Libyan Journal of Medicine

REVIEW ARTICLE

Prevalence of *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium* spp. in Libya: 2000–2015

Khalifa Sifaw Ghenghesh[1]*, Khaled Ghanghish[2], Elloulu T. BenDarif[3], Khaled Shembesh[3] and Ezzadin Franka[3]

[1]Independent Scholar, Cairo, Egypt; [2]Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya; [3]Faculty of Medicine, University of Tripoli, Tripoli, Libya

Introduction: The intestinal protozoa *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium* spp. are the causative agents of giardiasis, amebiasis, and cryptosporidiosis, respectively. Adequate knowledge of the geographical distribution of parasites and the demographic variables that influence their prevalence is important for effective control of infection in at-risk populations.

Methods: The data were obtained by an English language literature search of Medline and PubMed for papers using the search terms 'intestinal parasites and Libya, *G. lamblia* and Libya, *E. histolytica* and Libya and *Cryptosporidium* and Libya' for the period 2000–2015.

Results: The data obtained for the period 2000–2015 showed prevalence rates of 0.8–36.6% (mean 19.9%) for *E. histolytica/dispar*, 1.2–18.2% (mean 4.6%) for *G. lamblia* and 0.9–13% (mean 3.4%) for *Cryptosporidium* spp. among individuals in Libya with gastroenteritis (GE). On the other hand, prevalence rates of 0.8–16.3% (mean 8.3%), 1.8–28.8% (mean 4.8%), and 1.0–2.5% (mean = 2.4), respectively, were observed for individuals without GE. The mean prevalence rate of *E. histolytica/dispar* was significantly higher among individuals with GE compared with those without GE ($p < 0.0000001$, OR = 2.74). No significant difference in prevalence rate of the three organisms was found according to gender, but most of infections were observed in children aged 10 years or younger.

Conclusion: The reviewed data suggest that *E. histolytica*, *G. lamblia,* and *Cryptosporidium* spp. may play a minor role in GE in Libya. The observed high prevalence rates of *E. histolytica/dispar* reported from Libya could be due mainly to the non-pathogenic *E. dispar* and *E. moshkovskii*. However, more studies are needed in the future using *E. histolytica*-specific enzyme immunoassays and/or molecular methods to confirm this observation.

Keywords: *intestinal protozoa*; Entamoeba histolytica; Giardia lamblia; Cryptosporidium; *gastroenteritis*; *Libya*

*Correspondence to: Khalifa Sifaw Ghenghesh, El-Nakheel Compound, El-Sherouk, Cairo, Egypt, Email: ghenghesh_micro@yahoo.com

Received: 28 April 2016; Accepted in revised form: 9 June 2016; Published: 29 June 2016

Entamoeba histolytica, *Giardia lamblia* (synonymous with *G. intestinalis* and *G. duodenalis*), and *Cryptosporidium* spp. are the causative agents of amebiasis, giardiasis, and cryptosporidiosis, respectively. These organisms are among the most common intestinal protozoa associated with diarrhea in developed and developing countries (1, 2). The Global Burden of Disease Study (GBDS) estimated that amebiasis was associated with more than 55,000 deaths and 2.2 million disability-adjusted life years (DALYs), and cryptosporidiosis with more than 99,000 deaths and 8.3 million DALYs in 2010 (3, 4). Most of these deaths and DALYs occurred in developing countries. Table 1 shows reservoirs, modes of transmission, and clinical manifestations of *E. histolytica*, *G. lamblia,* and *Cryptosporidium* spp.

Recently, Muhsen and Levine (5) carried out a systematic literature review investigating the correlation of *G. lamblia* infection with pediatric diarrhea in developing countries. Their findings indicate that giardiasis may not be associated with acute diarrhea in children, except in early infancy. However, their findings suggest that *G. lamblia* is positively correlated with persistent diarrhea in such populations.

Adequate knowledge of the geographical distribution of parasites and the demographic variables that influence their prevalence is important for effective control of

 Citation: Libyan J Med 2016, **11**: 32088 - http://dx.doi.org/10.3402/ljm.v11.32088

Table 1. Reservoirs, modes of transmission and clinical manifestations of *Entamoeba histolytica*, *Giardia lamblia,* and *Cryptosporidium* spp.

Organism	Disease	Reservoir	Mode of transmission	Clinical manifestations
Entamoeba histolytica	Amebiasis	Infected humans	Contaminated water, fecal–oral, contaminated food	Dysentery (bloody diarrhea), diarrhea, intestinal, and/or liver abscesses
Giardia lamblia	Giardiasis	Infected human and other mammals	Contaminated water, fecal–oral	Acute and persistent diarrhea, bloating, gas, steatorrhea, and malabsorption
Cryptosporidium spp.	Cryptosporidiosis	Infected human and a variety of animal hosts (zoonosis)	Contaminated water, swimming pools, fecal–oral	Self-limited diarrhea; chronic diarrhea in immunocompromised individuals (e.g. AIDS)

infection in at-risk populations (6). The aim of this minireview is to report on *E. histolytica*, *G. lamblia,* and *Cryptosporidium* spp. in Libya for the period 2000 – 2015. The data were obtained by an English language literature search of Medline and PubMed for papers using the search terms 'intestinal parasites and Libya, *G. lamblia* and Libya, *E. histolytica* and Libya, and *Cryptosporidium* and Libya'. Additional data were obtained from a Google search using the aforementioned terms.

For statistical analysis, Epi Info 2000 software (Centers for Disease Control and Prevention) was employed. *P*-values were calculated using the Chi-square test. $p < 0.05$ was considered to be statistically significant.

Prevalence of *E. histolytica*, *G. lamblia,* and *Cryptosporidium* spp. in people with or without gastroenteritis in Libya

The search of the published literature for the period 2000–2015 yielded seven studies that investigated stool samples only from individuals with gastroenteritis (GE, including some combination of diarrhea, abdominal pain, and vomiting) (7–13), eight studies only from individuals without GE (14–21), and one study from individuals with or without GE (22). Four studies that dealt with intestinal parasites in Libya were not included in this work. Two of the excluded studies were carried out in Benghazi, one of which examined stool specimens from diarrheic children during 1998–2002 and was published in 2002, and the other investigated persons without GE during 1998–1999 and was published in 2003 (23, 24). Also excluded was a study performed in 2006 in Tripoli and reporting results for 949 school children because the investigators examined only 591 stool samples (25). Moreover, a study performed in Benghazi between 1999 and 2000 on people of different nationalities was published in two different journals in 2004 (26) and 2005 (15), and so only the later study was included in this minireview.

More than 90% of the studies (15 out of 16) included in this review used more than one technique of microscopic examination of stool samples for cysts of *E. histolytica*, *G. lamblia,* and *Cryptosporidium*. There are three species, among others, in the genus *Entamoeba* that can be found in the human intestine (27). These include the pathogenic *E. histolytica* and the nonpathogenic *E. dispar* and *E. moshkovskii*. The use of microscopy cannot distinguish *E. histolytica* from the latter two species. Unless stated otherwise, we use the term 'E. histolytica/dispar' because it was used in the majority of studies covered in this review.

Of the GE studies, four examined stools from children and four examined stools from children and adults. These eight studies showed prevalence rates of 0.8–36.6% (mean 19.9%) for *E. histolytica/dispar*, 1.2–18.2% (mean 4.6%) for *G. lamblia*, and 0.9–13% (mean 3.4%) for *Cryptosporidium* spp. among individuals in Libya with GE. However, when only the data from children with GE were analyzed, the mean prevalence rate of *E. histolytica/dispar* dropped significantly from 19.9 to 4.0% ($p < 0.000001$, odds ratio [OR] 5.96). No significant differences were observed in the mean prevalence rate of *G. lamblia* (3.2%) and of *Cryptosporidium* spp. (mean 4.2%) in children with GE compared with mean prevalence rates for both organisms in children and adults with GE. Table 2 shows the prevalence rates of *E. histolytica/dispar*, *G. lamblia,* and *Cryptosporidium* spp. in individuals with GE in different Libyan cities.

Of the nine studies that examined individuals without GE, five included children, three included children and adults, and one study included adults only. The nine studies showed prevalence rates of 0.8–16.3% (mean 8.3%) for *E. histolytica/dispar,* 1.8–28.8% (mean 4.8%) for *G. lamblia,* and 1.0–2.5% (mean 2.4%) for *Cryptosporidium* spp. from individuals without GE. The mean prevalence rate of *G. lamblia* among children without GE (15.4%) was significantly higher than the mean prevalence rate of combined children and adults without GE (4.8%) ($p < 0.000001$, OR 3.65). There was no significant difference between the mean prevalence rate of *E. histolytica/dispar* among children (7.7%) and that in adults and children without GE combined (8.3%). A comparison of the mean prevalence rate of *Cryptosporidium* spp. among children and children and adults was not possible due to the small number of studies that examined the organism in stools of individuals without GE. Table 3 shows the

Table 2. Prevalence of *Entamoeba histolytica/dispar*, *Giardia lamblia*, and *Cryptosporidium* spp. in subjects with gastroenteritis* in different Libyan cities

City	Population studied	No. positive/No. examined (%) *Entamoeba histolytica/dispar*	*Giardia lamblia*	*Cryptosporidium* spp.	References
Brack	Children and adults	29/305 (9.5)	4/305 (1.3)	7/305 (2.3)	(7)
Nalout	Children and adults	168/800 (21)	20/800 (2.5)	NE	(8)
Sirt	Children and adults	323/700 (46.1)	65/700 (9.3)	NE	(9)
Sirt	Children	128/350 (36.6)	36/350 (10.3)	NE	(10)
Tripoli[1]	Children	4/110 (3.6)	20/110 (18.2)	1/110 (0.9)	(22)
Tripoli	Children	2/239 (0.8)	3/239 (1.3)	5/239 (2.1)	(11)
Zawia	Children	19/605 (3.1)	11/605 (1.8)	NE	(12)
Zliten	Children	20/169 (11.8)	2/169 (1.2)	13/100 (13)	(13)

*Gastroenteritis = some combination of diarrhea, abdominal pain, and vomiting. [1]Tripoli and surrounding areas.
NE = Stool samples were not examined for *Cryptosporidium* spp.

prevalence of *E. histolytica/dispar*, *G. lamblia*, and *Cryptosporidium* spp. in people without GE in different Libyan cities and localities.

A significantly higher mean prevalence rate of *E. histolytica/dispar* was found among children and adults with GE compared with the mean prevalence rate of *E. histolytica/dispar* in children and adults without GE ($p < 0.0000001$, OR 2.74). On the other hand, significantly higher mean prevalence rates of *E. histolytica/dispar* and *G. lamblia* were found in children without GE compared with the mean prevalence rates of *E. histolytica/dispar* and *G. lamblia* in children with GE ($p < 00004$, OR 2.02, and $p < 0.0000001$, OR 5.51, respectively).

High prevalence rates for *E. histolytica/dispar* and *G. lamblia* were reported mainly from the city of Sirt (Table 2 and 3), regardless of whether the individuals had or did not have GE.

Age, gender, and other factors associated with the prevalence of intestinal protozoa

Infections due to *E. histolytica/dispar*, *G. lamblia,* and *Cryptosporidium* spp. are prevalent in developing countries and depend on several factors, including age. Amebic infections mostly affect children below 15 years of age, with a significant increase in those aged five to nine years (28). In Arab countries, *Cryptosporidium*-associated diarrhea occurs mainly in younger children and inversely correlates with age, being more prevalent in children aged one year or less (29). Also, in developing countries, giardiasis is positively correlated with persistent diarrhea in children and with acute diarrhea in early infancy (5). On the other hand, no significant differences in the distribution of cases between males and females were reported from such countries.

In Libya, a few studies examined the influence of age and gender on the prevalence of *E. histolytica/dispar* and

Table 3. Prevalence of *Entamoeba histolytica/dispar*, *Giardia lamblia*, and *Cryptosporidium* spp. in subjects without gastroenteritis* in different Libyan cities/localities

City	Population studied	No. positive/No. examined (%) *Entamoeba histolytica/dispar*	*Giardia lamblia*	*Cryptosporidium* spp.	References
Alkhoms	Children and adults	289/2,383 (12.1)	61/2,383 (2.6)	NE	(14)
Benghazi	Children and adults	436/3,659 (11.9)	109/3,659 (2.9)	NE	(15)
Benghazi	Adults	139/3,150 (4.4)	60/3,150 (1.9)	NE	(16)
Derna	Children	69/1,039 (6.6)	132/1,039 (12.7)	NE	(17)
Sirt	Children	98/601 (16.3)	173/601 (28.8)	NE	(18)
Tripoli	Children	2/50 (4.0)	1/50 (2.0)	NE	(19)
Tripoli	Children	4/486 (0.8)	28/486 (5.8)	NE	(20)
Tripoli	Children	3/100 (3.0)	17/100 (17)	1/100 (1.0)	(22)
WadiAl-Shati localities[1]	Children and adults	14/1,192 (1.2)	21/1,192 (1.8)	30/1,192 (2.5)	(21)

*Gastroenteritis = Some combination of diarrhea, abdominal pain, and vomiting.[1]Stool samples were collected from 17 rural localities of Wadi Al-Shati in southwestern Libya.
NE = stool samples were not examined for *Cryptosporidium* spp.

G. lamblia, but none for *Cryptosporidium* spp. A study from Nalout in western Libya investigated children and adults with GE aged less than 10 years to more than 20 years for intestinal parasites (8). The researchers found that the prevalence rate of *E. histolytica/dispar*, but not *G. lamblia*, was significantly higher in children of less than 10 years compared with children of 10 to 20 years or more than 20 years of age. Nevertheless, they found no meaningful differences in the prevalence rates of the organisms among the examined children in regard to sex. Kasssem et al. (10) investigated the presence of intestinal parasites in children aged less than 16 years with GE in the city of Sirt. They detected 13 different intestinal protozoan parasites with the predominance of *E. histolytica/dispar*. The majority of *E. histolytica/dispar* and *G. lamblia* infections occurred in children 1–4 years old. However, no significant difference was observed in intestinal protozoa infection between male and female patients. Also, Alsirieti et al. (9) in Sirt city studied patients with GE aged 2–60 years. They reported intestinal parasites, including *E. histolytica/dispar* and *G. lamblia*, most commonly in those aged 2–10 years compared to other age groups, and higher infection rate in males than in females.

Anuar et al. (30) carried out a cross-sectional study examining the prevalence and risk factors associated with *E. histolytica/dispar/moshkovskii* infection among different Orang Asli ethnic groups in Malaysia. They found higher prevalence rates among subjects aged less than 15 years. Furthermore, multivariate analysis proved that not washing subsequent to playing with soil or gardening and the presence of other family members infected with *E. histolytica/dispar/moshkovskii* were significant risk factors of infection among all groups examined.

A study from the city of Sirt examined school children aged between 7 and 14 years without GE for factors associated with intestinal parasitic infection (18). The investigators found a statistically significant higher prevalence rates of *E. histolytica/E. dispar* among males than females, among those aged 7–10 years than those of 11–14 years, and among children who sometimes washed their hands than those who always washed their hands. The prevalence rates of *E. histolytica/E. dispar* among children with educated mothers were closely similar to those with non-educated mothers. They found no significant differences in the prevalence rate of *G. lamblia* between females and males, or between children 7–10 years old and those 11–14 years old. However, they observed statistically significant higher prevalence rates among children with non-educated mothers than those with educated mothers; and among children who sometimes washed their hands than children who always washed their hands.

Another study, from Derna city, examined the presence of intestinal parasites in primary school children aged 6–12 years, selected by systematic random sampling using the master list of eight schools (17). They found no significant difference in the prevalence rates of *E. histolytica/E. dispar* and *G. lamblia* in relation to the gender and age of children. However, contrary to the study from Sirt, they found a significantly higher prevalence rate of intestinal parasites in children with educated than non-educated parents. In addition, they reported that children from lower income families, eating food from the school canteen and drinking water from metal tanks, were significantly associated with intestinal parasites, including *E. histolytica/E. dispar* and *G. lamblia*.

Prevalence of *E. histolytica*, *G. lamblia,* and *Cryptosporidium* spp. in food handlers and foreign workers

The main modes of transmission of intestinal protozoa are water- and food-borne. In general, there is lack of information regarding the prevalence of intestinal protozoa in water, fresh fruits, and vegetables in Libya. Only one study from Libya examined *Giardia* cysts in 126 specimens of fresh tomatoes, cucumber, lettuce, and cress from wholesale and retail markets in Tripoli. It reported a prevalence rate of 10% (31).

None of the few reported outbreaks of amebiasis in developed countries were food-borne (32). There are no reports of outbreaks of amebiasis due to food handlers in developing countries. However, several food-borne outbreaks of cryptosporidiosis and giardiasis were attributed to direct contamination by food handlers (33). Kubti et al. (16) investigated 3,150 food handlers in Benghazi for intestinal parasites (1,563 Sudanese, 1,304 Egyptians, and 283 Libyans). The investigators found that the prevalence rate of *E. histolytica/dispar* among the Sudanese was significantly higher than among the Egyptian food handlers but not among the Libyan food handlers. On the other hand, the prevalence rate of *G. lamblia* was higher among the Egyptians than among the Sudanese and the Libyan food handlers, but the differences were not significant.

A study from Alkhoms found a significantly higher prevalence rate of *E. histolytica/dispar* among foreign workers, mainly from Egypt and other African countries, than among Libyan individuals without GE. However, they observed a significantly higher prevalence rate of *G. lamblia* among Libyans compared with foreign workers.

Diagnosis of *E. histolytica*, *G. lamblia,* and *Cryptosporidium* spp. in subjects with or without gastrointestinal disturbances in Libya

The microscopic examination of stool specimens remains the backbone of the diagnosis of intestinal protozoa, particularly in developing countries. However, microscopic methods require well-trained medical technologists who can identify and differentiate pathogenic from non-pathogenic protozoa and discriminate artifacts. In Libya,

Citation: Libyan Journal of Medicine 2016, **11**: 32088 - http://dx.doi.org/10.3402/ljm.v11.32088

there is a clear shortage of skilled technologists mainly due to a lack of training programs. In recent years, several enzyme immunoassays (EIAs) that detect antigens of *E. histolytica*, *G. lamblia,* and *Cryptosporidium* spp. in fresh or frozen stool specimens with 85 – 100% sensitivity and 93 – 100% specificity have become commercially available (34). Only one study in Libya used second-generation EIAs to investigate stool samples from diarrheic children in Tripoli (11). The investigators observed low prevalence rates of *E. histolytica* (0.8%), *G. lamblia* (1.3%), and *Cryptosporidium* spp. (2.1%). They reported that enteric viral and bacterial pathogens were significant causative agents of childhood diarrhea in Tripoli, but not the intestinal protozoa examined. Their findings contradict the high prevalence rates of *E. histolytica/dispar* reported by other studies from Libya using microscopy, particularly from the city of Sirt (Table 2 and 3). However, molecular studies have shown that many infections previously considered to be due to *E. histolytica* are caused by non-pathogenic *E. dispar* or *E. moshkovskii* (27). As mentioned previously, these three different species of *Entamoeba* cannot be differentiated by microscopy. Furthermore, a study from Saudi Arabia investigated 156 stool samples from diarrheic children for *E. histolytica* (35). The investigators detected *E. histolytica/dispar* in 65% of the samples by microscopy and in 2.6% of the samples by second generation *E. histolytica*-specific EIA. The authors concluded that the high prevalence of *Entamoeba* infections among the examined children was mainly due to *E. dispar* (i.e. *E. dispar/E. moshkovskii*). Therefore, the high prevalence rates of *E. histolytica/dispar* reported previously from Libya should be interpreted carefully.

Prevention and treatment

In developing countries, poor hygiene and the use of untreated human feces (i.e. fecal sludge) are important factors that contribute to the contamination of food and water. In such countries, prevention of infection with *E. histolytica*, *G. lamblia,* and *Cryptosporidium* spp. can be attained by improved community health education, sanitation, hygiene, and water treatment. To reduce enteric pathogens, including intestinal protozoa that may be present on fresh vegetables and fruits, the produce can be washed by clean tap water and soaked in a solution of acetic acid or vinegar for 10 to 15 minutes before consumption (36). Infected food handlers and caregivers in day-care centers and health institutions for the disabled and the elderly should be suspended from work for at least 3 months after completion of treatment with anti-parasitic agents.

The nitroimidazoles (i.e. metronidazole, tinidazole, and ornidazole) are the drugs of choice for the treatment of amebiasis and giardiasis. More than a decade ago, the US Food and Drug Administration (FDA) approved nitazoxanide for the treatment of cryptosporidiosis in children and adults. Nitazoxanide is also effective against *G. lamblia* as well as other intestinal parasites.

Conclusion

The reviewed data suggest that *E. histolytica*, *G. lamblia,* and *Cryptosporidium* spp. may play a minor role in GE among the population in Libya and that infections occur mainly in children 10 years old or younger. In addition, the previously reported high prevalence rates of *E. histolytica/dispar* reported from Libya could have been due mainly to *E. dispar* and *E. moshkovskii*. However, more studies are needed using *E. histolytica*-specific EIAs and/or molecular methods (i.e. PCR) to confirm this observation. The use of such methods will stop unneeded drug therapy of patients infected with *E. dispar*, *E. moshkovskii* or both.

Conflict of interest and funding

The authors have not received any funding or benefits from industry or elsewhere to conduct this study.

References

1. Davis AN, Haque R, Petri WA Jr. Update on protozoan parasites of the intestine. Curr Opin Gastroentrol. 2002; 18: 10–14.
2. Fletcher SM, Stark D, Harkness J, Ellis J. Enteric protozoa in the developed world: a public health perspective. Clin Microbiol Rev. 2012; 25: 420–49.
3. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012; 380: 2095–128.
4. Murray CJL, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012; 380: 2197–223.
5. Muhsen K, Levine MM. A systematic review and meta-analysis of the association between *Giardia lamblia* and endemic pediatric diarrhea in developing countries. Clin Infect Dis. 2012; 55(Suppl 4): S271–93.
6. Brooker S, Clements AC, Bundy DA. Global epidemiology, ecology and control of soil-helminth infections. Adv Parasitol. 2006; 62: 221–61.
7. Mergani MH, Mohammed MA, Khan N, Bano M, Khan AH. Detection of intestinal protozoa by using different methods. Dent Med Res. 2014; 2: 28–32.
8. Al Kilani MK, Dahesh SM, El Taweel HA. Intestinal parasitosis in Nalout popularity, western Libya. J Egypt Soc Parasitol. 2008; 38: 255–64.
9. Alsirieti SRA, Elahwel AM, Elamari AA. Intestinal Protozoa in Libyan patients in Sirt. Jamahiriya Med J. 2006; 6: 59–61.
10. Kasssem HH, Zaed HA, Sadaga GA. Intestinal parasitic infection among children and neonatus admitted to Ibn-Sina Hospital, Sirt, Libya. J Egypt Soc Parasitol. 2007; 37: 371–80.
11. Rahouma A, Klena JD, Krema Z, Abobker AA, Treesh K, Franka E, et al. Enteric pathogens associated with childhood

diarrhea in Tripoli-Libya. Am J Trop Med Hyg. 2011; 84: 886–91.
12. Elsaid MMA, Shaktur AT, Elsaid SM, EL-Alem MDEE, Traish KO, Kahbar F. Prevalence of intestinal protozoa in primary schools in Zawia City, Libya. Nat Sci. 2014; 12: 67–71.
13. Ali MB, Ghenghesh KS, Ben Aissa R, Abuhelfaia A, Dufani MA. Etiology of childhood diarrhea in Zliten-Libya. Saudi Med J. 2005; 26: 1759–65.
14. El Ammari NE, Nair GA. Critical evaluation of the intestinal Protozoan parasites among Libyan and other African residents of Al-Khoms, Libya. J Entomol Zool Stud. 2015; 3: 42–6.
15. Nowara EE, Nair GA. Intestinal protozoan parasites among Libyan, non-Libyan African and non-African residents of Benghazi, Libya. Rev Ibérica Parasitol. 2005; 65: 15–20.
16. Kubti Y, Ali MY, Khan AH, Daw A. Prevalence of intestinal parasites among food handlers in Benghazi, Libya. Sebha Med J. 2011; 10: 22–4.
17. Sadaga GA, Kassem HH. Prevalence of intestinal parasites among primary schoolchildren in Derna District, Libya. J Egypt Soc Parasitol. 2007; 37: 205–14.
18. Abdel-Magied AA, Elahwel AM. Factors associated with intestinal parasitic infection among school children in Sirt, Libya. Benha Med J. 2006; 23: 821–32.
19. Ben Musa NA. Intestinal parasites in school aged children and the first case report on amebiasis in urinary bladder in Tripoli, Libya. J Egypt Soc Parasitol. 2007; 37: 775–84.
20. Ben Musa N, Sehari A, Hawas A. Intestinal parasitic infections among school children in Tripoli, Libya. J Egypt Soc Parasitol. 2007; 37: 1011–16.
21. Saad GS, Khan A, Ajaili A, Abdulsalam AM, Al-Shebani M, Kubti Y. A study of prevalence of human intestinal parasites in Wadi Al-Shati region. Sebha Med J. 2009; 8: 51–8.
22. Ben Rashed MB, Abulhassan M, Tabit A, Hawas A. Demographic features of intestinal parasitic infection among Libyan children. Jamahiriya Med J. 2006; 6: 138–40.
23. Al-Tawaty AH, Khan AH, Naida IE, El-Buni AA. Screening of *Cryptosporidium* oocysts in clinically immunocompetent children. Garyounis Med J. 2002; 19: 26–33.
24. EI-Ammari NE, Nair GA. Occurrence and prevalence of intestinal protozoan parasites in male and female Libyan nationals residing in Benghazi, Libya. Rev Ibérica Parasitol. 2003; 63: 47–54.
25. Ben Musa NA, Ibrahim R. Long term formalin preserved stool specimens for detection of intestinal parasites from school aged children in Tripoli, Libya. J Egypt Soc Parasitol. 2007; 37: 1049–54.
26. El-Ammari NE, Nair GA, Kassem HH. Intestinal protozoan and helminth parasites among Libyans, non-Libyan Arabs and non-Arabs living in Benghazi, Libya. Jordan J Appl Sci. 2004; 6: 72–81.
27. Faust DM, Guillen N. Virulence and virulence factors in Entamoeba histolytica, the agent of human amebiasis. Microbes Infect. 2012; 14: 1428–41.
28. Ximénez C, Morán P, Rojas L, Valadez A, Gómez A. Reassessment of the epidemiology of amebiasis: state of the art. Infect Genet Evol. 2009; 9: 1023–32.
29. Ghenghesh KS, Ghanghish K, El-Mohammady H, Franka E. *Cryptosporidium* in countries of the Arab world: the last decade (2002–2011). Libyan J Med. 2012; 7: 19852, doi: http://dx.doi.org/10.3402/ljm.v7i0.19852
30. Anuar TS, Al-Mekhlafi HM, Abdul Ghani MK, Osman E, Yasin AM, Nordin A, et al. Prevalence and risk factors associated with *Entamoeba histolytica/dispar/moshkovskii* infection among three Orang Asli ethnic groups in Malaysia. PLoS One. 2012; 7: e48165, doi: http://dx.doi.org/10.1371/journal.pone.0048165
31. Abougrain AK, Nahaisi MH, Madi NS, Saied MM, Ghenghesh KS. Parasitological contamination in salad vegetables in Tripoli-Libya. Food Cont. 2010; 21: 760–2.
32. Lawley R, Curtis L, Davis J. The food safety hazard guidebook. Cambridge, United Kingdom: The Royal Society of Chemistry; 2008. p. 146–7.
33. Smith HV, Caccio SM, Cook N, Nichols RAB, Tait A. Cryptosporidium and Giardia as foodborne zoonoses. Vet Parasitol. 2007; 149: 29–40.
34. McHardy IH, Wu M, Shimizu-Cohen R, Couturier MR, Humphries RM. Detection of intestinal protozoa in the clinical laboratory. J Clin Microbiol. 2014; 52: 712–20.
35. Al-Harthi SA, Jamjoom MB. Diagnosis and differentiation of *Entamoeba* infection in Makkah Al Mukarramah using microscopy and stool antigen detection kits. World J Med Sci. 2007; 2: 15–20.
36. Dhawan VK. Amebiasis; 2016. Available from: http://emedicine.medscape.com/article/212029-overview [cited 29 March 2016].