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Research Article

Prevalence of Zoonotic Parasites in Drinking Water and Sanitation Practices in Rural Areas of Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Access to safe drinking water remains a critical public health challenge in rural regions, where water scarcity, inadequate sanitation, and dependence on multiple water sources increase the risk of waterborne and zoonotic parasitic infections. Tehsil Daraban Kalan, District Dera Ismail Khan (Khyber Pakhtunkhwa, Pakistan), represents a vulnerable rural setting where drinking water quality and sanitation conditions may contribute significantly to disease transmission. This study aimed to determine the prevalence of zoonotic parasites in different drinking water sources and to evaluate household water accessibility and sanitation practices in the study area. A cross-sectional study was conducted in six rural union councils comprising 24 villages and 600 households, and a total of 450 water samples were collected from ponds, tube wells, and drainage sources. Samples were processed using filtration and centrifugation techniques. Parasitic organisms were identified through modified Ziehl–Neelsen staining for acid-fast parasites (*Cryptosporidium* spp., *Toxoplasma gondii*) and Lugol's iodine wet mount for non-acid-fast parasites (*Entamoeba histolytica*, *Giardia lamblia*, *Balantidium coli*, and *Fasciola hepatica*). Statistical analysis was performed using SPSS, and differences in prevalence were analyzed using the Chi-square test at a significance level of $p < 0.05$. Tube wells were identified as the predominant source of drinking water, while flush toilets were the most commonly reported sanitation facility. Drainage water exhibited the highest prevalence of parasitic contamination, particularly *Entamoeba histolytica*, *Cryptosporidium*, and *Giardia* ($p < 0.01$), followed by pond water. In contrast, tube well water showed comparatively lower levels of contamination, although some samples tested positive for *Giardia* and *Entamoeba histolytica*. Household water accessibility varied significantly, with most families spending approximately 15 minutes to collect drinking water. Surface and drainage water sources pose substantial zoonotic and public health risks in the study area. Immediate interventions including improved water treatment systems, enhanced sanitation infrastructure, and community-based hygiene education are essential to reduce waterborne parasitic infections and improve public health outcomes in rural communities.

Keywords: Prevalence; Zoonotic Parasites; Drinking Water; Sanitation Practices; & Dera Ismail Khan.



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INTRODUCTION

Water pollution has become a major health problem for the general population in most developing countries, despite the fact that safe and clean drinking water is essential for both human and animal health. Contaminated water is a primary source of a wide range of harmful bacteria, viruses, and parasites.

Zoonotic parasites are among the most dangerous since they may infect both humans and animals and are frequently spread through polluted water sources. According to the World Health Organization (WHO), drinking contaminated water causes millions of instances of waterborne illnesses annually, particularly in rural and underdeveloped regions with inadequate sanitation (WHO, 2020) (Khalid et al., 2025).

Protozoa and helminths are most often associated with waterborne parasitic infections include *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium spp.*, *Balantidium coli*, *Fasciola hepatica* and *Toxoplasma gondii*. When the environment is conducive, these parasites survive in water as cysts, oocysts or eggs that are infectious over a long period of time. When these parasites are consumed through tainted drinking water, they can cause systemic and gastrointestinal illnesses in both humans and animals. According to the literature, water sources such as ponds, rivers and drainage water have been found to be especially susceptible to parasite contamination by the runoff of agricultural lands, farm waste and poor waste disposal (Asghar et al., 2025).

In the rural areas where there is minimal access to purified municipal water systems, consumer usually use the ground water sources like tube wells, hand pumps and even the lakes and rivers water. Despite the fact that, ground water is usually considered to be safer as compared to lakes and rivers water, it is because several studies have indicated that it is also prone to contamination due to improper well construction; leaking of the septic systems and poor sanitation practices. These contaminations pose the risk of contracting zoonotic parasites and lead to the waterborne disease burden among the rural population (Khan, 2024).

In Pakistan, particularly in rural Khyber Pakhtunkhwa, water quality issues are exacerbated by rapid population expansion, a lack of sanitary services, and the close proximity of humans and animals. There is little scientific data on the prevalence of zoonotic parasites in different drinking water sources in the majority of the nation's rural areas, despite the fact that the problem of waterborne parasitic illnesses has grown to be a serious concern. Similarly, the Tehsil Daraban Kalan in District Dera Ismail Khan has not yet been the subject of comprehensive study on water quality, especially in terms of parasite contamination.

The current study was conducted, to determine the prevalence of the zoonotic parasites in the various sources of drinking water such as pond water, tube well water and drain water in Tehsil Daraban Kalan. This study should provide crucial baseline data about the risk of parasites in water and aid in the development of efficient water management and health initiatives for the local population.

MATERIALS AND METHODS

Study area and sampling design

The present study was conducted in Tehsil Daraban Kalan, District Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan, to investigate the prevalence of zoonotic parasites in different drinking water sources. According to the Pakistan Bureau of Statistics (2023), the population of Tehsil Daraban Kalan is approximately 149,447. The tehsil consists of eight union councils, of which six rural union councils were purposively selected based on reported issues such as water scarcity, inadequate sanitation infrastructure, and a high reliance on untreated groundwater and surface water sources.

From these selected union councils, 24 villages were included in the study, and a total of 600 households were surveyed to assess drinking water sources, sanitation practices, and water accessibility. Households were distributed equally among the selected villages. Within each village, households were selected using a simple random sampling technique to minimize selection bias and ensure a representative sample of the local population (Nasir et al., 2026).

The distribution of the selected union councils, villages, and surveyed households in Tehsil Daraban Kalan is presented in Table 1.

Water sample collection

In order to establish the prevalence of zoonotic parasites in drinking water, 450 water samples were collected from three major sources of water that are an extensively used by the local population, which include pond water, tube well water, and drain water. The sterile glass bottles were used to collect the water samples under the standard water sampling methods as prescribed by APHA (2017) and WHO (2011) (Murtaza et al., 2025a).

Each sample bottle was properly labeled with details regarding the sampling date, water source, and sampling location. In order to prevent contamination or deterioration of the samples prior to analysis, they were subsequently transported under controlled circumstances to the Microbiology and Biotechnology Laboratory, Faculty of Veterinary and Animal Sciences, Gomal University, Dera Ismail Khan (Murtaza et al., 2024).

Table 1. Distribution of Selected Union Councils, Villages, and Surveyed Households in Tehsil Daraban Kalan

No.	Union Council	Distribution of Selected Union Councils, Villages, and Surveyed Households	
		Selected Villages	Selected Households
1	Daraban Kalan	4	100
2	Mosa Zai Sharif	4	100
3	Chodhwan	4	100
4	Bhukhi	4	100
5	Gandi Ashiq	4	100
6	Saggu	4	100
	Total	24	600

Water sample processing

Filtration and concentration

In order to detect parasites, 250–500 mL of each water sample were filtered using Whatman filter paper No. 42 using a sterile glass funnel to concentrate parasitic cysts, oocysts, and eggs (Orlandi and Lampel, 2000). To extract the parasite infiltrates, the filter paper was filtered and then washed with 10–15 mL of phosphate-buffered saline (PBS, pH 7.2). To further concentrate the washings, they were placed in sterile containers and then moved into 15 mL centrifuge tubes (Baquer *et al.*, 2018).

Centrifugation

The filtrates were centrifuged at 3000 rpm for 10 to 15 minutes in order to precipitate the parasites. The centrifugation tube was handled carefully; the supernatant was discarded and the pellet containing the concentrated parasitic material was suspended again in 1- 2 mL PBS to form a homogenous suspension to examined under the microscope (Orlandi and Lampel, 2000).

Parasite detection and identification

Smear preparation

A small smear of one drop of the concentrated solution was applied to a spotless glass slide. To preserve the parasites' morphology, the smear was first allowed to air dry before being heat-fixed using a Bunsen burner (Uzun Ozsahin *et al.*, 2022).

Staining and microscopy

Two staining techniques were used for parasite identification. Modified Ziehl–Neelsen staining was employed to detect acid-fast parasites, particularly *Cryptosporidium* spp. (Rigo and Franco, 2002). *Giardia* In this method, smears were stained with carbol fuchsin, decolorized with an acid–alcohol solution, and counterstained with methylene blue. Under light microscopy with oil immersion, the oocysts appeared as bright red structures against a blue background, indicating acid-fast characteristics.

The Lugol's iodine wet mount technique was used to detect non–acid-fast parasites, including *Entamoeba histolytica*, *Giardia lamblia*, *Balantidium coli*, and *Fasciola hepatica*. The prepared slides were examined under light microscopy at 10x, 40x, and 100x magnifications. Parasites were identified based on their morphological characteristics, including size, shape, and internal structures, using standard parasitological identification keys (Bassad, 2023); WHO, 2020).

Determination of parasite prevalence

The prevalence of parasites in water samples was calculated by finding the ratio of samples where parasitic stages were detected. This method allowed to estimate the prevalence of each parasite species in different water sources (Riaz *et al.*, 2020). The following formula was used to calculate the percentage of prevalence:

Prevalence (%) = (Number of positive samples / Total number of samples examined) × 100

Statistical analysis

Data obtained from the study were analyzed using *IBM SPSS Statistics* (Version 26.0). The prevalence of parasites in different water sources (pond water, tube well water, and drain water) was expressed as percentages. Differences in parasite prevalence among the water sources were analyzed using the Chi-square test, which is appropriate for categorical data. A p-value of less than 0.05 was considered statistically significant. The analyzed data were presented in graphical form to illustrate variations in parasite occurrence among the different water sources.

RESULTS

Prevalence of various drinking water sources in rural households

The distribution of different drinking water sources among the research population is shown in figure 1 (a). The tube

well water was the most often utilized source, with a significantly high proportion ($p < 0.01$) as compared to other sources. A moderate number of respondents utilized the pressure pumps, hand pumps and rainwater, whereas the well water was used the least. According to the statistical analysis, the various water sources ($p < 0.05$) reveals that tube wells are the major source of drinking water.

Accessibility of drinking water based on fetching time

Figure 1 (b), illustrates the time spent by the respondents to procure drinking water. The estimated time needed by most households to get water was about 15 minutes and this was much longer periods ($p < 0.01$). The lowest number of responders cited 60 minutes to gather water, while a smaller percentage claimed 30 minutes. The found difference between these groups was statistically significant ($p < 0.05$), indicating that the houses under study had varying levels of water accessibility.

Efficacy of six local plant powders at various concentrations was assessed against pulse beetle on different parameters under controlled laboratory conditions. The data obtained were statistically evaluated and explained below:

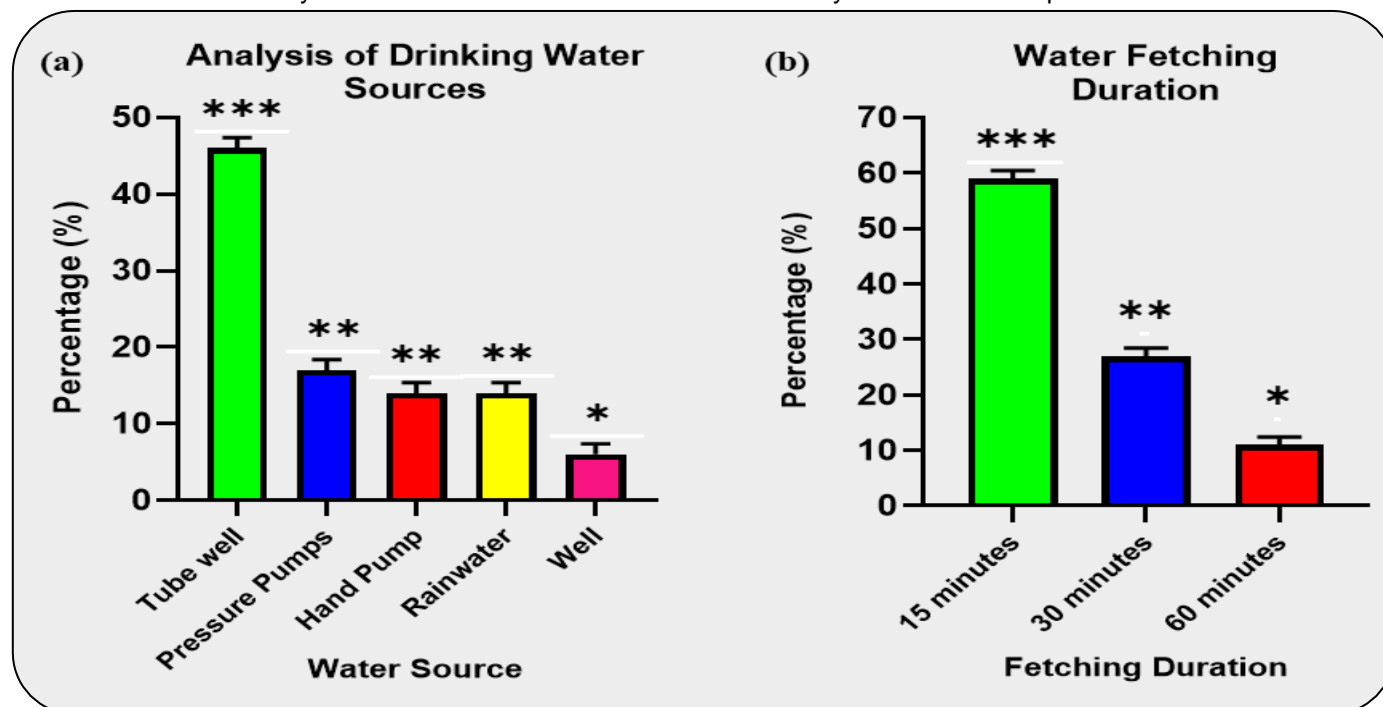


Figure 1 (A, B). Percentage distribution of primary drinking water sources, and (b) the corresponding time duration required for water fetching.

Distribution of household sanitation facilities

Figure 2 (a) demonstrates the distribution of sanitation facilities among the surveyed households. The majority of households used flush toilets, representing the highest proportion and showing a statistically significant difference compared with other sanitation types ($p < 0.01$). Pit latrines were the second most common sanitation facility, indicating moderate adoption among respondents. In contrast, open defecation and temporary sanitation structures were reported by a very small proportion of households. Statistical analysis revealed a significant variation among sanitation practices ($p < 0.05$). These results suggest that although improved sanitation facilities are widely available, a small segment of the population still relies on less hygienic sanitation practice.

Household access to safe drinking water

Figure 2 (b) indicate the household access to safe drinking water. The proportion of respondents with access to clean drinking water was significantly higher ($p < 0.01$) than the proportions of households reported the lack of access to safe drinking water or restricted access. Statistical comparison of these two groups revealed that there was a significant difference ($p < 0.05$). These results indicate that despite the access to safe drinking water in the majority of households, the apparent minority is still struggling with receiving reliable water supplies.

Sources of domestic water supply

The distribution of residential water supply sources among dwellings is depicted in figure 2 (c). The most common source was tube wells with the highest utilization among the respondents and statistically significant difference with

other sources ($p < 0.01$). They utilized pressure pumps and hand pumps, which are indicative of their reliance on ground water extraction technologies. Less used sources were rainwater harvesting and well water. The statistical analysis showed that there was a significant difference between the water sources ($p < 0.05$). These findings suggest that the study's area is significantly dependent on groundwater-based water delivery systems.

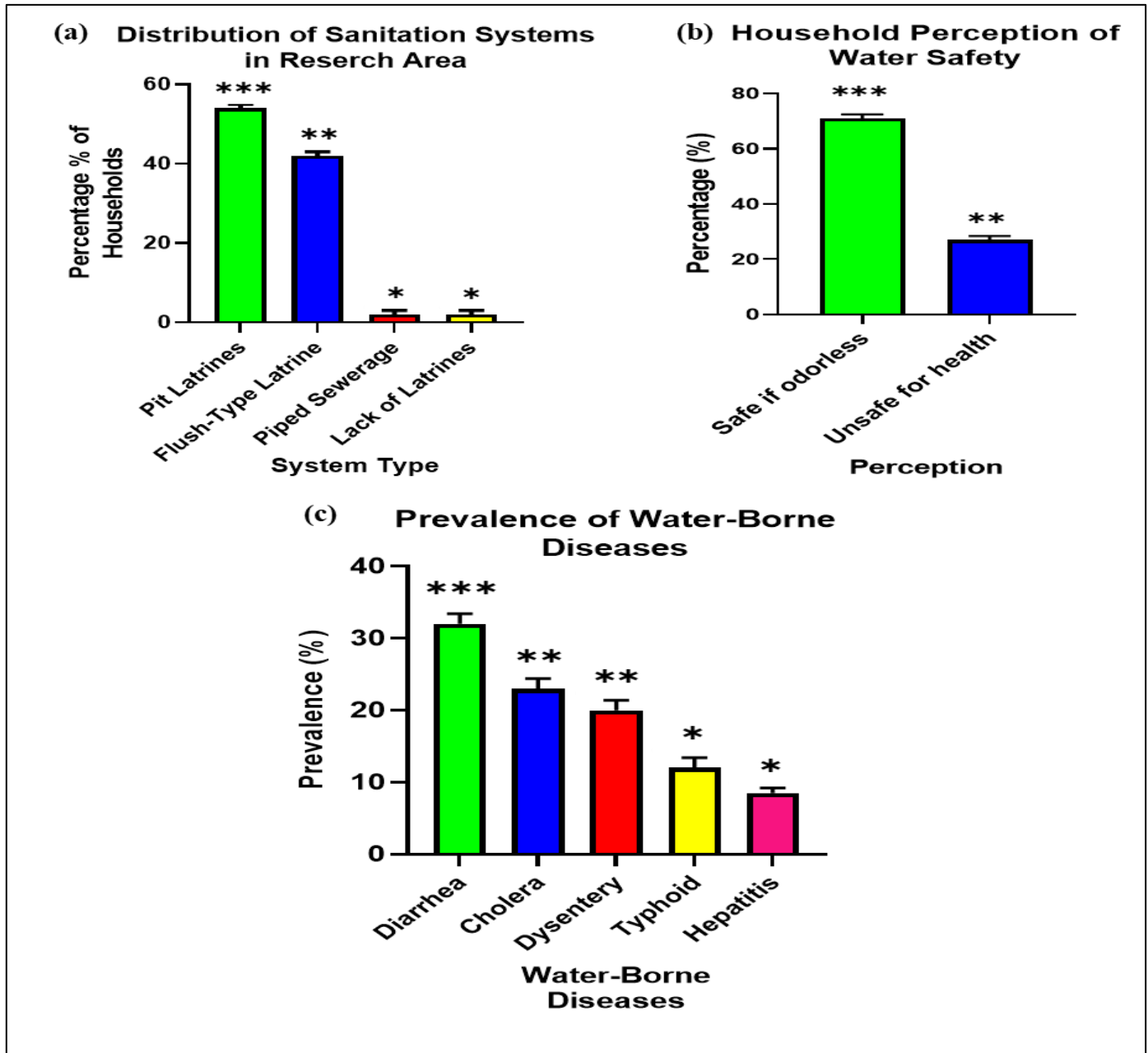


Figure 2 (A, B, C). Distribution of Sanitation System in Research Area, (b) Household Percentage of Water Supply & (c) Prevalence of Water-Borne Diseases.

Prevalence of zoonotic parasites in different drinking water sources

Figure 3 shows major prevalence of Zoonotic parasites found in various water sources such as pond water, tube well water and drain water. It was found that drain water was the most contaminated and had a much higher prevalence of *Entamoeba histolytica*, *Cryptosporidium*, and *Giardia* as compared to other water sources ($p < 0.01$). The pond water was also found to be highly contaminated especially with *Cryptosporidium* and *Entamoeba histolytica*, and therefore, it may be a risk of environmental exposure. In contrast, the amount of parasites in tube well water had been extremely low, but *Giardia* and *Entamoeba histolytica*, were detected. *Balantidium coli*, *Fasciola hepatica*, and *Toxoplasma gondii* had a relatively low prevalence among all sources of water yet were statistically significant ($p < 0.05$). The results have shown that the water sources of surface and drainage pose a higher risk of contamination in zoonotic parasitism as

compared to ground water sources. These findings indicate that adequate water management and hygiene practices should be employed in order to reduce the risks of water borne parasitic infections within the study site.

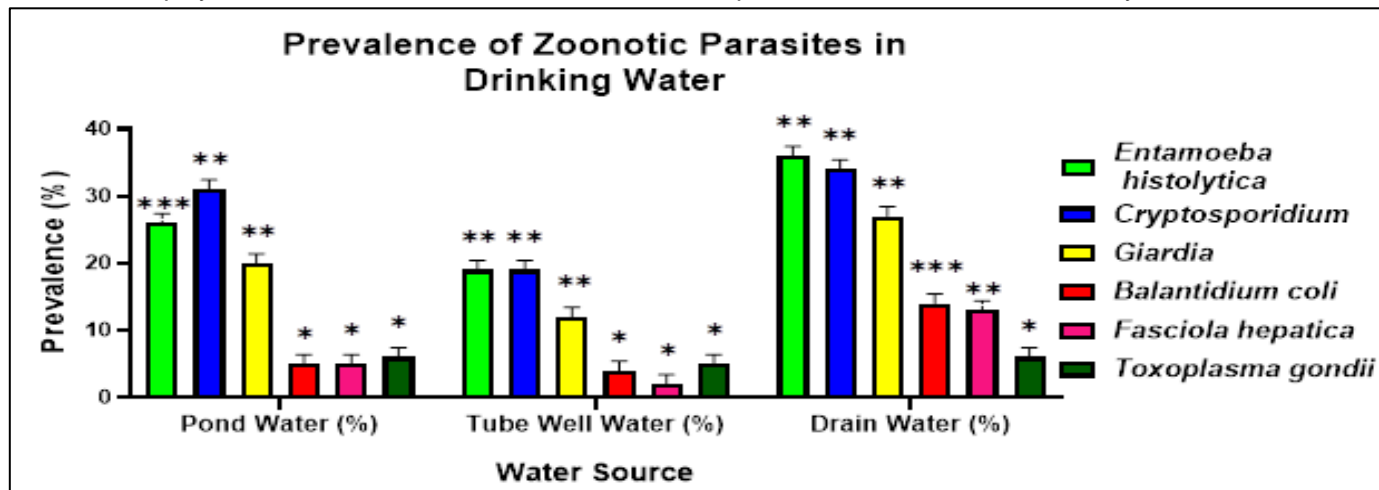


Figure 3. Prevalence of zoonotic parasites in different drinking water sources (pond water, tube well water, and drain water).

DISCUSSION

The current research examined the prevalence of zoonotic parasites in various drinking water types which are pond water, tube well water and drain water in Tehsil Daraban Kalan, District Dera Ismail Khan. The findings showed the high prevalence of parasitic contamination in surface water sources, specifically drain, and pond water rather than tube well water. These results delineate the possible health hazards of untreated or polluted water consumption in rural areas.

Entamoeba histolytica, *Cryptosporidium* and *Giardia* were more prevalent parasites in the present study especially in the drain water. The prevalence rates of these protozoan parasites in drainage waters can be explained by the fact that these parasites can be directly contaminated by human and animal waste, poor sanitation standards, as well as inadequate systems of waste disposal in villages. Murtaza et al. (2025) also reported similar results when they pointed out that protozoan parasites (including *Giardia* and *Cryptosporidium*) are widely spread in the global population through contaminated water sources and are significant agents of waterborne diseases (Murtaza et al., 2025b). Similarly, Sajid et al. (2024) noted that surface water bodies (river & lakes) in most cases are reservoirs of protozoan parasites because of the environmental contamination (Sajid et al.).

This study also showed a high parasitic contamination of pond water. It may have been related to the openness of the environment where parasites are likely to occur, livestock accessibility, and run-offs into water that carry fecal content. Such patterns of contamination of the rural water sources have also been found in previous studies. As an example, Moslen et al. (2024) cited that ponds and other still water bodies are extremely prone to being contaminated by parasitic cysts and oocysts of animal and human excretions (Moslen et al., 2024).

On the contrary, in the present study, tube well water exhibited relatively lower rates of parasitic contamination. This could be because the sources of ground water are comparatively shielded against direct contamination by the environment. Nevertheless, the presence of certain parasites at even the tube well water indicate the potential presence of groundwater contamination by seepage, improper construction of wells or by the lack of the proper sanitation systems surrounding the water sources. WHO (2020) also made similar observations, stating that even in regions with insufficient sanitation infrastructure, ground water has the potential of getting contaminated (Bull et al., 2020).

The identification of other parasites like *Balantidium coli*, *Fasciola hepatica* and *Toxoplasma gondii* in the sampled water also evidence the possibility of water sources in the transmission of zoonotic parasitic infections. These parasites are generally linked with domestic animals and livestock, which implies that animal waste can cause a contamination of this pollution to the environment through water sources (Sobsey et al., 2006).

In general, the results of the current research indicate the high risk of parasitic zoonotic infections related to the contaminated drinking water sources in rural regions. The findings highlight the importance of better water management practices, sanitation systems and periodic water quality monitoring in a bid to ease the burden of water borne parasitic diseases in the area.

CONCLUSION

This study highlights the significant public health concern associated with parasitic contamination of drinking water sources in rural areas of Tehsil Daraban Kalan, District Dera Ismail Khan. Although tube wells were identified as the primary source of drinking water and showed relatively lower contamination, surface water sources such as ponds and drainage water exhibited a considerably higher prevalence of zoonotic parasites, including *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium*. These findings indicate that untreated surface water sources may pose substantial health risks to rural communities. Variations in water accessibility and sanitation practices further emphasize the need for improved water management strategies. Therefore, strengthening water quality monitoring systems, improving sanitation infrastructure, and promoting community awareness regarding safe water handling and hygiene practices are essential to reduce the burden of waterborne parasitic infections and ensure sustainable access to safe drinking water in rural populations.

AUTHOR CONTRIBUTIONS

Khalid Muhammad and Tooba Muhammad: Conceptualization of the study, research design, and supervision of the project. Ghulam Murtaza and M. Inam Ullah Malik: statistical analysis, and data interpretation. Inam Ullah Khan and Ayesha Haleem Shah Data collection, laboratory experimentation, and preparation of the initial manuscript draft. Jawad Ullah Shah and Ali Zaman: Manuscript review, critical editing, project supervision, and final approval of the manuscript.

COMPETING OF INTEREST

The authors declare no competing interests.

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