Open Veterinary Journal, (2024), Vol. 14(9): 2421-2432
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Research Article
DOI: *10.5455/OVJ.2024.v14.i9.31*

Submitted: 19/06/2024 Accepted: 23/08/2024 Published: 30/09/2024

Molecular detection and phylogenetic analysis of microsporidia in water and soil in Mosul city

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ABSTRACT

Background: Microsporidia are opportunistic intracellular parasites that cause a variety of illnesses in humans. There is little information available regarding the frequency of this parasite in human cases of diarrhea and cancer.

Aim: This study's primary objectives are to identify microsporidia in soil and water samples, determine the relationships between microsporidia by using sequencing analysis in the targeted area, and look into the genetic diversity of microsporidia originating from domestic, farm, and wild animals.

Methods: The current investigation was conducted from March to December of 2023. In total, 35 water samples and 20 soil samples have been obtained from 12 and 5 locations in Mosul city, respectively, for this investigation. Every sample was moved to the main laboratory of the College of Science and Veterinary Medicine at the University of Mosul, Iraq.

Results: The results of the present study showed that Microsporidia were found in water at a prevalence rate of 45.7% (16/35) and in soil at a rate of 45% (9/20). In Mosul, the various regions have varying rates of Microsporidia prevalence. Microsporidia having a molecular weight of 1,300 bp were found using the polymerase chain reaction technique, which was attributed to the small subunit ribosomal RNA gene. Furthermore, the results of the current investigation indicate that three of the Microsporidia were *Enterocytozoon bieneusi*, which has a molecular weight of 400 bp, based on the ITS gene utilizing the inner primer, while none was found in soil. With a molecular weight of 260 bp, the SSU rRNA gene of Microsporidia was found in water and in soil. However, in accordance with each species' unique DNA discovered in soil and water, none of the *Encephalitozoon intestinalis*, *Encephalitozoon cuniculi*, or *Encephalitozoon hellem* were found. Twelve novel sequences found 9 sequences of Microsporidia and 3 sequences of *E. bieneusi*).

Conclusion: The study demonstrated a water is the main source of Microsporidia and is highly similarity to some of the microsporidia detected in this study. Furthermore, Microsporidia found in our study showed a close relationship with Microsporidia isolated from different countries in the world.

Keywords: Phylogenetic analysis, Microsporidia, Water, Soil.

Introduction

Microsporidia are small, obligatory, spore-forming, intracellular eukaryotes that infect a wide variety of vertebrates such as humans and animals, despite being once classified as a protozoan genus and species, new reports indicate that they more closely resemble fungus (Franzen and Müller, 1999). These various sources are regarded as a reservoir of microsporidia that causes infection in humans and animals. Historical: more than a century ago, microsporidia were discovered. In 1959, the first study reported the discovery of microsporidia in humans (Matsubayashi *et al*., 1959). in the last decades, many researchers focused on the organism heightened which is associated with HIV and AIDS (Modigliani *et al*., 1985; Desportes *et al*., 1985). Numerous human diseases, including those of the intestines, eyes, sinuses, lungs, muscles, and kidneys, can be brought on by microsporidia in both immunocompetent and immunocompromised individuals (Weber *et al*., 1994). Human infections with up to six different genera of microsporidia have been documented. The majority of microsporidia discovered in immunocompromised hosts with a wide range of clinical manifestations were unclassified microsporidia and microsporidia belonging to the genera Enterocytozoon, Encephalitozoon, Nosema, Pleistophora, Trachipleistophora, and Vittaforma (Weber *et al*., 1994). At least 14 microsporidian species have been identified as harmful to humans to date, with *Enterocytozoon bieneusi* being the most common

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species globally, followed by *Encephalitozoon intestinalis* (Matos *et al*., 2012), and *Encephalitozoon cuniculi* (Deplazes *et al*., 1996). The primary means of transmission are fecal-oral pathways, and other infected persons and animals can serve as sources of infection, contaminated water, and food (Didier *et al*., 2004; Galván *et al*., 2013). All microsporidia produce spores that are resistant to the environment and have the ability to extrude their internal polar filament, or polar tube, which allows them to infect neighboring host cells with their contents. Finding the polar filament, which is distinct in both structure and function, is diagnostic for the phylum. Owing to their diminutive size, numerous species that infect humans have a diameter of 1–2 µm (Franzen and Müller, 2001). There are two techniques for identifying and diagnosis microsporidia, firstly transmission electron microscopy (TEM) has historically been used in the diagnosis of microsporidiosis to identify the polar filament and other ultrastructural features peculiar to a given phylum and species. While TEM is still the gold standard, it is a labor- and time-intensive process that needs a dedicated histology crew working over several days, expensive equipment, and a great deal of specialized knowledge (Weber *et al*., 1992). Typical histological stains like modified trichrome stain, which can be used either alone or in conjunction with other stains such as Warthin-Starry silver or Gramme, are among these techniques (Garcia, 2002). There are numerous molecular identification techniques, such as loop-mediated isothermal amplification, real-time polymerase chain reaction, and polymerase chain reaction (PCR). Microsporidia have been found and identified using PCR; however, these investigations have only amplified DNA from biopsy specimens, and all prior researchers have employed species-specific primers in their PCR assay (Visvesvara *et al*., 1994).

The main goals of this study were to employ the molecular and microscopic techniques to identify microsporidia in water and soil samples and to find the relationship between microsporidia using sequencing analysis of the specific region, we also investigated the genetic diversity of microsporidia from animal sources, including domestic, farm, and wild animals.

Materials and Methods

Sampling

In the current study, the water and soil samples were collected from a different surface water source (a canal and a river) that was used directly (with no prior treatment) and were obtained from March to December 2023. Throughout the study period, 35 water samples were gathered from 12 locations in Mosul city, whereas the number of soil samples gathered from 5 locations was 20. Each water sample was 2–2.5 L in volume, and each soil sample weighed 250–500 g. Both samples were taken in sterile containers. After being quickly cooled with CO_2 ice in a cold box, the samples were transferred to the central laboratory of the University of Mosul, Iraq's College of Science and Veterinary Medicine.

Water and soil sample preparation

Water samples were placed in the container (150 ml) to separate the supernatant and sediment materials using the refrigerator centrifuge at high speed for 20 minutes. To stop the sediment from adhering to the parasite, 10 ml of distilled water was added to the sediment after the supernatant was ignored and transferred to a tube. Subsequently, the mixture was moved to a fresh tube and centrifuged at a high speed for 10 minutes. The supernatant was then ignored in order to remove any remaining sediment. The parasite was purified by repeating this step multiple times, after which it was transported to a 1.5 ml Eppendorf tube. For 3 minutes, the Eppendorf tube was spun at 13,500 rpm in a micro-centrifuge. The sediments should then be saved for use in parasite coloring and DNA extraction.

The procedures described by Ardila-Garcia *et al*., (2013) and Kim *et al*., (2015) called for mixing 20 g of dirt with 500 ml of distal water using a vortex [DRAGON LAB, Germany). Using the filter sheets, the mixture was filtered, and the centrifuge device was used to centrifugal the filtered water. The soil samples for this study were prepared according to the above-mentioned water preparation procedures.

Microscopic examination

The modified trichrome staining technique was used to examine microsporidia in water and soil under a microscope (Ryan *et al*., 1993). In 3 ml of glacial acetic acid, 6 g of chromotrope 2R (CDH, New Delhi, INDIA) was dissolved along with 0.5 g of aniline blue (Solarbio Life Sciences, Beijing, China) and 0.25 g of phosphotungstic acid to create the staining solution used in this investigation. After 30 minutes of standing at room temperature, 100 ml of distilled water and 1 M/L of HCl were added to this solution to create a pH 2.5 solution. Chromatope 2R solution was used to stain methanol-fixed smears for 30 minutes at 37°C. After that, the smears were rinsed with acid alcohol for 10 seconds (4.5 ml of acetic acid in 995.5 ml of 90% ethyl alcohol). Following a 10-second rinse in 95% ethyl alcohol, the smears underwent two 5-minute incubations in 95% ethyl alcohol, a 10-minute incubation in 100% ethyl alcohol, and a 5-minute incubation in xylene (or a replacement for xylene) to dry them. Then, a microscope (100X) was used to look at these smears.

DNA extraction

To extract and analyze the doubtful microsporidia, the following procedures were carried out. For examination of the suspected microsporidia, all microsporidia staining using the modified trichrome staining technique to detect the morphology of the parasite. According to the instructions of the FavorPrep™ Stool DNA Isolation Mini Kit (Taiwan) was applied to extract DNA from microsporidia. Next, for estimation

Table 1. The sequence Primers and PCR program used for detecting of the genes.

Microsporidia SPP.	**Gene**	**Primers**	**Sequence of the primers (5-–3-)**	**Size (bp)**	**Reference**
Enterocytozoon bieneusi Nested PCR	*ITS* outer primers	EBITS3	5-GGTCATAGGGATGAAGAG-3	400	(Del Coco *et al.,* 2014)
		EBITS4	5-TTCGAGTTCTTTCGCGCTC-3		
	ITS inner primers	EBITS1	5-GCTCTGAATATCTATGGCT-3		
		EBITS2	5-ATCGCCGACGGATCCAAGTG-3		
E. bieneusi and *Encephalitozoon*	*SSU rRNA*	FP	5-CAGGTTGATTCTGCCTGACG-3	260	(Notermans *et al.*, 2005)
		RP	5-ATCTCTCAGGCTCCCTCTCC-3		
E.bieneusi and *E.intestinalis*	*Small subunit ribosomal RNA*	PMP1	5'-CACCAGGTTGATTCTGCCTGAC-3'	250–270	(Fedorko *et al.*, 1995)
		PMP2	5'-CCTCTCCGGAACCAAACCTG-3'		
Microsporidian species Nested pcr	*Small subunit ribosomal RNA*	F1	5-CACCAGGTTGATTCTGCCTGACG-3	1,300	(David *et al.,* 1996)
		R1	5-TTATGATCCTGCTAATGGTTCTCC-3		
		F1	5-GCCTGACGTAGATGCTAGTC-3	1,265	(Liguory *et al*., 1997)
		R1	5-ATGGTTCTCCAACTGAAACC-3		
Enc. cuniculi, Enc. hellem, Enc. İntestinalis and E. Bieneusi	Small subunit ribosomal RNA	PMP1	5-CACCAGGTTGATTCTGCCTGAC-3	*E. bieneusi* 250 bp, *E. cuniculi* 268 bp, *E. intestinalis* 270 bp *E. hellem* 279 bp	(20)
		PMP2	5-CCTCTCCGGAACCAAACCCTG-3		

PCR program**:** EBITS1 = 35 cycles (94 °C–60 seconds, 57°C–60 seconds, 72°C–60 seconds), EBITS2 = 35 cycles (94°C–60 seconds, 55°C–60 seconds, 72°C–60 seconds), *p* = 35 cycles (94°C–60 seconds, 58°C–60 seconds, 72°C–60 seconds), PMP = 35 cycles (94°C–60 seconds, 60°C–60 seconds, 72°C–60 seconds), MI 1 = 35 cycles (94°C–60 seconds, 57°C–60 seconds, 72°C–60 seconds), MI 2 = 35 cycles (94°C–60 seconds, 55°C–60 seconds, 72°C–60 seconds).

of the DNA concentration of extracted DNA, we used the English Genova Nano instrument (Jenway/UK), which offers precise measurement. The isolated DNA from microsporidia was then kept in storage at −20°C to maintain its purity and stability during additional examinations.

Amplification of the genes

The PCR method was used to amplify each gene sequence (Table 1). The complete 30 µl PCR reaction was run. 15 µl of 2 × GoTaq (Green Mix Master) from Promega Corporation (USA), 1 µl each of primer F and R, 9 µl of double distillate water from Promega Corporation (USA), and 4 µl of the microsporidia DNA template were included in the reaction mixture. After that, gel electrophoresis was used to visualize the target sequence amplicons.

In order to conduct gel electrophoresis, a 2% agarose gel from Peqlab (Germany) was produced, and the DNA samples were loaded into wells together with a 100 bp ladder DNA marker. The amplified DNA fragments were separated and visualized using electrophoresis, and their sizes were estimated by comparing them to the DNA ladder. After adding the entire mixture, the volume was adjusted to 30 µl in an Eppendorf tube. The PCR amplification was carried out using the proper heat cycling conditions. The particular conditions varied based on the PCR procedure that was employed, including denaturation, annealing, and extension temperatures and durations. These are usually tailored to work best with the particular primer set and amplified DNA template.

DNA sequencing

Eight PCR amplicons that were obtained from water and soil samples—all of which had previously been determined to be microsporidia -positive by traditional PCR—were purified and sequenced by Macrogen, a South Korean commercial sequencing business. The ITS and ribosomal RNA target genes were the ones being sequenced. The ITS and ribosomal RNA gene sequences were then collected and subjected to a comparison analysis using the NCBI BLASTn tool, which can be accessed at http://www.ncbi.nlm.nih.gov, against previously published microsporidia sequences that are available on GenBank. Using CLUSTALW from GenomeNet (available via http://www.genome.jp/tools/clustalw/), an online multiple sequence

alignment tool, the alignment and comparability of these sequences were further investigated.

The same GenomeNet CLUSTALW tool was used in conjunction with the Neighbor-Joining program to generate phylogenetic trees. In order to increase robustness, 500 repetitions of the microsporidia ITS and ribosomal RNA gene sequences were included as an out group for building the phylogenetic tree. Through the use of purification, sequencing, and subsequent bioinformatics studies, this all-encompassing strategy sought to shed light on the genetic links between microsporidia from soil and water, ultimately improving our comprehension of the evolutionary context of these isolates.

Ethical approval

Not needed for this study.

Results

Based on microscopic analysis, the current study's results indicated that 45.7% (16/35) of the microsporidia identified in the water samples the microsporidia's morphology (Fig. 1). The high concentration of microsporidia was found in all seven of the following: Bawira, Al Rashidiya, Forests of Mosul, sewage of Old Bridge, sewage of Mosul University Campus, Suker, and Zuhuer. The present study reports a greater percentage rate of microsporidia in the sewage of Old Bridge, Mosul University Campus, Suker, and

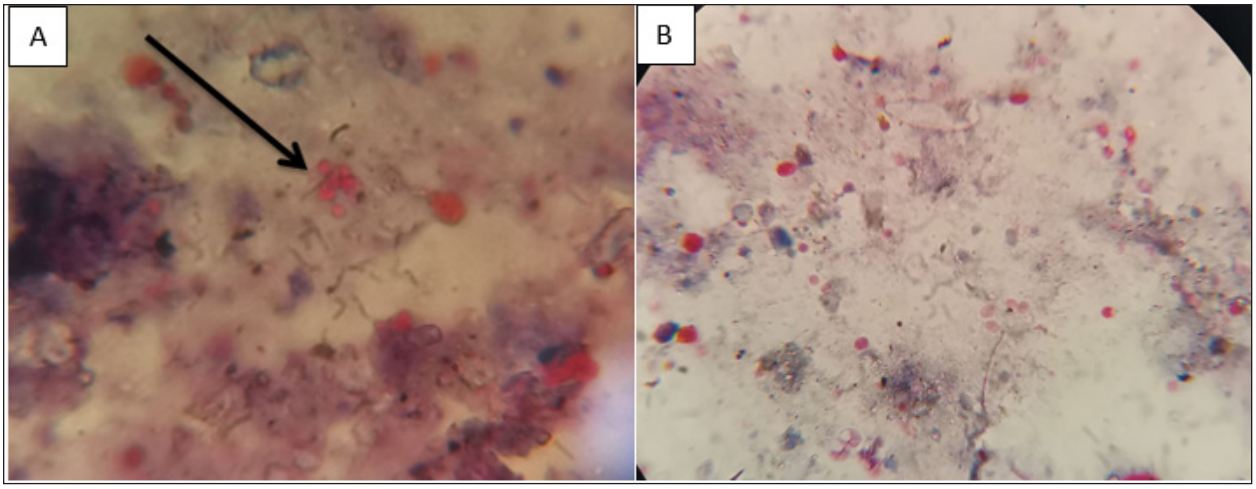

Fig. 1. Microscopic examination revealed the presence of microsporidia in the soil (A) and water (B) samples. The power of magnification was 100X. The microsporidia spores are indicated by the arrows. The spores were pink, shaped like an oval, and stayed in clusters.

Table 2. The number and percentage rate of microsporidia identified in water using microscope and PCR examination.

Location	No. of samples	Results of positive microscopic exam No. (%)	Result of positive PCR exam No. (%)
Bawira	6	5 (83.3%)	5 (83.3%)
Besan	2	–	–
Al Rashidiya	4	3 (75%)	3 (75%)
Forests of Mosul	3	1 (33.3%)	1 (33.3%)
Sewage of Old bridge	2	2(100%)	2 (100%)
Sewage of Mosul University Campus	1	1 (100%)	1 (100%)
Sewage of Suker	2	2 (100%)	2 (100%)
Sewage of Zuhuer	2	2 (100%)	1 (50%)
Domestic water tanks	5	–	–
Well water	3	–	–
Home filter water	3	–	–
Water filters in water shops	2	–	–
12 Locations	35	16 (45.7%)	15(42.9%)

Zuhuer, which was 100%. In contrast, the percentage of microsporidia found in the forests of Bawira, Al Rashidiya, and Mosul was 33.3%, 75%, and 83.3%, respectively. However, there were no microsporidia discovered in Besan, Domestic water tanks, well water, home filter water, and water filters sold in water shops. Additionally, by employing the PCR technique, all of the microscopic analysis's findings were highlighted, and that appeared to be agreement between the results of the PCR and the microscopic investigation (Table 2). According to the microscopic investigation, microsporidia are present in 30% (9/20) of the soil. Microsporidia were 80% (4/5) highly distributed in Al Rashidiya, and 55.5% (5/9) in Bawira. Nevertheless, no Microsporidia were found in Suker or Old Bridge (Table 3). Furthermore, all of the results of the microscopic inspection were emphasized by using the PCR technique. The results of the study revealed that Microsporidia were detected in 33.3% (2/6) of the soil samples using ITS primers and small subunit ribosomal RNA. The high percentage of Microsporidia was 50% (2/4) in Bawira and no Microsporidia were discovered in any other areas of Mosul city (Table 3).

Table 3. The number and percentage rate of microsporidia identified in soil using microscope and PCR examination.

Location	No. of samples	Results of positive microscopy exam No. (%)	Result of positive PCR exam No. (%)
Bawira	9	5 (55.5%)	2 (50%)
Al Rashidiya	5	4 (80%)	-
Old bridge	2	–	–
Suker	2	–	–
Zuhuer	2	–	–
5 Locations	20	9 (45%)	2 (33.3%)

The microscopic examination of all the spores in the water confirmed their identification as Microsporidia. This was further supported by the PCR technique, which highlighted that all the spores belonged to the genus Microsporidia, with a molecular weight of 1,300 bp, as determined by the small subunit ribosomal RNA gene. However, none of the Microsporidia were detected by this type of primer (Fig. 2).

Furthermore, based on the ITS gene using the external primer, the current study's results indicated that three of the microsporidia were *Enterocytozoon (E.) bieneusi*, which has a molecular weight of 400 bp, three *E. bieneusi* were discovered in the Bawira, Mosul University Campus, and Zuhuer Sewage regions (Fig. 3). While none was found in soil (Fig. 4).

The SSU rRNA gene in Microsporidia was detected in the present investigation, and the results further demonstrated that all Microsporidia discovered in water were Microsporidia, with a molecular weight of 260 bp as shown in Figure 5.

The results further showed that only two *Enterocytozoon bieneusi* were studied in soil with a molecular weight of 260 bp, based on the SSU rRNA gene found in the current experiment (Fig. 6).

While, not detecting any of the *E. intestinalis*, *E. cuniculi*, and *Encephalitozoon hellem* based on the

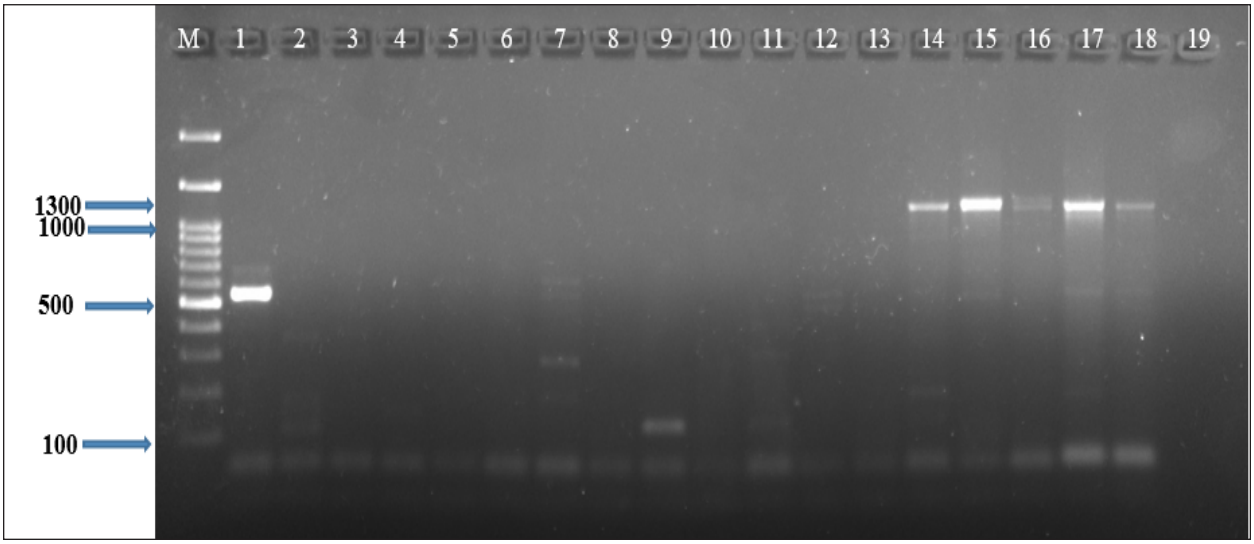

Fig. 2. Agarose gel electrophoresis (2%) showing the typical amplicon of the small subunit ribosomal RNA gene product of microsporidia. The amplification of DNA appears as a ladder-like pattern. Lanes 1–12 represent negative microsporidia isolated from soil, Lane 13 is a non-template control, Lanes 14–18 represent positive microsporidia isolated from water, and Lane 19 is a non-template control. Lanes M are DNA Marker 100 bp ladder (GeneDirex, Korea).

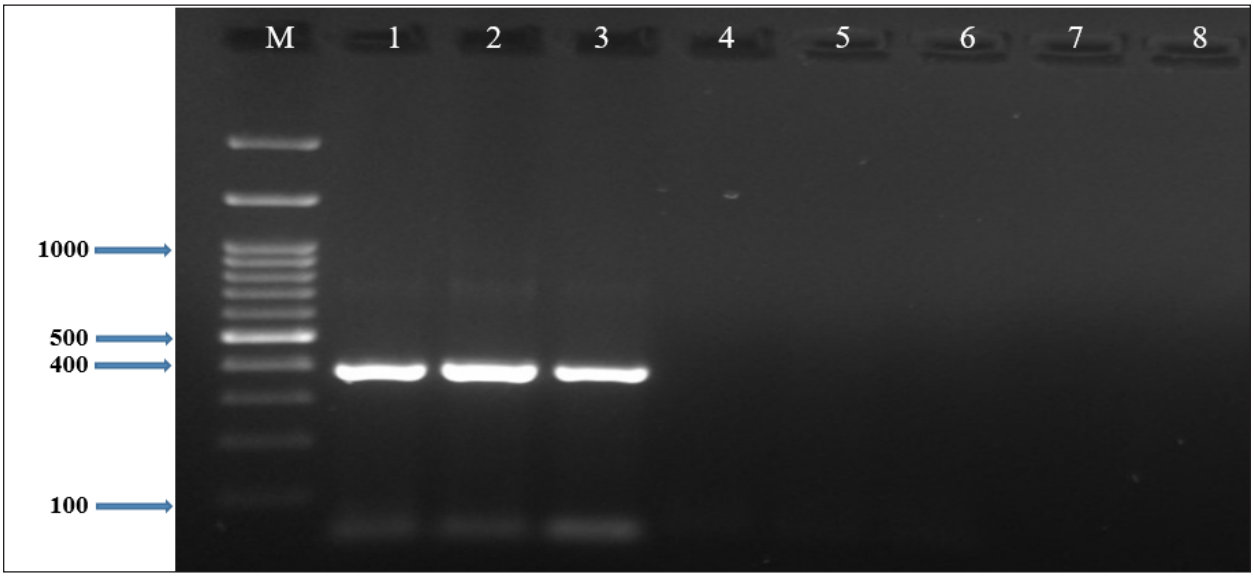

Fig. 3. Agarose gel electrophoresis (2%) showing the typical amplicon of the ITS gene to detect *E. bieneusi* in water. The amplification of DNA appears as a ladder-like pattern. Lanes 1–3 represent positive *E. bieneusi* isolated from water, Lanes 4–7 represent negative *E. bieneusi*, and Lane 8 is a non-template control. Lanes M are DNA Marker 100 bp ladder (GeneDirex, Korea).

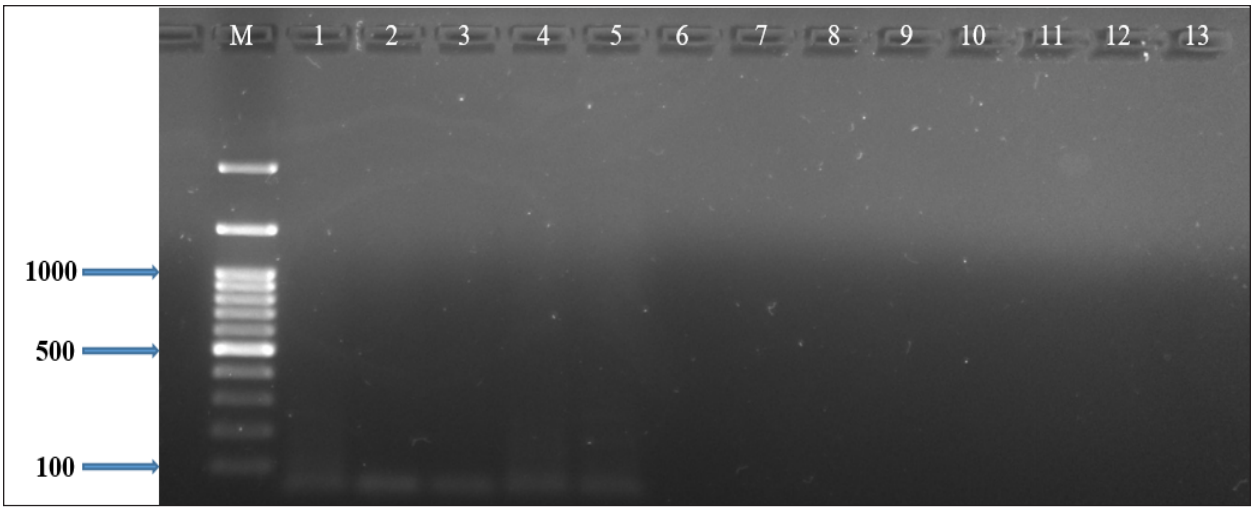

Fig. 4. Agarose gel electrophoresis (2%) showing the typical amplicon of the ITS gene to detect *E. bieneusi* in soil. Lanes M are DNA Marker 100 bp ladder (GeneDirex, Korea).

specific species genes of each of them in water and soil (Fig. 7).

The sequencing results and individual sequencing analysis (BLASTn) were subjected to 12 novel sequences (9 sequences of Microsporidia and 3 sequences of *E. bieneusi*), 8 sequences of Microsporidia that were gathered from water, one sequence of Microsporidia that was found from soil, and 3 sequences of *E. bieneusi* that were collected from water. The sequences of Microsporidia found in the NCBI Genbank are indexed under the following accession numbers (PP625131, PP625132, PP625133, PP625134, PP626147, PP626148, PP626149, PP626150, and PP600314), while the sequences of *E. bieneusi* found in the NCBI Genbank are indexed under the following accession numbers (PP599451, PP599452, and PP599453) as shown as in the Table 4.

Furthermore, a phylogenetic tree analysis using the maximum likelihood technique in MegAlign software (version 15.3) revealed that local SSU rRNA gene sequences showed a different relationship from those available in the GenBank database that was previously

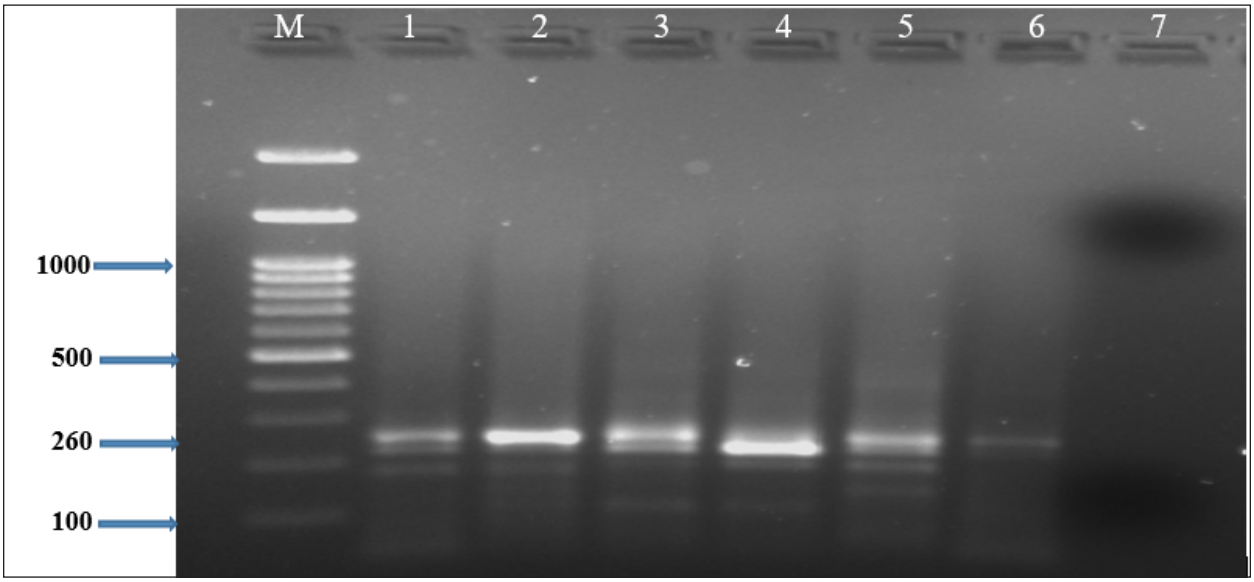

Fig. 5. Agarose gel electrophoresis (2%) showing the typical amplicon of the *SSU rRNA* gene product of microsporidia. The amplification of DNA appears as a ladder-like pattern. Lanes 1–6 represent positive microsporidia isolated from water, Lane 7 is a non-template control, and Lanes M are DNA Marker 100 bp ladder (GeneDirex, Korea).

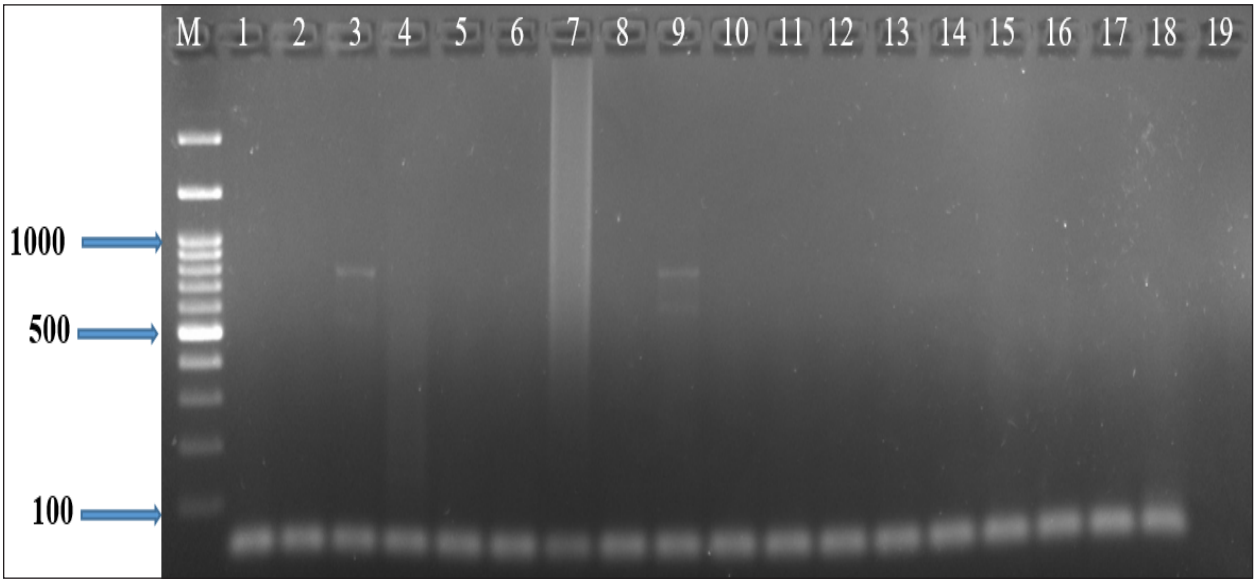

Fig. 6. Agarose gel electrophoresis (2%) no showing the typical amplicon of DNA product of microsporidia in water and soil. Lanes M are DNA Marker 100 bp ladder (GeneDirex, Korea).

reported. The relationship between my sequence types of microsporidia PP626147 and PP626149, and the relationship between my sequence types of microsporidia PP600314 (soil) and MG241422 (Italy) was 100%. In addition, the relationship between my sequence types of microsporidia, PP626150 and HQ999007 (Carbbean region) was 98.6%. Furthermore, the relationship between my sequence types of microsporidia, PP626148 and OR500624 (Poland) was 90%. Additionally, the relationship between my sequence types of microsporidia PP626147, PP626149, and MG241417 (Norway) was 94.4% (Fig. 8).

Discussion

Microsporidia is one of the most significant parasites within cells that infect humans and animals. There are

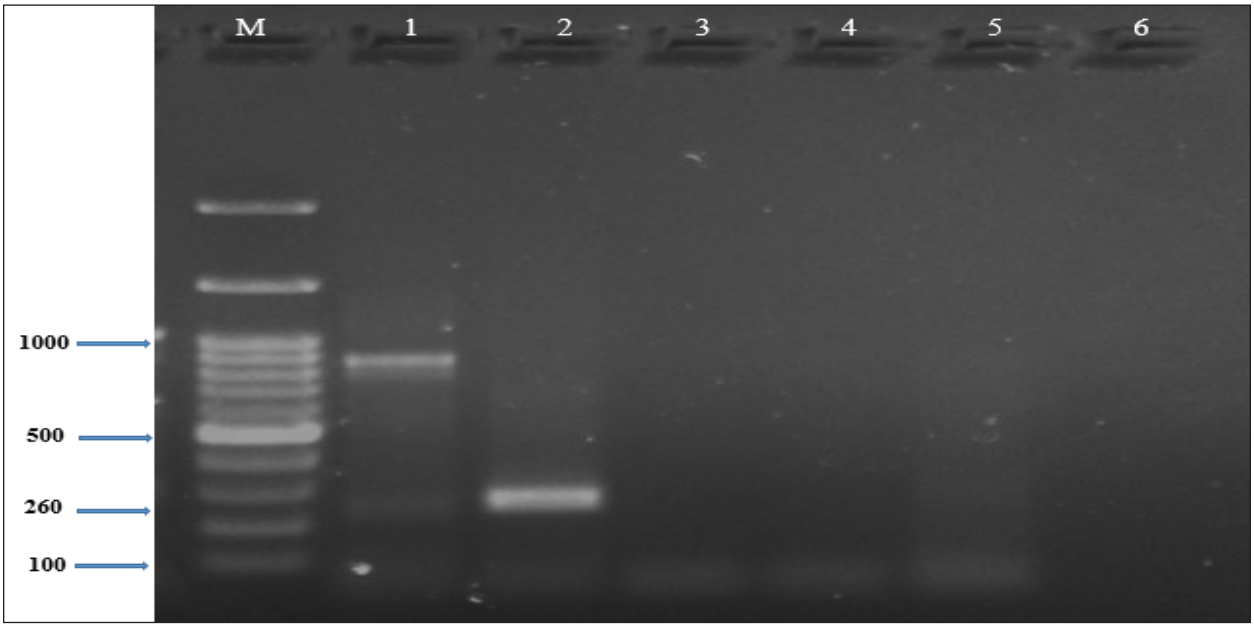

Fig. 7. Agarose gel electrophoresis (2%) showing the typical amplicon of the SSU rRNA gene product of microsporidia in soil. The amplification of DNA appears as a ladder-like pattern. Lanes 1–2 represent positive *Enterocytozoon bieneusi* isolated from water, Lanes 3–5 represent negative *Enterocytozoon bieneusi*, Lane 6 is a non-template control, Lanes M are DNA Marker 100 bp ladder (GeneDirex, Korea).

Table 4. The NCBI GenBank accession numbers for the microsporidia and *E. bieneusi* sequences in water and soil.

Accession numbers	Parasite	Types of samples
PP625131	Microsporidia	Water
PP625132	Microsporidia	Water
PP625133	Microsporidia	Water
PP625134	Microsporidia	Water
PP626147	Microsporidia	Water
PP626148	Microsporidia	Water
PP626149	Microsporidia	Water
PP626150	Microsporidia	Water
PP600314	Microsporidia	Soil
PP599451	*E. bieneusi*	Water
PP599452	*E. bieneusi*	Water
PP599453	*E. bieneusi*	Water

over a thousand species, and 12 of them have been associated with human infection (Franzen and Müller, 1999). There are a variety of ways that Microsporidia are transmitted to people. One major way is by water, which can be contaminated while drinking or swimming in a pool of humans or male homosexuality, which is the fecal-oral mode of transmission (Hutin *et al*., 1998). According to the results of the current investigation, 45.7% (16/35) of the Microsporidia were found in water from various parts of Mosul city. Numerous studies have concluded that the Microsporidia identified in the water samples obtained from rivers (Sparfel *et al*., 1997; Dowd *et al*., 1998; Franzen and Müller, 1999). Human Microsporidia have been found in groundwater, suggesting that the parasites could spread underground and contaminate drinking water sources (Dowd *et al*., 1998). Several features of Microsporidia include their ability to survive and remain infectious for days or weeks in ordinary environmental conditions. Organisms in water can live for almost a year at 4°C. For the reason that Microsporidia are so tiny, it is necessary to remove them from water by using the proper filtration techniques. Furthermore, the direct pumping of surface water from a summertime swimming area and the use of filtration, ozoflotation, and flocculation rather than chlorination for water treatment could have further contributed to the pollution. Furthermore, waste water from developing countries is sometimes dumped straight into rivers, where Microsporidia may grow, and then treated before being administered to humans, creating an environment that invites the spread of Microsporidia infections. Although the infectious dose needed to produce disease in humans is unknown, it might be low because athymic mice may get infected with as little as 100 spores (Didier *et al*., 1994). It is now recognized, that it is impractical to provide drinking water that is totally free of pathogens so the proposed revision of the directive requires that: "water intended for human consumption does not contain pathogenic microorganisms and parasites in numbers

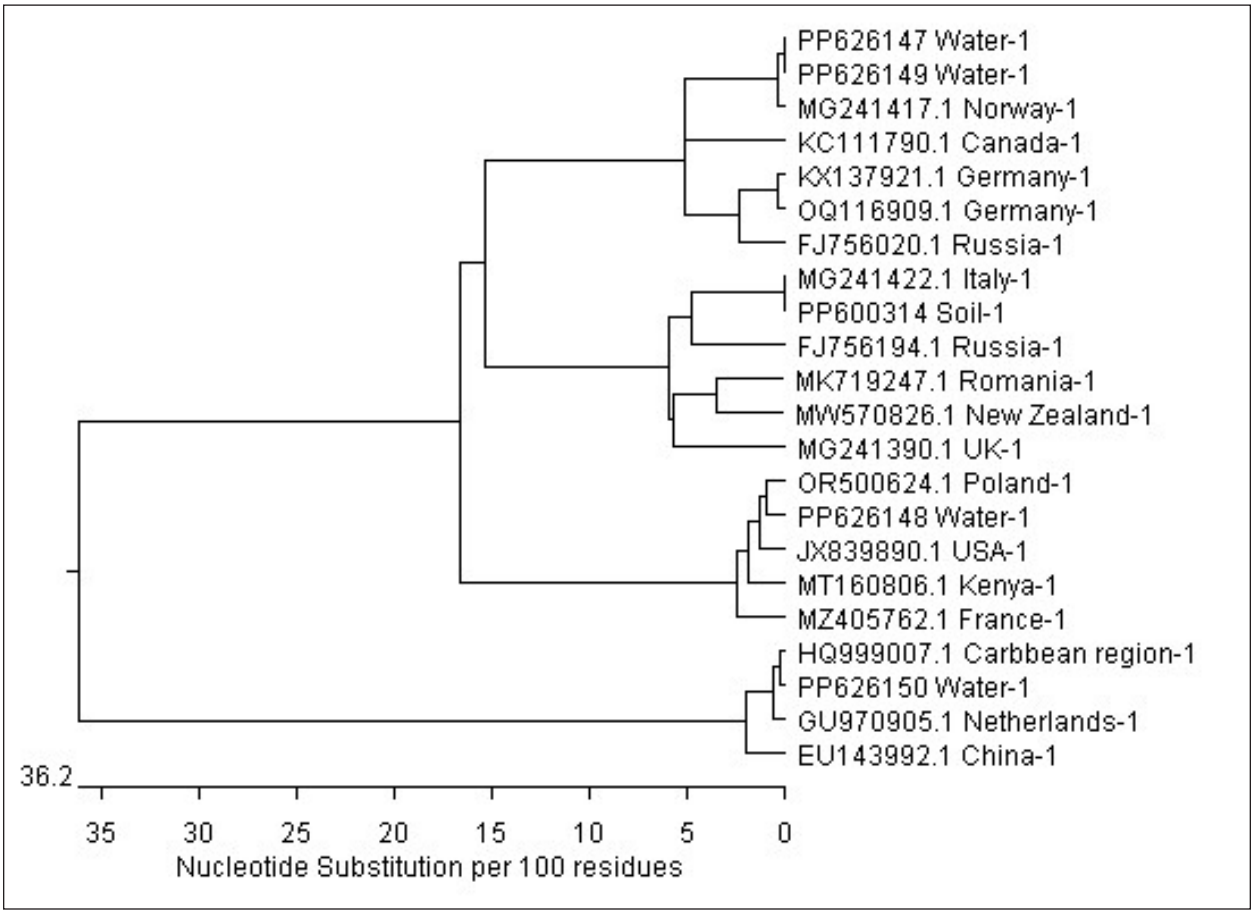

Fig. 8. Clustering analysis of the *SSU rRNA* gene sequence of microsporidia and other *SSU rRNA* gene sequences of microsporidia retrived from NCBI GenBank. The designation in the parentheses indicates the NCBI accession number.

that constitute a potential danger (Smith and Rose, 1998)".

The investigation findings also revealed that 45% (9/20) of the soil has Microsporidia. According to earlier research, Microsporidia are thought to be preserved in animals like dogs, goats, and rabbits (Aguila *et al*., 1999; Lores *et al*., 2002), pigeons (Haro *et al*., 2005), and soil and fecal samples from urban parks (likely from cats and dogs). Distribution of Microsporidia according to data gathered using both pigs and dogs had the greatest number of positive samples for these parasites, and they also played a role in the spread Microsporidia in the soil (Galván-Díaz *et al*., 2014). Spores are released into the environment by infected animals through their feces, urine, and respiratory excretions, all of which have the potential to spread infection in soil (Bornay-Llinares *et al*., 1998). Rainfall can contaminate canals, river streams, and wells used as irrigation water sources with the excrement of diseased animals that have Microsporidia contamination from domestic, wild, and agricultural animals (including birds) (De Roever, 1998).

Enterocytozoon bieneusi was detected in water and soil based on the SSU rRNA gene. Findings from recent molecular research appeared to suggest that raw sewage, tertiary effluents, surface water, and groundwater in France and the US contained *E. bieneusi* (Dowd *et al*., 1998). The most common, *E. bieneusi* is one of most five species of Microsporidia complicated in human infections, *E. bieneusi* can cause severe, severe diarrhea and has a prevalence of 7%–50% (Weber *et al*., 1994). *Enterocytozoon bieneusi* has been identified in AIDS patients suffering from chronic diarrhea and wasting that lead to the onset of the AIDS epidemic (Didier and Todd Bessinger, 1999). The feces of those with immunocompromised and immunocompetent people have been identified to have intestinal microsporidia, which may have been caused by contamination of the drinking water supply in France, where an outbreak of the illness was recently reported (Cotte *et al*., 1999). According to the results of the earlier study, children were exposed to *E. bieneusi* through live in their social environments, which in turn caused the children to become contaminated (Bretagne *et al*., 1993).

There are several molecular biology methods used to detect Microsporidia based on the DNA sequence of Microsporidia. The Microsporidium found in the Seine is *E. bieneusi*, as evidenced by DNA sequencing of the PCR product produced from the sample of the river Seine, which revealed 98% similarity with a known *E. bieneusi* sequence (Sparfel *et al*., 1997). Additionally, the PCR method was applied to recognize *E. bieneusi* in surface water and detect Microsporidia DNA (Sparfel *et al*., 1997). *Enterocytozoon bieneusi* had been detect based on the Target sequence of DNA using the PCR technique (Hartskeerl *et al*., 1993). There are various types of primers used to identify of Microsporidia using the PCR technique. Additionally, the result of our research, was found in 12 novel sequences—9 Microsporidia sequences and 3 *E. bieneusi* sequences. Furthermore, eight Microsporidia sequences had been obtained from water, one from soil, and three from water were *E. bieneusi* sequences. Based on the phylogenetic tree analysis, there is a close relationship between Microsporidia in this study. Microsporidia can infect many different types of hosts, such as people, domestic animals, poultry, companion animals, birds, and wildlife. The fecal-oral pathway is the primary means of transmission (Desportes *et al*., 1985; Qin *et al*., 2022). Water can get contaminated by animals excreting their waste in it or by rain carrying the Microsporidia-laden waste into rivers and water.

Conclusion

Microsporidia contaminate the soil and water close to rivers. The Microsporidia are primarily transmitted to people through soil and water. Only Microsporidia is found in water and soil based on the SSU rRNA gene. The current study's findings reveal that PCR testing using a powerful DNA extraction procedure on water and soil samples is a sensitive and practical tool for diagnosing intestinal microsporidiosis and distinguishing between species. PCR and other molecular techniques appear to be completely sensitive and specific for the diagnosis and differentiation of species of Microsporidia. Microsporidia found in this study have relationships with each other and with Microsporidia found worldwide.

Acknowledgment

The author expresses sincere gratitude to Mosul University, specifically the College of Veterinary Medicine and the College of Science, for generously providing the facilities essential for the completion of a recent study.

Authors' contributions

Narmen Tariq Fadhel Tekeli, Senaa Abdullah Ali Al-jarjary, and Omar Hashim Sheet: Conceptualization. Senaa Abdullah Ali Al-jarjary and Omar Hashim Sheet: Study design. Narmen Tariq Fadhel Tekeli collected data and sampling. Omar Hashim Sheet: Statistical Analysis. Narmen Tariq Fadhel Tekeli and Omar Hashim Sheet: Writing.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency.

Data availability

All relevant data are provided in the manuscript.

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