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.

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
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.
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

| Country   | Sample Source (stool) | Investigation Method | Method and sequenced molecular markers | Study Period | *Cryptosporidium* spp. | Cryptosporidium spp. Subtype | Prevalence among species % | Age | Reference         |
|-----------|------------------------|----------------------|----------------------------------------|--------------|------------------------|----------------------------|-----------------------------|-----|------------------|
| Iraq      | Displaced Population   | ELISA, modified ZiehlNeelsen, Flotation | --                                      | 2008-2014    | 780                    | 780                        | --                          |     | Salman et al., 2015 |
|           |                        |                      |                                        |              | n positive             | n total                    | % (CI)                      |     |                  |
| Iran      | Children (Hospitalized)| Ziehl-Neelsen        | --                                      | 2014-2016    | 18                     | 200                        | --                          |     | <4                |
|           |                        |                      |                                        |              | C. sp.                 | --                         | 8                           |     | Dabirzadeh et al., 2017 |
| Iran      | Children (Diarrhoea patients) | Ziehl-Neelsen staining, | PCR                                      | 2005-2007    | 19                     | 794                        | --                          |     | Taghipour et al., 2011 |
|           |                        |                      |                                        |              | C. parvum              | C. hominis                 | IaA15G2R1 IIdA18G1 IIdA20G1a IaA15G1 IfA22G1 | 89.47 | 10.52 |                  |
|           | Farmworkers Calves    | Ziehl-Neelsen, modified staining | PCR (18S rDNA)                          | 2009-2010    | 31                     | 218                        | --                          |     | Izadi et al., 2014  |
|           |                        |                      |                                        |              | 36                     | 422                        | C. parvum                   |     |                  |
|           | Children               | Acid-fast            | PCR (18S)                               | 2015         | 2                      | 113                        | --                          | 1.7 | 3-12 | Mahdavi           |
| Study Location  | Study Group                      | Methodology                                      | Year Range | Cysts Per 100 | Species       | Age Range | Notes               |
|----------------|----------------------------------|--------------------------------------------------|------------|----------------|---------------|-----------|---------------------|
| Kuwait         | Children                         | ELISA, PCR-RFLP (SSU rRNA)                       | 2005-2008  | 87             | C. parvum     | --        | 3.4/73.5/80.3       | Iqbal et al., 2011 |
|                |                                  |                                                  |            | 2548           | C. hominis    |           | 41.4/26.5           |                      |
|                |                                  |                                                  |            |                | C. spp.       |           | 80.3/36.4/54.5      |                      |
|                |                                  |                                                  |            |                | ---           |           | 26.5/36.4/54.5      |                      |
|                |                                  |                                                  |            |                |               |           | 4-8 years           |                      |
| Lebanon        | Children and Hospitalized patients | Ziehl-Neelsen staining, microscopy               | 2017       | 16             | C. hominis    | 10.4      | 11 (Children)       | Osman et al., 2018  |
|                |                                  |                                                  |            | 25             | C. parvum     |           | (Patients)          |                      |
|                |                                  |                                                  |            | 163            | IdA19, IbA10G2, IaA18R3, IIaA15G1R1 |           |                      |                      |
|                |                                  |                                                  |            | 249            |               |           |                     |                      |
|                |                                  |                                                  |            |                |               |           |                     |                      |
|                | Children                         | Modified Zhiel-Neelsen, ELISA                    | 2017       | 77             | C. oocysts    | 3.8       | 5.2                | Poor et al., 2015   |
|                | Immunosuppressed patients        |                                                  |            | 84             |               |           |                     |                      |
|                |                                  |                                                  |            | 3630           |               |           |                     |                      |
|                |                                  |                                                  |            | 54.8-3.9       |               |           |                     |                      |
|                |                                  |                                                  |            |                |               |           |                     |                      |
|                | Gastroenteritis Patients         | Acid fast staining, Auramine phenol fluorescence | 2010-2011  | 8              | C. oocysts    | 32.8      | 32.7/36.8           | Kalantari et al., 2018 |
|                |                                  | PCR-RFLP (SSU rRNA)                              |            | 348            |               |           | 10-20/21-40/40      |                      |
|                |                                  |                                                  |            |                |               |           |                     |                      |
|                |                                  |                                                  |            |                |               |           |                     |                      |
| Country | Group | Method | Year | Age | Genus/Species | PCR Result | Reference |
|---------|-------|--------|------|-----|---------------|------------|-----------|
| Saudi Arabia | Children (Diarrhoea and asymptomatic) | Microscopy | 2000 | 7 | C. sp. | -- | Al Braiken et al., 2004 |
| Yemen | Children (Diarrhoea patient) | ELISA’ modified Ziehl-Neelsen staining | 2006-2007 | 247 | C. spp. | -- | Al-Shamiri et al., 2010 |
| Patients (colon adenocarcinoma) | Microscopy, immunofluorescence | PCR (18S rRNA) | 2016 | 28 | C. spp. | 1.44-89.02 | Osman et al., 2017 |
| Hospitalized patients | Modified Ziehl-Neelsen staining, microscopy | PCR | 2013 | 15 | C. spp. | C. parvum, C. hominis | IdA19, IlaA15G1R1, IlaA15G2R1 | Osman et al., 2015 |

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

| Country                  | Sample Source                          | Method (and sequence molecular markers) | Duration of study | N positive | N total | % (CI) | Genotype                  | Prevalence among species | Age | Reference               |
|--------------------------|----------------------------------------|----------------------------------------|-------------------|------------|--------|--------|---------------------------|-------------------------|-----|-------------------------|
| Iraq                     | Displaced Population (patients)        | Microscopy                             | 2014-2015         | 40         | 417    | --     | *G. lamblia*              | 10.31                   | --  | Salman et al., 2016     |
| Children (Diarrhoeal)    | PCR                                    | 2017                                   | 108               | 160        |       | --     | *G. lamblia*              | 26.25                   | --  | Hussein et al., 2018    |
| Patients (acute or chronic Diarrhoea) | Flotation and PCR                 | 2014-2015                             | 73                | 200        |       | --     | Genotype A                | 30.14                   | >1 - 40 | Turki et al., 2015     |
| Children (*Giardia lamblia*is) | ELISA                             | 2016-2017                             | 21                | 39         |       | --     | Genotype A                | 23.80                   | 0-11 | Taha et al., 2018       |
| Children and Adults      | microscopy                             | 2015-2016                             | 31                | 780        |       | --     | *G. lamblia*              | 3.9                      | 1-20 >50 | Bazzaz et al., 2017   |
| Children and Adults      | microscopy                             | 2011-2012                             | 97                | 97         |       | --     | *G. lamblia*              | 25.77                   | 1-10 11-20 21-30 | Khudhair, 2020 |
| Iran                     | Humans                                 | microscopy                             | 2015              | 222        | 4788   | 0.2-2.1 | *G. lamblia*              | 3.8                      | <5   | Siyadatpanah et al., 2018 |
| Category                          | Method           | Year  | N    | Assemblage | A II | B II | B IV | Mixed A & B | Reference            |
|----------------------------------|------------------|-------|------|------------|------|------|------|-------------|----------------------|
| Patients (Giardia lamblia)       | PCR-RFLP         | 2010  | 174  | 205        | 3.11 | 3.11 | 3.31 | 3.31        | 74.41                |
|                                  |                  |       |      |            | 3.11 | 3.11 | 3.31 | 3.31        | Hijjawi et al., 2015 |
| Food Handlers                    | Microscopy, PCR-RFLP | 2015  | 47   | 3653       | 74.5 | 17.44| 3.49 | 4.66        | Hooshyar et al., 2017|
| Patients                         | Microscopy, PCR (TPI) | 2015-2016 | 23   | 1383       | 1.66 | 52   | 48   | 1.66        | Bahrani et al., 2017 |
| Children (Childcare centre)      | Microscopy       | 2012  | 134  | 632        | 13.93| 1.74 | 10.76| 14.24       | Faraji et al., 2015  |
| Jordan                           | Patients         | 2014-2015 | 49   | 49         | 46.4 | 50   | 3.6  | 43.7        | Hijjawi et al., 2015 |
| Country         | Type               | Methodology                                      | Year | Sample Size | Prevalence | Age Range | Assemblage | Source                          |
|-----------------|--------------------|--------------------------------------------------|------|-------------|------------|-----------|------------|---------------------------------|
| 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 | 67.5 1-16 | Skhal et al., 2016             |
|                 |                    |                                                  |      |             |            |           | Assemblage B | 10 22.5 |                   |
|                 |                    |                                                  |      |             |            |           | Mixed A +B | 22.5 1-16 |                   |
| Yemen           | General Population (rural communities) | microscopy                                       | 2012-2014 | 170     | 605        | 0.73-1.48 | G. lamblia | 28.1 33.3 | Al-Mekhlafi et al., 2017       |
|                 |                    |                                                  |      |             |            |           |             | 13.5 <5 |                   |
|                 |                    |                                                  |      |             |            |           |             | >40    |                   |
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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