Assessment of the parasitological quality of water stored in private cisterns in rural areas of Tunisia

Layla Ben Ayed, Khaoula Belhassen, Sonia Sabbahi, Panagiotis Karanis and Issam Nouiri

ABSTRACT

Limited access to safe water enhances poverty, hunger and diseases. This study evaluated the drinking water quality stored in home-based systems, located in rural areas of Tunisia. Water management was also documented as an improvement of good practice as most of the water contamination could be related to behavioral patterns. Thirty-nine water samples from five different sources (rainwater, truck cistern, mix, etc.) were screened for enteric parasitic contamination with the modified Bailenger technique. This technique allowed the detection of three protozoa: *Giardia* spp.; *Entamoeba histolytica/dispar/moshkovskii; Entamoeba coli* and one helminth specie: *Ascaris* spp. The overall prevalence of these intestinal parasites was approximately 97% with a relatively high frequency of protozoa over helminths and more specifically *E. histolytica/dispar/moshkovskii*. This incidence could be correlated to the lack of hygiene practices, sanitation and education. This situation requires the need for frequent monitoring of the water quality and management in these areas.

Key words | cisterns, parasites, rural areas, Tunisia, water quality

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INTRODUCTION

Access to safe drinking water is compulsory to assure the required health conditions and the economic development of countries. This evidence has enhanced the worldwide investment in water systems construction and management to meet stringent water quality standards (Trevett *et al.* 2004). Of the six billion people in the world, more than one billion are lacking access to safe drinking water and about 2.5 billion do not have adequate sanitation services. These two shortcomings spawn waterborne diseases, leading to approximately 1.8 million deaths per year, among or within nations, located mainly in Latin America, Asia and Africa (Trevett *et al.* 2004; Xavier *et al.* 2011). In fact, Africa recorded the highest burden disease, where almost a quarter of the sub-Saharan people are lacking these commodities. This situation leads to an important prevalence of child mortality, particularly those less than five years of age, by infectious diarrhea, the fourth largest child killer in that continent (Squire & Ryan 2017). In North Africa, Tunisia, has given priority to the importance of sanitation, water management and supply by developing strategies to satisfy the increasing needs of irrigation, drinking water, tourism and industry sectors. Most of the Tunisian urban water for human consumption is tap water issued from surface or ground water and treated with advanced techniques. However, in rural areas, good quality tap water is not always easy to access. The rural community water system often consists of public or private wells, stand pipes, or rain water. One of the most important and actual challenges is to meet the basic needs of safe drinking water by finding alternatives to ease its access with the faced growing scarcity. Thus, storing

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water in adequate home-based systems is being encouraged by the Tunisian government. This water is either from rain as source or bought from water suppliers. During a period of prolonged drought this water becomes an important commodity for everyday use and survival. However, the main constraint for the stored water is its microbiological quality, which could be deteriorated as it is closely related to: (i) the system collection (pipes, gutters, unclean containers, etc.), (ii) the original water-source quality, (iii) the transport conditions, (iv) the unhygienic and/or inadequately domestic water-handling practices (open, uncovered or partially covered cisterns, children playing with the household water container, etc.) (Tambekar *et al.* 2008).

In developing and developed countries, waterborne pathogens, such as bacteria, viruses, parasites (helminths and protozoa), are frequently associated with a high prevalence of morbidity and/or mortality, particularly in children. Their spread is closely related to the sanitary, environmental, educational conditions, socio-economic status and the absence of safe drinking water supplies.

Parasites are of public health interest as they are characterized by an important persistence to hostile conditions, acquired thanks to their protective walls. Their transmissible stages can survive for long periods outside of the host in a moist environment and all water sources (lakes, surface water, wells, tap water, dam, etc.). This reflects the increasing health threat of populations using these water bodies for drinking, recreational and agriculture purposes. *Giardia* cysts could survive in water for up to four months at temperatures lower than 8 °C, while *Entamoeba histolytica* could survive up to one year in water and sludge at temperatures lower than 4 °C (Ben Ayed & Sabbahi 2017). They are transmitted through fecal, oral, zoonotic and human to human pathways, with food and water the most common transmission routes (Efstratiou *et al.* 2017).

Giardia, *Cryptosporidium* and *Entamoeba histolytica* represent the etiological agents for more than 900 waterborne outbreaks of epidemic and endemic human diseases (Karanis *et al.* 2007; Efstratiou *et al.* 2017). Even if the presence of parasites is correlated with poor hygiene behaviors, the majority of the outbreaks have been reported in industrialized countries (Aldeyarbi *et al.* 2016; Efstratiou *et al.* 2017). In Tunisia, waterborne or foodborne outbreaks of giardiasis, amoebiasis or cryptoporidiosis have not been reported yet, but their presence has been reported in epidemiological data. In the Tunis region, the prevalence of *Giardia duodenalis* and *Cryptosporidium* spp. in diarrheic persons was found to be 46.2 and 8% respectively (Bouratbine *et al.* 2000). *Cryptosporidium* spp. prevalence was significantly higher in immunocompromised than in immunocompetent children without any differences between rural or urban communities (Essid *et al.* 2008). The prevalence of helminth infections was estimated to be 0.03% for *Ascaris lumbricoides* and 10.3% for *Enterobius vermicularis* (Chaker *et al.* 1995). There is no functional surveillance system to report waterborne disease outbreaks in Tunisia in comparison with well-established reporting systems in European countries and only indirect and limited prevalence data, reporting incidence, together with the level of contamination may be used as risk predictors.

The parasites usually cause acute gastrointestinal disorders, chronic diseases and may contribute to poor growth in children. *Giardia duodenalis* and *Cryptosporidium* species may also adversely affect young livestock, with negative impact in their rates of growth and production. Infections by these parasites can induce nausea, abdominal pain, watery diarrhea, cramps and weight loss.

All these issues subsequently give rise to an urgent need for evaluating the microbiological quality of drinking water stored in such systems to assure the user's health, safety and protection.

The objectives of the present study were: (a) first, to evaluate the parasitological quality of the water stored in cisterns or underground tanks and used as a unique source for drinking water in rural areas of Kairouan in Tunisia; (b) second, to document the normal practices of storage and use, thus providing directive guidelines in Tunisia for improving drinking water quality in such communities.

MATERIALS AND METHODS

Study sites

This research was carried out in rural areas of Kairouan governorate, which is located in the center of Tunisia and covers an area of 6,712 km², the equivalent of 4.1% of the whole country (Figure 1). This governorate was selected due to the relatively high presence of poor populations and the precarious state of the water supply and sanitation infrastructure.

In order to achieve the objectives of this study, the main activities undertaken in rural areas of this governorate were: (i) the geo-localization of homes using cisterns for drinking water or for other usages (cleaning, cooking, bathing, etc.) in order to determine their numbers and distribution as this information is lacking from local responsible authorities, (ii) the elaboration of surveys and observations of the cisterns in order to report the water storage practices and management, and (iii) the water analysis for parasitological contamination with the modified Bailenger technique (WHO 1989).

In total, 150 houses were investigated for these purposes. They were located throughout the seven-governorate's delegation: Sbikha, El Oueslatia, El Alaa, Haffouz, Hajeb et Ayoun, Echrarda and Nasrallah as summarized in Figure 1.

Among them, 39 representative samples of private cisterns were rigorously selected and were attributed a code to preserve the household identities. All these 39 selected households are using the water, stored in their private cisterns, exclusively for drinking water.

Surveys and observations

On all the 39 households selected, the following information was collected: (1) the date of the construction of the cisterns in order to determine their age (years), (2) their internal construction material, (3) their capacity (m^3), (4) the origin and method of filling of the stored water (rain, suppliers, mix of both, etc.), (5) the method and frequency of cleaning, (6) the presence and type of the cistern's cover, (7) the type and preservation of the instrument used for water collection, (8) relevant observations of hygiene behavior and water storage (e.g. hand washing, presence of animals in the house, etc.),

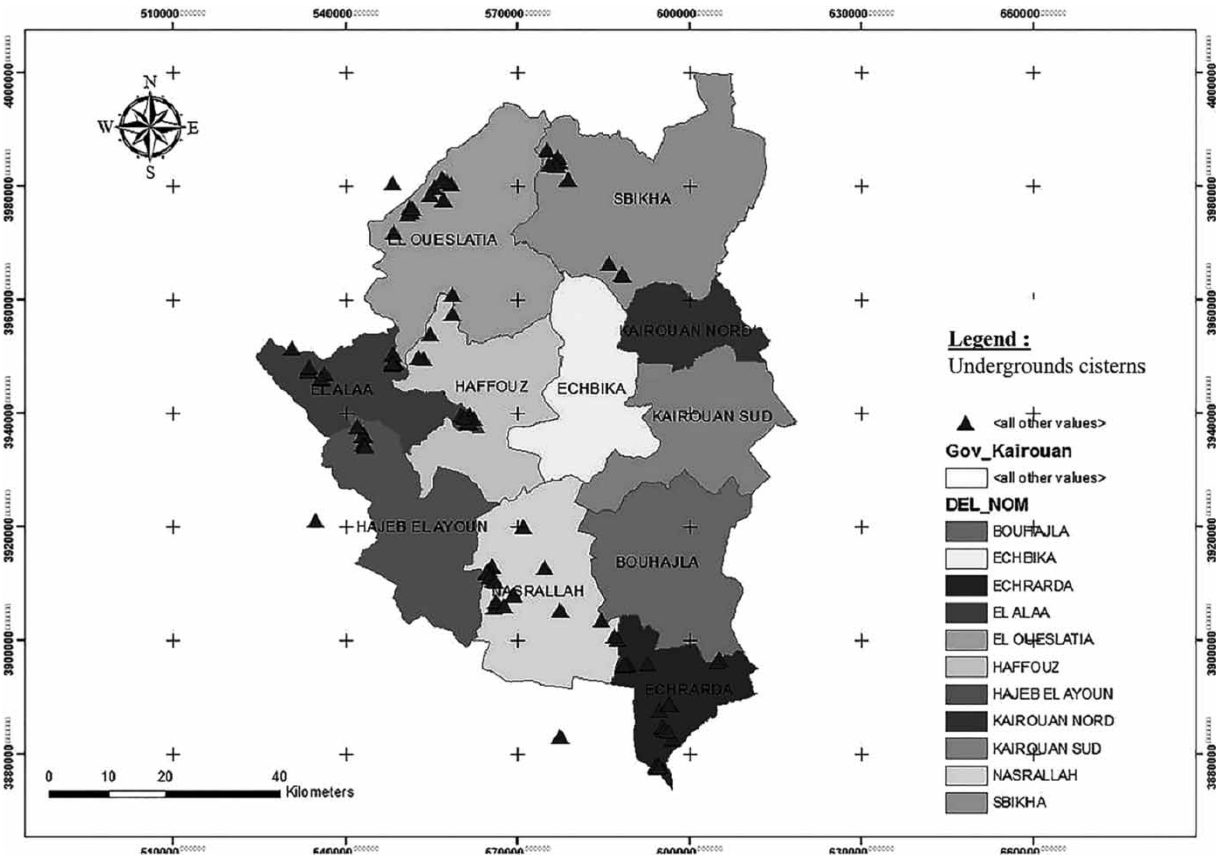

Figure 1 | Map of the geographic location of the 150 cisterns and underground tanks investigated in the seven delegations of Kairouan, in the central part of Tunisia.

(9) the respect of a construction plan enacted by the Regional Commissariat for Agricultural Development, (10) the method used for water serving (manually or pumping) and (11) the user's satisfaction of the water quality stored (Table 1).

According to Table 1, three categories were formed regarding the cistern's capacity, which is closely related to the financial household resources. The first one is a cluster of houses with a cistern's capacity not exceeding 10 m^3. The second category, composed of 16 representative samples, is concerning houses with a cistern's capacity ranging between 12 and 27 m^3. The last one is a cluster of homes with cistern's capacity higher than 30 m^3.

All the investigated cisterns were private except the cistern referenced 22, which is a collective one. Any of them did respect the specificities of the plan enacted by the Regional Commissariat for Agricultural Development for their construction.

These 39 selected cisterns were filled by five ways, as follows. (1) From the roof harvested rainwater. (2) From truck's metallic cisterns that fills directly into the home-based cisterns. The water is not placed in any intermediate containers. These kinds of cisterns are not connected to the roof. (3) Mixture of roof rainwater and truck cisterns or rainwater with tap water or rainwater with well water. In this case, householders do not have any idea about the right proportion of each source. (4) From tap water, as it is not continuously provided in rural areas. It is filled into the cisterns for storage when available. (5) From well water.

Considering all the cisterns capacities, the water samples analyzed were as follow: 16 bought from suppliers and filled directly in the home based cisterns (41%), 13 roof harvested rainwater (impluvium) (33.3%), four water mixed samples (10.25%), five issued from the tap and stored in the underground tanks (12.8%) and only one sample was filled from well water.

For water collection, the selected sites were using a plastic bucket of approximately 25 L capacity, not well preserved, old, always kept outside the cisterns and placed directly on the floor. All the cisterns surveyed were protected with a metallic cover, which was rusted and non-hermetic.

Water sampling

Five liters per sample were collected from the 39 selected private cisterns or tanks with the same utensil or bucket used by households for serving water. This volume was defined by the WHO (1989) and Ben Ayed et al. (2009) as representative for parasitic identification from environmental samples.

To avoid disrupting the householders with further visits to their home, only one sample was collected from March to April 2017, after the rainy season.

For this investigation, only a parasitic detection analysis was performed. No bacteriological analysis was undertaken due to the absence of necessary and required equipment.

Quantitative detection of enteric parasites

Upon arrival in the laboratory, the water samples were left to settle undisturbed for 24 h at room temperature without addition of any reagents. The supernatant was then removed and the sediment was used for the microscopy analysis.

Nsom Zamo et al. (2003) noticed after testing many techniques, namely Teleman-Rivas, Willis, Janisco-Urbanyi and Bailenger, that the latter method was the most efficient. Thus, samples were examined for parasites according to the modified Bailenger method (WHO 1989). Briefly, the sediment was centrifuged at 1,000 × g for 15 min. After that, the pellet was suspended in an equal volume of acetoacetic buffer, pH 4.5 (15 g sodium acetate anhydrous 99%, 3.6 mL acetic acid) (LOBA Chemie, India and Chem-Lab NV, Belgium, respectively). This pH was considered as the most favorable to concentrate parasites. Then, two volumes of ether (Biotechnica, Tunisia) were added and the sample was mixed for 10 min. The sample was then centrifuged at 1,000 × g for 15 min (Jouan E82, France). The pellet was resuspended in five volumes of zinc sulfate solution (AnalaR NORMAPUR, Prolabo, Belgium) with a gravity of 1.3 (density 33%) and mixed thoroughly. This density was considered as adequate, leading to a good purification of parasites. Quickly, 50 µl were transferred to a slide for microscopic counting (magnification of ×400) by light microscopy (Olympus, China).

The number of ova or cysts per liter of wastewater was calculated using following the equation:

$$C = \frac{N.X}{P.V}$$

where C = the number of ova or cysts per liter of water; N = the number of ova or cysts counted under microscopic

Table 1 | Main characteristics raised of the 39 representative private cisterns categories selected

Category	Cisterns capacity (m^3)	Sampling code	Construction material	Age of the cistern (y)	Water source	Pumping type	Period between water collection and sampling (weeks)	User's satisfaction
1	C <=10	1	Stone	16	Water tanker	Manually	2–3	Yes
		2	Stone	8	Water tanker		2–3	No
		3	Stone	N.S	Water tanker		2–3	No
		4	Reinforced concrete	3	Water tanker		2–3	No
		5	Stone	N.S	Rain water		Use until finish	Yes
		6	Stone	7	Water mix (Rainwater + well water)			No
2	12 < =C<=27	7	Stone	5	Water tanker	Manually	2–3	Yes
		8	Stone	5	Water tanker	Manually	2–3	Yes
		9	Stone	47	Water tanker	Engine	2–3	No
		10	Stone	11	Water tanker	Manually	2–3	No
		11	Stone	4	Water tanker	Manually	2–3	No
		12	Stone	11	Water tanker	Manually	2–3	Yes
		13	Stone	12	Water tanker	Engine	2–3	No
		14	Reinforced concrete	4	Water tanker	Manually	2–3	Yes
		15	Reinforced concrete	1	Rain water	Manually	Use until finish	Yes
		16	Stone	11	Rain water	Manually	Use until finish	Yes
		17	Stone	13	Rain water	Manually	Use until finish	No
		18	Stone	6	Rain water	Manually	Use until finish	Yes
		19	Stone	11	Rain water	Manually	Use until finish	Yes
		20	Stone	5	Water mix (rainwater + water tanker)	Manually		No
		21	Stone	16	Wells	Manually		No
		22	Reinforced concrete	15	Stored tap water	Engine		Yes
		23	Stone		Stored tap water	Manually		Yes
3	C>=30	24	Stone	N.S	Water tanker	Manually	2–3	No
		25	Reinforced concrete	5	Water tanker	Engine	2–3	Yes
		26	Other	37	Water tanker	Manually	2–3	Yes
		27	Stone	N.S	Water tanker	Manually	2–3	No
		28	Reinforced concrete	1	Rain water	Manually	Use until finish	Yes
		29	Stone	N.S	Rain water	Manually	Use until finish	Yes
		30	Stone	32	Rain water	Manually	Use until finish	Yes
		31	Stone	2	Rain water	Manually	Use until finish	Yes

(*continued*)

Table 1 | continued

Category	Cisterns capacity (m^3)	Sampling code	Construction material	Age of the cistern (y)	Water source	Pumping type	Period between water collection and sampling (weeks)	User's satisfaction
		32	Other (Dried clay bricks)	47	Rain water	Manually	Use until finish	No
		33	Other (Dried clay bricks)	42	Rain water	Manually	Use until finish	Yes
		34	Stone	N.S	Rain water	Manually	Use until finish	Yes
		35	Stone	31	Water mix (rainwater + water tanker)	Manually		No
		36	Stone	11	Water mix (Rainwater + tap water)	Engine		Yes
		37	Stone	16	Stored tap water	Manually	Use until finish	No
		38	Reinforced concrete	7	Stored tap water	Engine	Use until finish	Yes
		39	Stone	15	Stored tap water	Manually	Use until finish	No

N.S. = not specified.

observation; X = the volume of the final product (mL); P = the volume placed on the slide (50 µl); and V = the original sample volume (5 L).

Statistical analysis

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

RESULTS

Surveys and observations of the hygienic practices

Questions were applied to the 39 selected households to report their practices regarding water storage, serving and handling. It was observed from the 12 raised questions that 100% of the households do not wash their hands when handling water and that they are not applying any treatment to preserve the quality of the water stored in their cisterns. The bucket used for water serving is not well preserved, is never washed and is put directly on the ground. Moreover, the covers of the wide-mouthed tanks were rusted and not hermetic. In all the rural households investigated, domestic animals were seen inside the houses (Table 2).

Prevalence and distribution of parasites

A preliminary investigation into the occurrence of parasites was conducted in order to estimate the potential risks for the rural population using water stored in underground tanks or cisterns for drinking water. In the rural households of Kairouan, the parasitological analysis showed that out of the 39 water samples collected and used exclusively for drinking water, 97% were contaminated and only one house contained suitable drinking water in its cistern.

The modified Bailenger technique (WHO 1989) applied to the 39 sampled waters allowed the detection of four parasites. Among them, three protozoa were detected and were represented by one flagellate (*Giardia* spp.), and two amoeba species (*Entamoeba histolytica/dispar/moschovskii* and *Entamoeba coli*). This technique also allowed the detection of one helminth represented by a nematode which is *Ascaris* spp. This latter parasite was found only in two samples (5%):

Table 2 | Reports of the hygienic status of the 39 investigated households

	Hygienic practices	Number of houses applying the practice
1	Hand washing before water use	0
2	Presence of a unique bucket for the water serving	0
3	There is only one person who serves water	0
4	Bucket wash before water use	0
5	Bucket wash after water use	0
6	Bucket preservation	0
7	Hermetic closing of the underground tank	0
8	Frequency of cleaning	0
9	Application of a disinfection treatment	0
10	Presence of domestic animals	39 (cats, dogs, chickens, livestock)
11	Filtration before water use	0
12	Presence of prophylaxis and drug treatment	0

one in water issued from a truck cistern (sample 10) and the second from roof harvested rain water (sample 31).

As the parasitic identification was based only on the morphological observation, the pathogenic amoeba *E. histolytica* will be called *E. histolytica/dispar/moshkovskii* (Ben Ayed & Sabbahi 2017).

The chi square tests applied showed that these four parasites presence were not correlated to any of the studied parameters, namely the low or high cistern capacity, the construction material (stone, reinforced concrete, etc.), the cistern age (old or new), the type of water supplied (manually or pumping) and the type of water stored (rain or from suppliers, etc.) with $p > 0.05$ for all the considered cases.

Giardia spp. was present in 92% of the samples investigated with a concentration ranging between 13 and 393 cysts/L (Figure 2(a)). The pathogenic (*E. histolytica/dispar/moschkovskii*) and the non-pathogenic amoeba (*E. coli*) were present in 97% of the samples with concentrations ranging respectively from 25–394 and 25–250 cysts/L (Figures 2(b) and 2(c)).

These three protozoa were present together with the same concentration in samples 5, 8 and 21 where they were detected respectively with 25, 50 and 57 cysts/L.

Quality of the ingested water stored in the underground tanks

In order to clearly investigate the parasitic contamination of the ingested water quality in the 39 investigated rural cisterns, each water tank was studied separately.

The water tanker

Sixteen investigated rural homes were filling their cisterns exclusively from the cistern's truck. Among them the four detected parasites were present, an exception was made for sample 7, where they were all absent (Figure 3). The

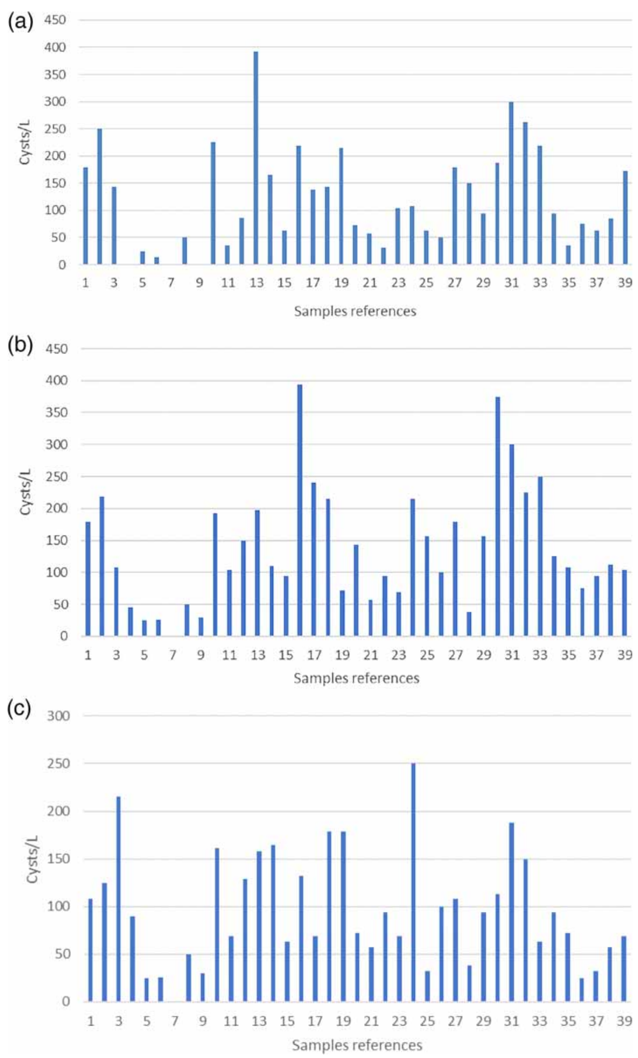

Figure 2 | Variation in concentrations in the 39 investigated water cisterns of: (a) *Giardia* spp.; (b) *Entamoeba histolytica/dispar/moshkovskii*; (c) *Entamoeba coli*.

contamination prevalence rate of the tanker water was approximately 94%.

Throughout this kind of water, three categories of the cistern's capacity were covered. The first four samples (1–4) are cisterns of category 1 with a capacity less than 10 m^3 that are extracting water manually from their underground tanks. The eight following (7–14) are cisterns of the second category (12–27 m^3). The last four are from cisterns with a capacity higher than 30 m^3.

Based on this criterion, the respective concentrations of *Giardia*, *Entamoeba histolytica/dispar/moshkovskii*, *E. coli* were ranging, for the first category, between 0–250, 45–219 and 90–215 cysts/L; while *Ascaris* spp. was absent. For the second category where the cistern's capacities vary between 12 and 27 m^3, the concentrations of the protozoa were ranging between 0–393, 0–197 and 0–165 cysts/L respectively for *Giardia* spp., *E. histolytica/dispar/moschovskii* and *E. coli*. Protozoa contamination of the last category was reported with concentrations ranging between 50–179, 100–215 and 32–250 cysts/L respectively for *Giardia* spp., *E. histolytica/dispar/moshkovskii* and *E. coli*. *Ascaris* spp. was only detected in this cistern's category with 33 ova/L.

Considering the capacity of all the cisterns, the highest concentrations of the protozoa were detected in sample 13 for *Giardia* spp. (393 cysts/L) where the water is electrically pumped, sample 2 for *E. histolytica/dispar/moschovskii* (219 cysts/L) and sample 24 for *E. coli* (250 cysts/L).

Water mixture

The four cisterns investigated and filled by water mixture were only infected by protozoa. The highest concentrations detected was 75 cysts/L for *Giardia* for sample 36; 143 cysts/L for *E. histolytica/dispar/moshkovskii* and 72 cysts/L for *E. coli* in the two samples 20 and 35 (Figure 4).

Among them, one sample was from the first cluster of cistern's category (sample 6), one from the second category (20) and two from the third (35 and 36). The protozoa concentrations were variable according to the cistern's capacity. In fact, for the first category (capacity less than 10 m^3), the protozoa *Giardia, E. histolytica/dispar/moschovskii* and *E. coli* were present respectively with 13 and 26 cysts/L. For the second category, with the cistern's capacity of 12–27 m^3, protozoa concentrations were higher and, respectively, 72, 143 and 72 for *Giardia, E. histolytica/dispar/moshkovskiii* and *E. coli*. The last two samples that belong to the third category showed a contamination ranging between 36 and 75 cysts/L for *Giardia* spp., 75 and 108 cysts/L for *E. histolytica/dispar/moshkovskii* and between 25 and 72 cysts/L for *E. coli*.

The roof harvested rainwater

The roof water systems were also monitored for parasitic presence (Figure 5). The protozoa were present in all samples,

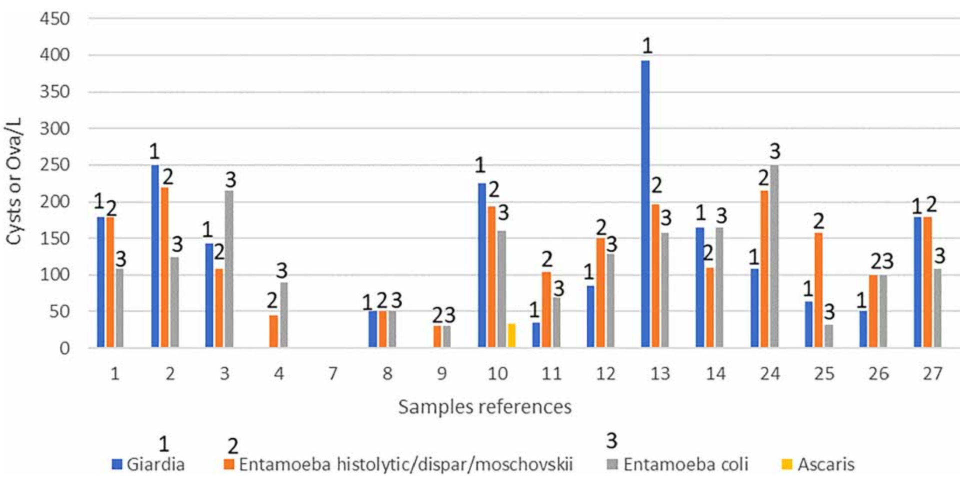

Figure 3 | Parasites concentrations in the 16 water samples originated from truck cisterns in the three considered underground tanks capacities (1–4: first category; 7–14: second category; and 24–27: third category).

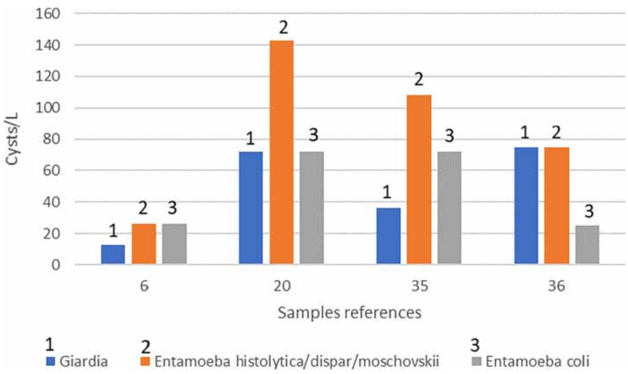

Figure 4 | Protozoa concentrations in the water mixture samples in the three considered underground tanks capacities (6: first category; 20: second category; and 35–36: third category).

while *Ascaris* spp. was detected once in the sample referenced 31. The roof rain water samples were represented by one water sample from the cistern capacity less than 10 m^3; five from the second category and seven from the last one. The water collected from the sample reference 5 (first category) presented the same concentration for the three detected protozoa (25 cysts/L). For the second category, concentrations of *Giardia* spp. ranged between 63–219 cysts/L while the amoeba concentrations were 72–394 for *E. histolytica/dispar/moshkovskii* and 63–179 cysts/L for *E. coli*.

For the last category, the concentrations were ranging between 94 and 300 for *Giardia* spp., 38 and 375 cysts/L for *E. histolytica/dispar/moshkovskii* and 38 and 188 cysts/L for *E. coli*.

The stored tap water

Tap water is available for some rural homes but not for the whole time. Thus, householders are storing it, when available, in their cisterns (Figure 6). Among the 39 selected cisterns, this type of water was stored only in cisterns of the second (samples 22 and 23) and third categories (samples 37, 38 and 39). *Ascaris* spp. was not present in the tap water stored samples, while the detected protozoa were present in all the samples with concentrations ranging from 32–172 cysts/L for *Giardia* spp., 69–113 cysts/L for *E. histolytica/dispar/moshkovskii* and 32–94 cysts/L for *E. coli*.

Well water stored

Only the sample referenced 21 was filled exclusively from well water. *Ascaris* spp. was absent while the three protozoa were detected with the same concentration (57 cysts/L).

DISCUSSION

In arid countries, limited access to good quality and adequate quantity of water is of public health concern as it represents a spreading vector of waterborne parasites, the most common cause of infection worldwide. Drinking

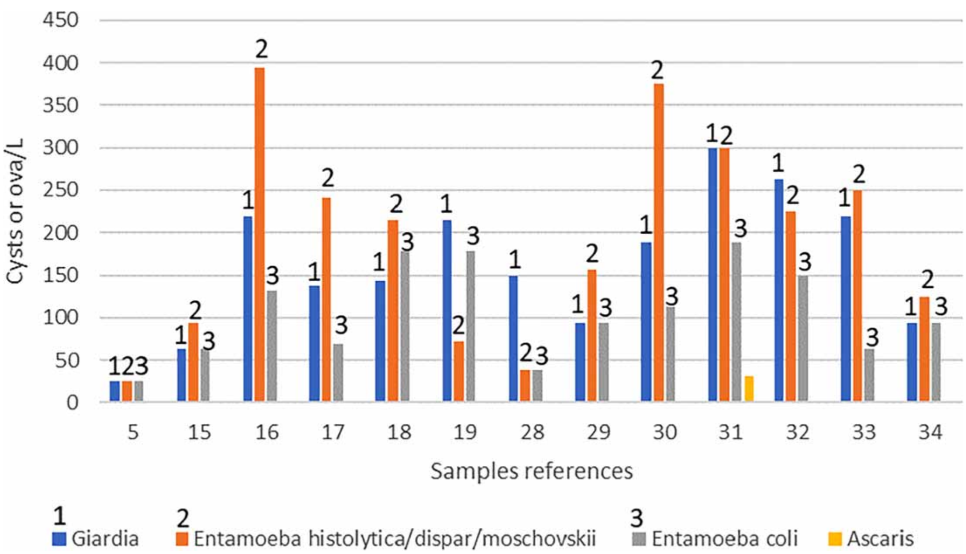

Figure 5 | Parasite concentrations in roof rainwater in the three considered underground tanks capacities (5: first category; 15–19: second category; and 28–34: third category).

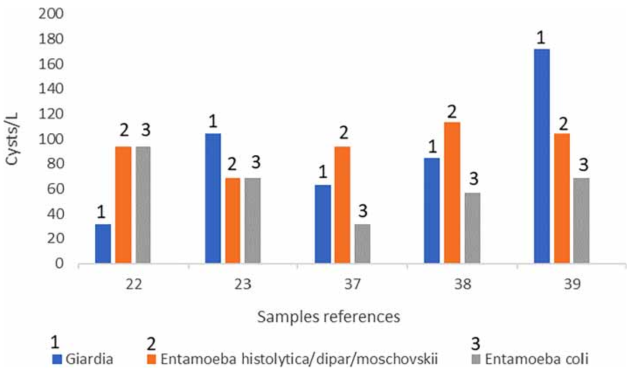

Figure 6 | Protozoa concentrations in stored tap water in two considered underground tanks capacities (22–23: second category and 37–39: third category).

water free of pathogens is compulsory to block one of the principal routes of their transmission.

Improvements in water, sanitation, and hygiene would improve life quality, decrease enteropathogen transmission and reduce the disease burden, actually estimated to be 1.7 billion episodes of diarrheal illness in children less than five years of age and approximately 15% of deaths in this age group (Black *et al.* 2010). According to Hunter *et al.* (2009), reducing the number of people without access to safe drinking water is one of the key development goals and health improvements. Therefore, water quality studies should be continuously performed in order to partly characterize this health hazard, which is difficult as there is an important 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, also useful in periods of prolonged drought.

This study was conducted in order to evaluate the presence of parasites in water stored in underground tanks and used exclusively for drinking water. It could also be used for preparing food, maintaining personal hygiene and cleaning utensils, within or between rural householders. 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 the first assessment of water use practices.

Water quality could be investigated by the monitoring of the presence of fecal indicators and parasitic presence. For this study, as we do not have the necessary equipment to undertake the coliforms, enterococci and *Escherichia coli* presence in drinking water samples, we focused only on parasitic detection. We did not undertake bacteriological analysis due to an absence of correlation between their presence and the parasitic contamination.

Although the modified Bailenger technique applied is specific to helminth ova detection, it also allowed the detection of protozoa, which were more frequent. This corroborates both epidemiological data (Bouratbine *et al.* 2000) and previous studies (Ben Ayed *et al.* 2009, 2010).

The parasitological analysis showed that out of the 39 water samples collected in cisterns from the rural households of the Kairouan, there was 97% of contamination, pointing to problems of water management, lack of hygiene and possible fecal contamination from domestic animals and human excreta; and only one house presented suitable drinking water. This high prevalence is according to the observations and answers to the surveys, showing an absence of water treatment, a lack of prophylaxis and drug treatment, the poor hygiene habits and sanitary conditions, as observed in the study of Xavier *et al.* (2011) in Brazil. The lack of education among households could also be considered an important factor of risks. Thus, the application of efficient sanitation and the improvement of drinking water supplies could represent a safety measure against pathogens hazards.

Due to a lack of access to private taps, rural households are managing this limited situation by using five kinds of water, which were sampled and analyzed for parasitic contamination: 16 from truck cisterns depending on water vendors, 13 from roof harvested rain water, four of a water mix, five from tap water and one from wells.

The detected parasites in the examined 39 water samples were *Entamoeba histolytica/dispar/moshkovskii* (97%); *E. coli* (97%); *Giardia* spp. (92%) and *Ascaris* spp. (5%). The high reported prevalence could be attributed to the sampling period where the recorded temperatures were adequate for microbial proliferation.

Even if *Ascaris* spp. possesses higher persistence in the environment (up to one year), very low prevalence was reported, as already stated in the work of Jarallah (2016) in drinking water sources in Iraq. The detected parasites are of particular relevance due to their persistence in the environment, their ability to survive outside the hosts, their minimal infective dose and their potential for zoonotic transmission.

E. coli was as frequent as *E. histolytica/dispar/moshkovskii*. However, as it is a non-pathogenic enteric protozoa, it does not represent a public health concern. *E. histolytica/dispar/moschovskii* was the most frequent pathogenic protozoa present in all types of water stored. This amoeba is responsible, after malaria and schistosomiasis, for the highest mortality rate from parasites. This highest rate in water is consistent with the study of Yousefi *et al.* (2009) in Iran, Ayaz *et al.* (2011) in Pakistan and Christiana & Levi (2015) in Nigeria. Between 1946 and 1980, this parasite was responsible for six water and foodborne outbreaks in the United States and up to year 2010, *E. histolytica* caused 10 reported waterborne outbreaks in China, Japan, Thailand, Sweden, UK, USA and Taiwan. Moreover, this protozoa is prevalent in children under five years of age, teenagers and immuno-suppressed patients.

Rafiei *et al.* (2014) reported the predominance of *E. histolytica/dispar/moshkovskii* in two of the three kinds of water surveyed (raw and treated river water or water refiner, tap water and water selling station) with a presence in 22 among the 48 samples. This represents the only concordance found with this study as Rafiei *et al.* (2014) not only reported its absence from water selling stations, but also detected the presence of other protozoa such as *Cryptosporidium* spp. (27.3%) and *Blastocystis* spp. (13.6%) that were not detected in this study and they reported a lower rate of *Giardia* spp. contamination (9.1%).

In this work, four parasitic species were reported. However, in well drinking water, Yousefi *et al.* (2009) reported the presence of 21 species. Among the 197 parasitic contaminated samples, 2 and 2.3% were respectively contaminated by *Giardia* spp. and *E. histolytica/dispar/moshkovskii*. They also argued their resistance to chlorination. In Iraq, Kassim *et al.* (2015) reported drinking water contamination by 10 parasitic species with the highest prevalence rate of *E. histolytica/dispar/moshkovskii* (14.4%) as it was detected in 15 over 104 water samples. Similar important incidence of this amoeba was reported in well water in Nigeria in Bishop & Inobo (2015) work where *Giardia* spp. and *Ascaris* spp. were equally present (1.9%) in the surveyed water samples.

Cryptosporidium spp. has been reported in rural and urban populations worldwide. In this study, oocysts were not detected. While it is likely that they could be present, they may have remained undetected by the Bailenger method. Due to their small size relative to that of the other parasites, they may have settled less efficiently and may have been overlooked because no specific staining was applied (Ben Ayed *et al.* 2009, 2010). A more efficient detection of *Cryptosporidium* oocysts could have been achieved using flocculation by ferric sulfate in combination with an immunofluorescence test, as described by Karanis & Kimura (2002).

In Australia, a study on the assessment of the health risks linked to the use of roof harvested rainwater for potable, argued its microbiological contamination and reported 9.8% of *G. lamblia* infection (Chubaka *et al.* 2018). In rural communities of Tuparetama in Brazil, drinking rain water, conserved in tanks and pots, was also contaminated by six protozoa (*Cryptospodium* spp., *Giardia* spp., *E. coli, E. histolytica/dispar, Endolimax nana* and *Isospora belli*) that were not affected by sodium hypochlorite addition (Xavier *et al.* 2011).

Underground tank or cistern capacity did not influence the parasitic distribution as no differences were reported from lower or higher capacities. Older or new cisterns and manual or mechanical water management did not influence the protozoa and helminth contamination. 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 & Inabo (2015).

This first report indicated that the hygienic quality of water in the rural areas investigated is not satisfactory. *E. histolytica/dispar/moshkovskii* and *Giardia* are water- and foodborne pathogens that are of potential risk for human infection. Poor sanitation and hand hygiene, contaminated and lack of sufficient water increases the diarrheal incidences. In fact, at the water collection sites, it was observed that all the householders did not wash their hands before water serving. They washed neither their containers nor their buckets before collecting water, which is thrown away on the floor and not in a safe place. They are also using it for other purposes and sharing it between neighbors, corroborating the reported parasitic prevalence. Tambekar *et al.* (2008) emphasized that the water quality deterioration is closely related to hygiene practices and that the most involved factors are hand and water drawing utensil. Thus, it is advised to avoid hand water contact in normal household management. Trevett et al. (2004) reported an immediate deterioration in water quality as collection containers were filled, presumably caused by its inadequate washing, or hand contact.

It is also important to manage the presence of domestic animals in the houses, as they are a source of contamination. This study was only based on morphological analysis due to a lack of necessary conditions to undertake further investigations. The application of molecular tools for the identification of specific parasites of contamination sources and seasonal evolution is recommended.

Some of the households investigated are maintaining or cleaning their underground tanks well. The cover of the underground tanks or cisterns must be changed and painted to avoid the risks of eventual uncontrolled water run-off. Moreover, proper cleaning of the tanks, the roof and the material used to collect the water (pipes, gutters, etc.) is compulsory to ensure safe water storage and quality preservation. Further studies may be undertaken in order to determine the most efficient physical or chemical disinfection method (boiling, heating, settling, filtering, exposing to UV radiation in sunlight, chlorination). Xavier et al. (2011) argued that even if a chlorination treatment was applied, the prevalence of *Giardia* and *Ascaris* remained high in rain water stored in pots and in tanks.

As the water bought could be more expensive than tap or rain water, rural households unable to afford to purchase water as often tend to store water for longer times, increasing the possibility of risk among consumers. The investigated communities consisted of several extended families, living as neighbors, sometimes even without borders or limits. It was common to observe close relationships and interactions between them. They are sharing water, meals and even the utensils used for water serving. This fact could enhance the parasitic transmission and could represent a potential transmission route as is the case of several rural communities such as in Thailand (Pinfold 1990). Most contaminations are the result of behavioral patterns, which will lead to a reduction or elimination of health risks and inter-family disease transmission as they are using the same water source (Trevett et al. 2005). In order to preserve the water quality, hygiene education and behavioral change should be provided to rural householders. The aim of education is to inform and educate people to include regular hand washing and proper collection, storage and handling of water. Nala et al. (2003) reported that behavioral changes are complex and that hygiene education alone may not be sufficient to change people's behavior as education is closely related to the household's income. In situations of extreme poverty, the household's ability to improve or maintain the sanitary environment of the home will be limited (Trevett et al. 2005). The investigated women claimed that soap is too expensive to be frequently used and does not last long, therefore they are managing its use.

One of the best ways for quality surveillance and preservation is regular analysis, community participation, education and responsibility of households in water storage and use.

CONCLUSIONS

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

The obtained results reflect the complexity of interactions of several factors involved in the fecal–oral disease transmission. We recommend that considerable attention must be accorded to water management practices in rural households in Tunisia.

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