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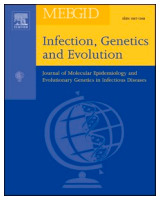

Review

# A review of the molecular epidemiology of *Cryptosporidium* spp. and *Giardia duodenalis* in the Middle East and North Africa (MENA) region

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ABSTRACT

*Cryptosporidium* spp. and *Giardia duodenalis* are important protozoan parasites which are associated with diarrheal diseases in humans and animals worldwide. Relatively little is known about the molecular epidemiology of *Cryptosporidium* spp. and *Giardia duodenalis* in the Middle East Countries and North Africa (MENA region). Therefore, this review aimed to inspect published genotyping and subtyping studies on *Cryptosporidium* spp. and *Giardia duodenalis* in the MENA region. These studies indicate that both anthroponotic and zoonotic transmission of *Cryptosporidium* occurs with the predominance of zoonotic transmission in most countries. Seven *Cryptosporidium* species were identified in humans (*C. parvum*, *C. hominis*, *Cryptosporidium meleagridis*, *C. felis*, *Cryptosporidium muris*, *C. canis* and *C. bovis*), with *C. parvum* by far being the most prevalent species (reported in 95.4% of the retrieved studies). Among *C. parvum gp60* subtype families, IIa and IId predominated, suggesting potential zoonotic transmission. However, in four MENA countries (Lebanon, Israel, Egypt and Tunisia), *C. hominis* was the predominant species with five subtype families reported including Ia, Ib, Id, If and Ie, all of which are usually anthroponotically transmitted between humans. In animals, the majority of studies were conducted mainly on livestock and poultry, 15 species were identified (*C. parvum*, *C. hominis*, *C. muris*, *Cryptosporidium cuniculus*, *C. andersoni*, *C. bovis*, *C. meleagridis*, *C. baileyi*, *C. erinacei*, *C. ryanae*, *C. felis*, *C. suis*, *Cryptosporidium galli*, *C. xiaoi* and *C. ubiquitum*) with *C. parvum* (IIa and IId subtypes) the dominant species in livestock and *C. meleagridis* and *C. baileyi* the dominant species in poultry. With *G. duodenalis*, five assemblages (A, B, C, E and F) were identified in humans and six (A, B, C, E, D and F) in animals in MENA countries with assemblages A and B commonly reported in humans, and assemblages A and E dominant in livestock. This review also identified a major knowledge gap in the lack of *Cryptosporidium* spp. and *Giardia duodenalis* typing studies in water and food sources in the MENA region. Of the few studies conducted on water sources (including drinking and tap water), ten *Cryptosporidium* species and four genotypes were identified, highlighting the potential role of water as the major route of *Cryptosporidium* spp. transmission in the region. In addition, three *G. duodenalis* assemblages (A, B and E) were detected in different water sources with AI, AII and BIV being the main sub-assemblages reported. More research is required in order to better understand the molecular diversity and transmission dynamics of *Cryptosporidium* spp. and *Giardia duodenalis* in humans, animals, water and food sources in MENA region.

## 1. Background

Diarrheal diseases are among the leading causes of death globally especially in children <5 years old, accounting for 1.6 million death in 2017 (Dadonaite et al., 2018) with an extensive burden reported in many recent studies (Roth et al., 2018; Khalil et al., 2018; Kotloff et al., 2019; Ugboko et al., 2020). In general, poor hygiene, access to unsafe drinking water, poverty and overcrowding are key factors responsible for the increase in the transmission of diarrheal diseases, especially in developing countries and Middle East and North Africa (MENA region) (Squire and Ryan, 2017; Ahmad et al., 2020; Yang et al., 2021). The socio-economic impact of these diseases are even greater in developing countries such as the MENA region. This is mainly due to contributing factors such as lack of adequate sanitation and access to clean water,

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lack of transport to medical facilities and a shortage of skilled health care workers and inadequate reporting and lack of surveillance systems (WHO, 2016; Ryan et al., 2017; Squire and Ryan, 2017; Ahmad et al., 2020).

The protozoan parasites *Cryptosporidium* spp. and *Giardia duodenalis* (syn. *Giardia lamblia* and *Giardia intestinalis*) are important causative agents of diarrhea in a wide range of vertebrates including humans, domestic and wild animals with a worldwide distribution (Ahmed et al., 2018; Robertson et al., 2020; Yang et al., 2021). Both parasites are transmitted by the oral faecal route upon the ingestion of oocysts/cysts via contaminated food or drink, or through direct contact with infected persons (person-to-person transmission) or animals (zoonotic transmission) (Gorcea et al., 2020; Krumkamp et al., 2021). The Global Enteric Multicentre Studies (GEMS) has shown that *Cryptosporidium* is a major cause of moderate-to-severe diarrhea (MSD) and mortality in young children in African and Asian countries (Delahoy et al., 2018; Kotloff et al., 2019; Levine et al., 2020). *Giardia duodenalis* infects approximately 200 million individuals worldwide (Feng and Xiao, 2011), however asymptomatic infections are common and it has been inversely associated with MSD (Kotloff et al., 2019; Messa Jr et al., 2021), but is associated with irritable bowel and chronic fatigue syndromes (Lalle and Hanevik, 2018). In addition, both parasites are frequently associated with vomiting, weight loss, fever, abdominal pain and can result in growth stunting and cognitive impairment in children (Berkman et al., 2002; Rogawski et al., 2017; Iglói et al., 2018; Plutzer et al., 2018).

In animals, both parasites (*Cryptosporidium* spp. and *Giardia duodenalis*) can cause severe diarrhea and mortality, particularly in young ruminants, resulting in substantial economic losses (Innes et al., 2020; Santin, 2020; Shaw et al., 2020). Currently, nitazoxanide is the only FDA approved drug to treat cryptosporidiosis but is ineffective in immunocompromised individuals (Fox and Saravolatz, 2005). 5-nitroimidazoles and benzimidazoles derivatives, quinacrine, furazolidone, paromomycin and nitazoxanide have been approved for the treatment of human giardiasis, but treatment failures are increasingly common (Lalle and Hanevik, 2018). Halofuginone (Halocur) is approved in some countries to treat cryptosporidial infections in calves and lambs, while albendazole, fenbendazole, and paromomycin have been used to treat giardial infections in animals, however treatment outcomes are variable (Santin, 2020).

To date, 44 *Cryptosporidium* species and more than 100 genotypes have been recognised within the genus *Cryptosporidium,* with *C. hominis* and *C. parvum* responsible for the majority of infections in humans (Firoozi et al., 2019; Zahedi and Ryan, 2020; Guo et al., 2021; Zahedi et al., 2021). Other species and genotypes that have been reported in humans includes *C. andersoni*, *C. bovis*, *C. canis*, *Cryptosporidium cuniculus*, *C. ditrichi*, *C. erinacei*, *C. fayeri*, *C. felis*, *Cryptosporidium meleagridis*, *Cryptosporidium muris*, *C. scrofarum*, *C. suis*, *Cryptosporidium tyzzeri*, *C. ubiquitum*, *C. viatorum*, *C. xiaoi*, *Cryptosporidium* chipmunk genotype I, *Cryptosporidium* skunk genotype, *Cryptosporidium* horse genotype and *Cryptosporidium* mink genotype (Ryan et al., 2017; Zahedi et al., 2016; Feng et al., 2018; Zahedi and Ryan, 2020; Braima et al., 2021).

*Giardia duodenalis* is a species complex consisting of eight distinct assemblages (A–H); with assemblages A and B having a wide host range and being responsible for the majority of human giardiasis cases, while assemblages C, D, E, F, G and H are mostly considered to be more adapted to animals (domestic mammals, cats, rodents, and seals), however a few cases of assemblages C, D, E and F have been reported in humans (Broglia et al., 2013; Štrkolcová et al., 2015; Scalia et al., 2016; Zahedi et al., 2017; Cacciò et al., 2018). Assemblage A can be subtyped into sub-assemblages AI, AII, and AIII by multilocus sequence typing (MLST), with sub-assemblages AI and AII being major contributors to human and animal giardiasis worldwide, and sub-assemblage AIII more commonly infects hoofed animals (Cacciò et al., 2018). Assemblage B has previously been subtyped into BIII, BIV but these groupings are now considered invalid (Feng and Xiao, 2011: Brynildsrud et al., 2018) and a new typing system for Assemblage B has been developed (Seabolt et al., 2021). However, for the purposes of this review, we have referred to BIII and BIV subtypes as they have been reported in the literature.

A recent excellent review examined the epidemiology of cryptosporidiosis in low and middle-income countries (Yang et al., 2021). In that study, a much wider range of countries was examined including Asia, Africa and South America in addition to some countries in the Middle East. The aim of the current review is to summarise available information on the molecular epidemiology of *Cryptosporidium* spp. and *Giardia duodenalis* in humans, animals and water/food samples specifically in the MENA region (defined below) and discuss the transmission dynamics particular to that region.

## 2. Search strategy

For the purpose of this review, electronic databases consisted of published articles in databases including Google Scholar, ResearchGate, PubMed, ScienceDirect, Scopus and some relevant articles that were published in local journals were accessed. The screened articles which were published between 2005 and 2021 were included in the 20 countries which comprise the MENA region: Algeria, Bahrain, Egypt, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Libya, Morocco, Oman, Palestine, Qatar, Saudi Arabia, Syria, Turkey, Tunisia, United Arab Emirates and Yemen. Although microscopy is still the international standard for detection of *Cryptosporidium* spp. and *Giardia duodenalis* in water and food samples, it cannot identify species and subtypes. Therefore, only studies which reported the detection, prevalence and or typing of *Cryptosporidium* spp. and *Giardia duodenalis* using molecular tools (PCR-based assays) in humans and animals were included. An exception to this search strategy was made for water and food samples as there were so few studies on these topics conducted in the MENA region that studies which screened by microscopy followed by molecular characterisation were included. In general the literature search strategy was limited to title, abstract and key words: *Cryptosporidium* or cryptosporidiosis, *Giardia duodenalis* or giardiasis, molecular prevalence, genotyping and subtyping (human, animal, water or food) for any country in MENA region.

A total of 65 and 68 peer reviewed research articles with a focus on molecular prevalence, characterisation, typing and subtyping of *Cryptosporidium* spp. and *Giardia duodenalis* in humans respectively in the MENA region were retrived from databases (Tables 1 and 2). The highest number of articles for studies on the molecular analysis of *Cryptosporidium* spp. was retrieved from Iran (20) followed by Egypt (9), Iraq (7), Saudi Arabia (5), Lebanon (4), Jordan and Tunisia (3 each), Turkey, Kuwait, Qatar, United Arab Emirates, and Israel (2 each), Algeria, Palestine, Yemen and Syria (1 each), however, no studies were found for Oman, Bahrain, Libya and Morocco (Table 1). Regarding molecular analysis of *Giardia duodenalis* in the MENA region, the highest number of articles was retrived from Iran and Egypt (19 each), Iraq (12), Turkey (6), Algeria, Saudi Arabia and Jordan (2 each), Morocco, United Arab Emirates, Lebanon, Syria, Qatar and Yemen (1 each), with no studies found for Oman, Libya and Bahrain (Table 2).

A total of 54 and 17 research articles were retrieved from databases that used molecular tools to study the prevalence and subtypes/assemblages of *Cryptosporidium* spp. and *Giardia duodenalis* in animals in MENA region, respectively (Tables 3, 4). The highest number of *Cryptosporidium* articles was retrieved from Iran (17) followed by Egypt (11), Algeria (10), Iraq (7), Turkey (4), Jordan, Kuwait, Syria, Israel and Tunisia (1 each) and no studies could be found in the rest of 10 countries of MENA region. Only few studies from six countries in the MENA region used genotyping tools to study *Giardia duodenalis* in animals; with the highest number of studies conducted in Turkey (5) followed by Egypt (4), Iran (3), Algeria and Iraq (2 each) and one from Jordan (1).

Very few reports on the molecular characterisation and prevelance of *Cryptosporidium* spp. and *Giardia duodenalis* in water and enviromental samples in the MENA region were extracted from the databases (10 for

**Table 1**
Molecular characterisation and prevalence of species and subtypes of *Cryptosporidium* in humans in countries of MENA region (2005–2021).

| Country | Study population | Age group | Percentage positive for *Cryptosporidium* based on screening by PCR (No. positive/No. tested) | Genotyping method (Target gene) | Species and subtypes (where available) and their percentage % | Reference |
|---|---|---|---|---|---|---|
| Algeria | HIV/AIDS[a] infected patients | < 10–90 years old | 9.4% (33/350) | PCR[b] (18S rRNA[c] and *gp60*[d]) 22 isolates genotyped | *C. parvum* 68.2% (15/22)<br>IIa 31.8% (7/22)<br>IId 36.3% (8/22)<br>*C. hominis* 22.7% (5/22)<br>Ia 0.09%(2/22)<br>Ib 1.3% (3/22)<br>*C. felis* 0.09% (2/22) | Semmani et al., 2020 |
| Egypt | Patients with liver cirrhosis | 29–46 years old | 3.3% (2/60) | Copro-nPCR/ RFLP assays[e] (COWP) | *C. parvum* 100% | Abo-Mandil et al., 2020 |
| Egypt | Children with diarrhea | < 8 years old | 1.4% (8/585) | PCR- RFLP[f] (SSU-rRNA[g] and *gp60*) | *C. parvum* 37.5% (3/8)<br>IIdA20G1 12.5% (1/8)<br>IIaA15G2R1 12.5% (1/8)<br>IIcA5G3a 12.5% (1/8)<br>*C. hominis* 62.5% (5/8)<br>IbA6G3 25% (2/8)<br>IdA17 12.5% (1/8)<br>IdA24 12.5% (1/8)<br>IfA14G1R5 12.5% (1/8) | Naguib et al., 2018a |
| Egypt | Children | 2–12 years old | 14% (14/100) | PCR- RFLP (COWP[h]) | *C. parvum* 28.6% (4/14)<br>*C. hominis*50% (7/14)<br>Mixed *C. parvum* + *C. hominis* 21.4% (3/14) | Gharieb et al., 2018 |
| Egypt | Children with and without diarrhea | NS [i] | 19.5% (84/431) | PCR- RFLP (HSP90[j]) | *C. parvum* 7.1% (6/84)<br>*C. hominis* 89.3% (75/84)<br>Failed to amplify 3.6% (3/84) | El-badry et al., 2017 |
| Egypt | Patients with and without diarrhea | 1–60 years old | 19% (55/290) | Nested PCR- RFLP (COWP and *gp60*) | *C. parvum* 25% (5/20)<br>IIdA20G1<br>*C. hominis* 75% (15/20) | Ibrahim et al., 2016 |
| Egypt | Children with diarrhea | 1–14 years old | 11.63% (50/430) | PCR-RFLP (TRAP-C2 gene[k]) | *C. parvum* 82% (41/50)<br>*C. hominis* 12% (6/50)<br>Mixed *C. parvum* + *C. hominis* 6% (3/50) | Eraky et al., 2014 |
| Egypt | Children with diarrhea | 3 months - 8 years old | 10.9% (12/110) | Nested PCR- RFLP (COWP) | *C. hominis* 88.9% (8/9)<br>1 isolate did not digest | Sadek, 2014 |
| Egypt | Children with diarrhea | <10 years old | 49.1% (81/165) | Nested PCR and PCR-RFLP (18S rDNA[l] and *gp60*) | *C. parvum* 38.3%<br>IIa 50%<br>IIaA15G1R1<br>IIaA15G2R1<br>IId 50%<br>IIdA20G1<br>*C. hominis* 60.5%<br>Mixed *C. parvum* + *C. bovis* 1.2% | Helmy et al., 2013 |
| Egypt | Patients with gastrointestinal discomfort | 1 month −70 years old | 4.6% (18/391) | PCR-RFLP (COWP) 15 isolates genotyped | *C. parvum* 20% (3/15)<br>*C. hominis* 60% (9/15)<br>Mixed infection 20% (3/15) | Abd El Kader et al., 2012 |
| Iran | Patients with diarrhea | 1 day - 33 years old | 12% (6/50) | Nested PCR (*gp60*) | *C. parvum* 100% (6/6)<br>IIdA14G1 83.3% (5/6)<br>IIdA15G1 16.7% (1/6) | Najafi-Asl et al., 2020 |
| Iran | Immunocompromised patients (HIV/ Cancer, and organ transplants patients) | > 15 years old | 3.46% (12/346) | Hemi-nested PCR sequencing (SSU-rRNA) | *C. parvum* 8.3% (1/12)<br>*C. hominis* 91.6% (11/12) | Izadi et al., 2020 |
| Iran | Individuals with and without tuberculosis (TB) | NS | 0.87% (3/342) | Nested PCR- RFLP (*gp60*) | *C. parvum* 100% (3/3)<br>IIa 66.7% (2/3)<br>IId 33.3% (1/3) | Taghipour et al., 2019 |
| Iran | Children with diarrhea | NS | 14.8% (27/182) | PCR- RFLP (SSU-rRNA) | *C. parvum* 63% (17/27)<br>*C. hominis* 37% (10/27) | Mohammadian et al., 2019 |
| Iran | Children with acute diarrhea | Children <10 years old | 0.91% (7/764) | Nested-PCR (18S rRNA) | *C. parvum* | Mirshekari et al., 2019 |
| Iran | HIV/AIDS patients | <18 years old | 10.8% (27/250) | Nested PCR- RFLP (18S rRNA) | *C. parvum* 70.37% (19/27)<br>*C. hominis* 25.93% (7/27<br>*Cryptosporidium meleagridis* 3.7% (1/27) | Ghafari et al., 2018 |
| Iran | Patients with gastrointestinal complaints | 22–90 years old | 1.3% (17/1301) | Nested PCR (*gp60*) 17 isolates genotyped | *C. parvum* 100%<br>IId 64.7% (11/17)<br>lldA26G1, lldA20G1<br>IIa 35.3% (6/17)<br>llaA15G2R1, llaA16G3R1 | Kiani et al., 2017 |
| Iran | | NS | 0.42% (7/1685) | Nested PCR (*gp60*) | | Ranjbar et al., 2016 |

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**Table 1** (*continued*)

| Country | Study population | Age group | Percentage positive for *Cryptosporidium* based on screening by PCR (No. positive/No. tested) | Genotyping method (Target gene) | Species and subtypes (where available) and their percentage % | Reference |
|---|---|---|---|---|---|---|
| | Patients with gastrointestinal complaints | | | | *C. parvum* 100%<br>IIa 71.4% (5/7)<br>IIaA16G2R160% (3/5)<br>IIaA17G1R1 40% (2/5)<br>IId 28.6% (2/7), IIdA17G1d | |
| Iran | Randomly collected samples | Birth-50 years old | 0.12% (1/820) | Nested PCR (*gp60*) | *C. parvum* 100%<br>IIa<br>IIaA16G2R1 | Shahdoust et al., 2016 |
| Iran | Children with diarrhea | NS | 2.74% (15/547) | Nested PCR (*gp60*) | *C. parvum*100%<br>IIa 46.7% (7/15)<br>IIaA16G2R1 28.6% (2/7)<br>IIaA17G1R1 14.3% (1/7)<br>IIaA22G3R1 57.1% (4/7)<br>IId 53.3% (8/15)<br>IIdA17G1d 100% (8/8) | Sharbatkhori et al., 2015 |
| Iran | Hospitalized children with diarrhea | Average 3 years old | 1.76% (2/113) | Nested PCR (SSU-rRNA) | *C. parvum* 100% | Mahdavi Poor et al., 2015 |
| Iran | Farm workers (96) and their household members (326) | NR | 8.5% (63/422) | Nested PCR (18S rRNA) | *C. parvum* 78% (28/36)<br>*C. hominis* 22% (8/36) | Izadi et al., 2014 |
| Iran | Patients with gastroenteritis | NS | 2.3% (8/348) | Nested PCR- RFLP (SSU-rRNA) | *Cryptosporidium spp* | Gholami et al., 2014 |
| Iran | Children with diarrhea | NS | 3.4% (29/850) | Nested PCR (18S-rRNA) | *Cryptosporidium spp* | Kuzehkanan et al., 2011 |
| Iran | Children with diarrhea | NS | 2.40% (19/794) | Nested PCR (*gp60*) | *C. parvum* 89.47% (17/19)<br>IIa 35.3% (6/17)<br>IIaA15G2R1 35.3% (6/17)<br>IId 64.7% (11/17)<br>IIdA18G1 17.6% (3/17)<br>IIdA20G1a 41.2% (7/17)<br>IIdA15G1 5.9% (1/17)<br>*C. hominis* 10.52% (2/19)<br>IfA22G1 | Taghipour et al., 2011 |
| Iran | Children with diarrhea | NS | NS | Nested PCR (SSU-rRNA and *gp60*) 25 isolates genotyped | *C. parvum* 88% (22/25)<br>IIa 28% (7/22)<br>IIaA15G2R1 85.7% (6/7)<br>IIaA16G3R1 14.3% (1/7)<br>IId 60% (15/22)<br>IIdA15G1 6.67% (1/15)<br>IIdA18G1 20% (3/15)<br>IIdA20G1a 60% (9/15)<br>IIdA21G1a 6.67% (1/15)<br>IIdA26G1 6.66% (1/15)<br>*C. hominis* 12% (3/25)<br>IdA20 33.3% (1/3)<br>IfA22G1 66.7% (2/3) | Nazemalhosseini-Mojarad et al., 2011a |
| Iran | Children with diarrhea | NS | 2.5% (12/469) | Nested PCR- RFLP (TRAP-C2 gene) | *C. parvum* 83.3% (10/12)<br>*C. hominis* 8.3% (1/12)<br>Mixed *C. hominis* + *C. parvum* 8.3% (1/12) | Nazemalhosseini-Mojarad et al., 2011b |
| Iran | Children with diarrhea | < 12 years old | 2.45% (31/1263) | Nested PCR- RFLP (SSU-rRNA) | *C. parvum* 80.6% (25/31)<br>*C. hominis* 16.1% (5/31)<br>Mixed *C. parvum* + *C. hominis* 3.2% (1/31) | Keshavarz et al., 2008 |
| Iran | Patients with diarrhea | 1–21 years old | NS | Nested PCR- RFLP (18S rRNA) 21 isolates genotyped | *C. parvum* 81% (17/21)<br>*C. hominis* 19% (4/21) | Pirestani et al., 2008 |
| Iran | Children with HIV and normal children with diarrhea | NS | NS | Nested PCR- RFLP (18S rRNA) 15 isolates genotyped | *C. parvum* 73.3% (11/15)<br>HIV patients 46.7(7/11)<br>Normal children 36.4% (4/11)<br>*C. hominis* 26.7 (4/15)<br>HIV 7.1% (1/4)<br>Normal children 75% (3/4) | Meamar et al., 2007 |
| Iraq | Patients with diarrhea | NS | 34% (34/100) | Nested PCR (18S rRNA) | *C. parvum* 100% (34/34) | Al-Yasary and Faraj, 2021 |
| Iraq | Patients with gastroenteritis (1 year to >40 years) | < 1 year old to >40 years old | 35.63% (41/150) | Nested PCR (*gp60*) 10 isolates genotyped | *C. parvum* 80%<br>IIa 80% (8/10)<br>IIaA15G2R1 20% (2/10)<br>and IIaA22G1R1 60% (6/10) | Merdaw et al., 2018 |

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**Table 1** (*continued*)

| Country | Study population | Age group | Percentage positive for *Cryptosporidium* based on screening by PCR (No. positive/No. tested) | Genotyping method (Target gene) | Species and subtypes (where available) and their percentage % | Reference |
|---|---|---|---|---|---|---|
| | | | | | *C. hominis* 20%<br>Ia 20% (2/10) | |
| Iraq | Children with acute and persistent diarrhea | 1 month <1 year old | 18% (18/100) | RT-PCR (18S rRNA) | *C. parvum* | Jomah and Mallah, 2016 |
| Iraq | NS | NS | 24% (12/50) | PCR (HSP gene) | *C. parvum* | Ahmed et al., 2016 |
| Iraq | Children with diarrhea | < 6 months - 2 years old | 8.35% (39/467) | Nested and RT-PCR (18S rRNA) | *C. parvum* 69.2% (27/39)<br>*C. hominis* 23.1% (9/39)<br>Mixed *C. parvum* + *C. hominis* 5.1% (2/39)<br>Unknown isolate 2.6% (1/39) | Abdul-Sada, 2015 |
| Iraq | Children with diarrhea | < 5 years old | 6.25% (4/64) | Nested PCR (TRAP-C2 gene) | *C. parvum*75% (3/4)<br>Mixed *C. parvum* + *C. hominis* 25% (1/4) | Jawad., 2015 |
| Iraq | Children with gastrointestinal complains | 5–15 years old | 7.37% (7/95) | RT-PCR (NS) | *C. parvum* | Shakir and Hussein, 2015 |
| Israel | Hospitalized children after diarrhea outbreak and sporadic *Cryptosporidium* cases | Birth - 9 years | 23% (33/146) | Nested PCR (18S rRNA and *gp60*) 36 isolates genotyped. | 33 isolates from an outbreak<br>*C. parvum* 11% (4/36)<br>IIdA20G 50% (2/4)<br>IIaA15G2R1 50% (2/4)<br>*C. hominis* 100%<br>IbA6G3 30% (10/30)<br>70% (23/ 30) IeAIIG3T3<br>36 sporadic *Cryptosporidium* cases sequenced.<br>*C. hominis* 88.9% (32/36)<br>IeAIIG3T3 75% (24/32)<br>IdA16 21.9% (7/32)<br>IbA10G2 3.1% (1/32) | Grossman et al., 2019 |
| Israel | Hospitalized children with gastrointestinal symptoms | 0.3–14 years old | 3.1% (9/291) | RT-PCR | *C. parvum* 22% (2/9)<br>*C. hominis* 78% (7/9) | Shaposhnik et al., 2019 |
| Jordan | Paediatric oncology patients and paediatric non-oncology patients with diarrhea | 1–5 years old | 9.76% (29/297)<br>Paediatric oncology patients 13.75% (22/160)<br>Paediatric patients 5.1% (7/137)<br>29 isolates typed to species and subtype level | Nested PCR (18S rRNA and *gp60*) | *C. parvum* (100%)<br>Paediatric oncology patients<br>IIaA16G2R1 4.5% (1/22)<br>IIaA17G2R1 95.5% (21/22)<br>Paediatric patients<br>IIaA16G2R1 28.6% (2/7)<br>IIaA17G2R1 71.4% (5/7) | Hijjawi et al., 2017 |
| Jordan | Hospitalized children with gastrointestinal symptoms | 10 months - 56 years old | 8.33% (4/48) | Quantitative PCR (18S rRNA and *gp60*) | *C. parvum* 50% (2/4)<br>IIdA20G1 50% (1/2)<br>IIaA15G2R1 50% (1/2)<br>*C. hominis* 50%, (2/4)<br>1bA9G3 50% (1/2)<br>1bA10G2R2 50% (1/2) | Hijjawi et al., 2016a |
| Jordan | Children suffering from gastrointestinal illness | 2 months - 12 years old | NS | Quantitative PCR (18S rRNA and *gp60*) 44 isolates genotyped 29 isolates subtyped at *gp60* | *C. parvum* 50% (22/44)<br>IIa 13.64% (3/22),<br>IIaA15G1R1 9.1% (2/22),<br>IIaA20G3R1 4.5% (1/22)<br>IIc 9.1% (2/22)<br>IIcA5G3a 9.1% (2/22)<br>IId 36.4% (8/22)<br>IIdA14G1 4.5% (1/22)<br>IIdA20G1 22.7% (5/22)<br>IIdA24G1 4.5% (1/22)<br>IIdA291G 4.5% (1/22)<br>*C. hominis* 45.46% (20/44)<br>Ib 40% (8/20)<br>IbA6G3 15% (3/20)<br>IbA9G3 15% (3/20)<br>IbA10G2 5% (1/20)<br>IbA20G2 5% (1/20)<br>Id 35% (7/20)<br>IdA21 10% (2/20)<br>IdA24 25% (5/20)<br>*C. meleagridis* 2.27% (1/44)<br>IIIaA12G3R1<br>*C. canis* 2.27% (1/44) | Hijjawi et al., 2010 |
| Kuwait | Children with diarrhea | < 2 years - 16 years old | 3.4% (83/2548) | PCR-RFLP (18S rRNA and *gp60*) | *C. parvum* 73.5% (61/83)<br>IId 32.8% (20/61)<br>IIa 47.5% (29/61)<br>IIc 19.7% (12/61)<br>*C. hominis* 26.5% (22/83)<br>Ia 36.4% (8/22) | Iqbal et al., 2011 |

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**Table 1** (*continued*)

| Country | Study population | Age group | Percentage positive for *Cryptosporidium* based on screening by PCR (No. positive/No. tested) | Genotyping method (Target gene) | Species and subtypes (where available) and their percentage % | Reference |
|---|---|---|---|---|---|---|
| Kuwait | Children with diarrhea and gastrointestinal symptoms | 1–19 years old | NS<br>63 isolates genotyped | PCR-RFLP and nested PCR (SSU-rRNA and *gp60*) | ld 54.5% (12/22)<br>le 9.1% (2/22)<br>Mixed *C. parvum* + *C. hominis* 1.2% (1/83)<br>*C. parvum* 94% (59/63)<br>IId 49% (29/59)<br>IIa 47.5% (28/59)<br>7 other alleles IIc, IIf<br>*C. hominis* 4.8% (3/63)<br>Ib, Id, Ie<br>Mixed *C. parvum* + *C. hominis* 1.6% (1/63) | Sulaiman et al., 2005 |
| Lebanon | Symptomatic hospitalized patients and children | NS | Total 10.4% (43/412)<br>Symptomatic hospitalized patients 11% (18/163)<br>Children 10% (25/249) | Nested PCR (18 rRNA and *gp60*) | *C. parvum*<br>IIaA15G1R1<br>IIaA15G2R1<br>*C. hominis*<br>IdA19<br>IbA10G2<br>IaA10G2<br>IaA18R3 | Osman et al., 2018 |
| Lebanon | Patients with neoplasia/ adenocarcinoma (colon neoplasia, stomach neoplasia) compared with patients without neoplasia but with persistent gastrointestinal symptoms | 18–92 years old | Total 11% (24/218)<br>21% (15/72) Patients with colon neoplasia/ adenocarcinoma<br>7% (9/146) Patients without neoplasia but with gastrointestinal symptoms | Real-time PCR (18S rRNA) | *C. parvum* 20.8% (5/24)<br>*C. hominis* 79.2% (19/25) | Osman et al., 2017 |
| Lebanon | School children | 3–16 years old | 10.4% (26/249) | Nested PCR (18 rRNA and *gp60*) | *C. parvum* 23% (6/20)<br>IIaA15G1R1<br>*C. hominis* 77% (20/26)<br>IaA18R3 20% (4/20)<br>IbA10G2 80% (16/20) | Osman et al., 2016 |
| Lebanon | Patients presented with gastrointestinal disorder | 1–88 years old | 9.2% (15/163) | Nested PCR/ 18 rRNA and *gp60* | *C. parvum* 33.3% (5/15)<br>IIaA15G1R1 100% (5/5)<br>*C. hominis* 66.7% (10/15)<br>IdA19 100% (10/10) | Osman et al., 2015 |
| Palestine | Patients with gastroenteritis | 1–36 years old | NS | Nested PCR-RFLP (18S rRNA)<br>30 samples genotyped | *C. parvum* | Hussein, 2011 |
| Qatar | Asymptomatic Immigrants | 18–56 years old | 4.5% (38/839) | qPCR-sequencing/ 18S rRNA, *gp60* | *C. parvum* 79% (30/38)<br>IId (IIdA20G1b)<br>*C. hominis* 2.6% (1/38)<br>Ie (IeA12G3T3)<br>Mixed *C. parvum* + *C. hominis* 10.5% (4/38)<br>Mixed *C. parvum* + *C. meleagridis* 7.9% (3/38) | Boughattas et al., 2019 |
| Qatar | Hospitalized children with chronic diarrhea | Children <5 years old | 15.1% (90/580) | PCR-RFLP (18S rRNA and *gp60*) | *18S rRNA PCR-RFLP*<br>*C. parvum* 92.22% (83/90)<br>*C. hominis* 4.44% (4/90)<br>*C. meleagridis* 1.11% (1/90)<br>Mixed *C. parvum* and *C. hominis* 1.11% (1/90)<br>Mixed *C. parvum* + *C. meleagridis* 1.11% (1/90)<br>*gp60*<br>35 isolates successfully sequenced.<br>*C. parvum* 88.6% (31/35)<br>IId<br>IIdA20G1 71% (22/31)<br>IIdA19G2 3.2% (1/31)<br>IIdA18G2 3.2% (1/31)<br>IIdA18G1 6.5% (2/31)<br>IIdA17G1 9.7% (3/31)<br>IIdA16G1 3.2% (1/31)<br>IIdA14G1 3.2% (1/31)<br>*C. hominis* 11.4% (4/35)<br>Ib<br>IbA9G3 75% (3/4)<br>IbA10G2 25% (1/4) | Boughattas et al., 2017 |
| | Children with diarrhea | | 1.6% (22/1380) | | | El-Malky et al., 2018 |

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**Table 1** (*continued*)

| Country | Study population | Age group | Percentage positive for *Cryptosporidium* based on screening by PCR (No. positive/No. tested) | Genotyping method (Target gene) | Species and subtypes (where available) and their percentage % | Reference |
|---|---|---|---|---|---|---|
| Saudi Arabia | | 1–14 years old | | PCR-RFLP (18S rRNA) | *C. parvum* 18.18% (4/22)<br>*C. hominis* 81.82% (18/22) | |
| Saudi Arabia | Children from different hospitals | < 10 years old | 11% (11/100) | AP-PCR sequencing (18S rRNA) | *C. parvum* 100% (11/11) | Shalaby et al., 2014 |
| Saudi Arabia | Young children | NS | 8.1% (12/148) | Real-time PCR (18S rRNA) | *C. parvum* | Elsafi et al., 2013 |
| Saudi Arabia | Asymptomatic children | NS | NS | Nested PCR and PCR-RFLP (18S rRNA, COWP and *gp60*)<br>35 samples genotyped | *C. parvum* 42.9% (15/35)<br>*C. hominis* 37% (13/35)<br>*C. meleagridis* 2.9% (1/35)<br>*Cryptosporidium muris* 2.9% (1/35)<br>Mixed *C. parvum* + *C. hominis* 2.9% (1/30) | Al-Brikan et al., 2008 |
| Saudi Arabia | Children with diarrhea and asymptomatic children | < 2 years old | 6.3% (103/1641) | PCR-RFLP sequencing/ (18S rRNA, *gp60* and HSP70)<br>101 isolates genotyped | *C. parvum* 78.2% (79/101)<br>IId, IIa, IIc<br>*C. hominis* 12.9% (13/101)<br>Ib, Ie | Areeshi, 2008 |
| Syria | Children with diarrhea | < 5 years old | 3.2% (7/213) | Nested PCR-RFLP (SSU-rRNA) | *C. parvum* | Kassouha et al., 2016 |
| Tunisia | Immunocomprimissed (Oncology and HIV children) and immunocompetent children | < 5 years old | 7.98% (42/526) | PCR- RFLP (18S rRNA and *gp60*) | *C. parvum* 45.24% (19/42)<br>IIa 42.1% (8/19)<br>IIaA15G2R1 26.3% (5/19)<br>IIaA14G2R1 5.26% (1/19)<br>IIaA16G2R15.26% (1/119)<br>IIaA20G1R1 5.26% (1/19)<br>IId 47.4% (9/19)<br>IIdA15G2R1 5.3% (1/9)<br>IIdA16G2R1 33.3% (3/9)<br>IIdA192R 5.3% (1/9)<br>IIdA22G2R1 44.4% (4/9)<br>IIc 10.5% (2/19)<br>IIcA5G3 100% (2/2)<br>*C. hominis* 47.62% (20/42)<br>Ia 80% (16/20)<br>IaA26G1R1 35% (7/20)<br>IaA27G1R1 25% (5/20)<br>IaA28 G1R1 5% (1/20)<br>IaA23G1R1 5% (1/20)<br>IaA12R3 5% (1/20)<br>IaA11G1R1 5% (1/20)<br>*C. meleagridis* 4.76% (2/42)<br>IIIbA26G1R1 100% (2/2)<br>Mixed *C. hominis* + *C. meleagridis* 2.38% (1/42) | Essid et al., 2018 |
| Tunisia | Oncology and HIV children | < 5 years old | 7.63% (39/511)<br>(Only 37 samples were genotyped) | Nested PCR and PCR- RFLP (18S rRNA and *gp60*) | *C. parvum* 43.24% (16/37)<br>*C. hominis* 48.65*% (18/37)<br>Ia 78% (14/18)<br>IaA26G1R1 38.9% (7/18)<br>IaA27G1R1 16.7% (3/18)<br>IaA28G1R1 5.6% (1/18)<br>IaA23G1R1 5.6% (1/18)<br>IaA12G1R1 5.6% (1/18)<br>IaA11G1R1 5.6% (1/18)<br>*C. meleagridis* 5.40% (2/37)<br>Mixed *Cryptosporidium* + *C. bieneusi* 2.71% (1/37) | Essid et al., 2017 |
| Tunisia | Symptomatic and non-symptomatic children | < 5 years old | 1.7% (7/403) | Nested PCR and PCR- RFLP (18S rRNA and *gp60*) | *C. parvum* 57.14% (4/7)<br>IIdA16G150% (2/4)<br>IIaA15G2R150% (2/4)<br>*C. meleagridis* 42.86 (3/7) | Rahmouni et al., 2014 |
| Turkey | Patients suffering from diarrhea and gastroenteritis | 2 months-78 years | 8.93% (5/56) | Nested PCR-RFLP (SSU-rRNA) | *C. parvum** 80% (4/5)<br>*C. hominis* 20% (1/5) | Yılmazer et al., 2017 |
| Turkey | School children | NS | 0.56% (4/707) | Nested PCR-RFLP (NS) | *C. parvum* 100% (4/4) | Tamer et al., 2007 |
| UAE | Expatriate workers | 20–60 years old | 16.3% (14/86) | PCR (18S rRNA) | *Cryptosporidium spp* | Al-Rifai et al., 2019 |
| UAE | Asymptomatic individuals | 25- > 40 years old | 19.4% (26/134) | RT- PCR (qPCR) (18S rRNA) | *Cryptosporidium spp* | ElBakri et al., 2018 |
| Yemen | Patients attended the outpatient's clinics in hospitals | 1–80 years old | 9.9% (33/335) | Nested PCR (18 s rRNA and *gp60*) | *C. parvum* 97.0% (32/33)<br>7 sequenced as IIaA15G2R1<br>*C. hominis* 3.0% (1/ 33)<br>IeA11G3T3 | Alyousefi et al., 2013a |

[a] AIDS Acquired immunodeficiency Syndrome.
[b] PCR, Polymerase Chain Reaction, PCR-RFLP, PCR restriction fragment length polymorphism.
[c] 18S rRNA, 18S ribosomal ribonucleic acid.
[d] *gp60*, hyper-variable 60 kDa glycoprotein gene.
[e] Copro-nPCR/ RFLP assay.
[f] PCR-RFLP.
[g] SSU rRNA, Small subunit ribosomal ribonucleic acid.
[h] COWP *Cryptosporidium* oocyst wall protein gene.
[j] HSP70, heat shock protein 70.
[k] TRAP-C1 gene, thrombospondin related adhesive protein 1 gene.
[l] 18S rDNA, 18S ribosomal deoxyribonucleic acid.
[i] NS, not specified.

*Cryptosporidium* spp. and 9 for *Giardia duodenalis*). The highest number of *Cryptosporidium* articles was retrieved from Iran and Turkey (3 each), Tunisia (2) and one from Kuwait and one from Egypt (Table 5). For *Giardia duodenalis* genotyping, two studies were retrieved from Egypt, Iran, Tunisia and Iraq and one from Turkey (Table 6).

## 3. Diagnosis of *Cryptosporidium* spp. and *Giardia duodenalis* in the MENA region

Routine diagnosis of *Cryptosporidium* spp. and *Giardia duodenalis* in different MENA countries relies mainly on morphological identification of the oocysts/cysts in faecal samples using microscopy, which undoubtedly contributes to the underdiagnosis and mis-diagnosis of these two parasites in the region (Hijjawi et al., 2010; Squire and Ryan, 2017; Ahmed and Karanis, 2020). Despite the relatively low cost for microscopic diagnosis, it is time consuming and lacks sensitivity and/or specificity and therefore immunological tools including enzyme-linked immunosorbent assay (ELISA) and immunochromatographic test are also employed (Gerace et al., 2019; Goudal et al., 2019; Dixon, 2021; O'Leary et al., 2021). The specificity and sensitivity of immunological tests for detecting these parasites however are variable (Lichtmannsperger et al., 2019; Dixon, 2021) and they cannot differentiate between different *Cryptosporidium* spp. and subtypes and *Giardia duodenalis* assemblages. Therefore, molecular tools, particularly PCR followed by restriction fragment length polymorphism (RFLP) and/or Sanger sequencing, are increasingly being used in many countries in the MENA region (Table 1).

### 3.1. Molecular detection and prevalence of Cryptosporidium spp. and Giardia duodenalis in humans in MENA region

Molecular characterisation is central to differentiating the sources of cryptosporidiosis and giardiasis, particularly in outbreaks (Fan et al., 2019; Rafiei et al., 2020; Yang et al., 2021). Commonly used genotyping tools for *Cryptosporidium* spp. in the MENA region are PCR and RFLP and/or sequence analysis of the most widely used and reliable locus, 18S rRNA (Table 1). A few studies have relied on other loci such as the heat shock 70 protein gene (*hsp-70*) (Areeshi, 2008; Ahmed et al., 2016), thrombospondin-related adhesive protein 2 (*trap-c2*) (Eraky et al., 2014; Jawad, 2015) and the *Cryptosporidium* oocyst wall protein gene (*cowp*) (Al-Brikan et al., 2008; Abd El Kader et al., 2012; Abo-Mandil et al., 2020). In the majority of studies where subtyping of *Cryptosporidium* spp. was performed, the glycoprotein 60 (*gp60*) gene was targeted (Table 1), however a few studies used *hsp-70* and *trap-c2* loci for subtyping purposes (Table 1). *Giardia* typing in the MENA region has been performed mostly using PCR/RFLP targeting the 18S rRNA, β-giardin (*bg*), glutamate dehydrogenase (*gdh*) and triose-phosphate isomerase (*tpi*) genes, either individually or two or three loci in combination (Table 2).

Only a few studies have exclusively used molecular approaches (PCR) to report on the prevalence of *Cryptosporidium* spp. and *Giardia duodenalis* within human populations in the MENA region countries (Tables 1 and 2). In those studies, the prevalence of *Cryptosporidium* spp. and *Giardia duodenalis* in humans within the regions ranged from 1.4%–49.1% and from 1.5–60.4% for *Cryptosporidium* spp. and *Giardia duodenalis*, respectively (Tables 1 and 2). The highest prevalence for *Cryptosporidium* (49.1%) was observed in Egypt among children and adults with diarrhea (Helmy et al., 2013), followed by a study in Iraq (35.63%) in patients suffering from gastroenteritis (Al-Yasary and Faraj, 2021), however, the lowest prevalence (1.4%) was observed in Egypt among children with diarrhea (Naguib et al., 2018a). The prevalence of *Giardia duodenalis* was unexpectedly the highest among healthy expatriates in the UAE (60.4%) (ElBakri et al., 2014), followed by an Iraqi study which reported a prevalence of 47.4% in children suffering from gastroenteritis (Shakir and Hussein, 2015), however the lowest prevalence (1.5%) was observed in adults and children in Egypt with gastrointestinal complaints (El Basha et al., 2016).

### 3.2. Cryptosporidium species/subtypes and Giardia duodenalis assemblages reported in humans in the MENA region

Seven species of *Cryptosporidium* infecting humans have been reported in the MENA region including *C. parvum*, *C. hominis*, *C. meleagridis*, *C. muris, C. felis*, *C. bovis* and *C. canis*, with *C. parvum* being the most prevalent species followed by *C. hominis* (Table 1). *Cryptosporidium parvum* was reported in nearly all the analysed genotyping studies conducted in the MENA region (95.4%; 62/65 studies) apart from one study which reported only *C. hominis* in children with diarrhea in Egypt (Sadek, 2014) and in other two studies where no species identification was performed (Kuzehkanan et al., 2011; Gholami et al., 2014). In particular, *C. parvum* was found to be the dominant species in Iran, Jordan, Syria, Kuwait, Qatar, Saudi Arabia, Yemen, and Algeria (Table 1). *Cryptosporidium hominis* was reported in 63% of the studies (41/65) and found to be the predominant species in Lebanon, Israel, Egypt and Tunisia (Table 1). Mixed infections with *C. parvum* and *C. hominis* were reported in 18.5% (12/65) of the retrieved studies (Table 1).

The third most prevalent species among humans in the MENA region was *C. meleagridis* 12.3% (8/65), which was reported in eight studies (Al-Brikan et al., 2008; Hijjawi et al., 2010; Rahmouni et al., 2014; Essid et al., 2017, 2018; Boughattas et al., 2017, 2019; Ghafari et al., 2018). Other species of *Cryptosporidium* such as *C. bovis*, *C. canis*, *C. felis* and *C. muris* were reported sporadically in humans (one study each) (Al-Brikan et al., 2008; Hijjawi et al., 2010; Helmy et al., 2013; Semmani et al., 2020). A mixed infection of *C. bovis* with *C. parvum* was detected in a child with diarrhea in Egypt (Helmy et al., 2013), *C. canis* was detected in a child suffering from gastrointestinal illness in Jordan (Hijjawi et al., 2010), *C. felis* was reported in a HIV positive patient in Algeria (Semmani et al., 2020) and *C. muris* was detected in an asymptomatic child in Saudi Arabia (Al-Brikan et al., 2008).

Subtyping analysis of *C. parvum* at the *gp60* locus in the MENA region identified four subtype families with subtype family IIa being the most dominant one in the region (reported in 18 studies) followed by IId (reported in 12 studies), while IIc and IIb were only reported in four and two studies, respectively (Table 1). The IIa subtype family is commonly reported in dairy calves, while the IId subtype family is more commonly

**Table 2**
Molecular characterisation and prevalence of *Giardia duodenalis* and its assemblages in humans in countries of the MENA region (2005–2021).

| Country | Study population | Age group | Percentage positive for *Giardia duodenalis* based on screening by PCR (No. positive/No. tested) | Genotyping method/ (Target gene) | *Giardia duodenalis* assemblages detected | Reference |
|---|---|---|---|---|---|---|
| Algeria | Children and adults | NS[a] | NS | Real-time PCR[b] (18S rRNA[c] and *tpi*[d])<br>48 samples genotyped | A % 46% (22/48)<br>AII 100%<br>B % 54% (26/48) | Belkessa et al., 2020 |
| Algeria | School children with and without diarrhea/gastroenteritis | 6–11 years old | NS | PCR- RFLF[e] (*bg*[f])<br>20 isolates genotyped | A 70% (14/20)<br>B 30% (6/20) | Rebih et al., 2020 |
| Egypt | Children | 2–16 years old | NS | PCR (*tpi*) | A 15.8% (9/57)<br>B 36.8%% (21/57)<br>Mixed A + B 47.4%% (27/57) | Elhadad et al., 2021 |
| Egypt | Children with diarrhea | NS | NS | Hemi-nested PCR, PCR-RFLP (*tpi* and *gdh*[g])<br>24 isolates genotyped | A (AII) 100% (24/24) | Abd El-Latif et al., 2020 |
| Egypt | Symptomatic children | 1 month - 15 years old | NS | Copro-nPCR (*tpi*) | A 16.2% (6/37)<br>B 83.8% (31/37) | Mohamed et al., 2020 |
| Egypt | Children with and without gastrointestinal symptoms | 3–12 years old | NS | Nested PCR (*tpi*)<br>35 isolates genotyped | A 45.7% (16/35)<br>B 31.4% (11/35)<br>Mixed A + B 22.8% (8/35) | Ahmad et al., 2020 |
| Egypt | Symptomatic patients | 2–40 years old | NS | Semi-nested PCR, PCR-RFLP (*gdh, tpi* and *bg*) | A 17.4% (4/23)<br>AI (1/4)<br>AII 75% (3/4)<br>B 69.6% (16/23)<br>BIII 80% (12/15)<br>BIV 26.7% (4/15)<br>2 isolates failed to subtyped | Ahmed et al., 2019 |
| Egypt | Patients with diarrhea | 1–65 years old | NS | Nested PCR (*tpi, gdh* and *bg*)<br>65 samples genotyped | A(AII) 40% (26/65)<br>B 49.2% (32/65)<br>23 subtyped<br>BIII 95.7% (22/23)<br>BIV 4.3% (1/23)<br>Mixed A + B 10.8% (7/65) | Yu et al., 2019 |
| Egypt | Children with diarrhea | < 8 years old | 11.3% (66/585) | Nested-PCR (*tpi, gdh* and *bg*) | A (AII) 47% (31/66)<br>B 51.5% (34/66)<br>Mixed A + B 1.5% (1/66) | Naguib et al., 2018a |
| Egypt | Children with and without gastrointestinal symptoms | 5–15 years old | NS | Nested IGS-PCR (*bg*)<br>60 isolates genotyped | Asymptomatic children<br>A(AI) 66.6% (20/30)<br>AII 13.4% (4/30)<br>B 20% (6/30)<br><br>Symptomatic children<br>AI 53.3% (16/30)<br>B 40% (12/30)<br>Mixed AI + B 6.6% (2/30) | Hussein et al., 2017 |
| Egypt | Children with diarrhea | 2 months-18 years | NS | Multiplex-PCR *(tpi)*<br>25 isolates genotyped | A 28% (7/25)<br>B 72% (18/25) | Hussein et al., 2016a |
| Egypt | Anaemic and non-anaemic children suffering from giardiasis | 1–6 years old | NS | IGS-PCR[h] (IGS ribosomal DNA (rDNA))<br>88 isolates genotyped | A 55.7% (49/88)<br>AI 33.2% (29/88)<br>AII 22.6% (20/88)<br>B 40.9% (36/88)<br>Mixed AI + AII 1.1% (1/88)<br>Mixed AI +AII+ B 2.3% (2/88) | Hussein et al., 2016b |
| Egypt | Children suffering from gastrointestinal symptoms | NS | 18.3% (42/229) | Nested PCR (*tpi*) | *Giardia duodenalis* | Ghieth et al., 2016 |
| Egypt | Children with diarrhea | NS | 18.9% (224/1187) | Copro-nPCR-RFLP[i] (*bg*) | A 18.3% (41/224)<br>B 81.7% (183/224) | Ismail et al., 2016 |
| Egypt | Children with gastrointestinal complains | 1.5–12 years old | NS | Nested PCR (*tpi*)<br>40 isolates | E 62.5% (25/40) | Abdel-moein and Saeed, 2016 |

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**Table 2** (*continued*)

| Country | Study population | Age group | Percentage positive for *Giardia duodenalis* based on screening by PCR (No. positive/No. tested) | Genotyping method/ (Target gene) | *Giardia duodenalis* assemblages detected | Reference |
|---|---|---|---|---|---|---|
| | | | | genotyped and sequenced | | |
| Egypt | Children and adults with gastrointestinal symptoms | 2–55 years old | 1.5% (60/400) | PCR-RFLP (*bg*) | A 36.7% (22/60)<br>B 63.3% (38/60) | El Basha et al., 2016 |
| Egypt | Children complaining from acute or chronic diarrhea | 8 months −16 years old | NS | Real- time PCR (*tpi, gdh* and *bg*) | A (AII) 27.3% (21/77)<br>B 70.1% (54/77)<br>Mixed A + B 2.6% (2/77) | Fahmy et al., 2015 |
| Egypt | Children with diarrhea | <10 years old | 21% (35/165) | Nested PCR (*tpi, gdh* and *bg*)<br>18 isolates sequenced (*gdh)* | A (AI+AII) 16.7% (3/18)<br>B 66.7% (12/18)<br>E 11% (2/18)<br>Mixed A + B 5.6% (1/18) | Helmy et al., 2014 |
| Egypt | Children and adult with diarrhea | 2–70 years old | NS | Real-time PCR (18S rDNA, *tpi, gdh* and *bg*)<br>15 isolates genotyped | A AII 6.67% (1/15)<br>B (BIII + BIV) 86.66% (13/15)<br>C 6.67% (1/15) | Soliman et al., 2011 |
| Egypt | Patient diagnosed with giardiasis | 4–65 years old | NS | Nested real-time PCR (*tpi*) | AI 58.5% (24/41)<br>AII 17.1% (7/41)<br>B 19.5% (8/41)<br>Mixed AII + B 5% (2/41) | Helmy et al., 2009 |
| Egypt | Randomly selected samples | NS | 34.6% (18/52) | PCR (18S rRNA and *tpi*) | A 5%<br>B 80%<br>E 15% | Foronda et al., 2008 |
| Iran | Patients with intestinal disorders | NS | NS | PCR-HMC[i] (*tpi*)<br>69 isolates genotyped | A(AI) 68.1% (47/69)<br>BIII 20.3% (14/69)<br>BIV 11.6% (8/69) | Sepahvand et al., 2020 |
| Iran | Patients with diarrhea | 8 months −5 years old | 2.1% (5/230) | PCR (*bg*) | *Giardia duodenalis* | Abbasi et al., 2020 |
| Iran | Patients with intestinal disorders | 18–60 years | NS | Semi-nested-PCR (*gdh*)<br>21 isolates genotyped | Mixed AII + BIII 42.8% (9/21)<br>BIII 28.6% (6/21)<br>AII 28.6%(6/21) | Kialashaki et al., 2020 |
| Iran | Patients with and without gastrointestinal symptoms | 1 - > 20 years old | NS | Semi-nested-PCR (*gdh*) | A (AII) 92.6% (38/41)<br>B (BIV) 7.4% (3/41) | Mahmoudi et al., 2020 |
| Iran | Individuals with and without gastrointestinal symptoms | 2–75 years old | NS | Nested PCR (18S rRNA, *gdh, tpi* and *bg*) | A 50% (12/24)<br>AII 41.7% (10/24)<br>AII/AIII 8.3% (2/24)<br>B 50% (12/24)<br>BIII 25.0% (6/24)<br>BIII/BIV 20.8% (5/24)<br>BIV 4.2% (1/24) | Rafiei et al., 2020 |
| Iran | Patients suffering from giardiasis | 7–44 years old | NS | PCR (18S rRNA, *gdh,* and *bg*)<br>40 isolates sequenced | A (AII) 57.5% (23/40)<br>B 42.5% (17/40) | Mirrezaie et al., 2019 |
| Iran | Patients with and without gastrointestinal symptoms | 0 - ≥ 40 years old | NS | Nested PCR (*gdh* and *tpi*) | A (AII) 78.2% (18/23)<br>B 21.7% (5/23) | Kashinahanji et al., 2019 |
| Iran | Food handlers | 30–60 yeas old | NS | PCR (*gdh*)<br>17 isolates genotyped | A 65% (AII)<br>B 35% (BIII) | Shahnazi et al., 2019 |
| Iran | Individuals with HIV or cancer and immunocompetent subjects | 9 months - 71 years old | 5.53% (11/199) | Nested PCR (*tpi* and *gdh*) | AI 54.5% (6/11)<br>AII 27.3% (3/11)<br>Mixed AI + B 19% (2/11) | Nooshadokht et al., 2017 |
| Iran | Random samples from health centers and laboratories | 3–78 years old | NS | Nested PCR (*tpi*) | A (AII) 58.3% (35/60)<br>B 28.3% (17/60)<br>BIII 82.3% (14/17)<br>BIV 17.7% (3/17)<br>Mixed A + B 11.6% (7/60) | Rahimian et al., 2018 |
| Iran | Children from day care centers | 1–7 years old | NS | PCR (18S rRNA and *tpi*)<br>9 isolates genotyped at (18S rRNA) and 8 at *tpi* | A (AII) 55.6% (5/9)<br>4 isolates untypable | Kasaei et al., 2018 |

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**Table 2** (*continued*)

| Country | Study population | Age group | Percentage positive for *Giardia duodenalis* based on screening by PCR (No. positive/No. tested) | Genotyping method/ (Target gene) | *Giardia duodenalis* assemblages detected | Reference |
|---|---|---|---|---|---|---|
| Iran | Food-handlers | 19–46 years old | NS | PCR-RFLP (*gdh*) | A (AII) 54.5% (24/44)<br>B 20.5% (9/44)<br>BIII 15.9% (7/44)<br>BIV 4.6% (2/44)<br>Mixed AII + B 25% (11/44) | Hooshyar et al., 2017 |
| Iran | Patients with gastrointestinal complaints | NS | NS | Semi-nested PCR (*gdh*)<br>40 isolates genotyped | A (AII) 80% (32/40)<br>B 20% (8/20)<br>BIII 37.5% (3/8)<br>BIV 12.5% (1/8)<br>Mixed BIII + BIV 50% (4/8) | Rayani et al., 2017 |
| Iran | Patients with gastrointestinal complaints | < 6 years old - > 50 years old | NS | Nested PCR (*tpi*) | A 52% (12/23)<br>AI (4/12)<br>AII (8/12)<br>B 48% (11/23)<br>BIII 45% (5/11)<br>BIV 36% (4/11)<br>Novel assemblage B 18% (2/11) | Bahrami et al., 2017 |
| Iran | Microscopically positive sample from laboratories | 5 months - 70 years old | NS | PCR (*tpi*)<br>67 isolaes genotyped | A 59.70% (40/67)<br>B 37.3% (25/67)<br>Mixed A + B 2.98% (2/67) | Pestechian et al., 2014 |
| Iran | Patients with gastrointestinal discomfort | NS | NS | PCR (18S rDNA[k] and *gdh*) | A(AII) 80% (23/50)<br>Mixed BIII + BIV 20% (8/50) | Rayani et al., 2014 |
| Iran | Symptomatic ($n = 50$) and asymptomatic cases ($n = 50$) confirmed with giardiasis | 4–65 years old | NS | Semi-nested PCR-RFLP (*gdh*)<br>100 samples *Giardia* genotyped | Asymptomatic (50)<br>Mixed AII and BIII 64% (32/50)<br>BIII 26% (13/50)<br>AII 10% (5/50)<br>Symptomatic (50)<br>54% (27/50)<br>Mixed AII + BIII<br>AII 18% (9/50)<br>BIII 28% (14/50) | Rafiei et al., 2013 |
| Iran | Symptomatic children | < 15 years old | NS | Semi-nested PCR-RFLP (*gdh*)<br>50 isolates genotyped | A (AII) 10% (5/50)<br>B 16% (8/50)<br>Mixed AII + B 74% (37/50) | Roointan et al., 2013 |
| Iran | Diagnosed giardiasis patients | 6 months −65 years old | NS | Semi-nested PCR and PCR-RFLP/ *gdh* | A(AII) 74.4% (128/172)<br>BIII 17.4% (30/172)<br>BIV 3.5% (6/172)<br>Mixed AII + BIV 4.7% (8/172) | Sarkari et al., 2012 |
| Iraq | Patients suffering from acute diarrhea | 2–72 years old | 27.7% (23/101) | PCR (*tpi*) | A 21.7% (5/23)<br>B 78.2% (18/23) | Al-Ani et al., 2020 |
| Iraq | Patients with gastrointestinal tract disturbance | NS | 10.7% (80/750) | Nested PCR (18S RNA and *tpi*) | A 22.5% (18/80)<br>B 52.5% (42/80)<br>Mixed A + B 10% (8/80)<br>F 5% (12/80) | Alhatemi et al., 2020 |
| Iraq | Children suffering from gastrointestinal discomfort | 1–10 years | NS | PCR (18S rRNA)<br>12 isolates genotyped | *Giardia duodenalis* | Alhayali et al., 2020 |
| Iraq | Patients with severe diarrhea | NS | 72% (36/50) | Nested PCR | A 22% (8/36)<br>B 78% (28/36) | Al-Asadi and Kadhum, 2018 |
| Iraq | Patients with gastrointestinal disturbances | NS | NS | Multiplex-PCR *(tpi)* | A 45% (9/20)<br>B 35% (7/20)<br>Mixed A + B 20% (4/20) | Rasheed and Jebur, 2018 |
| Iraq | Patients suffering from gastroenteritis and diarrhea | 1 - ≥19 | NS | Nested PCR *(tpi)* | A 23.8% (5/21)<br>B 71.42% (15/21)<br>Mixed A + B 4.76% (1/21) | Saleh et al., 2018 |
| Iraq | Children with gastroenteritis | 2–10 years old | NS | Nested PCR *(tpi)*<br>25 isolates genotyped | A 12% (3/25)<br>B 68% (17/25)<br>Mixed A + B 20% (5/25) | Al-Difaie, 2016 |
| Iraq | Patients suffering from acute and persistent diarrhea | 2–72 years old | 18.8% (19/101) | PCR-RFLP (*gdh*)<br>19 genotyped | AI 2% (2/19)<br>AII 3% (3/19) | Al-Fahadawi et al., 2017 |

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**Table 2** (*continued*)

| Country | Study population | Age group | Percentage positive for *Giardia duodenalis* based on screening by PCR (No. positive/No. tested) | Genotyping method/ (Target gene) | *Giardia duodenalis* assemblages detected | Reference |
|---|---|---|---|---|---|---|
| | | | | | BIII 4.9% (5/19)<br>BIV 1.9% (9/19) | |
| Iraq | Children with diarrhea | 2 months −15 years old | NS | Multiplex RT-PCR and nested PCR (*tpi*) | A 28.6% (8/28)<br>B 71.2% (20/28) | Hussein et al., 2016a |
| Iraq | Patients with gastroenteritis | NS | NS | RT-PCR (*tpi*)<br>59 isolates genotyped | B 25.4% (15/59)<br>Mixed A + B 74.6% (44/59) | Bedir et al., 2016 |
| Iraq | Children with gastroenteritis | 5–15 years old | 47.36% (45/95) | RT-PCR[l] (NS) | *Giardia duodenalis* | Shakir and Hussein, 2015 |
| Iraq | Patients suffering from acute and persistent diarrhea | 1–40 years | NS | Multiplex-PCR *(tpi)* | A 30.1% (22/73)<br>B 26% (19/73)<br>E 20.5% (15/73)<br>F 23.3% (17/73) | Turki et al., 2015 |
| Jordan | Patient suffering from diarrhea | 4 months - 60 years old | NS | Nested PCR (*gdh*) and qPCR[m] (*bg*)<br>62 isolates genotyped | A (AII) 42% (26/62)<br>B 58% (36/62)<br>BIII 30.6% (19/62)<br>BIV 27.4% (17/62) | Hijjawi et al., 2018 |
| Jordan | Patients suffering from giardiasis | 4 months - 57 years old | NS | Nested PCR (*tpi and gdh*)<br>49 isolates genotyped | At *tpi*:<br>A 46.4% (13/28)<br>B 50% (14/28)<br>Mixed A + B 3.6% (1/28)<br>At *gdh*:<br>A 43.7% (21/48)<br>AII 43.7% (21/48)<br>B 56.3% (27/48)<br>BIII 25% (12/48)<br>BIV 31.2% (15/48) | Hijjawi et al., 2016b |
| Lebanon | School children | 3–16 years old | 28.5% (71/249) | Real-time PCR 18 rRNA (*tpi)*<br>67 isolaes genotyped | A 3.0% (2/67)<br>B 95.5% (64/67)<br>Mixed A + B 1.5% (1/67) | Osman et al., 2016 |
| Qatar | Asymptomatic immigrants | NS | NS | PCR (*gdh, bg* and *tpi*)<br>54 isolates tested | A (AII) 16.7% (9/54)<br>B 55.5% (30/54)<br>Mixed A + B 27.8% (15/54) | Chourabi et al., 2021 |
| Morocco | School children | 5–14 years old | NS | Semi-nested PCR (18S rRNA[n] and *gdh*)<br>11 isolates genotyped | A (AII) 18.2% (2/11)<br>B 81.8% (9/11)<br>BIII 9.1% (1/11)<br>BIV 72.7% (8/11) | El Fatni et al., 2014 |
| Saudi Arabia | Young children | NS | 14.2% (21/148) | Real-time PCR (18S rRNA) | *Giardia duodenalis* | Elsafi et al., 2013 |
| Saudi Arabia | Gastrointestinal symptomatic and asymptomatic children | 6–12 years old | 2.67% (40/1500) | Real-time PCR (rDNA) | A 57.5% (23/40)<br>AI 30% (12/40)<br>AII 27.5% (11/40)<br>B 37.5% (15/40)<br>Mixed A and B 5% (2/40) | Al-Mohammed, 2011 |
| Syria | Symptomatic giardiasis patients | 4 months – 75 years | NS | Nested PCR- RFLP (*bg* and *gdh*)<br>40 samples sequenced | A 67.5% (27/40/)<br>AI 20% (8/40)<br>AII 45% (18/40)<br>B 10% (4/40)<br>BIII 5% (2/40)<br>BIV 12.5% (5/40)<br>BIII /BIV 10% (4/40)<br>Mixed A + B 22.5% (9/40)<br>AII + BIII 5% (2/40)<br>AI + BIV 2.5% (1/40) | Skhal et al., 2017 |
| Turkey | Asymptomatic and symptomatic individuals | <1–99 years | NS | qPCR (*bg*)<br>Semi-nested PCR (*gdh, tpi)*<br>30 isolates genotyped | A 53.3% (16/30)<br>AII 23.3% (7/30)<br>Discordant AII/AIII 23.3% (7/30)<br>B 43.3% (13/30)<br>Mixed AII + AIII 6.7% (2/30)<br>BIII 10%(3/30)<br>BIV 3.3% (1/30)<br>Dicordant BIII/BIV 23.3%(7/30)<br>Mixed A + B 3.3% (1/30) | Sarzhanov et al., 2021 |
| Turkey | Patients suffering from gastrointestinal complaints | 1–59 years old | NS | Nested PCR- RFLP | A 55.6% (15/27)<br>B 13.3% (2/15) | Küçük et al., 2019 |

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**Table 2** (*continued*)

| Country | Study population | Age group | Percentage positive for *Giardia duodenalis* based on screening by PCR (No. positive/No. tested) | Genotyping method/ (Target gene) | *Giardia duodenalis* assemblages detected | Reference |
|---|---|---|---|---|---|---|
| | | | | | Mixed B + E 66.7% (10/15) | |
| Turkey | Microscopically positive samples for *Giardia* | NS | NS | PCR (16S rRNA and *bg*) | A 80% (8/10)<br>AI 37.5% (3/8)<br>AII 37.5% (3/8)<br>AIII 25% (2/8)<br>B 20% (2/10) | Ertuğ et al., 2016 |
| Turkey | Symptomatic and asymptomatic children | 1–13 years old | NS | PCR (*bg*) | A 50%, (11/22)<br>B 31.8%, (7/22)<br>Mixed A and B 18.2%, (4/22) | Tamer et al., 2015 |
| Turkey | Symptomatic and asymptomatic patients | 1–74 years old | NS | PCR (*bg*) | A 53% (9/17)<br>AII 66.7% (6/9)<br>AIII 33.3% (3/9)<br>B 47% (8/17)<br>BII 75% (6/8)<br>BIII 12.5% (1/8)<br>BIV 12.5% (1/8) | Çiçek and Şakru, 2015 |
| Turkey | *Giardia* infected patients | NS | NS | PCR (*tpi* and *gdh*)<br>17 isolates genotyped | A 53% (9/17)<br>B 23.5% (4/17)<br>23.5% (4/17) failed to amplify | Değerli et al., 2012 |
| UAE | Healthy expatriates | NS | 60.3% (67/111) based on SSU-rRNA<br>41.4% (46/111) based on *tpi* | Nested-PCR (18S rRNA and *tpi*)<br>46 isolates genotyped | A 45.7% (21/46)<br>B 41.3% (19/46)<br>Mixed A + B 13% (6/46) | ElBakri et al., 2014 |
| Yemen | Patients attended the outpatient's clinics in hospitals | 1–80 years old | NS | PCR (16 s rRNA[o] and *bg*) | A 66% (43/65)<br>B 34% (22/65) | Alyousefi et al., 2013b |

[a] NS, not specified.
[b] PCR, Polymerase Chain Reaction.
[c] 18S rRNA, Small subunit ribosomal ribonucleic acid.
[d] *tpi*, triose phosphate isomerase gene.
[e] PCR-RFLP, PCR restriction fragment length polymorphism.
[f] *bg*, beta-giardin.
[g] *gdh*, glutamate dehydrogenase.
[h] IGS-PCR, intergenic spacer-PCR.
[i] Copro-nPCR.
[j] PCR-HMC, real-time polymerase chain reaction using the high-resolution melting curve analysis.
[k] SSU-rDNA.
[l] RT-PCR.
[m] qPCR, quantitative PCR.
[n] 18S rRNA, 18S ribosomal ribonucleic acid.
[o] 16S rRNA, 16S ribosomal ribonucleic acid.

reported in lambs and goats kids. The predominance of subtype families IIa and IId in the studies conducted in the MENA region countries supports potential zoonotic transmission of *C. parvum* among humans in the region and highlights the importance of dairy calves and small ruminants as a source of human infections. In contrast to the situation in low- and middle-income countries, where *C. parvum* infections are mostly caused by the anthroponotic IIc subtypes, which is largely a human pathogen, together with a few other human-adapted IIe and IIm subtype families (Feng et al., 2018), the anthroponotic IIc and IIb subtypes are rarely seen in humans in the MENA region, supporting further dominance of zoonotic transmission with the animal adapted IIa and IId subtypes.

Five *C. hominis* subtype families were reported in humans including Ia, Ib, Id, If and Ie with Ib being the most dominant (reported in 9 studies) followed by Ia (7 studies), Id (6 studies), Ie (5 studies) and If (3 studies) (Table 1). However, in some most recent studies, subtype family Id was reported to be more common in the Middle East followed by Ia and Ib, with only limited occurrence of Ie (Essid et al., 2018; Naguib et al., 2018a).

Five *G. duodenalis* assemblages infecting humans were identified (A, B, C, E and F) in the MENA region countries (Table 2). Assemblage A and B were reported in 58 and 57 out of the 68 subtyping studies retrieved respectively with 41 studies reporting mixed infections with A and B and no subtyping was carried out for three studies (Table 2). Assemblage C (commonly found in dogs) was detected in one study in Egypt in an immunocompromised adult male (Soliman et al., 2011) and assemblage E (normally found in livestock) was detected in humans in five studies (Table 2), highlighting the possibility of zoonotic transmission. In one study in Egypt in children with diarrhea, assemblage E was detected at a prevalence of 62.5% (Abdel-moein and Saeed, 2016), and in two additional studies in Egypt, at a prevalence of 15% and 11%, respectively (Foronda et al., 2008; Helmy et al., 2014). In the fourth report, assemblage E was reported in Iraq in patients suffering from acute and persistent diarrhea at a prevalence of 20.5% (Turki et al., 2015). In one study, a mixed infection with assemblage B and E (66.7%) was reported in Turkish patients suffering from gastrointestinal symptoms (Küçük et al., 2019). Assemblage F which usually infects cats was reported in two studies in Iraq, one among patients suffering from acute and persistent diarrhea (Turki et al., 2015) and the other one in patients with gastrointestinal disturbances (Alhatemi et al., 2020) at a prevalence of 23.3% and 5%, respectively. Subtyping studies of *G. duodenalis* in the MENA region have identified several sub-assemblages including AI, AII, BIII, BIV (Table 2) and a novel subassemblage B which was reported in two patients suffering from gastrointestinal symptoms in Iran (Bahrami et al., 2017) (Table 2).

**Table 3**
Molecular characterisation and prevalence of species and subtypes of *Cryptosporidium* in animals in countries of the MENA (2005–2021).

| Country | Host | Age group | Percentage positive for *Cryptosporidium* based on screening by PCR (No. positive/ No. tested) | Genotyping method (Target gene) | Species and subtypes (where available) and their percentage % | Reference |
|---|---|---|---|---|---|---|
| Algeria | Lambs | < 40 days old | 25.30% (21/83) | Nested PCR[a] and RFLP[b] (SSU rRNA[c] and *gp60*[d]) | *C. parvum* 76% (16/21)<br>IIa 26.67% (4/15)<br>IIaA13G2R1 6.67% (1/15)<br>IIaA21G2R1 20% (3/15)<br>IId 73.33% (11/15)<br>IIdA16G1 73.33% (11/15)<br>*C. ubiquitum* 24% (5/21) | Sahraoui et al., 2019 |
| Algeria | Calves | 2 days – 18 months | 15.8% (38/240) | PCR (SSU rRNA)<br>29 isolates genotyped | *C. parvum* 72.4% (21/ 29)<br>IIaA16G2R1 (19/21)<br>Unknown (2/21))<br>*C. bovis* 13.8%, (4/29)<br>*C. andersoni* 3.4% (1/29)<br>*C. ryanae* 3.4% (1/29)<br>7.0% (2/29) were only identified at the genus level due to poor sequence quality | Ouakli et al., 2018 |
| Algeria | Lambs and Goats | < 4 Weeks old | Total 11% (17/154)<br>Lambs: 14.52% (9/62)<br>Goat: 8.7% (8/92) | Nested PCR and RFLP (SSU rRNA and *gp60*) | Lambs<br>*C. parvum* 33.33% (3/9)<br>IIaA13G2R1<br>*C. xiaoi* 66.67% (6/9)<br>Goat<br>*C. xiaoi* 75% (6/8)<br>*C. ubiquitum* XIIa 25% (2/8) | Baroudi et al., 2018a |
| Algeria | Camels | < 90 days | 5.13% (2/39) | Nested PCR and RFLP (SSU rRNA) | *C. parvum*<br>If-like-A22G2 | Baroudi et al., 2018b |
| Algeria | Calves | < 8 weeks old | 18.18% (24/132) | Nested PCR (SSU rRNA and *gp60*) | *C. parvum* 16.67% (4/24)<br>IIaA13G2R1<br>*C. bovis* 58.33% (14/24)<br>*C. ryanae* 25% (6/24) | Benhouda et al., 2017 |
| Algeria | Domestic, captive, and wild birds in rural areas | All ages | 8.98% (31/345) | PCR (SSU rRNA actin and *gp60*) | *Cryptosporidium meleagridis* 45.16% (14/31)<br>IIIgA22G3R1 78.6% (11/14)<br>IIIgA23G2R1 21.4% (3/14)<br>*C. baileyi* 54.84% (17/31) | Laatamna et al., 2017 |
| Algeria | Pre-weaned dairy calves with or without diarrhea | < 90 days | 13.72% (14/102) | Nested PCR (SSU rRNA and *gp60*) | *C. parvum* 50% (7/14)<br>IIaA16G2R1 71.4% (5/7)<br>IIaA17G3R1 14.3 (1/7)<br>1 isolate did not amplify.<br>*C. bovis* 28.6% (4/14)<br>Mixed infections 21.4% (3/14)<br>*C. parvum* + *C. bovis* (2/3)<br>*C. bovis* + *C. andersoni* (1/3) | Baroudi et al., 2017 |
| Algeria | Horses and Donkeys | NS[e] | Total: 2.04 (7/343)<br>Horses: 2.3% (5/219)<br>Donkeys: 1.61% (2/124) | Nested PCR (SSU rRNA, *gp60*, TRAP-C1[f], COWP[g], and HSP70[h]) | Horses<br>*C. parvum* 40% (2/5)<br>IIaA16G1R1<br>*Cryptosporidium muris* 20% (1/5)<br>*C. hominis* 20% (1/5)<br>IkA15G1<br>*Cryptosporidium cuniculus*20% (1/5)<br>Donkeys<br>*C. parvum* 50% (1/2)<br>*C. muris* 50% (1/2) | Laatamna et al., 2015 |
| Algeria | Chicken and Turkey | 1–55 days old (Chicken)<br>1–75 days old (Turkey) | Total: 38.0 (56/147)<br>Chicken: 34.4% (31/90)<br>Turkey: 44% (25 /57) | Nested PCR and RFLP (SSU rRNA, *gp60*)<br>16 isolates amplified in chicken (8) and in turkey (8) | Chicken<br>*C. meleagridis* 28.9% (26/90)<br>IIIgA18G4R1 12.5% (1/8)<br>IIIgA19G5R1 25% (2/8)<br>IIIgA21G3R1 12.5% (1/8)<br>IIIgA24G2R112.5% (1/8)<br>IIIgA26G3R1 12.5% (1/8)<br>Unknown 25% (2/8)<br>*C. baileyi* 5.5% (5/90)<br>Mixed *C. meleagridids* + *C. baileyi* 1.1% (1/90)<br>Turkey<br>*C. meleagridis* 100% (25/25)<br>IIIgA18G4R1 12.5% (1/8)<br>IIIgA19G5R1 12.5% (1/8)<br>IIIgA20G4R1 12.5% (1/8)<br>IIIgA21G3R1 37.5% (3/8) | Baroudi et al., 2013 |

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**Table 3** (*continued*)

| Country | Host | Age group | Percentage positive for *Cryptosporidium* based on screening by PCR (No. positive/ No. tested) | Genotyping method (Target gene) | Species and subtypes (where available) and their percentage % | Reference |
|---|---|---|---|---|---|---|
| | | | | | IIIgA24G3R1 12.5% (1/8)<br>IIIgA26G2R1 12.5% (1/8) | |
| Algeria | Horses | NS | 3% (4/138) | Nested PCR (SSU rRNA and *gp60*) | *Cryptosporidium erinacei* | Laatamna et al., 2013 |
| Egypt | Camels | 4–9 years | 5.9% (6/101) | Nested PCR and RFLP (SSU rRNA, *gp60*)<br>5 isolates subtyped | *C. parvum* 40% (2/5)<br>IIdA19G1 and IIaA15G1R1<br>*Cryptosporidium* rat genotype IV 20% (1/5)<br>*Cryptosporidium* novel genotype 40% (camel genotype) (2/5) | El-Alfy et al., 2019 |
| Egypt | Dairy calves | 1 day - 6 months | 9.7% (24/248) | Nested PCR and RFLP (SSU rRNA and *gp60*) | *C. parvum* 66.67% (16/24)<br>IIaA15G1R1 93.75% (15/16)<br>IIaA15G2R1 6.25% (1/16)<br>*C. bovis* 20.83% (5/24)<br>*C. ryanae* 12.5% (3/24) | Naguib et al., 2018b |
| Egypt | Household dogs | 3 months −1 year | 24% (12/50) | Nested PCR and RFLP (COWP) | *C. parvum* | Gharieb et al., 2018 |
| Egypt | Cattle and buffaloes | < 2 months, 2 months-1 years, >1 year old | Total: 2.62 (16/610)<br>Cattle: 2.5% (12/480)<br>Buffalo: 3.07% (4/130) | Nested PCR and RFLP (COWP and *gp60*) | Cattle<br>*C. parvum* 100% (12/12)<br>IIdA20G1<br>Buffalo<br>*C. parvum* 100% (4/4)<br>IIdA20G1 | Ibrahim et al., 2016 |
| Egypt | Calves | 1 day to 6 months | 28% (14 /50) | Nested PCR (SSU rRNA) | *Cryptosporidium spp.* | Mahmoud et al., 2016 |
| Egypt | Buffalo, cattle and sheep | All ages | Buffalo: 1.28% (6/466)<br>Cattle: 6.90% (31/450)<br>Sheep: 2.5% (3/120) | Nested PCR and RFLP (SSU rRNA and *gp60*) | Buffalo<br>*C. parvum* 33.33% (2/6)<br>IIdA20G1<br>IIaA15G1R1<br>*C. ryanae* 66.67% (4/6)<br>Cattle<br>*C. parvum* 74.2% (23/31)<br>IIdA20G1<br>IIaA15G1R1<br>*C. ryanae* 12.90% (4/31)<br>*C. bovis* 6.45% (2/31)<br>*C. andersoni* 6.45% (2/31)<br>Sheep<br>*C. xiaoi* 100% (3/3) | Mahfouz et al., 2014 |
| Egypt | Calves | NS | 40% (40/100) | Nested PCR and RFLP (COWP) | *C. parvum* 100% (40/40) | Sadek, 2014 |
| Egypt | Dairy cattle | Pre-weaned calves, 2–4 months, Heifers and Adults | 13.63% (269/1974) | PCR and RFLP (SSU rRNA and *gp60*)<br>69 isolates genotyped | *C. parvum* 43.5% (30/69)<br>IIa 87% (26/30)<br>IIaA15G1R1 96.1% (25/26)<br>IIaA14G1R1r1b 3.9% (1/26)<br>IId 13%(4/30)<br>IIdA20G1 100% (4/4)<br>*C. ryanae* 18.8% (13/69)<br>*C. bovis* 10.2% (7/69)<br>*C. andersoni* 10.2% (7/69)<br>Mixed 17.3% (12/69)<br>*C. ryanae* and *C. bovis* 10.2% (7/12)<br>*C. parvum* and *C. bovis* 5.8% (4/12)<br>*C. parvum* and *C. ryanae* 1.5% (1/12) | Amer et al., 2013a |
| Egypt | Cattle and buffalos | 1 months −2 years | 32.3% (260/804) | Nested PCR and RFLP (18S rDNA[i] and *gp60*) | *C. parvum* 65.7%<br>IIa<br>IIaA15G1R1<br>IId* 81.1%<br>IIdA20G1*<br>IIdA19G1<br>*C. ryanae* 11.8%<br>*C. bovis* 4.2%<br>*C. parvum* + *C. ryanae* 11.2%<br>*C. parvum* + *C. andersoni* 1.8%<br>*C. parvum* + *C. bovis* 5.3% | Helmy et al., 2013 |
| Egypt | Water buffalos | 1 Week - 4 months calves and adults | 3.2% (17/538) | PCR and RFLP (SSU rRNA and *gp60*) | *C. parvum* 41.2% (7/17)<br>IIdA20G1 (5/7)<br>IIaA15G1R1 (2/7)<br>*C. ryanae* 58.8% (10/17) | Amer et al., 2013b |
| Egypt | Dairy calves | < 6 weeks of age | 27.08 (26/96) | | | Amer et al., 2010 |

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**Table 3** (*continued*)

| Country | Host | Age group | Percentage positive for *Cryptosporidium* based on screening by PCR (No. positive/ No. tested) | Genotyping method (Target gene) | Species and subtypes (where available) and their percentage % | Reference |
|---|---|---|---|---|---|---|
| | | | | PCR (SSU rDNA and COWP) | *C. parvum* 92.3% (24/26)<br>IIdA20G1 95.8% (23/24)<br>IIaA15G2R1 4.2% (1/24)<br>*C. andersoni* 7.7% (2/26) | |
| Iran | Broiler chickens | NS | 8% (8/100) | Nested PCR (18S rRNA[i]) | *C. parvum* 25% (2/8)<br>*C. baileyi* 75% (6/8) | Shahbazi et al., 2020 |
| Iran | Adult ruminants | All age groups | Total: 4.5% (22/484)<br>Cattle: 4.7% (9/192)<br>Sheep: 5.7% (11/192)<br>Goat: 2% (2/100) | PCR (18S rRNA and *gp60*) | Cattle<br>*C. andersoni* 77.8% (7/9)<br>*C. bovis* 22.2% (2/9)<br>Sheep<br>*C. ubiquitum* 45.5% (5/11) (subtype family XIIa)<br>*C. xiaoi* 54.5% (6/11)<br>Goats<br>*C. xiaoi* 100% (2/2) | Firoozi et al., 2019 |
| Iran | Household dogs and cats | NS | Dogs: 0.6% (2/315)<br>Cats: 0.7% (2/300) | Nested PCR (SSU rRNA) | Dogs<br>*C. canis* 100% (2/2)<br>Cats<br>*C. felis* 100% (2/2) | Homayouni et al., 2019 |
| Iran | Dogs | Average 27 months | 2.14% (3 /140) | Semi-nested PCR (18S rRNA) | *C. parvum* | Ranjbar et al., 2018 |
| Iran | Sheep | NS | 1.7% (22/1300) | Nested PCR and RFLP (18S rRNA) | *C. parvum* 9.10% (2/22)<br>*C. andersoni* 90.9% (20/22) | Dalimi et al., 2017 |
| Iran | Cattle | NS | 2.1% (5/240) | Nested PCR and RFLP (SSU rRNA) | *C. parvum* 100% (5/5) | Saki and Asadpouri, 2018 |
| Iran | Wild rodents | NS | 3% (3/100) | PCR and RFLP (18S rRNA) | *C. parvum* | Saki et al., 2016 |
| Iran | Chicken | NS | 0.7% (7/1000) | PCR and RFLP (18S rRNA) | *C. baileyi* | Hamidinejat et al., 2014 |
| Iran | Calves | < 6 months | 14.2% (31/218) | Nested PCR (18S rRNA) | *C. parvum* 64.6% (20/31)<br>*C. bovis* 29% (9/31)<br>*C. ryanae* 6.4% (2/31) | Izadi et al., 2014 |
| Iran | Cattle | < 1–24 months | 3.7% (8/217) | Nested PCR and RFLP (18S rRNA) | *C. parvum* | Mahami Oskouei et al., 2014 |
| Iran | Calves | 1–30 days | 28.3% (85/300) | Nested PCR and RFLP (SSU rRNA) | *C. parvum* | Asadpour et al., 2013 |
| Iran | Cattle | NS | NS | Nested PCR (*gp60*)<br>25 isolates genotyped | *C. parvum*<br>IIa 92% (23/25)<br>IIaA15G2R1 95.65% (22/23)<br>IIaA16G3R1 4.35% (1/23)<br>IId 8% (2/25)<br>IIdA15G1 100% (2/2) | Nazemalhosseini-Mojarad et al., 2011a |
| Iran | Healthy and diarrheic cattle | NS | 30% (30/100) | Nested PCR (18S rRNA) | *Cryptosporidium spp.* | Kuzehkanan et al., 2011 |
| Iran | Calves | 1–20 weeks | 18.8% (51/272) | Nested PCR and RFLP (SSU rRNA) | *C. parvum* 72.6% (37/51)<br>*C. parvum* novel genotype 1.9% (1/51)<br>*C. andersoni* 17.7% (9/51)<br>*C. bovis* 7.8% (4/51) | Keshavarz et al., 2009 |
| Iran | Adult cattle | NS | 10.5% (11/104) | Nested PCR and RFLP (SSU rRNA) | *C. parvum* 36% (4/11)<br>*C. andersoni* 64% (7/11) | Fallah et al., 2008 |
| Iran | Cattle | NS | 12% (35/292) | Nested PCR and RFLP (18S rRNA) | *C. parvum* 100% (35/35) | Pirestani et al., 2008 |
| Iran | Calves and turkey | NS | NS | Nested PCR and RFLP (18S rRNA)<br>Calves 9 isolates genotyped<br>Turkey 2 isolates genotyped | Calves<br>*C. parvum* 77.8% (7/9)<br>Turkey<br>*C. meleagridis* 100% (2/2) | Meamar et al., 2007 |
| Iraq | Domestic dogs | NS | 47% (47/100) | Nested PCR (18S rRNA)<br>10 isolates sequenced | *C. parvum* 70% (7/10)<br>*C. canis* 30% (3/10) | Al-Yasary and Faraj, 2021 |
| Iraq | Wild pigeon | NS | 11% (11/100) | Nested PCR (18S rRNA) | *C. baileyi* 81.81% (9/11)<br>*C. parvum* 18.18% (2/11) | Altamimi and Al-Zubaidi, 2020 |
| Iraq | Cattle | Different age groups | 38% (38/100) | Nested PCR (18S rRNA)<br>10 isolates genotyped | *C. parvum* 60% (6/10)<br>*C. andersoni* 20% (2/10)<br>*C. bovis* 10% (1/10)<br>*C. ryanae* 10% (1/10) | Alseady and Kawan, 2019 |
| Iraq | Cattle | < 1–4 years old | 35.63% (41/115) | Nested PCR (*gp60*)<br>16 isolates genotyped | *C. parvum*<br>IIaA15G2R1 25% (4/16)<br>IIaA16G2R1 12.5% (2/16)<br>IIaA16G3R1 25% (4/16)<br>IIaA17G2R1 12.5% (2/16) | Merdaw et al., 2018 |

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**Table 3** (*continued*)

| Country | Host | Age group | Percentage positive for *Cryptosporidium* based on screening by PCR (No. positive/ No. tested) | Genotyping method (Target gene) | Species and subtypes (where available) and their percentage % | Reference |
|---|---|---|---|---|---|---|
| | | | | | IIaA19G3R1 12.5% (2/16)<br>IIaA22G1R1 12.5% (2/16) | |
| Iraq | Sheep and goat | NS | Sheep 40% (24 /60)<br>Goat 32.5% (13/40) | Nested PCR (18 s rRNA)<br>10 isolates genotyped | Sheep<br>*C. parvum* 25% (6/24)<br>*C. hominis* 12.5% (3/24)<br>*C. suis* 4.35% (1/23)<br>Goat<br>*C. parvum* 38.5% (5/13)<br>*C. hominis* 7.7% (1/13)<br>*C. andersoni* 7.7% (1/13)<br>*C. ubiquitum* 15.4% (2/13)<br>*C. xiaio* 7.7% (1/13) | Alkhaled and Hamad, 2017 |
| Iraq | Camels | NS | 14% (7/50) | PCR (*hsp 70*) | *C. parvum* | Ahmed et al., 2016 |
| Iraq | Birds (turkey, chickens, ducks, quails, and pigeon) | NS | Total 58.1% (137/236)<br>Turkey 54.5% (12/22)<br>Domestic chickens 57.5% (23/ 40)<br>Broiler chickens 53.8% (28/52)<br>Ducks 62.5% (20/32)<br>Quails 76.7% (46/60)<br>Feral pigeon 26.7% (8/30) | PCR (18S rRNA)<br>32 isolates genotyped | Turkey, chickens, ducks, quails, and pigeon<br>*C. parvum*<br>Broiler chicken, ducks, quails, and Feral pigeon<br>*C. baileyi*<br>Turkey and quail<br>*C. meleagridis*<br>Domestic chicken<br>*Cryptosporidium galli* | Jasim and Marhoon, 2015 |
| Isreal | Preweaned dairy calves with diarrhea | NS | NS | PCR (18S rRNA and *gp60*) | *C. parvum*<br>IIa<br>IIaA15G2R1<br>IIaA12G1R1<br>IId<br>IIdA20G1 | Yasur-Landau et al., 2021 |
| Jordan | Cattle, sheep, horses, goat, chicken | Animals (>1 year old) | Overall prevalence 11.6% (33/ 284)<br>Cattle 18.7% (14/75)<br>Sheep 15.87% (10/63)<br>Horses 8.1% (6/74)<br>Goats 3.92% (2/52)<br>Chicken 4.8% (1/21) | Quantitative PCR and PCR (18S rRNA, C-type lectin and *gp60*)<br>18S sequences were obtained for 29 isolates | Cattle<br>*C. parvum* 7.14% (1/14)<br>IIaA16G1R1<br>*C. xiaoi* 14.3% (2/14)<br>*C. andersoni* 42.86% (6/14)<br>*C. ryanae* 35.7% (5/14)<br>Sheep<br>*C. parvum* 30% (3/10)<br>IIaA16G1R1<br>IIaA19G2R1<br>*C. xiaoi* 50% (5/10)<br>*C. andersoni* 10% (1/10)<br>NS 10% (1/10)<br>Horses<br>*C. parvum* 16.67% (1/6)<br>IIaA19G2R1<br>Novel *spp.* 33.33% (2/6)<br>NS 50% (3/6)<br>Goat<br>*C. xiaoi* 100% (2/2)<br>Chicken<br>*C. baileyi* 100% (1/1) | Hijjawi et al., 2016a |
| Kuwait | Sheep and goat | Pre-weaned (< 3 months) Post-weaned (> 3 months) | Sheep<br>6.9% (23/334)<br>20 genotyped<br>Goat<br>8.1% (18/222) 10 genotyped | PCR and RFLP (SSU rRNA and *gp60*) | Sheep<br>*C. parvum* 80% (16/20)<br>IIaA15G2R1 10% (2/20)<br>IIdA20G1 65% (13/20)<br>*C. ubiquitum* 15% (3/ 20)<br>XIIa<br>*C. xiaoi* 5% (1/ 20)<br>Goat<br>*C. parvum* 70% (7/10)<br>IIdA20G1<br>*C. ubiquitum* 20% (2/10)<br>XIIa<br>*C. xiaoi* 10% (1/10) | Majeed et al., 2018 |
| Turkey | Pre-weaned calves with diarrhea | ≤15–40 days | 53.6% (104/194) | Nested PCR-RFLP (SSU rRNA and *gp60*)<br>15 isolates genotyped | *C. parvum*<br>Ia 100% (15/15)<br>IIaA13G2R1 | Yildirim et al., 2021 |
| Turkey | Calves and heifers | NS | 35.5% (195/550) | Nested and real-time PCR (SSU rRNA and *gp60*) | *C. parvum* 70.8% (138/195)<br>IIaA13G2R1<br>IIaA14G1R1<br>*C. ryanae* 15.4% (30/ 195)<br>*C. bovis* 13.8% (27/ 195) | Yildirim et al., 2020 |
| Turkey | | 3 months old | One cat | | *C. felis* | Sursal et al., 2020a |

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**Table 3** (*continued*)

| Country | Host | Age group | Percentage positive for *Cryptosporidium* based on screening by PCR (No. positive/ No. tested) | Genotyping method (Target gene) | Species and subtypes (where available) and their percentage % | Reference |
|---|---|---|---|---|---|---|
| | Cat with severe diarrhea | | | Nested PCR (SSU rRNA) One isolate genotyped | | |
| Turkey | Dairy calves with diarrhea and Goat kids with diarrhea | 4-week-old (Calves) 10–15 days of age (goat) | NS | PCR and RFLP (SSU rRNA and *gp60*) Calves: 28 isolates genotyped Goats: 9 isolates genotyped Calves: 23 isolate subtyped Goats: 8 isolates subtyped | Calves *C. parvum* 96.4% (27/28) Ia (IIaA13G2R1) 66.7% (20/23) IId 13 (3/23) IIdA18G1 8.7% (2/23) IIdA20G1b 4.3% (1/23) *C. ryanae* 3.6% (1/23) Goats *C. parvum* 88.9% (8/9) IIaA13G2R1 37.5% (3/8) IIaA15G1R1 25% (2/8) IIdA18G1 12.5% (1/8) IIdA22G1 25% (2/8) | Taylan-Ozkan et al., 2016 |
| Syria | Calves with diarrhea and chickens | < 2 months (cattle) < 75 days old (chicken) | Cattle: 25.4% (17/67) Chicken: 9.9% (11/111) | Nested PCR and RFLP (SSU rRNA) | Calves *C. parvum* 100% (17/17) Chicken *C. baileyi* 81.8% (9/11) Unknown species 18.2 (2/11) | Kassouha et al., 2016 |
| Tunisia | Calves | < 5 months | 20% (14/70) | PCR and RFLP (18S rRNA and *gp60*) | *C. parvum* 100% (14/14) IIa (IIaA15G2R1) 85.7% (12/14) IId (IIdA16G1) 14.3% (2/14) | Rahmouni et al., 2014 |

[a] PCR, Polymerase Chain Reaction.
[b] PCR-RFLP, PCR restriction fragment length polymorphism.
[c] SSU rRNA, Small subunit ribosomal ribonucleic acid.
[d] *gp60*, hyper-variable 60 kDa glycoprotein gene.
[e] NS, not specified.
[f] TRAP-C1 gene, thrombospondin related adhesive protein 1 gene.
[g] *Cryptosporidium* oocyst wall protein gene.
[h] HSP70, heat shock protein 70.
[i] 18S rDNA, 18S ribosomal deoxyribonucleic acid.
[j] 18S rRNA, 18S ribosomal ribonucleic acid.

### 3.3. *Cryptosporidium spp. and Giardia duodenalis reported in animals in the MENA region*

*Cryptosporidium* spp. and *Giardia duodenalis* have been reported to infect numerous animal species including cattle, buffalo, sheep, goats, horses, donkeys, camels, rabbits, birds, poultry (chicken, ducks, quails, pigeons and turkeys), dogs, cats and wild rodents in the MENA region (Tables 3 & 4). A total of 15 *Cryptosporidium* species were identified from 54 genotyping studies conducted on animals in the region (*C. parvum*, *C. hominis*, *C. muris*, *C. cuniculus*, *C. andersoni*, *C. bovis*, *C. meleagridis*, *C. baileyi*, *C. erinacei*, *C. ryanae*, *C. felis*, *C. suis*, *Cryptosporidium galli*, *C. xiaoi* and *C. ubiquitum*), in addition to two genotypes (rat genotype IV and a camel genotype) and a novel species detected in horses in Jordan (Table 3). The majority of studies have been conducted on cattle with prevalences ranging from 2.1% to 40% with *C. parvum* the predominant species reported in 44 out of the 54 studies. *Cryptosporidium ryanae, C. bovis* and *C. andersoni* were also commonly reported, while *C. xiaoi* was only reported in one study in cattle in Jordan (Hijjawi et al., 2016a) (Table 3). In both sheep and goats, *C. parvum*, *C. ubiquitum, C. xiaoi, C. andersoni* were reported (Mahfouz et al., 2014; Hijjawi et al., 2016a; Taylan-Ozkan et al., 2016; Dalimi et al., 2017; Baroudi et al., 2018a; Majeed et al., 2018; Firoozi et al., 2019; Sahraoui et al., 2019), with *C. hominis* reported in one study in Iraq in both sheep and goats (Alkhaled and Hamad, 2017). Three studies in camels identified *C. parvum* (Ahmed et al., 2016; Baroudi et al., 2018b; El-Alfy et al., 2019). In equines, *C. parvum*, *C. muris*, *C. hominis*, *C. cuniculus, C. erinacei* as well as a novel species have been reported (Laatamna et al., 2013; Laatamna et al., 2015; Hijjawi et al., 2016a). *C. meleagridis* and *C. baileyi* are the two species most frequently reported in chickens, turkeys and other birds (Table 3), with *C. parvum* and *C. galli* only reported in individual studies in Iran (Shahbazi et al., 2020) and Iraq (Jasim and Marhoon, 2015), respectively.

Several species including *C. parvum*, *C. hominis*, *C. meleagridis*, *C. muris, C. felis*, *C. bovis* and *C. canis* were reported in both animals and humans indicating the possibility of zoonotic transmission and suggesting that some animals might act as a reserviour of infection for humans in some countries in the MENA region. The identification of *C. hominis* in some animals (sheep and horses) also supports a broader host range for this species (Widmer et al., 2020).

Subtyping studies for *Cryptosporidium* spp. at the *gp60* locus in animals from the region were mainly conducted on *C. parvum* but with some information available for *C. meleagridis, C. hominis* and *C. cuniculus*. Within *C. parvum,* subtype families IIa and IId dominated, with IIaA15G1R1, IIaA15G2R1 and IIdA20G1 the most common subtypes reported (Table 3). In addition, two unique subtypes IIaA14G1R1r1b and If-like-A22G2 were isolated from a calf in Egypt and from a camel in Algeria, respectively (Amer et al., 2013a; Baroudi et al., 2018b). The dominance of IIa and IId subtype families in both humans and animals further supports the possibility of zoonotic transmission. Only two studies subtyped *C. meleagridis* (Baroudi et al., 2013; Laatamna et al., 2017) with only subtype family IIIg identified and seven subtypes including subtype IIIgA22G3R1, previously identfiied in a human from Nepal (KU852730; unpublished). Only one study, subtyped *C. hominis* in horses and subtype IkA15G1 was identified (Laatamna et al., 2017), with recent studies suggesting that *C. hominis* is now endemic in horses and donkeys (Jian et al., 2016; Li et al., 2019). *C. cuniculus* subtypes VbA19 and VbA33 were reported in rabbits in a single study from Egypt (Naguib et al., 2021).

Six *G. duodenalis* assemblages were reported in the 17 typing studies conducted on animals within the MENA region (A, B, C, D, E and F), with

**Table 4**
Molecular characterisation and prevalence of *Giardia duodenalis* in animals in countries of the MENA region (2005–2021).

| Country | Host | Age group | Percentage positive for *Giardia duodenalis* based on screening by PCR (No. positive/No. tested) | Genotyping method (Target gene) | *Giardia duodenalis* assemblages | Reference |
|---|---|---|---|---|---|---|
| Algeria | Lambs | < 40 days old | NS[a] | Nested PCR[b] and RFLP[c] (*gdh*[d] and *tpi*[e]) 16 isolates amplified and genotyped | D 25% (4/16) E 62.5% (10/16) Mixed A + E 12.5% (2/16) | Sahraoui et al., 2019 |
| Algeria | Calves with or without diarrhea | < 90 days | 27.45% (28/102) | Nested PCR (*tpi*) | A 50% (14/28) AII E 21.4% (6/28) Mixed A + E 28.6% (8/28) | Baroudi et al., 2017 |
| Egypt | Dairy claves | 1 day-6 months | 13.3% (33/248) | Nested PCR (*tpi, gdh* and *bg*[f]) | A 3% (1/33) AII E 97% (32/33) | Naguib et al., 2018b |
| Egypt | Dairy cows and calves | NS | NS | Nested PCR (*tpi*) 4 isolates genotyped and sequenced | E 100% (4/4) | Abdel-moein and Saeed, 2016 |
| Egypt | Cattle and buffalo | 1 day >6 months | NS | Nested PCR (*tpi, gdh* and *bg*) and Sequencing | E > 81% | Helmy et al., 2014 |
| Egypt | Farmed and wild fish | NS | NS | Duplex PCR (*tpi*) | A 100% (6/6) | Ghoneim et al., 2012 |
| Iran | Cattle, sheep and goats | > 4 years | Total: 5.16% (25/484) Cattle 4.2% (8/192) Sheep 6.2% (12 /192) Goats 5% (5/100) | PCR (*tpi*) | E Sheep 50% (6/12) Goats 20% (1/5) | Kiani-Salmi et al., 2019 |
| Iran | Household dogs and cats | <1 and > 1 years old | Dogs 1.9% (6/315) Cats 1.3% (4/300) | PCR (SSU-rRNA[g]) | Dogs A (AII) 16.7% (1/6) C 50% (3/6) D 33.3% (2/6) Cats A (AI) 25% (1/4) F 75% (3/4) | Homayouni et al., 2019 |
| Iran | Sheep and goats | <12 months | Sheep: 19.8% (17/86) Goats: 15.9% (15/94) | Nested PCR (SSU-rRNA and *tpi*) and sequencing | E 100% (32/32) | Jafari et al., 2014 |
| Iraq | Calves | < 1 year old | NS | PCR (18S rRNA[h]) | *Giardia duodenalis* | Alhayali et al., 2020 |
| Iraq | Cattle | 6 months – 1 year | NS | Nested PCR (*tpi*) 25 isoltes genotyped | A 20% (5/25) B 64% (16/25) Mixed A + B 16% (4/25) | Al-Difaie, 2016 |
| Jordan | Horses (326) and donkeys (74) | Horses (2 months – 22 years) Donkey (1–9 years) | NS | PCR and RFLP[i] (*bg* and *tpi*) | A 60% (18/30) B 13.3% (4/30) E 26.7% (8/30) | Mukbel et al., 2017 |
| Turkey | Cattle | <1 and > 3 years | 30.2% (136 /450) | PCR (*bg*) and Nested PCR for subtyping (*tpi* and *gdh*) | A 11% (15/136) E 89% (121/136) | Onder et al., 2020 |
| Turkey | Cats | NS | 29.4% (30/102) | PCR (*bg*) | B 100% (30/30) | Sursal et al., 2020b |
| Turkey | Horses | 3–15 years | 16.6% (25/150) | Nested PCR (*bg*) | A | Demircan et al., 2019 |
| Turkey | Dogs with or without diarrhea | < 3 and > 3 months | NS | Nested PCR (*bg*) | B 100% (89/89) BIV Mixed A (AIII) + B 42% (38/89) | Gultekin et al., 2017a |
| Turkey | Calves with or without diarrhea | < 3 months | NS | Nested PCR (*bg*) | A 100% AIII | Gultekin et al., 2017b |

[a] NS, not specified.
[b] PCR, Polymerase Chain Reaction.
[c] PCR-RFLP, PCR restriction fragment length polymorphism.
[d] *gdh*, glutamate dehydrogenase.
[e] *tpi*, triose phosphate isomerase gene.
[f] *bg*, beta-giardin.
[g] SSU-rRNA, Small subunit ribosomal ribonucleic acid.
[h] 18S rRNA, 18S ribonucleic acid.
[i] RFLP, restriction fragment length polymorphism.

assemblage A being the most common assemblage (reported in 11/17 studies) followed by assemblage E (9/17) (Table 4). Sub-assemblage AI was reported in only one study in cats in Iran (Homayouni et al., 2019), while sub-assemblage AII was reported in two studies in calves in Algeria and Egypt (Baroudi et al., 2017; Naguib et al., 2018b), and in one study in household dogs in Iran (Homayouni et al., 2019). Sub-assemblage AIII was reported in two studies in Turkey (one in household dogs and the other one in calves) (Gultekin et al., 2017a, 2017b).

**Table 5**
Prevalence and molecular characterisation of *Cryptosporidium* species and subtypes in water and environmental samples from MENA region (2005–2021).

| Country | Water source | Sample concentration and oocyst recovery method | Prevalence % (positive /total number of samples collected) based on microscopy and/or screening by PCR | Genotyping method (Target gene) | Species and subtypes (where available) and their percentage % | Reference |
|---|---|---|---|---|---|---|
| Egypt | Tap water | Membrane filtration (1 μm) | 36.3% (29/80) based on positive samples detected by microscopy (MZN) | Nested PCR[a] (COWP[b]) | *C. parvum* 20.7% (6/29)<br>*C. hominis* 75.9% (22/29)<br>*C. parvum* and *C. hominis* 3.4% (1/29) | Hamdy et al., 2019 |
| Iran | River water contaminated with sewage | Membrane filtration - cellulose nitrate filter (1.2 μm) & sucrose flotation | 52.6% (10/19)<br>Based on screening by PCR | Nested PCR (18 rRNA[c])<br>5 isolates genotyped | *C. parvum* 30% (3/10)<br>*C. hominis* 10% (1/10)<br>*Cryptosporidium muris* 10% (1/10) | Mahmoudi and Karanis, 2020 |
| Iran | Human and livestock wastewater | Centrifugation followed by water-ether sedimentation procedure | 62.9% (34/54)<br>Based on microscopy (IFA)<br>70.5% (24/34)<br>Based on screening by PCR | PCR (18S rRNA)<br>91.6% (22/24) successfully sequenced | *C. andersoni* 95.4% (21/22)<br>*C. xiaoi* 4.6% (1/22) | Hatam-Nahavandi et al., 2016 |
| Iran | Rivers surface water | Membrane filtration (1.2-μm pore size) membrane filter or by Filta-Max filter followed by immunomagnetic separation or sucrose purification methods | 48.97% (24/49)<br>Based on screening by PCR | PCR (18S rRNA and *gp60*[d]) | *C. parvum* 45.8% (11/24)<br>IIdA15G1 72.7% (8/11)<br>IIdA20G1a 18.2% (2/11)<br>IIdA18G1 9.1% (1/11)<br>*C. hominis* 29.2% (7/24)<br>IdA20 100% (7/7)<br>*C. muris* 12.5% (3/24)<br>*C. andersoni* 8.3% (2/24)<br>*C. canis* 4.2% (1/24) | Mahmoudi et al., 2015a |
| Kuwait | Storage tank water | Filtration through an Envirochek HV filter (method 1623) *Cryptosporidium* oocysts pellet were detected by immunofluorescent microscopy (US EPA, 2001) | 20% (1/5)<br>based on positive samples detected by immunofluorescent microscopy | PCR-RFLP[e] (SSU rRNA[f], COWP and TRAP-C[g]) | *C. parvum* IIa 100% (1/1) | Iqbal et al., 2011 |
| Tunisia | Wastewater treatment plants | Microscopy using modified Bailenger method (MBM), immunomagnetic separation (IMS) followed by immunofluorescent assay microscopy | Total 50% (10/20)<br>Raw wastewater 72% (5/7)<br>Treated wastewater 12.5% (1/8)<br>Sludge 80% (4/5)<br>Based on positive samples detected by (IMS) followed by immunofluorescent assay microscopy | Nested PCR (SSU rRNA) | Raw wastewater<br>*C. parvum* (4/5)<br>*C. hominis* (2/5)<br>IaA27R3, IdA15G1<br>*C. muris/C. andersoni* (1/5)<br>*C. andersoni* (1/5)<br>Treated wastewater<br>*C. muris*<br>Sludge<br>*C. parvum* (4)<br>IIaA21R1<br>IIcA5G3b<br>*C. hominis* (3)<br>*C. andersoni* (1) | Khouja et al., 2010 |
| Tunisia | Wastewater and sludge | Centrifugation and storage of pellets in 2.5% potassium dichromate | Wastewater 220<br>Raw water 38.2% (42/110)<br>Treated water 12.7% (14/110)<br>Sludge 41.7% (5/12)<br>Based on PCR | Nested PCR (SSU rRNA and *gp60*)<br>232 waste water and sludge samples | *C. parvum* (10/19)<br>IIaA15G2R1,<br>IIaA17G2R1<br>IIaA18G3R1,<br>IIaA20G2R1 IIaA21R1,<br>IIaA21G2R1 IIcA5G3b<br>*C. hominis* (10/22)<br>IaA26R3, IaA27R4,<br>IdA14<br>*C. andersoni* (9/47)<br>Sheep genotype (1/1)<br>*C. ubiquitum* (4/6)<br>*C. muris* (4/11)<br>Rat genotype (1/1)<br>*Cryptosporidium meleagridis* (1/2)<br>Avian genotype II (2/4)<br>New genotype (1/1) | Ben Ayed et al., 2012 |
| Turkey | Environmental water sources | NS[h] | Environmental water sources 240 in 20 sampling sites 38.3% (92/240)<br>Environmental water sources 180 in 25 sampling sites | Loop mediated isothermal amplification (LAMP) targeting the S-adenosyl-L-methionine synthetase (SAM) gene & nested PCR (SSU rRNA) | *C. parvum* 61.1% (11/18)<br>*C. bovis* 33.3% (6/18)<br>*C. felis* 5.6% (1/18) | Koloren and Ayaz, 2016 |

(*continued on next page*)

**Table 5** (*continued*)

| Country | Water source | Sample concentration and oocyst recovery method | Prevalence % (positive /total number of samples collected) based on microscopy and/or screening by PCR | Genotyping method (Target gene) | Species and subtypes (where available) and their percentage % | Reference |
|---|---|---|---|---|---|---|
| | | | 38.8% (70/180)<br>Based IFA and PCR | 18 Isolates with the highest numbers of oocysts were sequenced | | |
| Turkey | Sea and tap water samples | NS[h] | 43.3% (26/70)<br>Sea water 68.6% (48/70)<br>Tap water 37.1% (26/70)<br>based on positive samples detected by immunofluorescence test (IFT) | Nested PCR (18S rRNA) | *C. parvum* 65.5%<br>*C. hominis* (NS[h])<br>*C. meleagridis* (NS) | Koloren et al., 2013 |
| Turkey | Water samples (70 taps, 50 wells and 15 sewage) | Membrane filtration through a filtration device equipped with a cellulose acetate membrane filter (0.45 μm) followed by centrifugation | 5.2% (7/135)<br>based on positive samples detected by microscopy (modified cold Kinyoun acid-fast) | PCR and RFLP | *C. parvum* 100% (7/7) | Aslan et al., 2012 |

[a] PCR, Polymerase Chain Reaction.
[b] COWP, *Cryptosporidium* oocyst wall protein gene.
[c] 18S rRNA, 18S ribosomal ribonucleic acid.
[d] *gp60*, hyper-variable 60 kDa glycoprotein gene.
[e] PCR-RFLP, PCR restriction fragment length polymorphism.
[f] SSU rRNA[f], Small subunit ribosomal ribonucleic acid.
[g] TRAP-C2 gene, thrombospondin related adhesive protein 2 gene.
[h] **NS, not specified.**

Sub-assemblage BIV was reported in only one study in household dogs in Turkey (Gultekin et al., 2017a).

### 3.4. *Cryptosporidium spp. and Giardia duodenalis reported in water and food in the MENA region*

The molecular characterisation of *Cryptosporidium* spp. and *Giardia duodenalis* in water and environmental samples were only reported in 10 and 9 studies, respectively (from 5 countries: Egypt, Iran, Iraq, Tunis and Turkey; Table 5), while no genotyping studies on the molecular characterisation of either parasite in food were retrieved. Analysed water samples included tap water, wastewater, storage tank water, treated water, sludge water and surface water and a variety of concentration and purification methods were used (Tables 5 and 6). The prevalence of *Cryptosporidium* spp. and *Giardia duodenalis* in different types of water ranged between 5.2% - 80% and 13.6% - 100%, respectively with the lowest prevalence reported in treated water and the highest in raw wastewater (Table 5 and 6).

Ten *Cryptosporidium* species including *C. parvum*, *C. hominis*, *C. muris*, *C. andersoni*, *C. canis*, *C. ubiquitum, C. meleagridis*, *C. bovis, C. xiaoi* and *C. felis* and three genotypes including the sheep genotype, rat genotype IV and a novel genotype (most closely related to *C. baileyi* and avian genotype I) were reported in water and environmental samples from the MENA region (Table 5). Subtyping was only conducted in three studies with *C. parvum* subtype families IIa, IIc and IId identified in wastewater in Tunisia, and *C. hominis* subtype families Ia and Id observed in surface water (river) in Iran and raw wastewater in Tunisia (Table 5). Three *G. duodenalis* assemblages A, B and E were recovered from different water sources, with assemblage A and B the most common assemblages reported in all the subtyping studies ($n = 8$), while assemblage E was only observed in two studies from raw wastewater in Iran and Tunisia (Ben Ayed et al., 2012; Hatam-Nahavandi et al., 2016; Table 6). *Giardia duodenalis* sub-assemblage AI was reported in two studies in wastewater in Tunisia (Khouja et al., 2010; Ben Ayed et al., 2012), sub-assemblage AII was reported in three studies including one report in drinking and raw water in Egypt (Abd El-Latif et al., 2020) and two reports in wastewater and sludge in Tunisia (Table 6) (Khouja et al., 2010; Ben Ayed et al., 2012), while sub-assemlage BIV was only reported in surface and river water in a single study from Iran (Mahmoudi et al., 2015b).

The high prevalence of *Cryptosporidium* spp. and *G. duodenalis* assemblages in different water sources (including those intended for human and animal consumption) in different countries of the MENA region indicates that waterborne transmission is a significant risk factor for the transmission of these two parasites (Tables 5 and 6). In several low and middle income countries including some countries in the MENA region, routine detection and monitoring of water and foodborne parasites is not conducted and the true burden of many parasitic diseases including cryptosporidiosis and giardiasis is underestimated (Dorny et al., 2009). Therefore, in order to decrease and potentially prevent future outbreaks of cryptosporidiosis and giardiasis in the MENA region, continuous and systematic monitoring of these parasites using genotyping tools is crucial.

## 4. Conclusions and future recommendations

The present review highlights the increasing recognition of *Cryptosporidium* spp. and *Giardia duodenalis* as infectious pathogens in countries of the MENA region. Analysis of the data obtained from a total of 20 countries in the MENA region showed that both *Cryptosporidium* spp. and *Giardia duodenalis* are widespread among humans and animals with both anthroponotic and zoonotic transmission cycles. Further studies are needed to assess the extent of molecular diversity of *Cryptosporidium* spp. and *Giardia duodenalis* in humans and animals among different age groups in different countries of the MENA region, in particular in countries where only few or no studies were retrieved from such as Bahrain, Libya and Oman. The continuous monitoring of infection is important to develop estimates of the regional burden of *Cryptosporidium* spp. and *Giardia duodenalis* infections. This knowledge is essential to support intervention and control strategies in the MENA region countries.

In general, most of the risk factors associated with the occurrence of cryptosporidiosis and giardiasis in the MENA region include poor hygiene, unclean drinking water, poverty, overcrowding, diarrhea in household and contact with animals (Khalil et al., 2018; Ahmad et al., 2020). Improved water, sanitation and hygiene (WASH) strategies including proper drinking water purification and water monitoring systems for *Cryptosporidium* spp. and *Giardia duodenalis* are critical (Omarova et al., 2018). More extensive molecular typing studies need to be carried out to better understand the epidemiology including transmission dynamics and differences in clinical presentations and to

**Table 6**
Prevalence and molecular characterisation of *Giardia duodenalis* in water and environmental samples from MENA region (2005–2021).

| Country | Water source | Sample concentration and oocyst recovery method | Prevalence % (positive /total number of samples collected) based on screening by PCR and/or after microscopy screening | Genotyping method (Target gene) | Species, sssemblage/ sub-assemblage | Reference |
|---|---|---|---|---|---|---|
| Egypt | Drinking and raw water | Membrane filtration - cellulose nitrate filter (0.8 μm), centrifugation and sedimentation | Drinking water 0% (0/40)<br>Raw water 100% (10/10)<br>Based on positive samples detected by microscopy using saline/iodine/ trichrome stain | Nested PCR[a] (*gdh*[b] and *tpi*[c]) | Raw water<br>A<br>AII 100% (10/10) | Abd El-Latif et al., 2020 |
| Egypt | Tap water | Membrane filtration (1 μm) followed by microscopy | 25% (20/80)<br>based on positive samples detected by microscopy (Lugol's iodine)) | Nested PCR and RFLP[d] (*bg*[e]) | A 15% (3/20)<br>B 85% (17/20) | Hamdy et al., 2019 |
| Iran | Livestoch and rawurban wastewater treatment plants | Centrifugation followed by water-ether sedimentation procedure and microscopy | 100% (54/54)<br>Based on immunofluorescence microscopy ((stained with mAb-conjugated FITC)<br><br>96.2% (52/54)<br>Based on screening by PCR | Nested PCR (*bg*, *gdh* and *tpi*) | A 73.1% (38/52)<br>E 26.9% (14/52) | Hatam-Nahavandi et al., 2017 |
| Iran | Surface water and river water ($n = 55$) | Membrane filtration (1.2-μm pore size) membrane filter or by Filta-Max filter followed by immunomagnetic separation or sucrose purification methods | 49% (27/55)<br>Based on screening by PCR | Semi-nested PCR (*gdh*)<br>10 isolates genotyped | B<br>BIV 100% (10/10) | Mahmoudi et al., 2015b |
| Iraq | Water supplies | Sucrose flotation methods | 16% (8/50)<br>Based on positive samples detected by microscopy | PCR (18S rRNA[f]) | *Giardia duodenalis* | Alhayali et al., 2020 |
| Iraq | Surface river water | Filtration (0.45 μm pore size filter), Centrifugation and storage of pellets in 2.5% potassium dichromate | 57.14% (4/7)<br>Based on PCR | RT-PCR (*tpi*)<br>4 isolates genotyped | A 25% (1/4)<br>B 50% (2/4)<br>Mixed A + B 25% (1/4) | Bedir et al., 2016 |
| Tunisia | Wastewater treatment plants | Microscopy using modified Bailenger method (MBM), immunomagnetic separation (IMS) followed by immunofluorescent assay microscopy | Raw wastewater 85.7% (6/7)<br>Treated wastewater 50% (4/8)<br>Sludge 100% (5/5)<br>Based on positive samples detected by (IMS) followed by immunofluorescent assay microscopy | Nested PCR (*tpi*)<br>18 Isolates with the highest numbers of cysts were sequenced | Raw wastewater<br>AI 16.67% (1/6)<br>AII 16.7% (1/6)<br>B 16.7% (1/6)<br>*Giardia duodenalis* (unknown genotype) 50% (3/6)<br><br>Treated wastewater<br>*Giardia duodenalis* 100% (4/4)<br>AI 25% (1/4)<br>AII 25% (1/4)<br>B 25% (1/4)<br>*Giardia duodenalis* (unknown genotype) 25% (1/4)<br>Sludge<br>AII 40% (2/5)<br>B 20% (1/5)<br>*Giardia duodenalis* (unknown genotype) 60% (3/5) | Khouja et al., 2010 |
| Tunisia | Wastewater and sludge | Sedimentation and storage of pellets in 2.5% potassium dichromate | Wastewater 220<br>Raw water 42.7% (47/110)<br>Treated 13.6% (15/110)<br>Sludge 25% (3/12)\<br>Based on PCR | Nested PCR (*tpi*) | A 29% (16/55)<br>AI 3.6% (2/55)<br>AII 25.5% (14/55)<br>B 10.9% (6/55)<br>E 1.8% (1/55) | Ben Ayed et al., 2012 |
| Turkey | Environmental and drinking water samples | Flocculation in Al2(SO4)3 followed by concentration using sucrose flotation | 50% (60/120)<br>Based on PCR | Nested PCR (18S rRNA)<br>45 samples were genotyped | A 22.2% (10/45)<br>B 33.3% (15/45) | Koloren et al., 2016 |

[a] PCR, Polymerase Chain Reaction.
[b] *gdh*, glutamate dehydrogenase.
[c] *tpi*, triose phosphate isomerase gene.
[d] RFLP, restriction fragment length polymorphism.
[e] *bg*, beta-giardin.
[f] 18S rRNA, 18S ribosomal ribonucleic acid.

develop more targeted intervention strategies. Sutyping of *G. duodenalis* assemblage B is particularly problematic due to high allelic sequence heterozygosity (Feng and Xiao, 2011). Recently validated loci (6-phosphogluconate dehydrogenase, phosphorylase B kinase gamma catalytic chain, and a hypothetical protein) with low sequence heterozygosity have been developed (Seabolt et al., 2021) need to be more widely used in future studies.

Subtyping of *C. parvum* in the MENA region showed the dominance of the zoonotic IIa and IId subtype familes in humans, which highlights the importance of cattle, sheep and goats in the epidemiology of human cryptosporidiosis in this region. However the transmission of *C. parvum* IIa and IId subtypes in the MENA region is still poorly understood largely due to lack of sufficient subtyping studies in ruminants. More systematic molecular epidemiological studies comparing subtypes in humans and their livestock are required. Another key knowledge gap is the lack of *Cryptosporidium* spp. subtyping and *Giardia duodenalis* sub-assemblage studies in animals other than livestock, which is also central to better understand the transmission dynamics. Studies on the prevalence and genetic diversity of *Cryptosporidium* spp. and *Giardia duodenalis* in drinking water supplies and food sources coupled with case-control studies are also important.

Due to the nature of human practices in the region and their close associations with animals which are regarded as a sustainable source of food, measures to control the zoonotic transmission of *Cryptosporidium* spp. and *Giardia duodenalis* in the MENA region is problematic. Therefore, in order to reduce the environmental contamination and to protect human and animal health in the region, improved disease prevention and control strategies in livestock need to be implemented including better hygiene and disinfection and fencing of livestock away from water sources to reduce run-off (Innes et al., 2020). The success of these "One Health" initiatives requires better communication and collaboration among doctors, veterinarians, and water utilizes across the MENA region.

## Author's contributions

NH: papers collection, tables design, manuscript preparation and revision. AZ: Tables preparation and manuscript revisions and editions. MA: papers collection, tables design. UR: manuscript revisions and editions.

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