Cryptosporidium and *Giardia lamblia* Epidemiology in Middle Eastern Countries: study of the proliferation problem in the aquatic environment

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Abstract. The aim is to present a summarized study of the available literature on *Cryptosporidium* and Giardia lamblia for Middle Eastern countries, in order to identify trends in human cryptosporidiosis and childhood morbidity, and to raise awareness among residents. This is necessary in order to address the gap in preventive measures required to mitigate the overall effect attributed to associated illness and its impact in the already water stressed Middle Eastern countries. To assess seroprevalence of Cryptosporidium and Giardia lamblia in Middle Eastern countries systematic review was carried out based on online articles published from 2010-2018. PubMed, Web of Science, Google Scholar, Science Direct, Scopus, World Bank and WHO report and scientific database were explored. The current study highlights the existing subsequent epidemiology, its seroprevalence distribution, genetic diversity across Middle Eastern countries since 2009. This study therefore will provide the platform for future research work and development in comprehending Cryptosporidium and Giardia lamblia epidemiology in Middle Eastern countries. It was found that lack of awareness, personal hygiene and sanitation facilities, poverty, indiscriminate eating habits are favourable infestation conditions for Cryptosporidium and Giardia lamblia infections. The prevalence for both Cryptosporidium and Giardia lamblia, is higher in developing countries as compared to developed countries. The originality is that it is the only study of its kind in the region, as such studies are still lacking in Middle East countries as compared to other European, Asian, American continents and countries.

Keywords: water resources, tap water contamination, epidemiology, hygiene and sanitation, protozoan parasites, seroprevalence, childhood morbidity.

1. Introduction

Water resources (surface and underground fresh water) must support the vital activity of humans, plants and animals on the planet. However, due to the negative technogenic influence (Ziarati et al., 2021; Khan et al., 2019a; Khan et al., 2019b; Deineka et al., 2018), as well as due to climatic factors (Dang et al., 2018; Liu et al., 2017), in some countries there are problems with the provision of water supply, safe for consumption.

There are many literatures on protozoan parasites around the world published in peer reviewed journal. However, literature from Middle Eastern countries is still lacking even though it is among one of the most water stressed region in the world. The pathogen entry to water resources is mainly attributed to improper water facilities, insufficient water purification processes and in water scarce region like Middle East water contamination and entry of protozoan parasites like *Cryptosporidium* and *Giardia lamblia* highly is highly susceptible (Mazzoni & Zaccagni, 2019). This review has covered available literature on *Cryptosporidium* and *Giardia lamblia* for Middle Eastern countries. This summarized study will be one of its kind in the region as such studies are still lacking in Middle Eastern countries as compared to other European, Asian, American continents and countries (Xu & May, 2017; Michel et al., 2012; Terink et al., 2013). Figure 1 presents the map of Middle Eastern countries covered in this study.

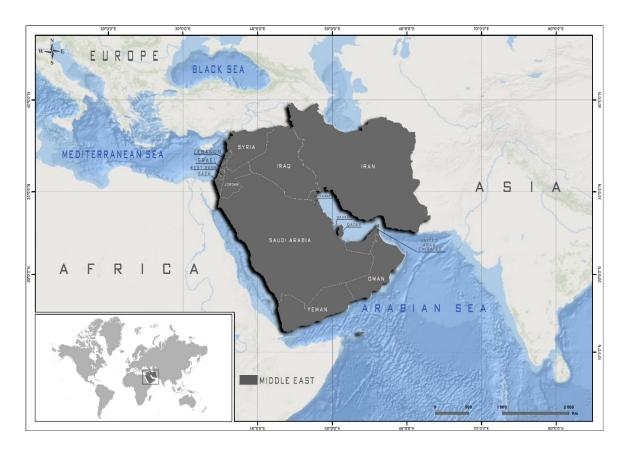


Figure 1. Map of Middle Eastern countries covered in this study

The second primary cause of death among children less than five years is diarrhoea. Each year about 525,000 deaths among kids are attributed to diarrhoea. Globally there are about 1.7 billion cases of diarrhoea each year (WHO, 2019; U.S.H.O.F., 2016). In children below five years mortality (no. of deaths) for the year 2016 in Middle Eastern countries was 1936 (Iraq), 870 (Iran), 117 (Jordan), 1 (Kuwait), 17 (Lebanon), 10 (Oman), 1 (Qatar), 87

(Saudi Arabia), 1519 (Syria), 5 (United Arab Emirates) and 3702 (Yemen) (WHO, 2019; U.S.H.O.F., 2016). *Cryptosporidium* is reported secondary cause of diarrhoea only behind rotavirus. The leading parasitic protozoa causing waterborne enteric disease outbreaks at global level are *Cryptosporidium* and *Giardia lamblia* (Efstratiou et al., 2017). Originating from faeces of infected hosts they can be transmitted to new hosts via oral-faecal route upon contact with contaminated soil, water, feed and food. Hence, the study has focused on each of this water borne protozoa.

2. Materials and Methods

In order to assess seroprevalence of *Cryptosporidium* and *Giardia lamblia* in Middle Eastern countries systematic review was carried out based on online articles published from 2010-2018. PubMed, Web of science, Google Scholar, Science Direct, Scopus, World Bank and WHO report and scientific database were explored to retrieve necessary information for assessment. The present review was performed using keywords, *Cryptosporidium*, *Cryptosporidiosis*, *Giardia lamblia* combined or as individual country for Middle Eastern countries. The literature distribution of *Cryptosporidium* and *Giardia lamblia* is presented in Figures 2 and 3.

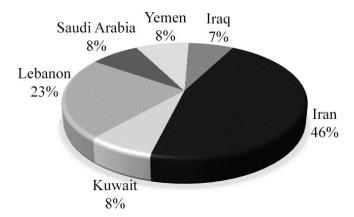


Figure 2. Cryptosporidium literature distribution in Middle Eastern countries

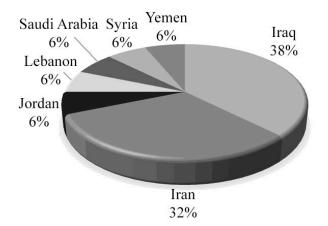


Figure 3. Giardia lamblia literature distribution in Middle Eastern countries

3. Results and Discussion

This study covered articles and reports published for Middle Eastern countries covering *Cryptosporidium* and *Giardia lamblia*. These studies were conducted based on host (Human (Infants, Children, Women, Elderly) or animals (bird, camel, sheep) for the identified *Cryptosporidium* and *Giardia lamblia*.

3.1. Cryptosporidium Seroprevalence in Middle East

The data obtained from literature was summarized and represented in Table 1. Majority of the studies infers that *C. hominis* and *C. parvum* are the primary *Cryptosporidium* species responsible for causing infection in Human population. In study from Kuwait, *C. parvum* was the dominant species present with its subtype prevailing in order of IIa, IId and IIc, while *C. hominis* followed with its subtype in prevailing order of Id, Ia and Ie, inferring that genetic of parasite may play a major role in human cryptosporidiosis (Iqbal et al., 2011). Other species studies did not identify genetics or sub species of *Cryptosporidium* species. This is primarily being due to non-uniform testing procedure and standards adopted across Middle Eastern countries along with lack of standardized infrastructure. PCR (Polymerase Chain Reaction) yielded highest rate of detection as compared to ELISA (Enzyme-linked immunosorbent assay) and MZN (modified ziehl neelsen) staining method. From three techniques; Direct Fluorescent Antibody (DFA), modified acid fast stain and Immune Card techniques used to detect *C. parvum*, modified acid fast stain performed better (Ali et al., 2014). Again, results of studies have variation depending upon the method used to identify genotype of *Cryptosporidium*.

In study from Kuwait; children in 4-8 years of age group are mostly affected (Iqbal et al., 2011). Iran reported, *Cryptosporidium* prevalence of 9.7% was higher in children under 4-5 years of age with *C. parvum* prevailing in diarrheic children (Mahdavi Poor et al., 2015; Dabirzadeh et al., 2017). Even the study in 2011 reported similar results of *C. parvum* dominance over other species of *Cryptosporidium* (Taghipour et al., 2011). Lebanon reported prevalence rate of children was 10.4%, and was peaking in children under five years of age with 11% (Osman et al., 2017; Osman et al., 2018; Osman et al., 2015). In Iraq; or children also Acid fast (AF) staining technique provided better results than immune cards (Ali et al., 2014).

The *Cryptosporidiosis* in Out patients, Hospitalized patients, immune suppressed patients are attributed to *C. hominis* and *C. parvum*. In Iran; among gastroenteritis patient, *Cryptosporidium* was reported for only 2.3% (Gholami et al., 2014). In Lebanon the reported prevalence rate was 21% in adenocarcinoma patients, 11% in symptomatic patients (Osman et al., 2017; Osman et al., 2018; Osman et al., 2015). *Cryptosporidium* was reported not to cause any effect on gastroenteritis but children under 10 year reported highest infection percent (Ghenghesh et al., 2016). Lack of data on age group in studies made it difficult to attribute any *Cryptosporidium* subspecies to specific age group. However, in displaced population (refugees) *C. parvum* was reported to be the cause of *Cryptosporidiosis*.

Among adult birds wild and domestic *C. meleagridis*, (IIIgA22G3R1) and *C. baileyi* (IIIgA23G2R1) were identified as *cryptosporidiosis* causing protozoa. While *C. bovis*, *C. ryanae* and *C. parvum* were identified as cause of cryptosporidiosis in Juvenile calves. *C. parvum* has been identified as causing infection in humans which was also detected in calves which can be responsible for zoonotic transmission of *cryptosporidiosis*.

Table 1. Cryptosporidium prevalence in various hosts by country of occurrence in Middle Eastern countries based on literature from the last one decade

	Sample Source (stool)		Method and		Cryptos	poridiun	n spp.		Cryptospori	Prevalence		
Country		Investigation Method	sequenced molecular markers	Study Period	n positive	n total	% (CI)	Cryptosporidi um spp.	<i>dium</i> spp. Subtype	among species %	Age	Reference
Iraq	Displaced Population	ELISA, modified ZiehlNeelsen, Flotation		2008- 2014	780	780		C. parvum		16.28 25.45 30.3	 <1-5 6-12	Salman et al., 2015
Iran	Children (Hospitalized)	Ziehl-Neelsen		2014- 2016	18	200		С. sp.		8 1	<4 >4	Dabirzadeh et al., 2017
	Children (Diarrhoea patients)	Ziehl-Neelsen staining,	PCR	2005- 2007	19	794		C. parvum C. hominis	IIaA15G2R1 IIdA18G1 IIdA20G1a IIaA15G1 IfA22G1	89.47 10.52		Taghipour et al., 2011
	Farmworkers Calves	Ziehl-Neelsen , modified staining	PCR (18S rDNA)	2009- 2010	31 36	218 422		C. parvum		Farmworker 8.5 9.6 8.3 Calves 14.2	 <5 >5	Izadi et al., 2014
	Children	Acid-fast	PCR (18S	2015	2	113		C. parvum		1.7	3-12	Mahdavi

	(Diarrhoea	staining,	rRNA, SSU									Poor et al.,
	patients)	microscopy	rRNA)									2015
	Children Immunosuppre ssed patients	Modified Zhiel- Neelsen, ELISA		2017	77 84	3630	54-83.9	C. oocysts		3.8 5.2		Kalantari et al., 2018
	Gastroenteritis Patients	Acid fast staining, Auramine phenol fluorescence	PCR-RFLP (SSU rRNA)	2010- 2011	8	348		C oocysts		32.8 32.7 36.8	10-20 21-40 >40	Gholami et al., 2014
Kuwait	Children	ELISA	PCR-RFLP (SSU rRNA)	2005- 2008	87	2548		C. spp. C. parvum C. hominis	IIa IIc IId Ia Id Ie	3.4 41.4 73.5 80.3 80.3 26.5 36.4 54.5 	 4-8 years	Iqbal et al., 2011
Lebanon	Children and Hospitalized patients	ZiehlNeelsen staining, microscopy		2017	16 25	163 249		C. hominis C. parvum	IdA19, IbA10G2, IaA18R3, IIaA15G1R1 ,	10.4 (Children) 11 (patients)		Osman et al., 2018

									IIaA15G2R1			
	Patients (colon adenocarcinom a)	Microscopy, immunofluoresc ence	PCR (18S rRNA)	2016	28	218	1.44- 89.02	C. spp.		21 (Cancer) 7 (No cancer)		Osman et al., 2017
	Hospitalized patients	Modified Ziehl- Neelsen staining, microscopy	PCR	2013	15	163		C. spp. C. parvum C. hominis	IdA19, IIaA15G1R1 , IIaA15G2R1	11 66.7 53.3	<5 year 	Osman et al., 2015
Saudi Arabia	Children (Diarrhoea and asymptomatic)	microscopy		2000	7	253		<i>C.</i> sp.		11 (Diarrhoea) 4.2 (asymptomat ic)	<5 years	Al Braiken et al., 2004
Yemen	Children (Diarrhoea patient)	ELISA' modified Ziehl- Neelsen staining,		2006- 2007	247	712		C. spp.		20.2 48.1 43.1	1 month - 2 year 2 - 6 year 6 - 12 year	Al-Shamiri et al., 2010

A = Adult, **J** = Juvenile, **PCR** = Polymerase Chain reaction, **ELISA** = enzyme-linked immunosorbent assay

3.2 Giardia lamblia seroprevalence in Middle East

Giardia duodenalis (*G. intestinalis* and *G. lamblia*) are morphologically similar terms which are used interchangeably in this study. From *Giardia lamblia* species only *G. duodenalis* can cause infection among humans. Majority of the reports have identified *G. duodenalis* as cause of Diarrhoea in hosts. However, among the reported prevalence of *Giardia lamblia* subspecies *Assemblage/Genotype A*. and *Assemblage B*. among various segment of population and age groups. Other subspecies *Assemblage E*. and *Assemblage F*. are sparsely reported.

Giardia lamblia infections in Saudi Arabia were reported to be prevalent more in children under five years old (Shalaby et al., 2011). In Iran, prevalence of *Giardia lambliasis* among children reported highest in children 4-6 years and lowest for children 0-2 years (Faraji et al., 2015). Also, in other study for diarrheic children; found *Assemblage B.*, B-IV and B-III predominant prevalence over *Assemblage A*. (El-Badry et al., 2018). *Assemblage B.* prevailed over *Assemblage A. G. lamblia* was prevalent in 1-10 years of age group in Iraq (Salman et al., 2015). The study in Lebanon reported, genotype *Assemblage B.* was predominant over *Assemblage A.* among school children (Osman et al., 2016). This may be due to Children infected with *Assemblage B.* released more cysts as compared to children infected with *Assemblage A.* (El Fatni et al., 2014).

The studies covering adult patients in Iraq, reported predominance of *Assemblage A*. over *Assemblage B*. Also study among food handlers depicted similar trend (Turki et al., 2015). The prevalence rate was affected by seasonal variation and was reported to be 25% for summer, 20% for spring, 15.15% for autumn and 0% in winter (Taha et al., 2018). Based on gender the prevalence in boys was higher than in girls (El Fatni et al., 2014). *Giardia lambliasis* infections are more prevalent more in children under five years old and in elder population (Shalaby et al., 2011). Human transmission was indicated but the need for more investigations has been suggested to determine the dynamics of the real transmission and epidemiology (Hijjawi et al., 2015). Adaptation of clean and hygienic practices was identifies as means to control transmission and morbidity attributed to *G. duodenalis* (Al-Mekhlafi, 2017).

Country	Sample	Method (and sequence	Duration of	N positive	N total	% (CI)	Genotype	Prevalence	Age	Reference
country	Source	molecular markers)	study	it positive	i totui	/0 (01)	Genotype	among species	inge	Reference
	Displaced Population (patients)	Microscopy	2014-2015	40	417		G. lamblia	10.31		Salman et al., 2016
	Children (Diarrhoeal)	PCR	2017	108	160		<i>G. lamblia</i> Genotype A Genotype B	26.25 28.57 71.43		Hussein et al., 2018
Iraq	Patients(acuteorchronicDiarrhoea)	Flotation and PCR	2014-2015	73	200		Genotype A Genotype B Genotype E Genotype F	30.14 26.03 20.55 23.29	>1 - 40	Turki et al., 2015
	Children (Giardia lambliasis)	ELISA	2016-2017	21	39		Genotype A Genotype B Mixed	23.80 71.42 4.76	0-11	Taha et al., 2018
	Children and Adults	microscopy	2015-2016	31	780		G. lamblia	3.9 7.3	1-20 >50	Bazzaz et al., 2017
	Children and Adults	microscopy	2011-2012	97	97		G. lamblia	25.77 22.68 22.68	1-10 11-20 21-30	Khudhair, 2020
Iran	Humans	microscopy	2015	222	4788	0.2-2.1	G. lamblia	3.8 9.4 4.9	<5 5-9 10-14	Siyadatpanah et al., 2018

 Table 2. Giardia lamblia prevalence in Middle Eastern countries by country of occurrence for the last one decade

								3.1	15-24	
								3.1	25-39	
								3.3	>40	
								74.41		
	Patients (<i>Giardia</i> <i>lamblia</i> sis)	PCR-RFLP	2010	174	205		Assemblage AII Assemblage BII	17.44		Sarkari et al.,
							Assemblage BIV Mixed A & B	3.49		2012
								4.66		
	Food Handlers	Microscopy, PCR- RFLP	2015	47	3653		G. lamblia	74.5		Hooshyar et al., 2017
		Microscopy, PCR (TPI)			1383		G. lamblia	1.66		Bahrami et al., 2017
	Patients		2015-2016	23			Assemblage A	52		
							Assemblage B	48		al., 2017
	Children							13.93	0-6	
	(Childcare		2012	134	632		G. lamblia	1.74	0-2	Faraji et al.,
		Microscopy	2012					10.76	2-4	2015
	centre)							14.24	4-6	
							At tpi locus			
							А	46.4		
T 1	Defente	PCR (TPI)	2014-2015	49			В	50	0>-45	Hijjawi et
Jordan	Patients				49		Mixed A & B	3.6		al., 2015
							At gdh locus			
							Assemblage AII	43.7		

							Assemblage B	56.3		
Lebanon	School Children	Microscopy, Modified Ziehl- Neelsen staining, PCR	2013	61	212		G. duodenalis	28.5	3-16	Osman et al., 2016
Saudi Arabia	General Population (Healthcare facility)	PCR-RAPD	2010	15	100		G. duodenalis	15		Shalaby et al., 2011
Syria	Patients	microscopy	2006	206	1469		Assemblage A Assemblage B Mixed A +B	67.5 10 22.5	1-16	Skhal et al., 2016
Yemen	General Population (rural communities)	microscopy	2012-2014	170	605	0.73- 1.48	G. lamblia	28.1 33.3 13.5	 <5 >40	Al-Mekhlafi et al., 2017

3.3 Discussion regarding the transmission of *Cryptosporidium* and *Giardia lamblia* in Middle Eastern countries

A significant relation for based on gender, socio- economy in rural areas and animal contact role was derived (Shalaby & Shalaby, 2015). High affinity of infections was associated with tap water contamination and animal contact in urban populations (Gawad et al., 2018). There is a potential risk of zoonotic transmission of Cryptosporidium and Giardia lamblia to human beings (Ghoneim et al., 2017). Based on the zoonotic patterns of isolates, it can be identified that the mode of transmission is direct or indirect animal contact (Taghipour et al., 2011). Lebanon reported molecular analysis as an important tool to identify mode of transmission (Osman et al., 2016). Kuwait reported genetic condition of parasites plays important role in human cryptosporidiosis (Iqbal et al., 2011). Report from Jordan identified human to human transmission (Hijjawi et al., 2015). When there is no significant demographic variation then modes of transmission can be ingestion of contaminated water, and human or animal contacts (Iqbal et al., 2001). Identification of the transmission routes will help in adopting mitigation measures. Hence, prevent spread of the infection is the most effective strategy (Abd El Kader et al., 2012). However, zoonotic transmission cannot be eradicated from Middle Eastern countries as whole region practices animal husbandry owing to extreme conditions prevailing in most of the region rendering animals as sustainable source of food and other products thereof. Lack of awareness, personal hygiene and sanitation facilities, poverty, indiscriminate eating habits are favourable infestation conditions for Cryptosporidium and Giardia lamblia infections. The prevalence for both Cryptosporidium and Giardia lamblia, is higher in developing countries as compared to developed countries (Mahmoudi et al., 2017; Efstratiou at al., 2017). Interesting is the future study of statistics regarding the measures taken to prevent the disease in children and to treat the sick. However, there is very little information in open sources, which does not allow drawing logical conclusions about the better mitigation of the consequences.

4. Conclusions

The literature in this study was collected to present an overall scenario of prevalence of *Cryptosporidium* and *Giardia lamblia* in Middle Eastern countries. The study is in lieu with literature from other region however in details it varies globally and at local Middle Eastern countries level. The variance of *Cryptosporidium* and *Giardia lamblia* follows similar pattern in human and animals as reported worldwide. Poor sanitation and improper hygiene condition are primary responsible for occurrence and thereby transmission of the parasite. However zoonotic transmission arising from animal to human contact is also a significant factor. The

most affected section of population comprised of under 5 years of age children, patients (Immune deficient) and weaker economic section of population, moreover, the prevalence among boys was higher than among girls. This is as per the global trend of high prevalence in developing countries than in developed countries.

The major hurdle that the study faced was non uniformity of literature availability. The number of studies varied greatly which impacted the overall study efficiency. For example, Gulf countries viz. Saudi Arabia, Kuwait, Qatar, Bahrain, Oman, United Arab Emirates primarily depend on desalination as drinking water source which limits the need of protozoa occurring research and relevant mitigation measures. In addition, there is heterogeneity in testing procedures and standards across the Middle East, as well as a lack of standardized infrastructure. Hence these countries produced least number of relevant researches works. The other reason for low frequency of published report is the regional instability (political, socio-economical) faced in various countries viz. Syria, Yemen, Iraq, Palestine, Libya and affecting the neighbouring countries thereof (refugees) especially in last one decade which has highly compromised the scope of research in these countries.

Future research work should be based on existing literature on *Cryptosporidium* and *Giardia lamblia*. Additionally, future work can be specific to research on molecular techniques which can provide important information on various strains present in Middle Eastern countries. Also, for the future mitigation of the consequences, it is necessary to investigate the appropriate measures that were applied to prevent the disease in children (types of sanitation and hygiene, ways to increase immunity, dietary habits, environment, etc.) and for the treatment of patients (types and schedule of medicinal treatment, restrictions, etc.). The lack of public awareness recognizing *Cryptosporidium* and *Giardia lamblia* responsible for Diarrhoea in children needs to be addressed by relevant authorities.

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