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Detection of Protozoa in Drinking Water of Kathmandu Valley, Nepal

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Abstract

Introduction: *Safe drinking water is a delicate matter, and its quality needs to be periodically checked. Although there has been microbial research on drinking water protozoan occurrence is uncommon in Nepal. The goal of the current study was to analyze the readily evaluable biological examination of tap water, tube well water and dug well water sources in the Kathmandu Valley of Nepal.*

Methodology: *A total of 720 samples were collected from residential colonies of Baneshwor, Chhetrapati, Bansbari, and Tripureshwor areas in the Kathmandu Valley of Nepal. The sample were collected in 2021 across the four seasons, spring (March-May), summer (June-August), autumn (September-November) and winter (December-February). To collect the drinking water samples, sterilized bottles with the capacity of 1000 ml were used and capped tightly. The Centrifugation method was employed for the identification of protozoa by routine parasitology with a light microscope.*

Results: *The major protozoa found in Nepal are Giardia lamblia, Entamoeba histolytica, Amoeba proteus, which are directly contaminated with fecal matter. The total number of positive cases reported was 42 (5.83%), from the tap water samples, there were the total five positive cases 5 (0.69%) in which 5 species were identified as non-pathogenic and 1 species as pathogenic. In the tube well samples total number of 11 protozoa were detected 11 (1.52%) in which 9 species were non-pathogenic and two species were pathogenic. Among dug well water samples, there were 26 positive cases (3.61%), where 34 species were identified as non-pathogenic and 12 species as pathogenic. Amoeba proteus 32 (4.44%), Paramecium caudatum 24(3.33%), Giardia lamblia, 8(1.11%) Entamoeba histolytica 6(0.83%) and Balantidium coli 1(0.13%). Chi square test suggest that there is no* *significant difference of positive samples among different locations (Chi- square = 0.30341, df = 3, p- value=0.9594). The similar test among seasons indicate significant difference (Chi square = 6.574, p-value=0.086). However notable significant difference was found in different types of water (tape water, tube-well water, and dug-well water) Chi- square = 17.75, df = 2, p-value = 0.00013990.*

Interpretation: *Overall the number of positive cases was more in summer than other seasons which could be due to obnoxious contamination of rain water in drinking water.*

Keywords: Drinking water, Kathmandu Valley, Occurrence, Protozoa, Uncommon.

Introduction

Access to safe drinking water is a fundamental human right and a critical component of public health. Despite significant progress, many regions worldwide still face challenges in ensuring a reliable supply of clean drinking water. This review outlines the current global conditions of drinking water, highlighting key statistics, major challenges, and progress made in improving water access quality. Globally, access to safe and sufficient water is a critical issue influenced by factors such as climate change, population growth, and socio-economic disparities **(Maharjan *et al*.,** 2018; Khadka *et al*., 2016). While progress has been made in increasing access to safe drinking water, significant challenges remain, particularly in developing regions where poor sanitation and inadequate water management systems contribute to widespread health problems. Effective water management, improved sanitation, and resilient infrastructure are essential to address these global water challenges (Samarrai *et al*., 2023; Sherchand *et al*., 2014). In 2021, over 2 billion people live in water stressed countries, which is expected to be exacerbated in some regions as result of climate change and population growth. In 2022, globally, at least 1.7 billion people use a drinking water source contaminated with faeces (WHO and UNICEF 2023). The majority of single-celled eukaryotes are known as protozoa, and they are essential to the environment because they help mineralize soil nutrients so that various plants and other soil microorganisms may use them. Nepal has made significant strides in improving access to drinking water over the past few decades. However, the influence of climate change on this sector remains inadequately documented. This review aims to consolidate the status of drinking water in Nepal through the lens of climate change by examining available secondary data from both published and gray literature (Sharma *et al*., 2021). Protozoa consume organic materials such as waste, biological tissues, and other microorganisms. They may live freely or as parasites (Tandukar *et al*., 2015; Adhikari *et al*., 2020). Binary fission is the most often used asexual reproduction process for protozoa. Protozoa can also reproduce sexually. Additionally, protozoa are crucial to the aquatic ecosystem's food chain. Numerous protozoa species inhabit water and consume they (Rai *et al*., 2023; Deng *et al*., 2021; Haramato *et.al.*, 2018). *Toxoplasma gondii, Entamoeba histolytica, Giardia lamblia,* an obligatory intracellular apicomplexan parasite that can infect all warm-blooded vertebrates, and *Giardia duodenalis*, a cosmopolitan organism and the cause of giardiasis, are the most common causes of waterborne parasite infections in developing countries (Shapiro *et al.*, 2019). These illnesses are caused by zoonotic protozoa, and the major means by which they proliferate include unhygienic conditions, incorrect waste management, and insufficient health education. These are zoonotic protozoan pathogens, and the main ways they spread are through unhygienic conditions, incorrect waste management, and inadequate health education (Cai *et al*., 2021; Djurkovic *et al*., 2019). The primary mode of human-to-human transmission of *G. duodenalis* and *T. gondii* is thought to be human consumption of contaminated food or water. Both humans and animals can eject *Giardia* cysts from their feces, and felids are the only known hosts of *T. gondii* oocysts (Xiao 2017). Water supplies can become contaminated by things like coming into direct touch with soil that has been contaminated by the feces of asymptomatic carriers of the infective stage of protozoa. (Utaaker 2017; Taghipour *et al*., 2022; Ismail 2016; Rousseau *et al*., 2018; Lee *et al*., 2021). Apart from consuming food and beverages, it has been discovered that exposure to contaminated soil poses a substantial risk factor for indirect water contamination (Hald *et al*., 2016; Ma *et al.,* 2022; Robertson. 2016). Protozoan parasites are reported to be the second most common etiological cause of mortality in children under five. They are to accountable for the 1.7 billion cases of diarrhea that occur globally each year, which lead to 842,000 deaths. Epidemics and endemic illnesses are caused by parasite infections disseminated by waterborne protozoa in both developed and underdeveloped countries. However, parasitic protozoa are often not considered to be the cause of these disorders because the former have higher hygienic conditions (Omarova *et al.*, 2018; Mtapuri *et al*., 2014; Bilung *et al* 2017). Globally, pathogenic protozoa such as *Giardia*, *Cryptosporidium*, and *Entamoeba* are known to cause gastrointestinal infections, especially in areas with poor sanitation and inadequate water treatment facilities (WHO, 2017). While the occurrence of protozoa in water sources is well-documented in many parts of the world, their presence in drinking water in Nepal's Kathmandu Valley has received relatively little attention. Limited studies have suggested that protozoan contamination in Nepal's water sources may contribute to recurring outbreaks of diarrheal diseases (Shrestha *et al*., 2015; Fernada *et al.*, 2018; Ghanashyam *et al.*, 2023; Giovanni *et. al.*, 2020). This highlights the need for a more in-depth investigation into the presence of protozoa in local water sources to better understand the health risks faced by the population (Artemis *et al.*, 2017; Karanis *et al*., 2027). The main objective of this study is to investigate the uncommon occurrence and prevalence of protozoa in drinking water sources of the Kathmandu Valley.

Materials and Methods

Study area

The Kathmandu Valley is located in the midland of the Himalayas, between 27°32' and 27°49' North and 85°12' and 85°32' East. It is almost round in shape, with a diameter 30 km E-W and 25 km north to south (Khanal *et al.,* 2023). Approximately 65% of the nation's economic activities takes place in Kathmandu Valley, has a population 2,517,023(CBS 2020). This study is conducted only in, Tripureshwor, Baneshwor, Bansbari and Chhetrapati (Fig 1) of the Valley. Since Kathmandu is the urban city of country and this study sites are highly populated and at least one of this water sources is used by people of these selected sites.

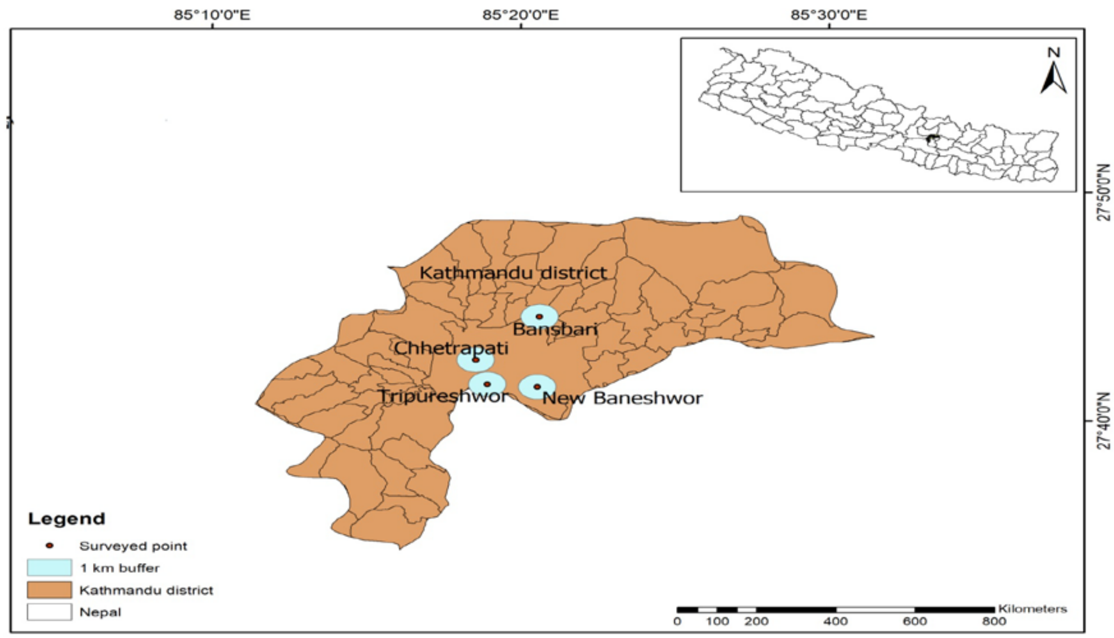

Figure.1: Map of study area and sampling sites in Kathmandu Valley, Nepal

Procedures

Water samples were collected from 720 houses in Tripureshwar, Baneshwor, Bansbari and Chhetrapati, in the Kathmandu Valley. The sample were collected from 240 taps (municipal supply) , 240 wells (2-20 m depth) and 240 deep tube-wells (10-35m depth) in 2021 covering four seasons; spring (March-May), summer (June-August), autumn (September-November) and winter (December-February). Water samples were normally examined within 2-6 hours of collection using the centrifugation method (2,500 r/min for 1-2 minutes) followed by light microscope to detect cysts and oocysts of the different parasites. Direct smears were examined under the light microscope at 100x magnification. Lugol's iodine stain and Modified Ziehl-Neelson Staining were applied to conform the presence of protozoan cysts and oocysts. Analysis of the samples was conducted in laboratory of Nepal Environmental and Scientific Services Pvt. Ltd. Prasuti Griha Marg, Kathmandu . The data were and analyzed in Microsoft Excel. Chi-square test was applied to test the significant differences among the areas, seasons and type of water. The diagrammatic presentations and some statistical analyses were conducted using Microsoft Excel 2016, while R Statistics (version V4.3.1)(R Core Team, 2023) was employed to perform the Chi-square test, which evaluated significant differences between area, seasons, and water types.

Results and Discussion

A total of 720 water samples were collected from houses in Tripureshwor, Baneshwor, Bansbari and Chhetrapati, Kathmandu Valley. The samples were collected throughout 2021, covering four seasons, spring (March-May), summer (June-August), autumn (September-November) and winter (December-February). In the spring season, the total number of protozoa was recorded only 6 but in the summer season, there were 17 positive cases. In the autumn there were 10 positive cases and in the winter it was 9 positive cases. In the spring season, a total 180 samples were taken; 60 samples were tap water, 60 samples of tube- well water and 60 samples of dug -well water. Out of 60 tap water samples, 1 (1.66%) were positive cases. However, in the tube-well water samples only 1(1.66%) positive cases was recorded out of 60. In the dug- well water samples 4(6.66%) positive cases were identified out of 60 (fig.2). In the autumn season, total 180 samples were taken 60 samples of tape water, 60 samples of tube- well water and 60 samples of dug –well water. Out of the 60 tap water samples, 4 (6.64%) were positive cases. In the tube-well water samples 4(4.64%) positive cases were identified out of 60. However, in the dug- well water samples 17 (28%) positive cases were identified out of 60 (fig.2). In the autumn season, total 180 samples were taken 60 samples of tap water, 60 samples of tube- well water, and 60 samples were from dug-well water. There were no positive cases in the tap water samples. However, in the tube-well water samples 3(5%) positive cases were recorded out of 60. In the dug-well water samples, 7 (11.66%) positive cases were identified out of 60 (fig. 2). In the winter season, a total 180 samples were taken 60 samples of tap water, and 60 samples of tube-well water, and 60 samples of dug –well water. Out of 60 tap water samples, there were no positive cases. However, in the tube-well water samples 3(5%) positive cases were recorded out of 60. In the dug-well water samples, 9 (15%) positive cases were identified out of 60 (fig.2). Chi square test suggest that there is no significance difference of positive samples among different locations with Chi square value = 0.30341, df = 3, p- value=0.9594. The similar test among seasons indicate significance difference with Chi square value = 6.574, p-value=0.086. However notable significance difference found in different types of water (tape water, tube-well water, and dug-well water) with Chi- square value = 17.75, df = 2, p-value = 0.0001399. On the basis of presence of pathogen and non pathogen out of 240 tap water samples, only 1(0.41%) pathogenic and 5(2.08%) of non pathogenic protozoa were detected. In the case of tube-well water samples out of 240, a total 2 (0.83%) pathogen and 9 (3.75%) non- pathogenic protozoa were detected. However, in the dug-well water samples out of 240, a total 12 (5%) pathogenic protozoa and 34 (14.16%) were non pathogenic protozoa were identified (fig.3). Regarding species, the maximum number of protozoa were *Amoeba proteus* with 32 (4.44%) identified. This was followed by *Paramecium caudatum*, with 24 (3.3%), *Giardia lamblia* was 8 (1.11%), *Entamoeba histolytica* with 6 (0.83%) and *Balantidium coli*, with 1 (0.13%) identified (fig.4). Comparatively the summer season shows more number of protozoa than the others seasons.

Table.1: Number of Protozoa in different site during the different seasons.

Season	Site	Tap water	Tube-well water	Dug- well water	Sub total	Seasonal total
Spring	Tripureshwor		-	1	1	6
	Baneshwor	-	-	1	1	
	Bansbari	-	1	1	2	
	Chhetrapati	1	-	1	2	
Summer	Tripureshwor	1	1	2	4	17
	Baneshwor	1	1	2	4	
	Bansbari	1	1	2	4	
	Chhetrapati	1	1	3	5	
Autumn	Tripureshwor	-	1	2	3	10
	Baneshwor	-	1	1	2	
	Bansbari	-	1	1	2	
	Chhetrapati	-	-	3	3	
Winter	Tripureshwor	-	-	2	2	9
	Baneshwor	-	1	2	3	
	Bansbari	-	1	1	2	
	Chhetrapati	-	1	1	2	
Total		5	11	26	42	42

Table. 2: Type of Protozoa in different site during the different seasons.

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Season	Site	Tap water	Tube-well water	Dug- well water	Total
Spring	Tripureshwor	Nil	Nil	*A. proteus, P. caudatum*	6 Samples
	Baneshwor	Nil	Nil	*A. proteus, G. lamblia*	
	Bansbari	Nil	*A. proteus*	*A. proteus, P. caudatum*	
	Chhetrapati	*Amoeba proteus*	Nil	*A. proteus, P. caudatum*	
Summer	Tripureshwor	*A. proteus*	*A. proteus P. caudatum*	*E. histolytica, A. proteus P. caudatum, A. proteus*	17 Samples
	Baneshwor	*A. proteus*	*A. proteus, P. caudatum*	*E.histolytica G. lamblia A.proteus,P.caudatum*	
	Bansbari	*A. proteus, Paramecium caudatum*	*A. proteus, P. caudatum*	*E.histolytica, G.lamblia A. proteus, P. caudatum G. lamblia, A. proteus*	
	Chhetrapati	*A. proteus Giardia lamblia*	*Entamoeba histolytica, G. lamblia*	*E. histolytica, A. proteus Balantidium coli, A. proteus, P. caudatum, E. histolytica*	
Autumn	Tripureshwor	Nil	*A. proteus*	*A. proteus, P. caudatum, P. caudatum*	10 Samples
	Baneshwor	Nil	*P. caudatum*	*A. proteus, P. caudatum*	
	Bansbari	Nil	*A. proteus*	*A. proteus, P. caudatum*	
	Chhetrapati	Nil	Nil	*A. proteus, P. caudatum, G. lamblia*	
Winter	Tripureshwor	Nil	Nil	*A. proteus, P. caudatum, G. lamblia. A. proteus*	9 Samples
	Baneshwor	Nil	*A. proteus, P. caudatum*	*A. proteus, P.caudatum*	
	Bansbari	Nil	*A. proteus, P. caudatum*	*A. proteus, P. caudatum*	
	Chhetrapati	Nil	*A. proteus, P. caudatum*	*A. proteus, P. caudatum*	
Total		5	11	26	42

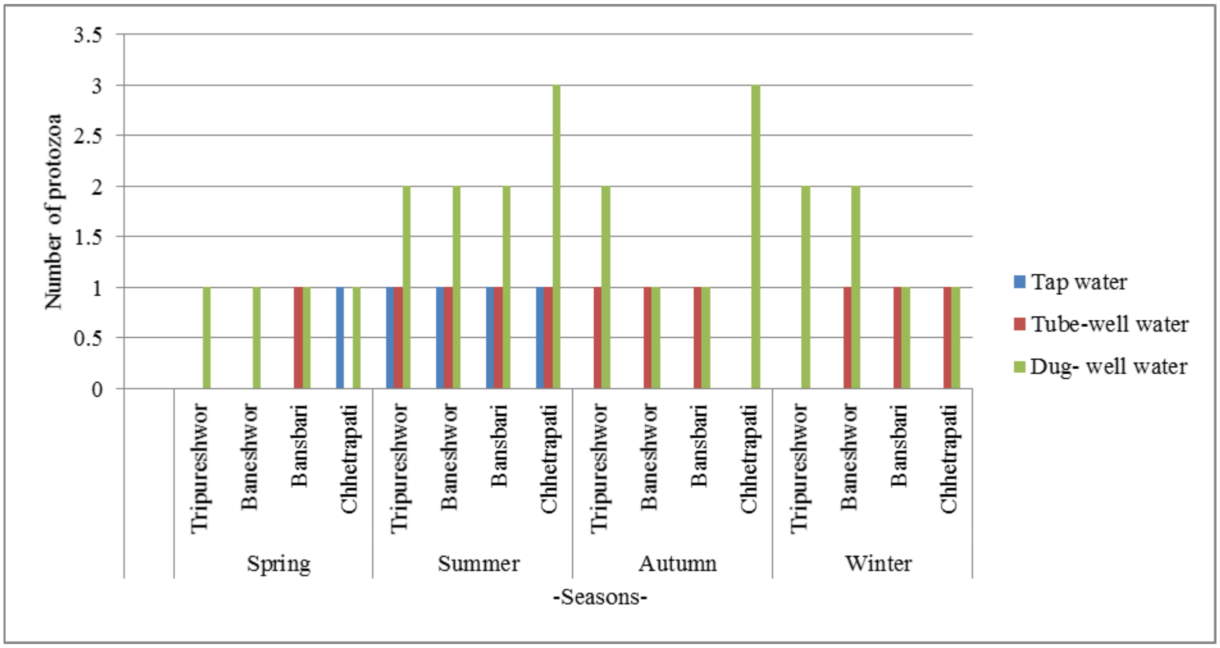
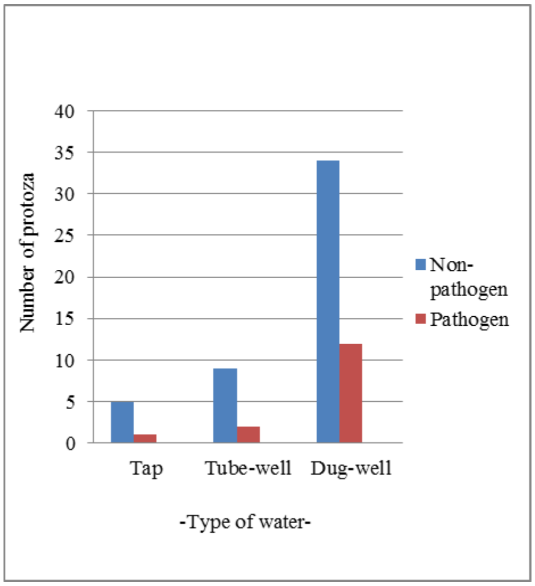


Figure. 2: Number of positive cases during four seasons.



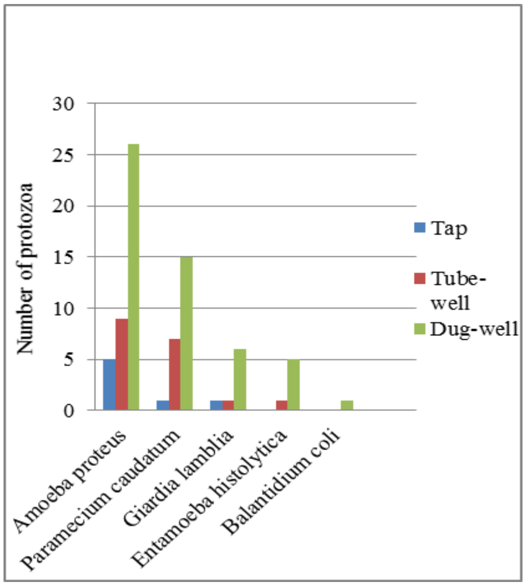


Figure.3: Number of pathogens and non-pathogens cases. **Figure.4**: Detection of different species during four seasons.

The term "seasonal protozoa" is not a specific scientific classification but may refer to protozoa that exhibit seasonal patterns in their abundance, activity, or life cycle. Seasonal changes, such as temperature, sunlight, and nutrient availability, can influence the distribution and dynamics of protozoa populations in various environments. The maximum number of pathogenic protozoa was found in dug wells, with six identified, while the least number was reported from tap water, with only two identified. The presented results indicate a seasonal variation in the occurrence of protozoa in water samples collected from different locations in Kathmandu Valley. A notable finding is that the summer season showed a higher number of positive samples for protozoa compared to the winter season. Studies by (Shanan *et al*., 2015) suggest that the findings align with or are consistent with existing literature on the seasonal variation of protozoa in water samples. The observed variation in the occurrence of protozoa between the summer and winter seasons in Kathmandu Valley could be influenced by a combination of environmental, climatic, and anthropogenic factors. In aquatic ecosystems, the abundance of certain protozoa may vary seasonally in response to factors like water temperature, sunlight, and nutrient levels. These variations can impact the ecological balance of the ecosystem and influence the interactions between protozoa and other organisms. In soil environments, the activity and population dynamics of soil protozoa can also be influenced by seasonal changes. Factors such as temperature, moisture, and organic matter availability can affect their growth, reproduction, and overall activity. It is important to note that different species of protozoa may respond differently to seasonal variations, and their life cycles and behaviors can be adapted to specific environmental conditions (Heitzinger *et al*., 2022). The comparison of protozoa in different water sources within the study area reveals notable differences in their abundance, with the maximum number recorded in dug wells and the least in tap water. The suitable reason could be dug wells are often more susceptible to contamination from surface water, runoff, and environmental debris. The presence of organic matter and nutrients in these wells may create a suitable environment for the growth of protozoa. In contrast, Municipal tap water is typically treated by chlorination, filtration and disinfectation to meet safety standards, and the lower number of protozoa may be attributed to water treatment processes that reduce microbial contamination. The identification and prevalence of isolated protozoa in the study show variation in the protozoans population. The identified protozoa are *Giardia lamblia* (2.22%), *Paramecium caudatum* (3.88%), *Entamoeba histolytica* (1.94%), *Amoeba proteus* (5.83%), and *Balantidium coli* (0.27%), *Giardia lamblia* (2.22%) is a common waterborne parasite associated with gastrointestinal infections (Omarava *et al*., 2018). A total of 12 out of 80 (15%) water samples were positive for different types of amoebic growth obtained from Magat and Ipo watersheds were positive for different types of amoebic growth (Masangkay *et al.*, 2022). Its presence in the water samples may indicate fecal contamination, often originating from an infected human or animal waste. Factors such as inadequate sanitation and improper waste disposal practices may contribute to its occurrence.

Furthermore, *Paramecium caudatum* is a free-living ciliate protozoa (Chatterjee 2022). Its presence suggests the natural occurrence of aquatic microorganisms. The abundance of *Paramecium caudatum* might be influenced by factors such as nutrient availability, water temperature, and the presence of suitable microhabitats. *Entamoeba histolytica* is a parasitic amoeba known to cause amoebic dysentery (Osman *et al*., 2016). Its presence in water samples is concerning, as it may indicate contamination with fecal matter containing the cysts of this pathogenic amoeba. Poor sanitation and sewage contamination are potential contributors. *Amoeba proteus* is a free-living amoeba commonly found in freshwater environments. Its higher prevalence may be attributed to its natural presence in aquatic ecosystems, thriving in environments with organic matter. The abundance might be influenced by water quality and availability of food sources. Finally, *Balantidium coli* is a ciliate protozoan that can infect the intestines of humans and animals. Its low prevalence suggests a relatively rare occurrence in the water samples (Zinyowera *et al*., 2014). Human or animal fecal contamination may be a potential source, emphasizing the importance of addressing sanitation issues. The result also shows variation in the number of pathogenics and non-pathogenics protozoa in different water sources, with an overall higher prevalence of non-pathogenic protozoa. The finding suggests that different water sources, such as, dug wells, tap water exhibit varying numbers of both pathogenic and non-pathogenic protozoa. This variability may be influenced by the specific characteristics of each water source, including factors such as contamination sources, water treatment, and environmental conditions. The higher prevalence of non-pathogenic protozoa suggests that the majority of protozoa present in the water samples are not directly harmful to human health (Medeiros *et al*., 2015). Non-pathogenic protozoa may include free-living species that are part of the natural microbial community in water ecosystems. The highest number of pathogenic protozoa being reported from dug wells may be due to the vulnerability of these types of water sources to various contaminants. Dug wells are often more exposed to surface water runoff and potential sources of fecal contamination, leading to a higher likelihood of harboring pathogenic protozoa. The lower number of pathogenic protozoa in tap water could be due to water treatment processes(Omarava *et al.*,2018; Almeida *et al.*, 2020). Municipal water treatment facilities typically employ methods such as chlorination and filtration to eliminate or reduce microbial contaminants, including pathogenic protozoa. This result suggests that water treatment measures are effective in minimizing the presence of harmful protozoa in tap water showed similar observations in previous research. According to the WHO, lack of sanitation, lack of safe water supply cause 80% all diseases in developing countries. In Nepal, morbidity and mortality rates from water borne diseases are considered high, especially among children five years and those who live under poor sanitation and in rural places. The data collected from the different study sites may be beneficial for the public in this region and around the world particularly for those whose water sources are tap, tube-well and dug well.

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