie et al., 1994; Newman et al., 1994; Andrew et al., 2004). Lack of safe drinking water and poor environmental sanitation, particularly in developing areas, contributes to more than 800 million annual cases of diarrheal diseases world-wide (Weitzel et al., 2006; Koskei et al., 2014).

Epidemiologic studies have described that diarrhea can be the presenting symptom of variety of etiological agents including bacteria (e.g., *Campylobacter* spp., enterohemorrhagic *E. coli*, and enteroadherent *E. coli*), viruses (e.g., norovirus, adenovirus, and astrovirus), and protozoa (e.g., *Giardia* and *Cryptosporidium*.) (Albert et al.,

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1995; Rahouma et al., 2011; WHO, 2012). Thus, multiple infectious agents must be suspected as causes of diarrhea in humans, particularly in children (Rahouma et al., 2011). However, little detail has been reported on pediatric diarrhea in Libya and other countries of the North Africa region (Rahouma et al., 2011).

Cryptosporidiosis and giardiasis are protozoan causes of childhood diarrhea in most developing countries as well as some industrialized countries and lead to significant morbidity and mortality, particularly in developing countries (Nkrumah and Nguah, 2011; Ajjampur et al., 2010; Espelage et al., 2010; Sanchez-Vega et al., 2006; WHO, 2005). The causative organism of enteric cryptosporidiosis is the coccidian parasite *Cryptosporidium* spp. and *Giardia* spp. for giardiasis. According to epidemiologic studies in undeveloped countries such as India, Ghana, Mexico, Uganda and Sri Lanka parasitic diarrhea in children is mainly due to *Giardia intestinalis* and *Cryptosporidium parvum* (Nkrumah and Nguah, 2011; Ajjampur et al., 2010; Sanchez-Vega et al., 2006; Rathnayake, et al., 2015).

Giardia is considered to be one of the leading causative agents of diarrhea, especially in children. Epidemiological surveys have shown that parasitic diarrhea in children is primarily due to *Giardia* infection, particularly in areas where fresh vegetables and drinking water sources are contaminated with sewage materials, and foodstuffs can be purchased from street vendors (WHO, 1992). It is estimated that up to 200 million people are chronically infected with *Giardia* globally, and 500,000 new cases reported annually. In the developed countries, overall prevalence rate of giardiasis is 2–5% (Noor Azian et al., 2007). However, in undeveloped countries, *Giardia* infects children early in life with prevalence as high as 15–20% in children younger than 10 years. Particularly children who are malnourished are more frequently infected (Al-Mekhlafi et al., 2005).

Information on cryptosporidiosis is lacking in some Arab countries, however available data show prevalence of the disease varies from < 1 to 43% (mean, 8.7%) in diarrheic immunocompetent persons to < 1–82% (mean, 41%) in immunocompromised individuals, in both children and adults. Reported prevalence rates differ widely among countries and within the same country (Ghenghesh et al., 2012).

Information on the prevalence of *Cryptosporidium* and *Giardia* in Libya is limited. Investigating *Cryptosporidium*- and *Giardia*-related diarrheas in Kufra City, Libya is interesting due to the remote agricultural location on economic and conflict driven refugee migration routes in the eastern Sahara. The infectious agents are likely to be prevalent for three reasons: First, various animals that are potential sources of transmission (sheep, goats, cats, and birds) share habitat with humans (Angus, 1983; Tzipori, 1983; Hall et al., 1988; Current and Garcia, 1991). *Cryptosporidium* species and *Giardia* species are established zoonotic enteric pathogens. Contact with animals is an important factor in *Cryptosporidium* and *Giardia* infection in children, particularly in rural areas (Moore et al., 1994; Thompson, 1994; Shoukry et al., 2009; Al-Dabbagh et al., 2010; El-Madawy et al., 2010; Ghenghesh et al., 2012); Second, ample opportunities exist for person-to-person contact. Also, foreign workers from sub-Saharan Africa who are in common contact with the local population, are a potential source of *Cryptosporidium* and *Giardia* transmission to both adults and children; Third, the likely occurrence of contaminated drinking water and fresh vegetables. Contaminated drinking water (El Shazly et al., 2007; Shoukry et al., 2009; Ghenghesh et al., 2012), and vegetables (Al-Binali et al., 2006; Ghenghesh et al., 2012) has been documented as an important source of *Cryptosporidium* and *Giardia* infections.

2. Materials and methods

The study was conducted in Kufra city in southeast Libya, about 1700 km by road from Tripoli, the capital city. The Kufra district, roughly the size of Spain, is the remote Southeastern quarter of Libya. It is at the end of the road connecting Kufra City to Bengazhi and Tripoli at least 1400 km to the North. No roads extend to the borders of Egypt

to the east, the Sudan and Chad to the South, each hundreds of km away across desert without roads. Migrants and refugees from troubled sub-Saharan areas travel poorly established routes to Al Kufra and the road leading to the Mediterranean.

This study was conducted at the local hospital in Kufra City, Libya for 10 months from September 2013 to end of June 2014. The local hospital is the main referral hospital, which serves the city of Kufra, and its surrounding urban population providing the source of both case and control populations described below

The objective was conducted to determine the occurrence of *Cryptosporidium* and *Giardia* among children with diarrhea, aged from 2 to 17 years. The enrolled sample population of 505 was accompanied by 100 controls. After receiving informed parental consent or consent of the guardian, a clinical history for each patient was obtained. Clinical symptoms from physical examination by medical doctors were recorded in a standard pro forma including: fever, nausea, abdominal pain, and blood in stool. The clinical history questionnaire included demographic information, area of residence, as well as information about the health status of the patient, previous parasitic infections, number of members in the household, level of education and occupations of both parents, socio-economic status of the parents, any symptoms of other diseases, contact with animals, hand washing practice using soap, the source of the household's drinking water and level of hygiene in food and fresh vegetables preparation was filled out by one of each patient's parent or guardian.

The presence of diarrhea was also determined by direct observation and by questioning the patients regarding the date of onset of diarrhea, number of stools per day (3 or more loose or liquid stools, or more frequently than is normal for the individual (WHO, 2012), duration of diarrhea, clinical course, complications, and previous antibiotic treatment. The control group of 100 children less than 14 years of age was matched for age, sex, area of residence and socio-economic status. Control candidates were selected, mostly attending the Outpatient Clinics of local Kufra hospital, presenting with complaints other than gastrointestinal symptoms.

Fresh fecal samples during diarrheal episodes were collected from each of 505 children in accordance with WHO guidelines on the collection of fecal samples (WHO, 1991). Each child was given a labeled plastic stool container in which an early morning stool specimen was to be collected. Samples were submitted and processed immediately for the detection of enteropathogenic bacteria, *Cryptosporidium*, *Giardia*, other identifiable protozoa, and nematodes. A portion of each sample was processed by the fecal parasitic concentration (FPC) method using formalin-ethyl acetate, centrifuged at 2000 rev/min for 2–4. Two slides were made from the resulting sediment. One slide was stained with Lugol's iodine and examined microscopically at 400× magnification for parasites. The second slide was processed for immunofluorescence assay including DAPI, to determine presence of *Cryptosporidium* oocysts and *Giardia* cysts and for visualization of nuclei as an aide to identification. *Cryptosporidium* and *Giardia* were identified based on the comparison with the fluorescent features of the (oo)cysts. Confirmation was based on the results of DAPI staining, and differential interference contrast microscopy. Each slide was examined entirely for bright apple-green fluorescence of the (oo)cyst wall, size, and shape using epifluorescence microscopy.

Stool samples were examined for bacterial pathogens as described previously (Albert et al., 1995). To isolate *Escherichia coli*, *Salmonella* spp. and *Shigella* spp., stool samples were inoculated directly onto MacConkey agar (MA), and *Salmonella*–*Shigella* agar (SSA). Stool specimens were cultured for *Campylobacter jejuni* using Campy-BAP. The lab was not equipped for virus detection.

3. Results

Fecal samples collected from the 505 children, presenting with diarrhea in the local Kufra hospital, were examined for *Cryptosporidium*,

Table 1
Pathogens present in 505 samples from Kufra diarrheic children under age 18.

Pathogen	Number + Alone	Number w/coinfectant	Total +	Total %
<i>Cryptosporidium</i>	13	6	19	4
<i>Giardia</i>	113	20	133	26.3
<i>Shigella</i> spp.	0	3	3	0.6
<i>Salmonella</i> spp.	0	5	5	1
<i>E. coli</i>	0	3	3	0.6
<i>B. hominis</i>	0	2	2	0.4
<i>E. histolytica</i>	0	2	2	0.4
<i>Ascaris ova</i>	0	3	3	0.6
total	126	26	148	29.3

Giardia, and accompanying pathogens. Out of these, the total number of samples positive for protozoan etiologic agents of diarrhea was 148/505 (29.3%), Table 1. Accordingly, among the 505 samples specifically collected as diarrheal, no etiologic agent was identified in 70%. Among the attributed samples, *Giardia* was the most frequently found intestinal protozoan, with 133/505 (26.3%) positive. *Cryptosporidium* was found less frequently, in 19/505 (4%) of all samples, Table 1.

Among the 148 samples positive for *Cryptosporidium* and *Giardia*, another 18 enteric pathogens were found including *E. coli*, *B. Hominis*, *E. histolytica*, *Shigella* spp, *Salmonella* spp, and *Ascaris ova*, Table 2. Of the 133 *Giardia* positive specimens 4 (3%) were found to contain *Cryptosporidium* along with *Giardia* but without any other pathogens. Another 16 (12%) of the positive specimens were found with pathogens other than *Giardia* (not including the 4 with *Cryptosporidium*). Of the 15 specimens positive for *Cryptosporidium* (excluding the 4 co-infected with *Giardia*) 2 (13.3%) had pathogens in addition to *Cryptosporidium*. The other pathogens found along with *Cryptosporidium* were *Shigella* spp., seen in 1 sample (6.7%) and *Salmonella* spp., seen in 1 sample (6.7%). Other pathogens found in the same samples of children with *Giardia* were: *Shigella* spp. in 2 samples (1.5%), *Salmonella* spp. in 4 samples (3%), *Ascaris ova* in 3 samples (2%), *E. coli* in 3 samples (2%), *B. hominis* in 2 samples (1.5%), and *E. histolytica* in 2 samples (1.5%). Neither *Cryptosporidium* nor *Giardia* was found in any fecal specimens from the 100 controls.

Additional (100) stool specimens were collected from children, who had no diarrhea or other gastrointestinal symptoms. The controls were recruited from children that came to the same health centre for other

Table 2
Concurrent pathogens found in *Cryptosporidium* & *Giardia* positive samples.

Pathogen	Number of children in age group, years								Total	%
	2 to 5		6 to 9		10 to 13		14 to 17			
	M	F	M	F	M	F	M	F		
<i>Giardia</i> : alone	13	10	38	26	12	9	5	0	113	22.4
<i>Giardia</i> with other pathogens										
<i>Cryptosporidium</i> spp.	0	0	3	1	0	0	0	0	4	0.8
<i>Shigella</i> spp.	0	0	2	0	0	0	0	0	2	0.4
<i>Salmonella</i> spp.	1	0	1	0	2	0	0	0	4	0.8
<i>E. coli</i>	0	0	1	0	1	1	0	0	3	0.6
<i>B. hominis</i>	2	0	0	0	0	0	0	0	2	0.4
<i>E. histolytica</i>	0	0	0	1	1	0	0	0	2	0.4
<i>Ascaris ova</i>	0	1	2	0	0	0	0	0	3	0.6
Total	16	11	46	28	16	10	5	0	133	26.3
<i>Cryptosporidium</i> : alone	0		10	3	0	0	0	0	13	2.5
<i>Cryptosporidium</i> with other pathogens										
<i>Giardia</i> (same as above)	0	0	3	1	0	0	0	0	4	0.7
<i>Salmonella</i> spp.	0	0	1	0	0	0	0	0	1	0.3
<i>Shigella</i> spp.	0	0	1	0	0	0	0	0	1	0.3
Total	0	0	15	4	0	0	0	0	19	3.8

Table 3
Age and sex distribution in Kufra City diarrhea positive sample population.

Age (years)	Number		Total	Percent
	female	male		
2–5	49	54	103	20.4
6–9	122	113	235	46.5
10–13	54	65	119	23.6
14–17	15	33	48	9.5
Total	240	265	505	–
Percent	47.5	52.5	–	100

reasons, e.g., immunization. All specimens were processed immediately for parasites and bacterial pathogens by culture, immunofluorescence assay, DAPI stain and microscopy, fecal parasitic concentration (FPC) method using microscopy of saline and iodine wet mount and formalin-ethyl acetate for other parasitic infections. The most common enteropathogenic bacteria isolated from the 100 healthy controls were *E. coli* (four subjects), *Salmonella* spp. (two) and *Shigella* spp. (two). The only parasites were *B. hominis*, and *Ascaris ova* which were isolated from two and four healthy controls, respectively. *Giardia* and *Cryptosporidium* were isolated either individually or in association with other microorganisms from patients, but not from the control subjects.

The gender distribution of the 505 children enrolled from Kufra City having diarrhea included 265 boys and 240 girls, Table 3. The population age range was from 2 years to 17 years with nearly uniform gender distribution except in the 14 to 17 group with twice as many males as females, Fig. 1. The fraction of *Giardia* infections found in male children compared to females was higher in of each of the age groups studied. The overall prevalence in males was 29.8% (79/265) compared to 22.5% (54/240) in females, Table 4. The most frequent *Giardia* positives for both male and females were found in the 6 to 9 age group.

Cryptosporidium was found in samples only from children in the 6 to 9 age group, Table 2. Prevalence of *Cryptosporidium* infection in male children was higher, 12 (4.5%) than in female, 3 (1.3%), Table 4. The higher prevalence of *Giardia* compared to *Cryptosporidium* by gender was apparent in each age group, Fig. 1.

Expressed in terms of prevalence, *Giardia* was 5 times higher than

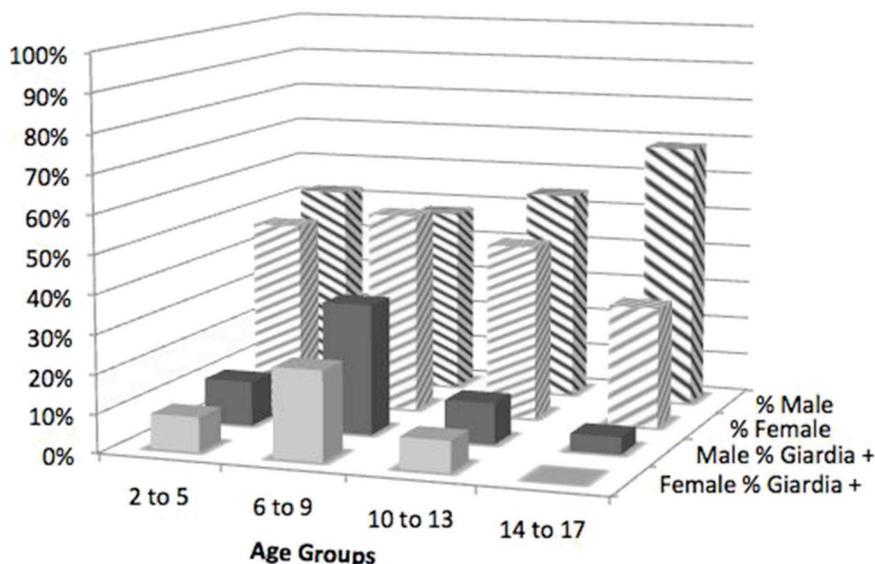


Fig. 1. Distribution of sample population and *Giardia* positives by gender and age.

Table 4
Giardia and *Cryptosporidium* prevalence by gender in Kufra City children presenting with diarrhea.

Gender	Number	Positive cases	<i>Giardia</i> (% positive)	<i>Cryptosporidium</i> (% positive)
Male	265	81	68 (25.6%)	13 (4.9%)
Female	240	46	45 (18.7%)	1 (0.4%)
Total	505	127	113 (22.4%)	14 (2.7%)

Table 5
Giardia and *Cryptosporidium* prevalence by age in Kufra City children presenting with diarrhea.

Age	Number of Children	Total Positive, <i>Cryptosporidium</i> and <i>Giardia</i>	Age Specific Prevalence, %
2 to 5	103	23	22.3
6 to 9	235	81	34.0
10 to 13	119	21	17.6
14 to 17	48	5	10.4
Total	505	130	25.7

Cryptosporidium in samples from male children at 25.6%, Table 4. The prevalence of *Giardia* in samples from female children was less, 18.7%, than that of male children. However, *Cryptosporidium* was found in only one sample from a female child, Table 4. The age distribution found in this population when of *Cryptosporidium* and *Giardia* combined increased from 22.3% in 2–5 year olds to a high of 34% in 6–9 year olds, decreasing to 17.6% in 10–13 year olds, then to 10.4% 14–17 year olds, Table 5.

In addition to the *Giardia* isolated from the stools of children with diarrhea small numbers were also positive for *Salmonella* spp., *Shigella* spp., *E. coli*, *B. hominis*, *E. histolytica*, and *Ascaris* ova, Tables 1 and 2. The other pathogens found in diarrhea samples positive for *Cryptosporidium* were *Salmonella* spp. and *Shigella* spp, Table 6.

Clinical manifestations on recorded for children with samples positive for *Giardia* ranged from 71% experiencing abdominal pain, decreasing to 58% with fever, 53% experiencing nausea and nearly half (48%) with blood in stool, Table 6. Among children with samples positive for *Cryptosporidium*, symptoms included 73% reporting fever, 67% experiencing abdominal pain, nearly half (47%) with blood in stool, and about 1/3 experiencing nausea, Table 6.

The information on risk factors collected from the sample

Table 6
Clinical manifestations among Kufra children with *Giardia* and *Cryptosporidium* positive diarrhea.

Information Category	<i>Giardia</i> positive	<i>Cryptosporidium</i> positive
	n = 133 (%)	n = 15 (%)
Clinical manifestations		
Abdominal pain	94 (70.7%)	10 (66.7%)
Fever	77 (57.9%)	11 (73.3%)
Nausea	71 (53.4%)	05 (33.3%)
Blood in stool	64 (48.1%)	07 (46.7%)
Other pathogens found		
<i>Shigella</i> spp.	6 (4.51%)	1 (6.67%)
<i>Salmonella</i> spp.	4 (3.01%)	1 (6.67%)
<i>E. coli</i>	3 (2.26%)	0
<i>B. hominis</i>	2 (1.5%)	0
<i>Entamoeba histolytica</i>	2 (1.50%)	0
<i>Ascaris</i> eggs	3 (2.26%)	0

Table 7
Exposure characteristics of Kufra children with diarrhea found positive for *Giardia* and *Cryptosporidium*.

Information Category	<i>Giardia</i> positive	<i>Cryptosporidium</i> positive
	n = 133 (%)	n = 15 (%)
Water source		
Public tap/piped water	83 (62.4%)	11 (73.3%)
Well	35 (26.3%)	4 (26.7%)
Filtered water	15 (11.3%)	–
Domestic animals		
Birds	122 (91.7%)	12 (80%)
Goat	117 (88%)	12 (80%)
Sheep	95 (71.4%)	9 (60%)
Cats	15 (11.3%)	–
African migrant contact	95 (71.4%)	11 (73.3%)

population on intake included the usual water source, presence of and contact with domestic animals, and contact with migrant workers from other areas of Africa. The information, Table 7, indicates a high rate of public water supply use and high rates of contact with birds (chickens?), goats, and sheep as well as a high rate of contact with workers from other parts of Africa. Little difference was apparent in the rates of contact between children found positive for *Giardia* and those positive for *Cryptosporidium*, Table 7.

Table 8
Prevalence of *Giardia* infection previously reported in Libya.

Prevalence	Population	Symptoms	Location	Reference
1.2%	children	diarrhea	Zliten	Ali et al. (2005)
2.0%	children		Tripoli	Ben Mousa et al. (2007)
3.8%	children		Benghazi	Al-Tawaty et al. (2002)
5.9%	children	gastroenteritis	Benghazi	Bugharara et al. (1999)
6.2%	children	in hospital	Benghazi	El-Buni et al. (1998)
7.2%	Libyan	patients	Sirte	Salem et al. (2006)
7.8%	emigrants		Benghazi	El-Buni and Khan (1998)
8.7%	patients	outpatients	Tripoli	Bolbol et al. (1981)
11.4%	children	<i>Giardia</i>	Benghazi	Saaed Frhat and Ongerth (2017)
12.7%	children	<i>Giardia</i>	North Libya	Sadaga and Kassem, 2007

4. Discussion

This is the first epidemiological study on *Cryptosporidium* species and *Giardia* infection in the city of Kufra, Libya. The importance of *Cryptosporidium* species as a widespread cause of diarrhea in humans is now increasing in the same way that *Giardia* was previously recognized to be increasing. The overall prevalence rate of cryptosporidiosis and giardiasis among immunocompetent children of Kufra city found in this study was 29.6%, the number of samples collected from the local Kufra hospital were equal to 505 and satisfactory for the size of the study.

In unpublished work, we found that more than 61% (427 out of 700) of fecal samples examined from foreign workers from Africa aged from 14 to 62 were positive for both organisms, roughly 25% positive for *Giardia*, 10% for *Cryptosporidium* and 3% coinfecting. In the same study, the organisms reported in a wide range of domestic animals including Goat, Sheep, Cats and birds with a prevalence rate ranging between 5% and 88%. Also *Cryptosporidium* and *Giardia* have been detected in 10%, and 8% of 100 samples each of tank water and underground water, respectively. Contact and presence of domestic animals, foreign workers from Africa and contaminated waters are the main modes of transmission of the two protozoa parasites. A previous study of *Cryptosporidium* and *Giardia* from the area described contamination of fresh vegetables as an important route of transmission (Saaed Frhat and Ongerth, 2017).

Table 9
Prevalence of *Giardia* and *Cryptosporidium* reported in Arab countries.

Prevalence	Population	Symptoms	Location	Refs
<i>Giardia</i>				
10%	children < 18	diarrhea	Rural central Ghana	Nkrumah and Nguah (2011)
22%	random general		Northern Ghana	Verweij et al. (2003)
24%			Karachi, Pakistan	Nkrumah and Nguah (2011)
26%	day-care children		Damghan, Iran	Heidari and Rokni (2003)
29%	random, urban slum, < 5		Karachi, Pakistan	Mehraj et al. (2008)
30%			Amman, Jordan	Shakkoury and Wandy (2005)
31%		Abdominal pain	Pakistan	Younas et al. (2008)
35%	children		Ethiopia	Ayalew et al. (2008)
39%			Dhouk, Iraq	Al-Saaed and Issa (2006)
44%	Aborigines		Pahang, Malaysia	Noor Azian et al. (2007)
78%	children		Amman, Jordan	Shakkoury and Wandy (2005)
<i>Cryptosporidium</i>				
4%	children 7-12	Asymptomatic	Makkah, Saudi Arabia	Ghenghesh et al. (2012)
5.3%	children		SW Nigeria	Reinthalder et al. (1987)
7.6%	Patients attending		Rural N. Kenya	Koskei et al. (2014)
7.6%	Infectious agent hospital		Mbagathi Hosp, Nairobi	Mbae et al. (2013)
9%	children < 5	Asymptomatic	Jeddah	Al-Braiken et al. (2003)
10%	children		Rwanda	Bogaerts et al. (1984)
12%	children, random		Ethiopia	Ayalew et al. (2008)

The present study showed a prevalence of giardiasis (26.3%) among children with diarrhea but no (0.0%) infections among the 100 controls. This differs with reports from other parts of the Libya as summarized in Table 8. Although other studies in parts of the world have reported higher prevalence comparable to this study, Table 9.

Although information on cryptosporidiosis is lacking in some Arab countries; however available data show prevalence of the disease varies from < 1 to 43% (mean = 8.7%) in diarrheic immunocompetent persons to < 1–82% (mean = 41%) in immunocompromised individuals in both children and adults. Prevalence rates differ widely among countries and within the same country (Ghenghesh et al., 2012).

The present study showed a lower prevalence of cryptosporidiosis (3%) than for giardiasis among children with diarrhea and none in 100 controls group. While this is contrary to the major recent studies (Kotloff et al., 2013; Platts-Mills et al., 2015) the isolation of the Kufra population and arid climate likely account for a majority of observed differences. Other studies have also reported *Cryptosporidium* species in diarrheic children and from none of controls (Nimri and Meqdam, 2004; Nimri et al., 2004; Shoukry et al., 2009; Ghenghesh et al., 2012).

The demographic data (ages, contact with animals, contact with foreign workers from Africa, contaminated fresh vegetables and drinking water sources) of the majority of the study children was recorded. The socio-demographic information suggests at least four possible modes of transmission of infection to children: person to person, contact with animals, contaminated fresh vegetables and drinking water stored in the overhead water tanks. Majority of houses in the agricultural areas of Kufra City have urbanized water supply system (piped-water) in their houses and then stored in storage tanks. All children found positive for *Cryptosporidium* and *Giardia* had a source of drinking water inside the homes through piped or taped water. Some of the population depends on wells water for drinking water. The well water used for drinking is untreated, potentially containing *Cryptosporidium* oocysts and *Giardia*, and other pathogens. No child was found positive for those organisms among those, who consumed drinking water brought from outside the home. Contaminated drinking water appears to be an important source of *Cryptosporidium* infections in Arab countries. Detection of *Cryptosporidium* in 9%, and 7% of 75 samples each of tank water, and underground water has been reported (Shoukry et al., 2009).

A recent study by the authors examined five different types of fresh salad vegetables in Kufra city for the prevalence of *Giardia* and *Cryptosporidium* (Saaed Frhat and Ongerth, 2017). They reported a

uniform high level (> 70%) of contamination among 50 samples each of lettuce, parsley, watercress (*Eruca Sativa*), tomato, and cucumber, *Giardia* and *Cryptosporidium*. This is a likely result of contamination from cultivation including watering, fertilizing, and harvesting procedures, in generally contaminated environments.

The occurrence giardiasis observed in Kufra appears due to the presence of large numbers of potential reservoir hosts around these children. As summarized above, children in Kufra have ample opportunity for contact with animals widely shown to have significant infection rates with both *Giardia* and *Cryptosporidium* (Bilagburn and Current, 1983; Current et al., 1983). As reported here, the highest *Cryptosporidium* and *Giardia* infection rate was among children aged between 6 and 9 years, and that 80% and 91.7%, respectively of infected children were in contact with animals compared with 20% and 8.2% of non-infected children.

There was a significant difference $p < 0.05$ in the prevalence of giardiasis and cryptosporidiosis and contact with animals by some children who lived in the agricultural areas. Children in contact with animals have more *Cryptosporidium* and *Giardia* infection than non-contact children similar to findings reported elsewhere in similar regions, e.g. in Egypt (Shoukry et al., 2009); or Iraq (Al-Dabbagh et al., 2010).

5. Conclusion

Cryptosporidium species and *Giardia* are important causes of diarrhea in children in the isolated, arid, agricultural community, demonstrated in Kufra City, Libya. Contact with animals, person to person, contaminated fresh vegetables and drinking water stored in the overhead water tanks are the most likely modes of transmission of the two protozoa parasites. The details of giardiasis and cryptosporidiosis etiology clearly have features specific to the environment of the observed population requiring care in considering generalization.

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