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Identification of Parasitic Contamination in Different Water Sources in Amedi District, Kurdistan Region

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ABSTRACT

Water is the primary component of the earth, essential for all known forms of life. It is a critical nutrient for maintaining homeostasis in the human body and exists in three states: liquid, solid, and gas. Ensuring drinking water remains clean, safe, and reliable is vital. Factors such as water contamination, heavy rainfall, and agricultural residues which can transfer parasites from soil to the water's surface contribute to the spread of parasitic diseases. This study focused on detecting different parasite stages in various water sources in the Amedi district of Duhok. A total of 250 water samples collected from 6 different sources including 80 samples from household tanks water, 45 samples from sewage water, 40 samples from small rivers canals, 35 samples from ponds, 25 samples from drinking municipal project and 25 samples from springs in Amedi district. For parasitological analysis, all water samples were transported in cold boxes to the Microbiology and Parasitology Laboratory at the Medical Laboratory Department of Amedi Technical Institute. The sediment from each sample was examined under a microscope to identify helminth eggs, trophozoites, and protozoa cysts. Staining was performed using Lugol's iodine and the modified Ziehl-Neelsen acid-fast method. Non-nutritive agar supplemented with *Escherichia coli* was used for the cultivation of Free-Living Amoebae. The rate of contamination of water in current study recorded as higher rate with *Giardia lamblia* 13,2 % followed by *Entamoeba histolytica* 11,6 %, while *Entamoeba coli* and *Ascaris lumbricoides* recorded 5,6 %, then both *Enterobius vermicularis* and *Cryptosporidium* spp. found in rate of 4%, the lowest rate of contamination found by free living amoeba *Naegleria fowleri* and *Acanthamoeba*. These findings underscore the urgent need for improved water quality management in the Amedi district. Policy recommendations include the implementation of routine water monitoring programs, stricter regulations on agricultural waste disposal, and investment in modernized water infrastructure to prevent contamination through pipe cracks and leaks. Additionally, community health education campaigns should be promoted to raise awareness about waterborne parasitic infections. Strengthening intersectoral collaboration between public health authorities, environmental agencies, and local governments is critical to ensuring a sustainable and safe water supply.

1. Introduction

Water is essential for human life. The Food and Agriculture Organization (FAO) recognizes access to clean drinking water as a fundamental human right necessary to safeguard human health (Acheson, 1992). Therefore, drinking water must meet safety standards and quality criteria to ensure it is safe for human consumption (Arora and Arora, 2010). Drinking water supplied for domestic use is commonly referred to as tap water or potable water. It is intended to be safe and clean for drinking, cooking, and other household purposes (Cotruvo et al., 2004; Yahooa and Mawlood, 2023). However, one of the greatest threats to public health is the consumption of contaminated drinking water (Azman et al., 2009; Mohammed et al., 2020). Globally, an estimated 1 to 2 billion people face a shortage of clean drinking water, and polluted water is responsible for approximately 30,000 deaths each week—surpassing the number of deaths caused by war (Cheesbrough, 2006). One of the most common ways to contract parasites is by consuming contaminated water or undercooked meat (Dheyab, 2016). Parasite infections are among the many conditions that can cause unexplained weight loss (Hambidge, 2001). The most common symptoms associated with parasite infections are those that resemble irritable bowel syndrome (Carmena, 2010). Intestinal parasites can cause a range of symptoms, including anemia, bloating, diarrhea, itching, bowel obstruction, and abdominal discomfort (Markell and Voge, 1976, Lallo, 2012). Standards for drinking water quality have existed since ancient times, particularly when changes in the taste and smell of water were observed. In Greek civilization, these standards were addressed by boiling the water, exposing it to sunlight, and filtering it through coal to remove suspended materials through coagulation (Robertson et al., 2006). Although various methods have been used to identify and distinguish parasites, DNA analysis has become increasingly essential as we move toward developing the most sensitive assays (AL-Kubaisy et al., 2014). The objective of this study is to assess and monitor the prevalence of parasite contamination in drinking

water across different locations within the Amedi district. By identifying the types and distribution of parasites present in water sources, this research aims to provide critical data that can inform public health policies and improve water quality management, ultimately reducing the risk of waterborne infections and illnesses.

2. Materials and methods

2.1 Study area

As shown in Figure 1, the area under investigation is the Amedi district, located in the Kurdistan region of the Duhok governorate in northern Iraq. Amedi is situated to the north of the Duhok Governorate, at an elevation of 4,600 feet (1,400 meters) above sea level. The district has a population of approximately 11,000 people.

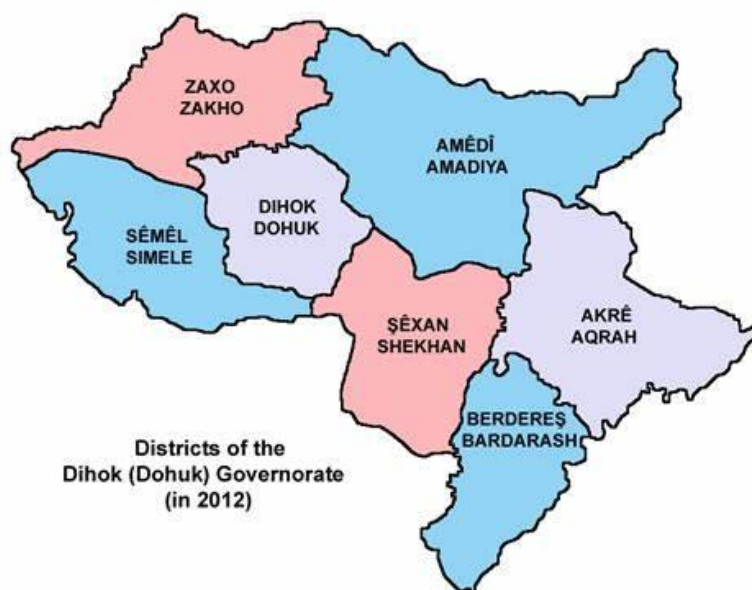


Figure 1: Map of Duhok Governorate showing site of Amedi district.

2.2 Data collection

The water sources of Amedi district are those from natural water sources and tap water, the sampling sites were selected based on their benefit and importance to public health important, physical water parameters including pH, percentage of chlorine and turbidity. A total of 250 water samples were collected in a clean and sterilized disposable plastic bottle, the samples were labeled with date and site of collection. The samples were collected from 6 different sources including; 80 samples from household water tanks, 45 samples from sewage

water, 40 samples from small rivers canals, 35 samples of ponds, 25 samples from springs and 25 samples from municipal drinking project of water in Amedi district, the water samples transported in cold box to the Microbiology and Parasitology Laboratory of Medical Laboratory Department, Amedi Technical Institute for parasitological for examination. A vacuum pump was used to filter the samples through a nitrocellulose membrane (0.45 μm pore size), and the samples were left undisturbed at room temperature for a full day (Arora and Arora, 2010). The supernatant was then removed and discarded. The sediments from each sample were examined under a microscope for parasite cysts, trophozoites, and helminth eggs using a 0.9% saline smear. The samples were stained using the trichrome method, modified Ziehl-Neelsen acid-fast stain, and Lugol's iodine (Dura et al., 2007, Carmena, 2010). The non-nutritive medium or nutrient agar with suspension of *E.coli* were used to cultivation of Free-Living Amoeba (FLA) by using centrifuge filtered sample for 10 minutes at 250 x g, removed suspension and resuspend sediment, then pipet 2-3 drops of suspension onto the middle of the plate surfaces of two Non-Nutrient Agar *E.coli* plate and not spread incubate each plate overnight at 37-42 c in an aerobic and examined the surface of the agar using traditional microscope with a 10 x objective to detection of amoeba trophozoites and cyst, or if feeding tracks are visible, ameba should be present at one end of each track if no tracks are visible, continue to examine daily for up to 10 days (Koloren and Ayaz, 2016, Dheyab, 2016, Ghasemi et al., 2015).

2.3 Statistical Analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS). Statistically significant differences were assessed using the t-test and the chi-square test. A P-value of less than 0.05 was considered indicative of a significant difference between the two groups.

3. Results

Out of 250 examined water samples, 120 (48%) were contaminated with parasitic stages, while 130 (52%) were uncontaminated. The contamination rate of all examined water

samples is presented in Figure 2.

A total of 250 water samples were collected from different sources, including 80 samples from household tank water, 45 from sewage water, 40 from small river canals, 35 from ponds, 25 from springs, and 25 from the municipal drinking water project in Amedi district. Among these, 120 (48%) samples were contaminated. The highest contamination rate was found in sewage water samples (41/45, 91.1%), followed by river samples (33/40, 82.5%), ponds (28/35, 80%), and springs (7/25, 28%). No contamination was detected in samples collected from the municipal drinking water project (Table 1). A statistically significant difference was observed in contamination rates across different water sources ($P = 0.012$).

Figure 3 displays the spread of parasite species found throughout the investigation. With a presence in 13.2% of the samples, *Giardia lamblia* was the most common parasite, closely followed by *Entamoeba histolytica* at 11.6%. In 5.6% of cases, *Ascaris lumbricoides* and *Entamoeba coli* were detected. Four percent of infections were caused by *Enterobius vermicularis* and *Cryptosporidium* spp. With respective prevalences of 2%, *Acanthamoeba* and *Naegleria fowleri* were the least prevalent. Eight different types of parasites were identified in the 120 contaminated samples, including *Cryptosporidium* spp., *Giardia lamblia*, *Enterobius vermicularis*, *Ascaris lumbricoides*, *Entamoeba histolytica*, *Entamoeba coli*, *Naegleria fowleri*, and *Acanthamoeba* (Figures 4).

Contamination in household tank water samples was relatively low, with *Giardia lamblia* detected in 5/80 (6.3%) samples, *Entamoeba histolytica* in 3/80 (3.75%), *Entamoeba coli* in 2/80 (2.5%), and *Cryptosporidium* spp. in 1/80 (1.25%). No contamination with *Enterobius vermicularis*, *Ascaris lumbricoides*, *Naegleria fowleri*, or *Acanthamoeba* was recorded (Table 2). There was no statistically significant difference in contamination rates between household tank water samples and other sources ($P = 0.220$).

Regarding contamination in spring water samples, *Acanthamoeba* spp. was found in 2/25 (8%), *Naegleria fowleri* in 1/25 (4%), *Entamoeba*

histolytica in 1/25 (4%), *Cryptosporidium* spp. in 1/25 (4%), and *Entamoeba coli* and *Giardia lamblia* in 1/25 (4%) each. No contamination with *Ascaris lumbricoides* or *Enterobius vermicularis* was detected (Table 3). The difference in contamination rates between spring water samples and other sources was not statistically significant ($P = 0.070$).

Among small river canal samples, *Giardia lamblia* was the most prevalent parasite (8/40, 20%), followed by *Entamoeba histolytica* (6/40, 15%). *Ascaris lumbricoides*, *Cryptosporidium* spp., and *Enterobius vermicularis* were detected in 4/40 (10%) samples each, while *Entamoeba coli* was present in 3/40 (7.5%) samples. *Naegleria fowleri* and *Acanthamoeba* spp. were detected in 2/40 (5%) samples each (Table 4). The contamination rate in river samples was significantly different from that of other sources ($P = 0.003$).

Pond water samples exhibited the highest contamination rates, with *Giardia lamblia* present in 9/35 (25.7%) samples, followed by *Entamoeba histolytica* (6/35, 17.1%). *Entamoeba coli* was

found in 4/35 (11.4%) samples, *Ascaris lumbricoides* in 3/35 (8.6%), and both *Naegleria fowleri* and *Cryptosporidium* spp. in 2/35 (5.7%). The lowest contamination rates were recorded for *Enterobius vermicularis* and *Acanthamoeba* spp. (1/35, 2.9%) (Table 5). The contamination rate in pond samples differed significantly from that of other sources ($P = 0.021$).

No contamination was detected in water samples collected from the municipal drinking water project in the Amedi district.

Sewage water samples used for agricultural irrigation exhibited the highest contamination rates, with *Entamoeba histolytica* detected in 13/45 (28.8%) samples, *Giardia lamblia* in 10/45 (22.2%), *Ascaris lumbricoides* in 7/45 (15.6%), *Enterobius vermicularis* in 5/45 (11.1%), *Entamoeba coli* in 4/45 (8.9%), and *Cryptosporidium* spp. in 2/45 (4.4%) (Table 6). The contamination rate in sewage water samples was significantly different from that of other sources ($P = 0.001$).

Table 1: Total number and percentage of contaminated Water from different sources in Amedi District (n =250)

Source of water	No. of samples examined	No. and % of contaminated samples	P value
Household tanks	80	11(13.8 %)	0.012
Small rivers canals	40	33 (82.5 %)	
Spring	25	7 (28 %)	
Ponds	35	28 (80 %)	
Sewage	45	4 (91.1 %)	
Project	25	0	
Total	250	120 (48 %)	

Table 2: Number and percentage of contaminated water collected from household tanks water.

Household tanks water Samples Examined (No=80)		
Species of parasite	No. of Positive & %	P value
<i>Giardia lamblia</i>	5 (6.3 %)	0.220
<i>Entamoeba histolytica</i>	3 (3.75 %)	
<i>Entamoeba coli</i>	2 (2.5 %)	
<i>Cryptosporidium</i> spp.	1 (1.25 %)	
<i>Enterobius vermicularis</i>	0	
<i>Ascaris lumbricoides</i>	0	
<i>Naegleria fowleri</i>	0	
<i>Acanthamoeba</i>	0	
Total	11 (13.8 %)	

Table 3: Number and percentage of contaminated water collected from spring.

Spring Water Samples Examined (No =25)		
Species of parasite	No. of Positive & %	P value
<i>Acanthamoeba</i>	2 (8 %)	0.070
<i>Naegleria fowleri</i>	1 (4 %)	
<i>Entamoeba histolytica</i>	1 (4 %)	
<i>Cryptosporidium spp</i>	1 (4 %)	
<i>Entamoeba coli</i>	1 (4 %)	
<i>Giardia lamblia</i>	1 (4 %)	
<i>Ascaris lumbricoides</i>	0	
<i>Enterobius vermicularis</i>	0	
Total	7 (28 %)	

Table 4: Number and Percentage of contaminated water collected from small rivers canals.

Small rivers canals water samples Examined (No=40)		
Species of parasite	No. of Positive & %	P value
<i>Enterobius vermicularis</i>	4 (10 %)	0.003
<i>Giardia lamblia</i>	8 (20 %)	
<i>Entamoeba histolytica</i>	6 (15 %)	
<i>Ascaris lumbricoides</i>	4 (10 %)	
<i>Cryptosporidium spp.</i>	4 (10 %)	
<i>Entamoeba coli</i>	3 (7.5 %)	
<i>Naegleria fowleri</i>	2 (5 %)	
<i>Acanthamoeba</i>	2 (5 %)	
Total	33 (82.5 %)	

Table 5: Number and Percentage of contaminated water collected from Ponds.

Ponds water Samples Examined (No=35)		
Species of parasite	Rate of Positive (%)	P value
<i>Entamoeba coli</i>	4 (11.4 %)	0.021
<i>Giardia lamblia</i>	9 (25.7 %)	
<i>Entamoeba histolytica</i>	6 (17.1 %)	
<i>Enterobius vermicularis</i>	1 (2.9 %)	
<i>Ascaris lumbricoides</i>	3 (8.6 %)	
<i>Cryptosporidium</i>	2 (5.7 %)	
<i>Naegleria fowleri</i>	2 (5, 7 %)	
<i>Acanthamoeba</i>	1 (2.9 %)	
Total	28 (80 %)	

Table 6: Number and Percentage of contaminated water collected from Sewage water.

Sewage Water Samples Examined (No=45)		
Species of parasite	Rate of Positive & %	P value
<i>Entamoeba coli</i>	4 (2.2 %)	0.001
<i>Giardia lamblia</i>	10 (22.2 %)	
<i>Enterobius vermicularis</i>	5 (11.1 %)	
<i>Ascaris lumbricoides</i>	7 (15.6 %)	
<i>Entamoeba histolytica</i>	13 (28.8 %)	
<i>Cryptosporidium spp.</i>	2 (4.4 %)	
<i>Naegleria fowleri</i>	0	
<i>Acanthamoeba</i>	0	
Total	41 (91.1%)	

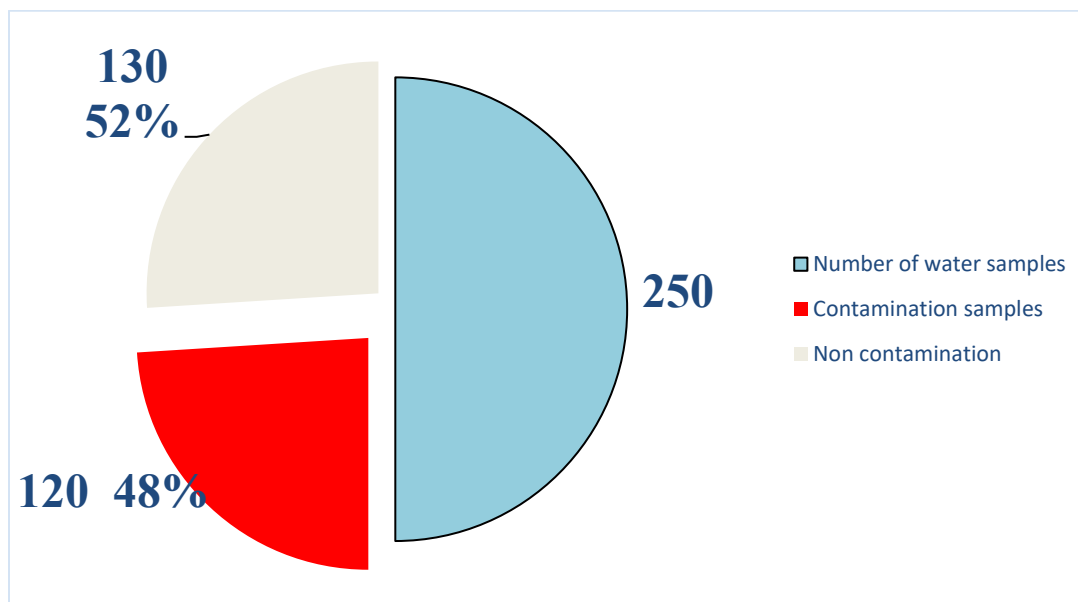


Figure 2: Total number and percentages of contaminated water samples in Amedi district.

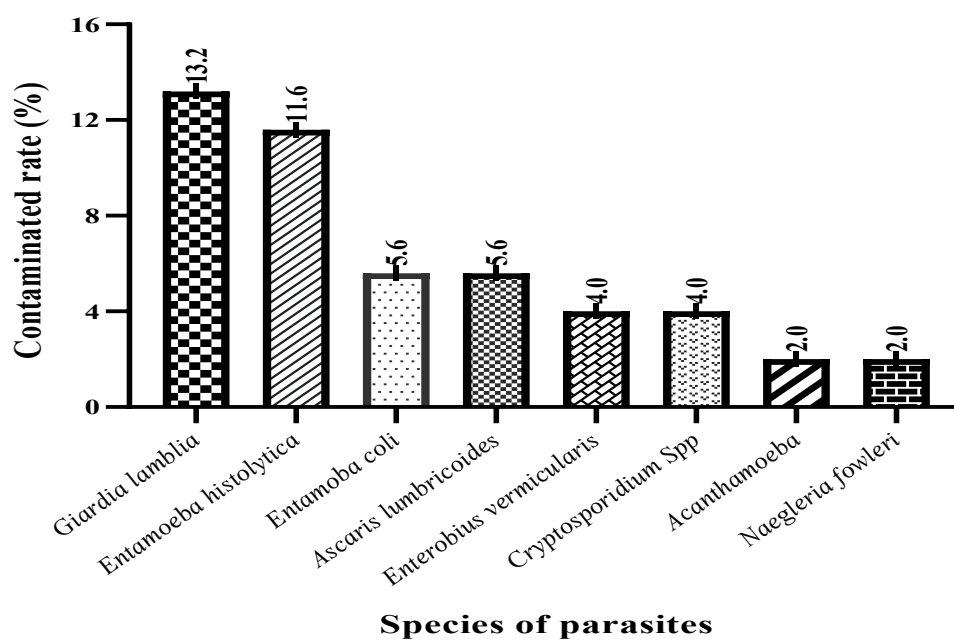


Figure 3: Contaminated rate of examined water with different parasitic Species in Amedi district.

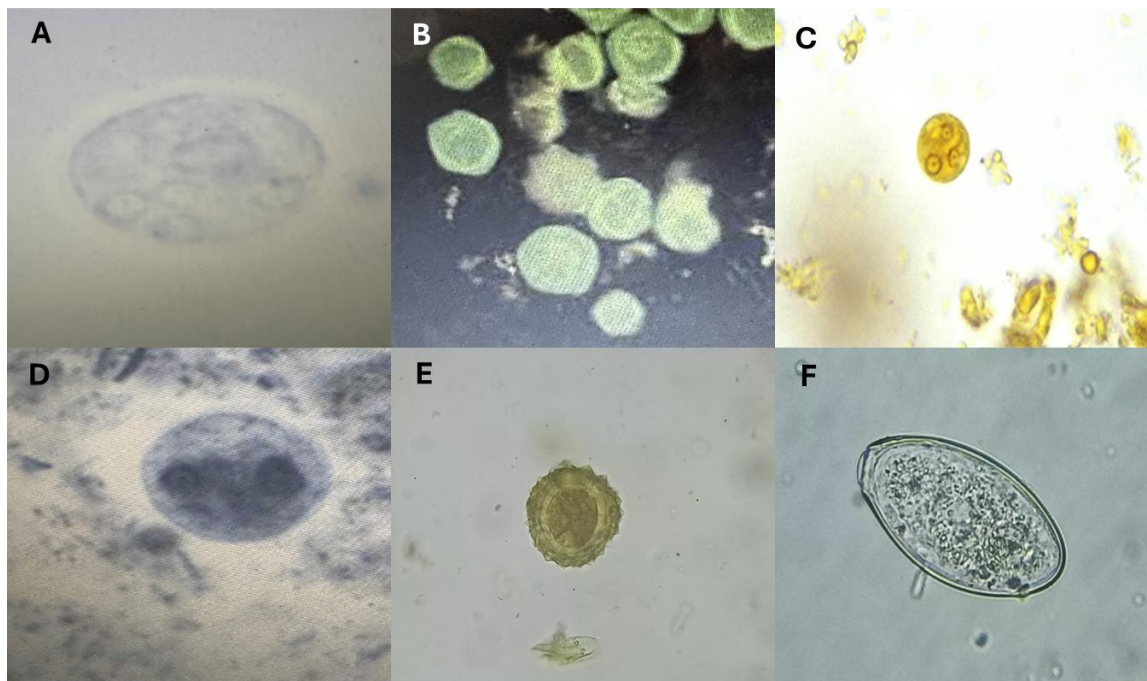


Figure 4: (A) Trophozoite of *Acanthamoeba* spp.; (B) Cyst of *Acanthamoeba* spp.; (C) Cyst of *Entamoeba histolytica*; (D) Cyst of *Giardia lamblia*; (E) Egg of *Ascaris lumbricoides*; (F) Egg of *Enterobius vermicularis* (40×).

4. Discussion

This study represents the first effort to detect parasitic contamination in water sources within the Amedi district. Unlike many previous investigations, which often focused on one or two water sources, this research examined a broad spectrum of sources, including household water tanks, springs, ponds, rivers, sewage water, and project water. This comprehensive approach highlights the utility and reliability of parasitological diagnosis in water samples, particularly in regions where waterborne diseases pose significant public health risks. The findings indicate that contamination rates varied depending on the water source. Among household water tanks, the contamination rate was 13.8%, with *Giardia lamblia* (11.4%) being the most prevalent parasite, followed by *Entamoeba histolytica*. These results align with (AL-Kubaisy et al. (2014), who reported a high prevalence of *Giardia lamblia* (45.54%) in Baghdad, followed by *E. histolytica* and other intestinal parasites. Similarly, (Karem and Khlaif (2016) observed a significant prevalence of *Giardia lamblia* and *E. histolytica* in Iraqi children with diarrhea, while Hussein (khudair Hussein (2010) identified a 33.3% infection rate of *Giardia lamblia* in Thi-Qar children, correlating it with risk

factors such as poor water quality and overcrowded living conditions.

Water sources such as rivers, ponds, and sewage showed considerably higher contamination rates (82.5%, 80%, and 91.1%, respectively), with *Giardia lamblia* (20%, 25.7%, and 22.2%) and *E. histolytica* (15%, 17.1%, and 28.8%) being the most frequently detected parasites. These findings deviate from those of Hadi and Faraj (2008), who reported a lower contamination rate (60%) in sewage water in Baghdad, and Jarallah Jarallah (2016), who found a 36.4% contamination rate in Basrah rivers. The higher rates observed in this study could be attributed to factors such as river size, proximity to human and animal activities, and inadequate sewage management in Amedi. Additionally, *Cryptosporidium* spp. were detected at a rate of 4%, while *Naegleria fowleri* and *Acanthamoeba* were each found at 2%. These findings are consistent with Baqer et al. (2018), who reported similar parasites in Baghdad's water sources. Variations in contamination rates between studies might stem from differences in environmental conditions, water sampling methods, and geographic factors. For instance, Koloren and Ayaz (2016) in Turkey reported significantly higher contamination rates with

Cryptosporidium spp., while Rafiei et al. (2014) found a 50% prevalence of *Entamoeba* and a 27.27% prevalence of *Cryptosporidium* in Iranian water samples. Contamination 28% was also notable in springs, likely due to the presence of stray animals, uncovered springs, and human activities. These factors underline the need for targeted measures to protect water sources. Seasonal variation in parasitic activity, particularly for free-living amoebae such as *Acanthamoeba* and *Naegleria*, as well as challenges in isolating and detecting parasites from complex water matrices, further complicate efforts to standardize findings across studies.

5. Conclusion

The study's findings underscore the importance of regular water quality monitoring and control measures to mitigate parasitic contamination. Recommendations include periodic screening of water sources, repairing cracks in water pipelines, improving sewage systems, and separating drinking water sources from animal activities. Additionally, improving water sanitation infrastructure in contaminated areas is crucial to reducing the risk of waterborne infections. Regular parasitological screening of both municipal and non-municipal water sources should be implemented to ensure early detection and intervention. Public awareness campaigns on waterborne parasitic diseases can further enhance community understanding of hygiene practices and safe water consumption. By addressing these factors, it is possible to reduce the risk of waterborne parasitic infections and improve public health outcomes in the Amedi district and similar regions.

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