

# Hygiene Practices and Investigation of Waterborne Parasites in Private Underground Tanks in Rural Areas of Tunisia <sup>†</sup>

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**Abstract:** Private cisterns or underground tanks may be at risk for certain types of contamination associated with adverse health effects. This first and preliminary study evaluated the parasitological quality of drinking water stored in home-based cisterns in rural areas in seven delegations of the Kairouan Governorate in the center of Tunisia. The practices of water management and hygiene were also surveyed in addition to details related to the age and the kind of material used to make these home-based tanks. To achieve this purpose, 39 samples from five different sources—(i) rain water, (ii) truck cisterns, (iii) a mix of these two, (iv) wells, and (v) stored tap water—were collected and examined for the presence of parasites using the Bailenger modified technique. Microscopic/morphological analysis showed the presence of three gastrointestinal protozoa: *Giardia duodenalis* (10–380 cysts/L), *Entamoeba histolytica/dispar/moshkovskii* (30–400 cysts/L), and *Entamoeba coli* (20–200 cysts/L). These protozoa were prevalent in all the five water sources examined. One species of helminths was also detected (*Ascaris* spp.). The high concentrations of the protozoa cysts found could be correlated with the lack of hygiene, sanitation practices, and education. No statistical correlations were demonstrated with the age or kind of water. Thus, the current situation highlights the need for the frequent monitoring of water microbiological quality in these rural areas. Further studies are needed to determine the true prevalence and pathogenic potential of these and other pathogenic species.

**Keywords:** cisterns; drinking water quality; enteric protozoa; rural areas; Tunisia

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## 1. Introduction

Access to safe drinking water and adequate sanitation services assure the health and the economic development of countries. Improved water, sanitation, and hygiene could prevent the deaths of more than two million children under the age of five annually. Moreover, the African and South-East Asia regions account for 78% (1.46 million) of all deaths due to diarrhea in developing countries (Boshi-Pinto et al., 2008 [1]).

Parasites are identified as the second most frequent etiological cause of mortality among children under five years old. Globally, they are responsible for 1.7 billion cases of diarrhea, which

leads to 842,000 deaths per year (Baldursson and Karanis, 2011 [2]; Efstratiou et al., 2017 [3]). Moreover, they are of public health interest, as they possess a protective wall that ensures them resistance to hostile conditions. They are transmitted by the oro-fecal routes and through zoonotic transmission. They can cause severe gastrointestinal illnesses. They also have been associated with stunted growth and malnutrition in children (Chekley et al., 1998 [4]). They can also affect the growth and the survival of neonatal calves and milk production (Olson et al., 2004 [5]).

In order to meet the basic needs of safe drinking water with the faced growing scarcity in rural areas, the Tunisian government is encouraging the storage of water in adequate home-based systems. However, the main constraint for the stored water is its microbiological quality. The owners of the private underground tanks are responsible for ensuring that their water supply is safe through performing testing, maintenance, and treatment (Colley et al., 2019 [6]). Water testing is the primary method by which cistern owners can identify potential contaminants, such as parasites, in their drinking water.

The investigation of parasitic contamination is of public health concern in Tunisia, because there is a lack of a well-established functional system that reports outbreaks and also a significant shortage of studies examining microbiological contamination in home-based water cisterns. Thus, it is necessary to take many measures, such as the identification of these microorganisms, to improve the quality and hygiene of water resources in Tunisia and other developing countries.

The objectives of this work were: (i) the evaluation of the parasitological quality of water stored in private cisterns, and (ii) the documentation of the normal practices of storage and use, thus providing a good quality of drinking water in the rural communities of Kairouan, Tunisia.

## 2. Material and Methods

This research was carried out in rural areas of Kairouan governorate in the center of Tunisia, which covers an area of 6712 km<sup>2</sup> (4.1% of the whole country).

In order to achieve the objectives of this study, these actions were undertaken: (i) the geo-localization of homes using cisterns for drinking water in that governorate; (ii) the parasitological analysis with the modified Bailenger technique (WHO, 1989); and (iii) the elaboration of surveys and the observations of water storage practices and management.

Among the 150 houses investigated in total, 39 representative samples of private cisterns were rigorously selected and were given a code to preserve the household identities.

In order to report the hygiene practices of water management and get information about the underground tanks, surveys and observations were undertaken. The collected information concerned: (i) the age and the construction material of the cisterns, (ii) their capacity (m<sup>3</sup>), (iii) the origin and the method of filling of the stored water, (iv) the method and frequency of cleaning, (v) relevant observations of hygiene behavior and water storage, (vi) the type of cistern cover, (vii) the instrument used for water collection, and (viii) the method used for water serving.

The 39 selected cisterns were filled using water from five different sources: (i) 13 (33.3%) from roof harvested rainwater; (ii) 16 (41%) from truck metallic cisterns; (iii) 4 (10.25%) with a mixture of roof rainwater with water from a truck's cistern, tap water, or well water; (iv) 5 (12.25%) from tap water; and (v) 1 from well water.

### 2.1. Water Sampling and Parasites Investigation

For parasites investigation, 5 L of water was sampled with the same bucket used by households for serving water.

The samples were examined for parasitic presence according to the modified Bailenger technique (WHO, 1989 [7]; Ben Ayed et al., 2018 [8]).

### 2.2. Statistical Analysis

A statistical package (SPSS 20) was used for the data analysis of water results. Categorical variables were compared using the chi square test;  $p \leq 0.05$  was considered statistically significant.

### 3. Results

#### 3.1. Hygiene Practices

In all the investigated houses, domestic animals were living in the houses. Moreover, it was observed that the bucket used for serving water was always kept outside and directly on the floor. All the investigated householders did not wash their hands when handling water, and they did not apply any treatment or disinfection process to preserve the stored water quality. Incidentally, the households had a low hygienic and educational level.

#### 3.2. Investigation of Parasitic Contamination

In the investigated sites, 97% of the drinking water samples were contaminated, and only one house (Sample 7) contained suitable drinking water in its cistern or underground tank.

Three protozoa were detected, one belonging to flagellated protozoan (*Giardia* spp.); this was the most prevalent parasite detected (92%). There were two amoeba species (*Entamoeba coli* and *Entamoeba histolytica/dispar/moshkovskii*), and one nematode was detected (*Ascaris* spp.) in two samples. According to statistical analysis, their presence was not correlated with any of the parameters mentioned above in Section 2—i.e., the cistern age and capacity, the construction material, the type of water supplied, etc.

The parasitic contamination was studied for each of the 39 methods used to fill the cisterns (see Section 2 above).

##### 3.2.1. Roof Harvested Rainwater

The 13 samples were contaminated by protozoa, and only one sample (sample 31) showed the presence of *Ascaris* spp. (25 eggs /L). Their concentrations ranged from 25 to 300 cysts/L for *Giardia* spp., 25 to 380 cysts/L for *E. histolytica/dispar/moshkovskii*, and 25 to 180 cysts/L for *E. coli*, as summarized in Figure 1.

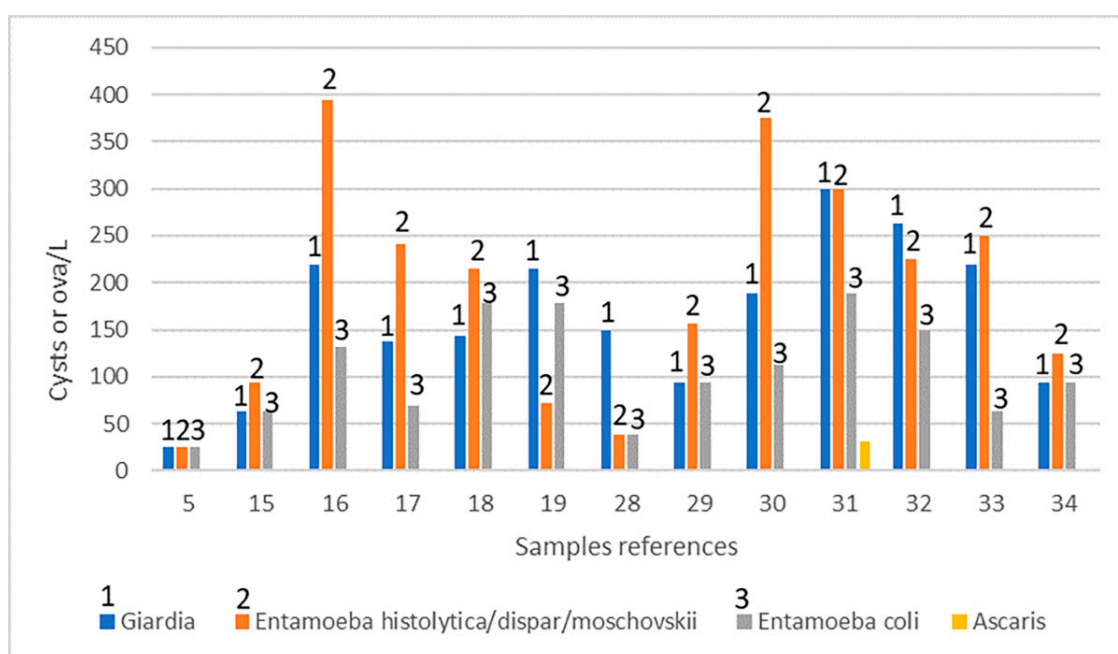


Figure 1. Parasitic concentrations in the 13 roof-harvested rainwater samples.

##### 3.2.2. Cistern's Truck Water Quality

Among the 16 investigated samples, only one (sample 7) was not contaminated. The overall contamination rate of this kind of water was approximately 94%.

For this category, *Giardia* spp., when present, was prevalent with concentrations varying between 25 and 393 cysts/L, *E. histolytica/dispar/moshkovskii* was present with 25 and 219 cysts/L, and *E. coli* ranged between 25 and 250 cysts/L (Figure 2). The highest concentrations were reported, respectively, in samples 13, 2, and 24.

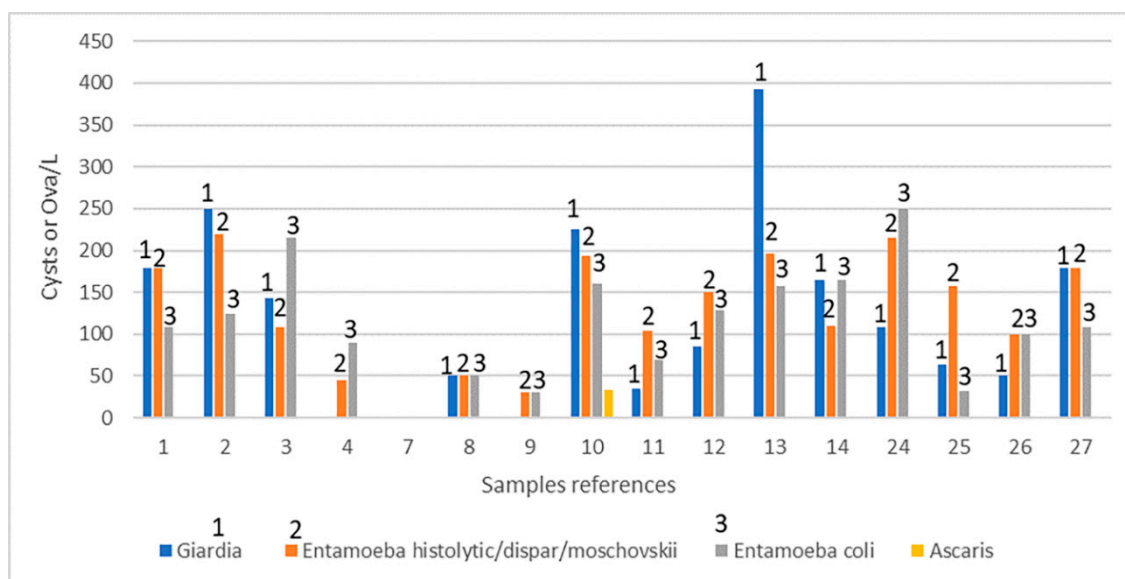


Figure 2. Parasitic concentrations in the 16 households using truck cistern water.

### 3.2.3. Water Mixture Quality

The four water cisterns filled with a mixture of water from different sources were only contaminated with protozoa. The highest concentrations reported were 75 cysts/L for *Giardia* spp. (sample 36), 143 cysts/L for *E. histolytica/dispar/moshkovskii* (sample 20), and 72 cysts/L for *E. coli* (sample 35), as summarized in Figure 3.

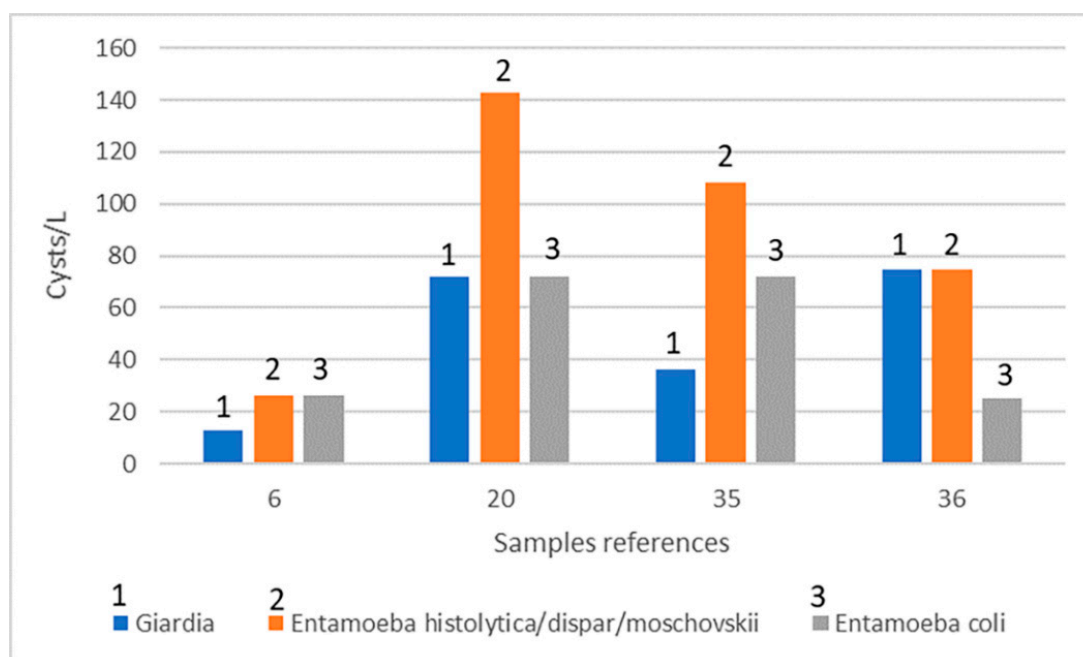


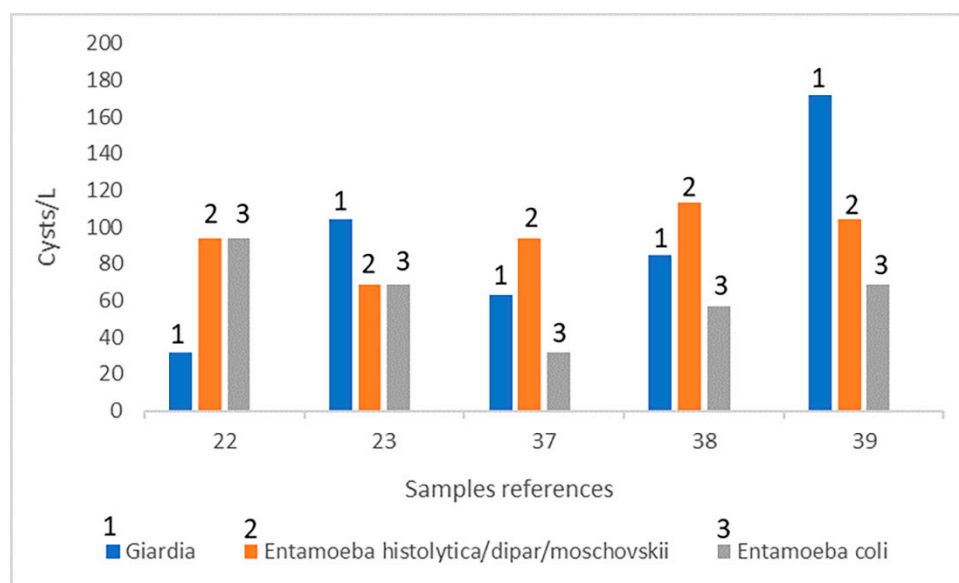
Figure 3. Parasitic concentrations in the 4 household cisterns using a mixture of water.

### 3.2.4. Well Water

In one cistern filled with well water (sample 21), only the three protozoa as above were detected. The concentration found was similar for all three—i.e., 57 cysts/L.

### 3.2.5. Stored Tap Water Quality

Tap water is available for some rural homes but not continuously, and therefore householders store it, when available, in their cisterns (Figure 4). *Ascaris* spp. was not detected in this kind of water, whereas the three protozoa were present in all the five samples, with concentrations ranging from 32 to 172 cysts/L for *Giardia* spp., 69 to 113 cysts/L for *E. histolytica/dispar/moshkovskii*, and 32 to 94 cysts/L for *E. coli* (Figure 4).



**Figure 4.** Parasitic concentrations in the 5 stored tap water samples.

## 4. Discussion

The unavailability of drinking water can lead to serious physical and mental discomfort arising from water shortage and waterborne diseases, which represent a major health burden, with an estimated 842,000 annual deaths linked to diarrhea alone (Chalchisa et al., 2017 [9]).

Water quality studies should be regularly performed in order to assess the risks to human health. However, this is difficult due to the lack of data (demographic statistics, incidence of waterborne diseases, etc.), particularly in rural communities. In this context, the Tunisian government is encouraging the construction of cisterns or underground tanks to store water in rural areas, which would also be useful in periods of prolonged drought (Ben Ayed et al., 2018 [8]).

To the authors' knowledge, this study represents the first investigative report of the quality of drinking water stored in cisterns in rural areas in Tunisia, and it is also the first assessment of water use practices.

The parasitological analysis showed that, out of the 39 water samples collected from the rural household cisterns of the Kairouan Governorate, 97% were contaminated and only one house presented suitable drinking water; this points towards problems of water management, lack of hygiene, and possible fecal contamination from domestic animals and human excreta.

The underground tank or cistern capacity did not influence the parasitic distribution, as no differences were reported between low- and high-capacity cisterns ( $p < 0.05$ ). The age of cisterns and manual or mechanical water management did not influence the level of contamination by protozoa and helminths. No significant implications on parasitic distribution, related to the type of water stored and the construction materials, were related with the chi square tests, as observed in Bishop and Inabo (2015) [10].

## 5. Conclusions

The results of this first assessment of water quality stored in underground tanks highlights the necessity of preventive measures, such as the periodic monitoring and improvement of the behavior and health education of rural communities.

The obtained results of this first report reflect the complexity of the interactions of several factors involved in oro-fecal and waterborne disease transmission. We recommend that considerable attention must be accorded to water management practices in rural households in Tunisia.

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## References

1. Boschi-Pinto, C.; Velebit, L.; Shibuyac, K. Estimating child mortality due to diarrhoea in developing countries. *Bull. World Health Organ.* **2008**, *86*, 710–717, doi:10.2471/BLT.07.050054.
2. Baldursson, S.; Karanis, P. Waterborne transmission of protozoan parasites: Review of worldwide outbreaks—An update 2004–2010. *Water Res.* **2011**, *45*, 6603–6614, doi:10.1016/j.watres.2011.10.013.
3. Efstratiou, A.; Ongerth, J.E.; Karanis, P. Waterborne transmission of protozoan parasites: Review of worldwide outbreaks—An update 2011–2016. *Water Res.* **2017**, *114*, 14–22, doi:10.1016/j.watres.2017.01.036.
4. Checkley, W.; Epstein, L.D.; Gilman, R.H.; Black, R.E.; Cabrera, L.; Sterling, C.R. Effects of *Cryptosporidium parvum* infection in Peruvian children: Growth faltering and subsequent catch-up growth. *Am. J. Epidemiol.* **1998**, *148*, 497–506, doi:10.1093/oxfordjournals.aje.a009675.
5. Oslon, M.; O’Handley, R.M.; Ralston, B.; Mcallister, T.A.; Thompson, R.C.A. Update on *Cryptosporidium* and *Giardia* infections in cattle. *Trends Parasitol.* **2004**, *20*, 185–191, doi:10.1016/j.pt.2004.01.015.
6. Colley, S.K.; Kane, P.K.M.; McDonalds Gibson, J. Risk Communication and factors influencing private well testing behavior: A systematic scoping review. *Int. J. Environ. Res. Public Health* **2019**, *16*, 1–23, doi:10.3390/ijerph16224333.
7. World Health Organization (WHO). *Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture*; WHO Technical Report Series, No. 778; WHO: Geneva, Switzerland, 1989.
8. Ben Ayed, L.; Belhassen, K.; Sabbahi, S.; Karanis, P.; Nouiri, I. Assessment of the parasitological quality of water stored in private cisterns in rural areas of Tunisia. *J. Water Health* **2018**, *16*, 737–749, doi:10.2166/wh.2018.117.
9. Chalchisa, D.; Megersa, M.; Beyene, A. Assessment of the quality of drinking water in storage tanks and its implication on the safety of urban water supply in developing countries. *Environ. Syst. Res.* **2017**, *6*, 1–6, doi:10.1186/s40068-017-0089-2.
10. Bishop, H.G.; Inabo, H.I. Incidence of *Entamoeba histolytica* in well water in Samaru-Zaria, Nigeria. *Int. J. Sci. Res. Environ. Sci.* **2015**, *3*, 0016–0022, doi:10.12983/ijres-2015-p0016-0022.



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