

The status of cryptosporidiosis in Jordan: a review

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Abstract

Background: *Cryptosporidium* is a waterborne intestinal parasite that causes diarrhoea in low and middle-income countries worldwide. Reports from Mediterranean countries have documented the prevalence of cryptosporidiosis in children at various ages, also among cancer patients, and in cases of chronic kidney disease, haemodialysis, and organ transplant. Until now, modified-acid staining preceded by stool concentration preparation remains the leading screening diagnostic test for the infection. In Jordan, few studies for cryptosporidiosis have been performed during the last 3 decades.

Aims: This paper reviewed the status of cryptosporidiosis in Jordan and tracked recent updates for this emerging protozoal infection among different population groups.

Methods: In this study, an online search was conducted on Google Scholar and PubMed databases using the keywords: Jordan, cryptosporidiosis and *Cryptosporidium* to inspect studies done on this parasite in Jordan.

Results: Only 9 articles were identified from 1994 to 2019. These were analysed in terms of population group, demography, clinical history and the diagnostic tools used.

Conclusion: Cryptosporidiosis is still neglected in Jordan as indicated by the low number of studies over the last 3 decades and the prevalence is diverse depending on the diagnostic test used and socioeconomic status.

Keywords: cryptosporidiosis, Jordan, diagnostic tools, identification

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Background

Cryptosporidium spp. are intracellular parasites of principal concern, especially in some Mediterranean countries with limited resources and in communities with low socioeconomic status (1,2). Globally, cryptosporidiosis has been ranked as the sixth most important foodborne parasitic infection of humans and domestic animals (3).

Human cryptosporidiosis was first reported in 1976; after that, it was identified as the major cause of chronic diarrhoea in HIV-positive patients and children and as a cause of zoonotic and waterborne diarrhoeal outbreaks (4). Later, *Cryptosporidium* infection prevalence was seen to be on the rise among malnourished children and a cause of premature death in low-resource settings (5–7). *Cryptosporidium* is acquired mainly via contaminated water and the ingested oocysts cause gastrointestinal symptoms before being shed in the stool and transmitted to another host via the faecal–oral route (8). Cryptosporidiosis causes acute diarrhoea in children (9), immunocompromised patients (3), cancer patients (10) and haemodialysis patients (11,12). The infection is usually self-limiting in immunocompetent individuals, however, it might be life-threatening in immunocompromised patients (2). The prevalence of cryptosporidiosis varies among different patient groups and this has been attributed to the endemicity of the parasitic infection in the region of sampling along with environmental, climatic and sanitary factors (13).

Microscopic examination of stool using acid-fast staining, with or without stool concentration, is the most frequently applied screening technique for cryptosporidiosis. Acid-fast staining microscopy is cheap and accessible in poor rural settings; it is the best parasitological method in terms of sensitivity and specificity (14) and shows good performance when compared with immunological and molecular methods (15).

In Jordan, *Cryptosporidium* has been investigated either alone or along with other intestinal parasites in various population groups (10,16), in the environment (17) and in domestic animals (18). Prevalence ranges from 4% to 19%. Modified acid-fast staining microscopy has frequently been used as the diagnostic method of choice. Cryptosporidiosis risk factors have been reported in Jordan, including drinking groundwater that is exposed to contamination from sewage (17), immunosuppression (10), contact with domestic animals and eating unwashed vegetables (19,20).

This paper reviews the status of cryptosporidiosis in Jordan and tracks recent updates for this emerging protozoal infection among different population groups.

Literature review

The search was performed on Google Scholar and PubMed databases using the keywords: Jordan, cryptosporidiosis and *Cryptosporidium*. Only 9 articles were identified from 1994 until 2019 and were downloaded;

this reflects the scarcity of studies on this parasite in Jordan.

The first study was published in 1994, it reported on the prevalence of various parasites in elementary school children in northern Jordan. Using various parasitological methods, *Cryptosporidium* spp. was detected in 40 out of 1000 stool specimens collected from symptomatic and asymptomatic elementary school children aged 6–14 years in northern Jordan (16). The same study reported a higher infection rate in the younger age group (< 9 years) than among children in the older age group, and a higher infection rate in villages than in cities. (16).

After that, and consistent with previous results relating to *Cryptosporidium* prevalence, the same author further explored cryptosporidiosis in pre-school children (less than 6 years old) who suffered from symptoms of gastroenteritis and who were living in Irbid city, 85 km north of Amman, the capital of Jordan (20). In this study, *Cryptosporidium* oocysts were investigated using the formalin–ether concentration technique and cold acid-fast staining followed by microscopy. *Cryptosporidium* oocysts were detected in 6.7% (20 out of 300) of the stool specimens collected from patients; 7/20 were also infected with other pathogens. Risk factors did not differ from those reported previously (16). However, it was noticed that breastfed children had a lower infection rate than those weaned early and those who were bottle-fed; this was attributed to passive maternal immunity as well as reduced exposure to contaminated water during bottle preparation (20).

Another study was done to explore different intestinal parasites associated with diarrhoea in a rural area in the northeast of Jordan. The researchers collected 200 stool samples from patients with symptomatic gastroenteritis and these were examined using a number of parasitological methods including formalin–ethyl acetate concentration, wet preparation and modified acid-fast staining. Diagnosis revealed the oocysts of

Cyclospora spp. and *Cryptosporidium* spp., *Entamoeba histolytica*, *Giardia lamblia*, *Blastocystis hominis*, *Endolimax nana*, *Hymenolepis nana* and *Ascaris lumbricoides*. Bacteria and other enteropathogens were also identified, including *Salmonella* spp., *Shigella* spp. and *Escherichia coli*. Oocysts of *Cryptosporidium* spp. were observed in the samples from 16 (8%) patients, distributed as follows: 10 in children ≤ 14 years old (mean 7.5 years) and 6 in adults 15–87 years (mean 51 years). Statistically significant risk factors for the infection were related to the source of drinking water, contact with animals and eating unwashed vegetables (19).

Ten years later, 2 studies were published from the same northern city; the first focused on drinking-water as a source of infection (17), while the second study explored diagnostic methods, children groups, infection seasonality and source of drinking water (14). Abo-Shehadeh et al. concluded that private groundwater reservoirs northern Jordan were significant enhancing risk factors for contamination with *C. parvum* in rural villages (17). They postulated the source of contamination was sewage disposal systems that were constructed near water reservoirs, which increased the risk of the leakage of their contents to be absorbed by the soil. Consequently, there is a health hazard through the contamination of drinking water stored in underground reservoirs. Therefore, the underground location of water reservoirs must be considered to avoid groundwater–faecal contamination.

Another survey was done to investigate for cryptosporidiosis among children from birth to 12 years at a hospital in Irbid (14). A single stool sample was collected from 300 children, 7 of whom were under chemotherapy treatment for cancer. This study compared several diagnostic methods, including direct wet mount microscopy, flotation concentration, cold Kinyoun Ziehl–Neelsen stain and direct immunofluorescence. Oocysts were detected in 112 samples using direct

Table 1 Cryptosporidiosis in selected Mediterranean countries

| Country | Population group | Diagnostic method | Prevalence (%) | Reference |
|-----------|---|--|------------------|-----------|
| Libya | Diarrhoeal patients aged 2–17 years | Lugol's iodine, immunofluorescence assay | 4 | 22 |
| Egypt | Children aged ≤ 8 years | Nested-PCR | 1.4 | 23 |
| Lebanon | Schoolchildren | Modified Ziehl–Neelsen staining and nested-PCR | 10.4 | 24 |
| Palestine | Gastroenteritis patients (outbreak) | Malachite green negative staining and nested-PCR | All ^a | 25 |
| Egypt | Adult immunocompetent diarrhoeal patients | Modified Ziehl–Neelsen stain, sandwich ELISA, nested-PCR | 21 | 26 |
| Egypt | Haemodialysis patients | Cold modified Ziehl–Neelsen staining | 40 | 13 |

PCR = polymerase chain reaction.

ELISA = enzyme-linked immunosorbent assay.

^aCryptosporidium detected in all outbreak cases.

immunofluorescence, which showed the highest sensitivity and specificity, 100% and 98%, respectively. A higher incidence rate was recorded during the rainy season (January–May). Also, *C. parvum* was detected in the stools of 4 among the 7 paediatric oncology patients, suggesting an association of immune status as a risk factor (14). Overall, these results concur with previous studies which indicated that contaminated drinking water is a main source for the infection as well as the increased incidence rate among rural areas (17,19,20).

Molecular epidemiology has been extensively studied in the current decade to inspect common and new genotypes of *Cryptosporidium* spp. in Jordan for a better understanding of the pathogen distribution, pathogenesis and transmission. Hijjawi et al. (21) genotyped 44 *Cryptosporidium* isolates from Jordanian children at the 18S rRNA locus and identified 4 *Cryptosporidium* spp.: *C. parvum* ($n = 22$), *C. hominis* ($n = 20$), *C. meleagridis* ($n = 1$) and *C. canis* ($n = 1$). Furthermore, subtyping for 29 isolates at the 60-kDa glycoprotein (GP60) locus revealed several rare and novel subtypes indicating unique endemicity and transmission of *Cryptosporidium* in Jordan. The same study showed that quantitative polymerase chain reaction (qPCR) increased the sensitivity of detection and confirmed the prevalence of the infection at up to 19% instead of the 1.8% that was obtained using microscopy.

Another genotyping study expanded the targeted populations to include several kinds of animals besides humans since associations of animals as a probable risk factor for cryptosporidiosis was postulated previously (16,19). A total of 284 stool samples from Jordanian cattle, sheep, goats and chickens and 48 human stool samples were screened via 18S-qPCR and lectin locus-specific qPCR. The age of the humans sampled ranged from 10 months to 56 years and all were immunocompetent, hospitalized with a history of diarrhoea, abdominal pain and gastroenteritis. *Cryptosporidium* DNA was amplified in 37/284 (13.0%) of animal and 4/48 (8.3%) of human samples. Speciation revealed 6 different *Cryptosporidium* species, including *C. xiaoi*, *C. andersoni*, *C. ryanae*, *C. parvum*, and *C. baileyi*. The study identified several novel and previously existing subtypes for *C. parvum* isolates at the 60-kDa glycoprotein (gp60) obtained from humans and animals (18).

Another genetic study was done in 2017 and assessed the prevalence and genotypes of *Cryptosporidium* among paediatric oncology and non-oncology patients (10). In brief, Hijjawi et al. reported a statistically significantly higher prevalence of cryptosporidiosis (14.4%; 23 out of 160) among symptomatic paediatric oncology patients than symptomatic paediatric nononcology patients (5.1%; 7 out of 137) using the modified acid-fast stain (10). All microscopy-positive stools were introduced for DNA extraction and were genotyped at species and subtype levels using the 18S and gp60 loci, respectively. *Cryptosporidium parvum* was identified in all samples. Clustering of the same subtype (IIaA17G2R1) was noticed in almost all species from the 2 patient groups, suggesting

the existence of a cryptosporidiosis outbreak during sampling. That study confirmed that chemotherapy and immunosuppression are considered risk factors for cryptosporidiosis as suggested previously (14).

The last published paper for cryptosporidiosis in Jordan was in October 2019 (12). This described the prevalence of cryptosporidiosis among haemodialysis patients from different areas. In that study a total of 133 stool samples were collected and screened for *Cryptosporidium* oocyst using formalin–ether concentration and a modified acid-fast staining technique. *Cryptosporidium* oocysts were recovered in 15/133 (11%) of the patients with higher positivity incidence reported in males and in rural villages. The age of the patients ranged from 25 to 80 (mean 57.8; standard deviation 12.2) years and the most frequently reported symptoms were gastrointestinal symptoms. The study ensured the importance of modified acid-fast stain as a primary screening method and has recommended increasing the awareness of this parasite in this population group, and to include patients with kidney transplantation as well (12).

Reports for cryptosporidiosis from Jordan among different population groups relatively concurs with those from neighbouring and countries and other countries in the Region, which reflects the endemicity of this parasite and the need for more extensive epidemiological studies. Table 1 summarizes selected studies on cryptosporidiosis, highlighting the status of cryptosporidiosis in other Mediterranean countries and illustrating the diversity of diagnostic methods applied as well as the variation in the prevalence among these countries, all of which indicates the relative harmony between these reports and the results and methods reported in Jordan.

Conclusion

Cryptosporidiosis is still neglected in Jordan as indicated by the low number of studies over the last 3 decades and the prevalence is diverse depending the applied diagnostic test and the socioeconomic status.

Recommendations

Extensive studies on cryptosporidiosis in Jordan should be conducted and may include patients with the following inclusion criteria: kidney transplantation, autoimmune disorders, immunocompetent and immunocompromised children and adults, gastroenteritis, low socioeconomic status, from rural areas of different locations in Jordan, and people depending on groundwater for drinking. Modified acid-fast staining is informative and less expensive as a diagnostic screening tool in Jordan. Development, validation, and evaluation of *Cryptosporidium*–stool antigen immunoassay might be successful in increasing the specificity and sensitivity of detection and in reducing the costs in comparison with PCR. Finally, gp60 genotyping is important to investigate and track suspected common-source *Cryptosporidium* outbreaks.

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Bilan de la situation de la cryptosporidiose en Jordanie

Résumé

Contexte : *Cryptosporidium* est un parasite intestinal d'origine hydrique qui provoque des diarrhées et que l'on retrouve dans les pays à revenu faible et intermédiaire du monde entier. Des rapports provenant de pays méditerranéens ont mis en évidence les taux de prévalence de la cryptosporidiose chez les enfants appartenant à divers groupes d'âge, les personnes atteintes de cancer ou d'insuffisance rénale chronique et les personnes ayant subi une hémodialyse ou une greffe d'organe. La coloration acido-résistante modifiée d'une préparation de selles concentrées reste à ce jour le principal test diagnostique utilisé pour le dépistage de l'infection. Peu d'études sur la cryptosporidiose ont été réalisées en Jordanie au cours des trois dernières décennies.

Objectifs : La présente étude visait à examiner la situation de la cryptosporidiose en Jordanie et à suivre les mises à jour récentes concernant cette infection à protozoaire émergente dans différents groupes de population.

Méthodes : Dans la présente étude, une recherche a été réalisée en ligne sur les bases de données Google Scholar et PubMed. Les mots clés suivants ont été utilisés : Jordanie, cryptosporidiose et *Cryptosporidium*. L'objectif était de recenser les études réalisées sur ce parasite en Jordanie.

Résultats : Seuls neuf articles publiés entre 1994 et 2019 ont été recensés. Les éléments suivants ont été analysés : groupe de population, démographie, histoire clinique et outils diagnostiques utilisés.

Conclusion : La cryptosporidiose est encore négligée en Jordanie, comme l'indique le faible nombre d'études réalisées au cours des trois dernières décennies. La prévalence varie par ailleurs selon le test diagnostique utilisé et le statut socio-économique.

حالة داء خفيات الأبواغ في الأردن: استعراض

عبد الرحمن زعيتر

الخلاصة

الخلفية: خفية الأبواغ هو طفيلي معوي ينتقل عن طريق الماء ويسبب الإسهال في البلدان النامية والمتخلفة في جميع أنحاء العالم. وقد وثقت تقارير واردة من بلدان البحر المتوسط انتشار داء خفيات الأبواغ بين الأطفال في مختلف الأعمار، وأيضاً بين مرضى السرطان، وفي حالات أمراض الكلى المزمنة، وغسيل الكلى، وزرع الأعضاء. وحتى الآن، لا يزال التلويين بالحمض المعدّل الذي يسبقه إعداد تركيز البراز هو الاختبار التشخيصي الرئيسي للتحري عن العدوى. وأجريت في الأردن بعض الدراسات عن داء خفيات الأبواغ خلال الثلاثة عقود الماضية.

الأهداف: هدفت هذه الدراسة إلى بحث حالة داء خفيات الأبواغ في الأردن، وتتبع آخر مستجدات عدوى الأولي الناشئة بين مختلف الفئات السكانية.

طرق البحث: أُجري بحث عبر الإنترنت في إطار هذه الدراسة على قاعدتي بيانات PubMed و Google Scholar باستخدام الكلمات الرئيسية التالية: الأردن، داء خفيات الأبواغ، خفية الأبواغ، لاستقصاء الدراسات التي أجريت على هذا الطفيلي في الأردن.

النتائج: حُدثت 9 مقالات فقط في الفترة من عام 1994 إلى عام 2019. وجرى تحليلها من حيث الفئة السكانية، والوضع السكاني، والسوابق السريرية، والأدوات التشخيصية المستخدمة.

الاستنتاجات: لا يزال داء خفيات الأبواغ مهماً في الأردن، ويشير إلى ذلك انخفاض عدد الدراسات التي أُجريت بشأنه على مدى العقود الثلاثة الماضية، ويتفاوت معدل الانتشار اعتماداً على الاختبار التشخيصي المستخدم والحالة الاجتماعية الاقتصادية.

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