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BACTERIOLOGICAL AND PARASITOLOGICAL ANALYSIS OF SELECTED WELL WATER IN KANGIWA TOWN, KEBBI STATE

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ABSTRACT

Wells can be susceptible to pollution because of harmful human activities, such as the practice of open defecation or the location of wells near pit latrines. Additionally, improper handling or the use of contaminated containers during the fetching of water can also contribute to well water pollution. In view of this, this study was carried out to analyse the

Introduction

Water is essential for life as without it, there would be no life. About 70% of the earth's surface is covered by water only a tiny fraction of this water is available to us as fresh water. About 97% of the total water available on earth is found in oceans and is too salty for drinking or irrigation. The remaining 3% is fresh water. Of this, 2.997% is locked in ice caps or glaciers. Thus only 0.003% of the earth's total volume of water is easily available to us as soil moisture, groundwater, water vapour, and water in lakes, streams, rivers, and wetlands (WHO, 2020). Water that is found in streams, rivers, lakes, wetlands, and artificial reservoirs is called surface water. Water that percolates into the ground and fills the pores in soil and rock is called groundwater. Porous water-saturated layers of sand, gravel or bedrock through which groundwater flows are called aquifers (Bitton, 2018).

Water is an inorganic universal solvent that contains only hydrogen and oxygen atoms. Water has the chemical formula of H_2O (WHO, 2020). Good quality water is

bacteriological and parasitological composition of water samples collected from well water sources in Kangiwa town of Kebbi State, Nigeria. A total of fifteen (15) distinct well water samples were employed in the present study. Standard protocol was followed to conduct basic plate counts, determine the total number of coliform bacterial counts, and perform parasite investigations. The findings of the study indicated that the bacterial load exhibited a range of 5.3 x10-4cfu/ml to 2.0 x10-5cfu/ml, whilst the total coliform counts varied from 3 to 160 MPN of coliforms per 100ml. Additionally, the study revealed the presence of *Escherichia coli* (*E. coli*) in three well-water samples. The findings also revealed cysts of two species of parasites, namely *Naegleria fowleri* and *Endolimax nana*. There was a significant correlation ($P<0.05$) between the location of the well and the occurrence of the parasites. The results indicated that well water samples obtained from a well situated in areas with potential for contamination exhibited the highest incidence, reaching 100%. The proportion of well water samples collected from a non-contaminated location was found to be the lowest, at 16.7%. The research findings underscore the significance of disseminating public health education regarding the potential hazards associated with the consumption of untreated water, particularly from well sources.

Keywords: Bacteria, Parasites, Kangiwa, Wells, Pollution, Public Health.

odourless, colourless, tasteless, and free from faecal pollution and chemicals in harmful amounts (WHO, 2017). Access to safe drinking water is a problem facing a large proportion of the inhabitants of developing nations (UNICEF, 2015). Water is inevitably the source of life on earth and without it, life sustenance becomes difficult. Water of good quality is of basic importance to human physiology, and man's continued existence depends very much on its availability (Okonko *et al*., 2008). Drinking water can be obtained from sources such as borehole/tap water, well water, dams, rivers, streams, lakes, municipal water, and rainwater. Every community of humans, animals, or plants has one or more of these as their source of drinking water (Chollom *et al*., 2013). Water is an essential component of all cells and a requirement for life. It represents about 45% to 95% of living cell (Lamikanra, 2019). The drinking water must be safe, and it is considered polluted if it contains toxic substances or pathogens (Shittu *et al*., 2018).

A well is an excavation or structure created in the ground by digging, driving, or drilling to access liquid resources, usually water. The oldest and most common kind of well is a water well, to access groundwater in underground aquifers (Adeyemi *et al*., 2007). The well water is drawn up by a pump, or using containers, such as buckets or large water bags that are raised mechanically or by hand. Water can also be injected back into the aquifer through the well. (Oluwasanya *et al*., 2011).

Microorganisms enter drinking water via humans' and animals' intestinal secretions in areas where sanitation conditions are poor or absent. When found in drinking water, microorganisms constitute a real indication that such waters should not be used for human consumption if these contaminants are found in excess of the maximum permissible level (1×10^2 CFU ml^{-1}) (Obiri and Jones, 2011; WHO, 2018).

Coliform bacteria are rod-shaped, non-spore-forming, Gram-negative, oxidase-negative, aerobic, or facultatively anaerobic bacteria that are able to grow in the presence of bile salts or other replacement surface active agents having an analogous growth inhibitory effect and that ferment lactose with gas and acid (or aldehyde) production within 48 h at 37 ± 1 C. Coliform bacteria include total coliforms, which are those bacteria that can be found everywhere in nature. They can be found in the soil, air, and water; Faecal coliforms are those bacteria that live in the intestinal tract of man and other warm-blooded animals. Their presence in water indicates recent faecal contamination of that water by man or other warmed-blooded animals (Schnabel, 2015). Water-borne parasitic infections are considered a threat and of public health importance especially in developing countries (Oyedeji *et al*., 2010).

Pollution of groundwater with pathogenic microorganisms is generally believed to be a consequence of migration or introduction of faecal material either from humans or animals into the subsurface. Faecal pollution can reach groundwater from many concentrated pond sources such as cesspools, landfills, leaking sewer lines, and filled septic systems (Sadallah and Al-Najar, 2014). These microorganisms induce water-borne parasitic infections such as schistosomiasis, giardiasis, cyclosporiasis, and amoebiasis (Chollom *et al*., 2013). Parasitic infections affect work and productivity as they are usually associated with a diminished capacity to carry out physical work (Chollom *et al*., 2013).

Despite the promotion of public health and improvement of lifestyle in recent years, parasitic diseases have remained a public health problem in many parts of the world. Water is a major source for acquiring several pathogenic agents including bacteria, viruses, and parasites. These biological agents can be transmitted via unsanitized water. According to the World Health Organization reports, more than 80 infectious diseases can be transmitted by water (Ayaz *et al*., 2011)

Well water serves as a source of possible portable water for people in rural areas. However, these wells are not free from being polluted due to detrimental human activities such as open defecation or siting well close to the pit latrines, poor handling poor handling, or fetching containers. Moreover, pollution of ground water is one of the sources of transmission of various waterborne diseases such as cholera, typhoid, dysentery, worm infection, polio, hepatitis etc. Such types of diseases are mainly related to the water, which is mixed up with microplastics, soil contaminants, and other various sources of pathogenic microorganisms (Ritabrata, 2019). It has been postulated that about 60% of the total diseases of humans are waterborne (Oluwasanya, 2019). Kangiwa suffers severe drinking water crises, particularly in the dry seasons every year (Sani *et al*., 2018). So, the residents are forced to use the water from the different nearly available natural spouts, wells, rivers, ponds, etc. to fulfil their works related for different purposes. Also, most people in Kangiwa use well water as their source of drinking water (Ritabrata, 2019). The growing imbalance between supply and demand has led to chronic shortages and competition that have resulted in pollution and environmental degradation (Schnabel, 2015). Apart from quantitative shortages, the quality of drinking water is becoming a serious public health issue for the village people (Nwabor *et al.,* 2016). In addition, most research works on waterborne diseases are limited to bacteriological analysis to determine the Coliform bacteria to the neglect of parasites (protozoans and helminths) which also cause waterborne diseases.

Based on studies carried out on water worldwide, reports show that water possesses diverse application and have been used for day-to-day activities by human and other animals. However, to the best of our knowledge, very few reports are available on bacterial and parasitic contamination of water in the study area so far. In this light, this study was conducted to analyse the bacteriological and parasitological status of water samples obtained from well-water in Kangiwa town of Kebbi State, Nigeria. The study serves as baseline information on the sanitary status of well water in the study area. It also suggests possible control measures to safeguard the health of the community members by protecting well water from microbial contamination.

MATERIALS AND METHODS

Study Area

This study was carried out in Kangiwa town, Arewa Dandi Local Government Area, Kebbi State, Nigeria. Kangiwa is a commercial, educational, and religious city. It is in northwestern Nigeria and located on Latitude and longitude of 12^{0}33`12``N and 3$^{0}$49`5``E respectively with a postcode 861106 (Mamma, 2020).

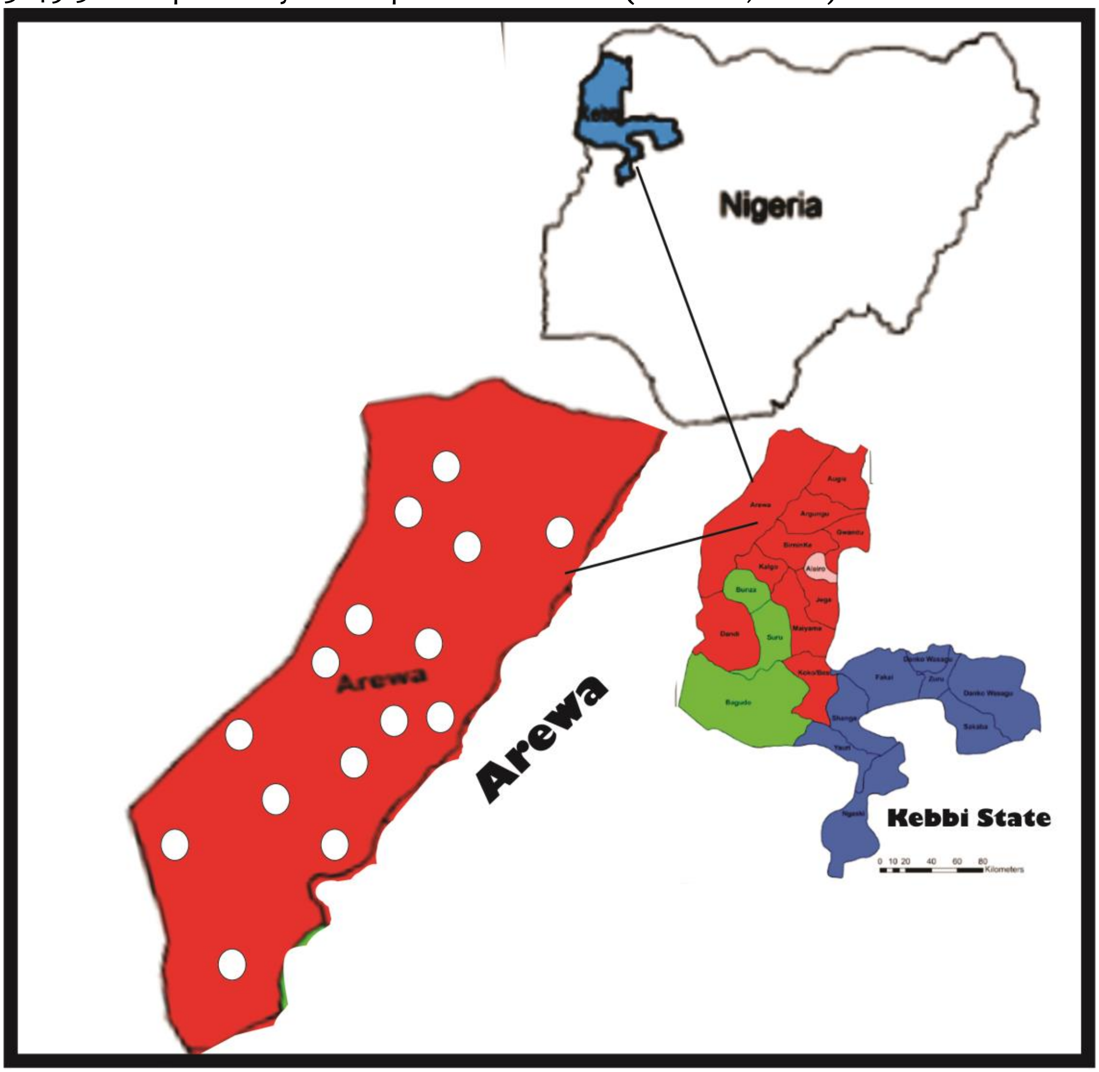

Figure 1: Map showing study area and location of sample collection (Mamma, 2020).

Ethical consideration

This study's approval was obtained from the ethical committee of Kebbi State Ministry of Health. Also, consent was obtained from various community heads where the investigation was carried out. The purpose and the proceedings involved in the study were explained to them.

Sample size

The sample size was determined according to Ugya *et al*. (2015). Water samples were collected from fifteen (15) different wells based on the division of Kangiwa town into different areas.

Sample Collection

Water samples were obtained by using a clean fetching bowl to draw out water. The first three bowls fetched were discarded, while the fourth sample was aseptically poured into a sterile sample bottle (Ugya *et al*., 2015)

Standard plate counts

The standard plate count was carried out to provide an estimate of the total number of bacteria in each of the samples that would develop into colonies during the period of incubation on Nutrient agar and Eosine methylene blue Agar plates. This test detects a broad group of bacteria including the pathogenic, non-pathogenic, and opportunistic pathogens.
Serial dilution of the sample in phosphate buffered dilution water was prepared (to prepare 0.1 ml of sample). The prepared media were allowed to cool to about 45°C before they were added to the dilution factors. The plates were incubated at 37°C for 24 h in an inverted position to prevent condensation from the lid to the agar, after which the number of colonies formed was counted. The acceptable value of the total number of Colony Forming Units (CFU) during the plate count for potable water was a total of less than 10^2 per ml.

Total number of Coliform Bacterial Count

Serial dilution of the sample in phosphate buffered dilution water was prepared (to prepare 0.1 ml of sample). The appropriate volumes of sample and diluted sample were pipetted into the tubes of the medium. The sample and the medium were shaken gently to mix them. The tubes were labelled with the sample numbers and the dilution and volume were added. The two batches of the tubes were incubated at 37◦ C for 24-48 hours (for the detection of total coliforms) and the others were incubated at 44°C for 24-48 hours (for detection of thermotolerant). After 24 hours the tubes which showed growth (turbidity and gas production or colour change) were regarded as positive. The number of positive tubes at each dilution was recorded, and then the tubes were returned to

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the incubator and re-examined after 48 hours of incubation. The pattern of positive results was compared with the Most Probable Number (MPN). The plates showing 30-300cfu/ml were counted (Mahmood *et al*., 2005)

Preparation and Isolation of parasites

One hundred millilitres of water samples were treated with 1g calcium carbonate and left for 2 h for water particles to settle at the bottom of the bottle. The supernatants were carefully decanted while the sediments were dissolved by adding 1ml of hydrogen sulphate (iv) acid and allowed to stand for another 2h. The dissolved sediments were centrifuged at 3000rpm for 15 minutes. The supernatants were discarded while the sediments were smeared on grease-free slides (Cheesebrough, 2016).

With the use of a pipette, the filtrate was obtained and used to prepare two slides for each sample: one was stained with Lugol's iodine and viewed directly under the microscope while the other was stained with acid-fast stain using Ziehl-Nelson's carbon fuchsin stain (FERN; certified suitable for staining acid-fast organisms in smears and tissue section) using the cold carbon fuchs in technique. A smear of the sediment was made on grease-free glass slides and were allowed to air-dry for a few minutes, and the smeared slide was fixed with methanol for 3mins to preserve the parasite on the slide after which the carbon fuchsin was used to stain the slides for 15min and rinsed under tap water. One percent of alcohol was used to decolorize the slide for 15 seconds, and the slides were again rinsed in tap water to remove excess acid alcohol (Cheesebrough, 2016).

Identification of Parasites

The isolated parasites on slides were counter-stained with 0.4% methylene blue for 60 seconds and rinsed under tap water, air dried, and examined under × 10 and × 40 objective lenses of a binocular light microscope (Cheesebrough, 2016). Images of all parasites viewed under the microscope were taken using a digital camera of high-resolution power and saved accordingly after which they were identified using taxonomic keys according to Cheesebrough (2016). And Bright *et al*. (2010). Platyhelminthes were identified using sizes and shapes of scolex and suckers among others (Ugya *et al*., 2015).

Determination of Well's Sanitary Condition

Physical inspection was conducted to determine whether each well was situated near a sock-away, gutter, pit latrine, or gutter. Furthermore, the determination of container suitability was contingent upon whether a single container or multiple vessels were employed for water retrieval. Additionally, an assessment of the environment was conducted to determine whether community members engaged in open defecation.

RESULTS

The results revealed a high bacterial load in all the water samples examined. The bacterial load ranged from 5.3 $x10^{-4}$cfu/ml to 2.0 $x10^{-5}$cfu/ml. Water samples collected from Rijiyar Shiyar Gidan Wan Muza and Rijiyar Kuka had the highest bacterial loads of 2.0 $x10^{-5}$cfu/ml each, while Rijiyar Shiyar Ketta water sample had the least bacterial loads of 5.3 $x10^{-4}$cfu/ml (Table 1). The presumptive test which indicates the total coliform counts for well-water showed that Rijiyar Kuka water samples had the highest total coliform counts of 160 MPN of coliforms per 100ml. while, Rijiyar Shiyar Kotu, Rijiyar Hodi, Rijiyar Maigi, and Rijiyar Gidan Barebari had the least total coliform counts of 3 MPN of coliforms per 100ml (Table 2). The presence of feacal coliforms was confirmed in all the samples examined. However, *E. coli* was confirmed in only three (3) well water samples collected from RijiyarYa'ari Babbaa, Rijiyar Kuka and Rijiyar Kuri. The gram reaction results confirm that all the Coliform bacteria were gram-negative (-) (Table 3). Table 4 shows the species of parasites recovered from sampled well waters. Four water samples were contaminated with parasites and indicated the presence of cysts of *Naegleria fowleri* and *Endolimax nana*. The occurrence of parasites in relation to the depth of the well is shown in Table 5. There was no significant association (P>0.05) between the depth of the well and the occurrence of the parasites. However, it showed that well water samples with 23.8$\pm$2m depth had the highest occurrence of 42.9%; followed by well water samples with 68.19$\pm$2m with 33.3%, while well water samples with 63.9$\pm$2m depth had the least occurrence with 20%. In relation to well's sanitary condition, the results showed significant association (P>0.05) between the location of the well and the prevalence of the parasites. It showed that well water samples collected from wells located in contaminated areas had the highest prevalence of 100%, while well water samples collected from wells situated in hygienic areas had the least parasite occurrence of 16.7% (Table 5).

Table 1: Total plate count of bacteria on the samples

S/N of Wells	Samples	Dilution Factor	No. of colony	Colony forming Unit/ml
1.	Rijiyar gidan mai arewa	10^{-3}	60	6.0 $x10^{-4}$

2.	Rijiyar yar gabas	10^{-3}	100	1.0 $x10^{-5}$
3.	Rijiyar Shiyar ketta	10^{-3}	53	5.3 $x10^{-4}$
4.	Rijiyar Kara	10^{-3}	173	1.73 $x10^{-5}$
5.	Rijiyar shiyar gidan wan muza	10^{-3}	200	2.0 $x10^{-5}$
6.	Rijiyar shiyar kotu	10^{-3}	100	1.0 $x10^{-5}$
7.	Rijiyar ya`ari karama	10^{-3}	81	8.1 $x10^{-4}$
8.	Rijiyar Honhon Nama	10^{-3}	94	9.4 $x10^{-4}$
9.	Rijiyar ya`ari babba	10^{-3}	174	1.74 $x10^{-5}$
10.	Rijiyar LEA	10^{-3}	100	1.0 $x10^{-5}$
11.	Rijiyar Hodi	10^{-3}	69	6.9 $x10^{-4}$
12.	Rijiyar Kuka	10^{-3}	200	2.0 $x10^{-5}$
13.	Rijiyar Maigi	10^{-3}	69	6.9 $x10^{-4}$
14.	Rijiyar Kuri	10^{-3}	160	1.6 $x10^{-5}$
15.	Rijiyar Gidan barebari	10^{-3}	161	1.61 $x10^{-5}$

Table 2: Presumptive Test

S/N of Wells	Samples	10ml	1ml	0.1ml	MPN/index/100ml
•	Rijiyar gidan mai arewa	0	0	0	3
•	Rijiyar yar gabas	0	2	0	6.2
•	RijiyarShiyar ketta	3	2	1	150
•	Rijiyar Kara	1	2	0	11
•	Rijiyar shiyar gidan wan muza	3	1	2	120
•	Rijiyar shiyar kotu	0	0	0	3
•	Rijiyar ya`ari karama	2	1	1	20
•	Rijiyar Honhon Nama	1	2	0	11
•	Rijiyar ya`ari babba	3	1	2	120
•	Rijiyar LEA	1	2	0	11
•	Rijiyar Hodi	0	0	0	3
•	Rijiyar Kuka	3	1	3	160
•	Rijiyar Maigi	0	0	0	3
•	Rijiyar Kuri	3	2	1	150
•	Rijiyar Gidan barebari	0	0	0	3

Table 3: Confirmatory and completed test for faecal Coliform Bacteria

S/N of Wells	Samples	Coliform	E. coli	Gram staining
•	Rijiyar gidan mai arewa	+	-	-
•	Rijiyar yar gabas	+	-	-
•	RijiyarShiyar ketta	+	-	-
•	Rijiyar Kara	+	-	-
•	Rijiyar shiyar gidan wan muza	+	-	-
•	Rijiyar shiyar kotu	+	-	-
•	Rijiyar ya`ari karama	+	-	-
•	Rijiyar Honhon Nama	+	-	-
•	Rijiyar ya`ari babba	+	+	-
•	Rijiyar LEA	+	-	-
•	Rijiyar Hodi	+	-	-
•	Rijiyar Kuka	+	+	-
•	Rijiyar Maigi	+	-	-
•	Rijiyar Kuri	+	+	-
•	Rijiyar Gidan barebari	+	-	-

Table 4: Species of Parasites recovered from sampled well water.

S/N of Wells	Samples	Parasite recovered
i.	Rijiyar gidan mai	Not seen
ii.	Rijiyar yar gabas	Not seen
iii.	Rijiyar Shiyar ketta	*Endolimax nana* cyst
iv.	Rijiyar Kara	Not seen
v.	Rijiyar shiyar gidan wan muza	*Endolimax nana* cyst
vi.	Rijiyar shiyar kotu	Not seen
vii.	Rijiyar ya`ari karama	Not seen
viii.	Rijiyar Honhon Nama	Not seen
ix.	Rijiyar ya`ari babba	Not seen
x.	Rijiyar LEA	Not seen
xi.	Rijiyar Hodi	Not seen
xii.	Rijiyar Kuka	*Naegleria fowleri*
xiii.	Rijiyar Maigi	Not seen

xiv.	Rijiyar Kuri	*Endolimax nana*
xv.	Rijiyar Gidan barebari	Not seen

Table 5: Occurrence of parasites in relation to the depth of the well and their sanitary conditions

Parameter	Number of Samples	Number of Positive	Percentage (%)	P-Value
Depth				
68.19 ±2m	3	1	33.3	X^2= 0.69 p-value=0.710
23.8 ±2m	7	3	42.9	
63.9±2m	5	1	20.0	
Total	**15**	**5**	**100.0**	
Sanitary condition				
Uncontaminated	12	2	16.7	X^2= 7.500 p-value=0.006
Contaminated	3	3	100.0	
Total	**15**	**5**	**100**	

DISCUSSION

The Total plate count of bacteria on the water samples examined indicated that bacterial contamination was significantly high, ranging from 5.3 x10^{-4}cfu/ml to 2.0 x10^{-5}cfu/ml. This is in consonance with the findings of Bello *et al*. (2013) who reported that all water samples from the well water were highly contaminated with bacteria and were above the permissible limit stated by WHO Standards. The higher bacterial loads in this study could be due to the proximity of the well to toilet and sock-away resulting in faecal contamination of the well water. It may also be due to the presence of dumping sites around the different wells examined, which may seep down during rainy season and contaminate the groundwater at the aquifer. The bacterial load in this study is higher than that obtained by Auta *et al*. (2021) who worked on the bacteriological and parasitological analysis of hand-dug well water in selected areas of Rigachikun community, Kaduna. It is also inconsistent with the findings of Orji *et al*. (2020) and Agwaranze *et al*. (2017) for well water analysed in Wukari, Nigeria.

The present study revealed that all sampled well water had varying numbers of coliform counts indicating high level of microbial contamination among well water samples examined. This could be due to the high level of contamination resulting from constant exposure and the lack of treatment. This is in agreement with the study of Shittu *et al*. (2018) in Abeokuta on well water and stream water and had Coliform counts ranged from 1-400 MPN of coliforms per 100ml. All the well waters examined had high coliform counts exceeding the standard given as zero total coliform per 100ml of water (EPA, 2012). The higher coliform count in this study could be attributed to the due to harmful human activities such as siting wells close to the pit latrines, poor handling of fetching containers and deposits of animal faecal matter around the wells.

The presence of faecal Coliform bacteria in all the samples examined could be attributed to the contamination with human wastes. For instance, *E. coli* was confirmed in some water samples, which is in line with the research of Zamxaka *et al*. (2014) who found similar coliform bacteria in dam water in South Africa. These organisms are important human pathogens associated with a variety of infectious diseases such as gastroenteritis, typhoid fever, dysentery, cholera, urinary tract infectious diseases (WHO, 2018).

The parasitological analysis revealed the presence of *Naegleria fowleri* and *Endolimax nana* in some sampled well water in the study area. *Naegleria fowleri* may cause a usually fatal infection of the brain called naegleriasis, primary amoebic meningoencephalitis (PAM), amoebic encephalitis/meningitis, or simple Naegleria infection (Sam-Wobo and Mariana, 2014). Infections most often occur when water containing *Naegleria fowleri* is inhaled through the nose (aspirated), where it then enters the nasal and olfactory nerve tissue, travelling to the brain through the cribriform plate. *Naegleria fowleri* cannot cause infection by swallowing contaminated water (Maikai *et al.,* 2018). Infections typically occur after swimming in warm-climate freshwater, although there have been cases in cooler climates such as Minnesota, US. In rare cases, infection has been caused by nasal or sinus rinsing with contaminated water in a nasal rinsing device such as a neti pot (Sanchez *et al.,* 2013)

Endolimax nana is transmitted through faecal-oral contamination of food or water. *Endolimax nana* cysts have been observed in drinking water from deep wells, and on raw consumed vegetables which have been suggested to be potential fomites (Sanchez *et al.,* 2013). However, *Endolimax nana* is considered a nonpathogenic commensal protozoon parasitizing the human colon (Sanchez *et al.,* 2013) These findings are in line with the results obtained by Vantsawa *et al*. (2020) in a study carried out on protozoan parasites associated with different water sources in Lokoja metropolis. The result is also in correlation with the report of Ani and Itiba (2015) who reported a high prevalence of parasites from drinking water sources in

Ebonyi State Nigeria. The high occurrence of parasites in the well water examined in this study may be attributed to unsatisfactory sanitary conditions associated with the wells. Specifically, it was observed that a significant number of wells were inadequately covered, located near latrines, and exposed to domestic animals such as chickens and goats that were observed feeding, drinking, and defecating in the vicinity of the wells. These factors are the likely primary sources of contamination for the wells. Agricultural activities such as the use of manure in which erosion could have washed some bacterial and parasitic contaminants present in manures into the stream could be another means through which the wells got contaminated. The findings of this study suggest a notable risk to the well-being of both humans and domestic animals that rely on the well water for drinking purposes, potentially resulting in the occurrence of waterborne disease epidemics such as diarrhoea, typhoid fever, amoebiasis, and various gastrointestinal illnesses.

Conclusion

The study assessed the bacteriological and parasitological quality of water samples from hand-dug wells in Kangiwa metropolis. Twelve wells were found to be free of *E. coli* contamination, while 10 showed no parasite presence. The water meets WHO standards, but three are unsafe. To minimize infection risk, treatment measures and proper sanitary practices must be implemented.

Recommendations

In the light of these findings, the following are recommended:

I. public health enlightenment on the risk of consuming untreated water, especially from Well water.

II. appropriate agencies should properly monitor the location and drilling of wells and boreholes by putting in place the correct measures and enforcing the right portable water policies.

III. Boiling of water for drinking, in addition to other methods (Household Water treatment), should be encouraged.

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