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MICROBIOLOGICAL POLLUTION FACTORS OF RURAL DRINKING WATER IN THE AGNEBY-TIASSA REGION, COTE D'IVOIRE

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ABSTRACT

Water is a vital resource for humanity, and its vulnerability to pollution is well known. Poor-quality drinking water can pose a health risk and affect the nutritional status of populations.The aim of this study was to assess the risks and factors involved in polluting well water intended for domestic consumption in rural Côte d'Ivoire. As regards the results of the work carried out, microbiological contaminants such as total coliforms, faecal coliforms, faecal enterococci, Escherichia coli, Clostridiums perfringens and Pseudomonas aeruginosa were identified and counted in most of the water samples analyzed. The quality of the water sampled varied significantly depending on the study locality and sampling period. The low level of sanitation, inadequate measures to protect and develop wells, lack of hygiene and unhealthy living conditions contributed significantly to the pollution of groundwater in the study area.

Keywords: Drinking water, Pollution, quality, health risk, rural areas

INTRODUCTION

Water is an indispensable resource for humans, who need it for their well-being. It is involved in many essential physiological functions such as digestion, waste elimination, absorption and thermoregulation [1]. In addition to its vital nature, water can also be a potential source of

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disease when it is subject to chemical or microbiological contamination that alters its quality and renders it undrinkable. Common water-related diseases include diarrhea, typhoid fever, dysentery and cholera [2]. The ingestion of waterborne pathogens that cause diarrhoea and parasitosis can lead to reduced nutrient absorption and, consequently, a deterioration in health and nutritional status [3]. According to the World Health Organization (WHO), 80% of diseases present on the earth's surface are waterborne, and drinking water is very often singled out for blame [4]. Worldwide, some 1.1 billion people do not have adequate access to safe drinking water. In Africa, 57% of the population has no access to potable water [5]. Particularly in Côte d'Ivoire, 35% of the population has no access to safe water [6]. Drinking water provision coverage has declined, due to damaged infrastructure weakened by years of underinvestment, conflict and rapid population growth [7]. The low rate of drinking water coverage and the growing needs of the population, particularly in rural areas, are forcing some of them to tap directly into groundwater through wells for their domestic water supply. These resources may be subject to contamination, and are not subject to any treatment or sanitary control by the user populations or the State. This situation is not without health consequences, as the quality of this water leaves much to be desired. Indeed, the results of several research studies on the subject, carried out in Ngaoundéré (Cameroon) [8], in Abidjan and Adiaké (Côte d'Ivoire) [9, 10] are unanimous on the chemical and bacteriological pollution of the surface groundwater captured by the wells. In these types of environment, there are many cases of diarrhoeal disease [11,12]. The poor quality of well water consumed by households is due to a number of factors, which to date have not been properly investigated. The aim of this study was therefore to fill this gap by assessing the risks and pollution factors of well water for domestic use in rural areas in the Agneby Tiassa region (Côte d’Ivoire).

MATERIALS AND METHODS

Study area

The study was carried out in four localities belonging to the Sub-prefecture of N'douci and three localities belonging to the Sub-prefecture of Gbolouville in Côte d'Ivoire. N'douci is a town in the south of Côte d'Ivoire. The population of the Sous-Préfecture of N'douci is estimated at 56,990 including 30,321 men and 26,669 women for a sex ratio of 113.7. Recognized as Gbolouville, the Sub-Prefecture is located in Binao-Boussoué. The latest population and housing census indicates a population figured at 28,854. Men are estimated at 16,594 and women at 12,260. The sex ratio is 135.4 [6]. The selected localities were Batera, Niamazra, Offa and Boussoukro in N'douci and Binao 2, N'drikro and Bandjoukro in Gbolouville.

Well selection

The water points to be tapped were selected on the basis of their spatial distribution and their relative importance for the user populations. The greater the volume of water drawn, the more important a water point is, and/or the more important the water drawn is for human consumption. The wells selected were hand-dug. Forty (44) wells were selected for this study. Table I shows the characteristics of the wells in the various localities. The water points were located using GPS. Each well was coded using the first letter of the well type, the first three letters of the locality and the chronological order of sampling, for each well type.

Table I: Description of some characteristics and environment of wells in different localities

Localities	Well condition	Operating mode	Probable source of pollution nearby
BATERA	-Presence of coping -No cover	Sump	Householdwaste
NIAMAZRA	- Presence of coping - No cover	Sump	Householdwaste
OFFA	-No coping - No cover	Sump	Water infiltration, agriculture, animals, household waste.
BINAO 2	-No coping - No cover	Sump	Water infiltration, agriculture, animals, household waste.
BOUSSOUKRO	- Presence of coping- No cover	Sump	Water infiltration, agriculture
N'DRIKRO	-No coping - No cover	Sump	Water infiltration, animals, Household waste.
BANDJOUKRO	- Presence of coping - No cover	Sump	Householdwaste

Well water sampling

In order to characterize the sanitary state of the well water, three sampling campaigns were carried out. The first took place in March, corresponding to the long dry season. The second took place in July, corresponding to the long rainy season, and the last in November, corresponding to the short rainy season in 2016 [13]. To comply with aseptic conditions, glass vials were used and

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sterilized in an autoclave in the laboratory. A quantity of 500 mL of water was collected per vial for each campaign. During transport to the laboratory, the samples were kept in a refrigerated cabinet (icebox) with ice packs and protected from solar radiation, as recommended by [14]. Vials containing water samples were labeled with information such as sampling site, GPS coordinates, date and time of collection and well code. Microbiological analyses were carried out as soon as the samples arrived at the laboratory.

Laboratory analysis

Microbiological analyses were carried out at the National Laboratory for Quality testing, Metrology and Analysis (LANEMA), in Côte d'Ivoire, according to ISO standard protocols and methods (Table II). Coliforms, Escherichia coli, Clostridium perfringens, Faecal Enterococci and Pseudomonas aeruginosa were identified and enumerated by filtering homogeneous 100 mL aliquots with or without dilution onto a membrane with a spore diameter of 0.45 µm. The membranes were then placed in selective media containing the nutrients and selective substances required for growth and identification of the bacteria of interest. They were then incubated. Microorganisms were identified and quantified by direct counting of bacterial colonies for a given medium and inoculum volume.

Table II: Different characteristics of the microorganisms tested

Microorganisms	Normative methods	Method used	Expression	Normative reference values
Total Coliforms	ISO 9308-1	Filtration on membrane	UFC/100mL	<1
FaecalColiforms	ISO 9308-1		UFC/100mL	<1
Escherichia coli	ISO 9308-1		UFC/100mL	<1
FecalEnterococci	ISO 7899-2		UFC/100mL	<1
Sulfito-reducing Clostridium	ISO 6461-2		UFC/20mL	<1
Pseudomonas aeruginosa	ISO 16268		UFC/100mL	<1

UFC: Unit Format Colonies

Statistical analysis

Before the statistical analyses of the microbiological results of the water samples, tests of normality (Shapiro-Wilk test) and homogeneity (Levene test) were carried out to check whether the data followed a normal distribution. The significance level of the probability value for each of these tests was P<0.05. The univariate tests used for the water sample results were non-parametric tests (the Kruskal-Wallis test and the Dunn multiple comparison test).Thus, the Kruskal-Wallis test followed by Dunn's multiple comparison test was used to check whether there were significant changes in water property between different sampling periods and different study localities using XL-STAT version 2015 software.

RESULTS

Microbiological quality of well water sampled in various localities

The results showed the presence of total coliforms in all water samples. Values ranged from 15 UFC/100mL in Niamazra in November to 9900 UFC/100mL in Boussoukro in March. Fecal coliform counts showed that 98% of water samples were contaminated with these germs. The lowest concentration, 4 UFC/100mL, was recorded in Batera in November, and the highest, 7500 UFC/100mL, in Boussoukro in March. Bacteriological analysis of water samples showed the presence of Escherichia coli in 93% of samples. Values ranged from 3 UFC/100mL in Niamazra in November to 2,100 UFC/100mL in Boussoukro in July. The search for and enumeration of faecal Enterococci showed the presence of these germs in all water samples. The minimum concentration, 6 UFC/100mL, was recorded in March in Batera, and the maximum concentration, 8900 UFC/100mL, was obtained in Offa in November. Microbiological analyses showed that 73% of water samples were contaminated with Clostridium perfringens. Values ranged from 3 UFC/20mL in N'drikro in March to 200 UFC/20mL in Boussoukro in July. Pseudomonas aeruginosa germs were identified in 89% of the water samples taken. Concentrations ranged from 5 UFC/100mL in N'drikro in July to 4300 UFC/100mL in Niamazra in March.

Inter-local variation in microbiological parameters

Table III shows the median values of microbiological parameters assessed in well water from different localities. The Kruskal-Wallis test indicates significant differences between the median values of the various microbiological parameters according to locality (p<0.05). The results of Dunn's post hoc multiple comparison test indicate significant differences between samples from the Boussoukro-Binao-Offa localities and those from other localities for total coliforms, faecal coliforms and Escherichia coli. The highest median values were observed in Boussoukro-Binao-Offa. The results of the post hoc test also show significantly higher median values for fecal enterococci in the Binao-Boussoukro localities than in the Bandjoukro-N'drikro-Offa-Niamazra-Batera localities. The medians for N'drikro and Bandjoukro are the lowest. The median values for Clostridium perfringens and Pseudomonas aeruginosa for the Bandjoukro-Binao 2-N'drikro-Batera locality groups and the Boussoukro and Offa localities are significantly different

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according to the Kruskal-Wallis comparison test. The highest median values were recorded in Boussoukro and Offa according to Dunn's multiple comparison test ($p<0.05$).

Seasonal variation in microbiological parameters

Table IV shows variations in total coliform, faecal coliform and Escherichia coli counts according to well water sampling periods. The results show significant differences according to well water sampling period. Dunn's post hoc test indicated a significant difference in median values for total coliforms, fecal coliforms and Escherichia coli, which were higher in July than in March and November ($p<0.05$). Similar results were observed for the median values of fecal enterococci. The Kruskal-Wallis test followed by Dunn's post hoc test indicated a significant difference between July and March and November ($p<0.05$). Median Clostridium perfringens values show significant variation ($p<0.05$) between March, with the lowest median, and July-November ($p<0.05$). Median Pseudomonas aeruginosa values showed no significant difference between sampling periods at the 5% threshold.

Table III: Median values for various microbiological parameters of well water by locality

Microbiologicalparameters	LOCALITIES						
	Bandjoukro	**Binao**	**N'drikro**	**Boussoukro**	**Offa**	**Niamazra**	**Batera**
Total Coliforms (UFC/100 mL)	1300^{a}	4800^{b}	1800^{a}	6800^{b}	5350^{b}	815^{a}	2700^{c}
FaecalColiforms (UFC/100 mL)	400^{a}	2000^{b}	210^{a}	3900^{b}	1700^{b}	390^{a}	960^{a}
Escherichia coli (UFC/100 mL)	55^{a}	795^{b}	90^{a}	750^{b}	450^{b}	45^{a}	120^{a}
FecalEnterococci (UFC/100 mL)	80^{a}	3450^{b}	340^{a}	2800^{b}	1950^{c}	1240^{c}	1200^{c}
Clostridium perfringens (UFC/20mL)	6^{a}	0^{a}	6^{a}	40^{b}	62^{b}	17^{ab}	4^{a}
Pseudomonas aeruginosa(UFC/100mL)	20^{a}	20^{a}	30^{a}	960^{b}	455^{b}	360^{b}	92^{a}

Kruskal-Wallis comparison test followed by Dunn's comparison test at 5% threshold. On the lines, the medians of the different parameters followed by a, b and c in different super scripts are significantly different ($P<0.05$).UFC : Unit Format Colonies

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Table IV: Median values of the different microbiological parameters of well water according to the sampling period

Paramètres microbiologiques	PERIODES D'ECHANTILLONNAGE		
	Mars	**Juillet**	**Novembre**
Total Coliforms (UFC/100 mL)	3100^a	5000^b	2500^a
FaecalColiforms (UFC/100 mL)	1100^a	2800^b	900^a
Escherichia coli(UFC/100 mL)	100^a	350^b	180^a
FecalEnterococci(UFC/100 mL)	1000^a	2200^b	850^a
Clostridium perfringens(UFC/20mL)	5^a	10^b	15^b
Pseudomonas aeruginosa(UFC/100mL)	10^a	20^a	25^a

Kruskal-Wallis comparison test followed by Dunn's comparison test at 5% threshold. On the lines, the medians of the different parameters followed by a, b and c in different super scripts are significantly different ($P<0.05$). UFC : Unit Format Colonies

DISCUSSION

Microbiological quality is a major health concern [15]. Microbiological germs have been identified and counted in water samples analyzed. The health impacts of this may be the high prevalence of diarrhea and intestinal parasitosis in children under 5, pregnant women and the elderly in the ecosystem studied. The abundance of pollution indicators such as coliform bacteria, Escherichia coli and faecal enterococci in well water is influenced by the total depth of the well, the thickness of the water column, the layout around the well and environmental conditions. Field observations show the presence of household garbage littering concession yards and streets, as well as wastewater from household cleaning, septic tanks and cesspits running off or forming stagnant puddles around wells and in uncleaned gutters. Microbiological pollution of Côte d'Ivoire's water resources has also been highlighted by several authors, such as [9,16]. This water is unfit for human consumption without physical or chemical treatment. The particular abundance of fecal enterococci and Escherichia coli in well water may indicate contamination by material of fecal origin. These species are very abundant in human and animal intestinal flora, and are also the only ones of strictly fecal origin. In fact, they are bio-indicators of fecal pollution. They are considered the best indicators of faecal contamination because of their specificity, and their seriousness lies in the fact that several other microbial, viral or parasitic pathogens, more or less serious, could be associated with these indicators in the human and/or animal organism and thus cause epidemics [17]. Although the concentrations of bacterial groups

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found in well water far exceeded the standard prescribed for drinking water, the analysis revealed relative differences between localities. Water from hand-dug wells in Boussoukro, Binao 2 and Offa was significantly more contaminated. This is undoubtedly due to the absence of appropriate sanitation systems to protect groundwater. Indeed, wells in these localities had more risk factors for contamination. The frequent presence of animals around wells in Binao 2 and Offa, who defecate and drink from them, and the lack of well maintenance in Boussoukro also contribute significantly to this pollution.

In Cameroon, studies have shown that poor maintenance of the well environment is also at the root of high concentrations of microbiological germs and pollution in some wells [18].

In terms of seasonal variation, germ concentrations were significantly higher in July (the main rainy season). The findings show that the latrines used by households are of the traditional type, also known as simple pit latrines. They can be understood as a simple excavation made in the ground to receive human excreta. The walls are not lined. The low cost of their construction certainly explains their adoption by households. Single-pit latrines are considered to be veritable cockroach nests, and breeding grounds for maggots, flies, mosquitoes and certain rodents. Because they're not watertight, these systems don't allow for proper decanting and processing of their contents. Also, in this area where rainfall is abundant, the rainy seasons reveal all the inadequacies linked to the precariousness of these facilities. Rainwater infiltrating the soil inevitably fills the pits. They very often overflow and, in the absence of drainage, spill the contents of the latrine onto the immediate surroundings.

Runoff water contributes to groundwater pollution by carrying numerous cells and particles into the soil and into the groundwater. Some of these particles may contain nutrients that can increase the growth rate of the bacteria present. Runoff can also be loaded with pathogenic microorganisms. Low levels of well protection and development encourage water pollution. Similar findings have been recorded at groundwater level in Kumassi (Ghana) [13] and in Korhogo (Côte d'Ivoire) [19]. Studies in Malawi also show that water from traditional wells during the rainy season has higher levels of contamination [17]. The microbiological quality of water intended for human consumption is a key health concern. The water consumed must be free from pathogenic organisms and hazardous chemical substances. Bacteriological analyses carried out on drinking water sources in Africa show that most wells and water sources are polluted. Drinking water and sanitation are crucial to the nutrition, health and dignity of every individual. Lack of access to safe, clean water for drinking and hygiene has long been recognized as a root cause of malnutrition, particularly among children [20]. The majority of cases of diarrhea of infectious etiology are caused by the consumption of food and the ingestion of contaminated drinking water [21]. In addition, infection affects nutritional status by reducing appetite and intestinal absorption of nutrients. Water is fundamental to food safety and nutrition.

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CONCLUSION

Variations in the different degrees of pollution showed that the sampling site and the sampling season have a significant effect on well water pollution. Lack of hygiene, absence of appropriate sanitation systems to protect groundwater, inadequate development and protection of wells, unhygienic practices and intense agricultural activity through the use of pesticides all contributed significantly to the different degrees of pollution observed. The contact of water with faecal matter and unhealthy substances poses a risk to the health of populations, one of the consequences of which is its impact on the nutritional status of individuals. Traditional wells are the most exploited water sources in the study area, although the data show that their sanitary condition is a cause for concern. It would be very interesting to extend this study to other localities in order to gain a better understanding of the factors contributing to microbiological pollution of well water.

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