Original Article

Comparative analysis of enteric bacteria in groundwater near poultry farm environments in Ijebu north, Ogun state, Nigeria

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Abstract

Background: Water-borne diseases are becoming more common because of inadequate water treatment procedures and unsanitary conditions.

Aim: This study involved performing comparative microbial analyses at five different sites to assess the presence of enteric bacteria in the soil and groundwater used by poultry farms.

Methods: Groundwater and soil samples obtained from four rural communities in Ijebu North region, Nigeria, were investigated using physicochemical and bacteriological analysis. Following the dilution plating method, enteric bacteria species were identified and characterized with the aid of colonial characteristics, gram-stain reaction, and biochemical tests

Results: The findings demonstrated high contamination of groundwater sources in these communities, the presence of enteric bacteria including Salmonella species (38%), Escherichia coli (27%), Klebsiella species (9%), Staphylococcus species (4%) and Shigella species (22%) were reported in water samples, soil sample analysis also revealed the presence of Enteric bacteria including Salmonella species (47%), Escherichia coli (24%), Klebsiella species (5%), Staphylococcus species (5%) and Shigella species (19%).

Conclusion: The analysis of water and soil samples provided strong evidence that soil can contaminate groundwater on poultry farms with enteric bacteria. To reduce the risk of enteric bacteria present, appropriate precautions such as proper poultry water treatment should be followed. [*Ethiop. J. Health Dev*. 2022; 36(4):00-00]

Keywords: Groundwater, Enteric Bacteria, Poultry

Introduction

Diarrheal diseases caused by enteric bacteria are a significant cause of fever, nausea, dehydration, and, in rare cases, death (1). Water is unsafe for domestic use when it contains enteric bacteria like *Escherichia coli*, *Salmonella*, *Shigella*, and others (2). Because of the third world's unsanitary conditions, dense population, and inadequate water treatment methods, the entire population is still largely at risk of infection (1). Sanitation and hygiene are major issues in most of Nigeria (3). It is noteworthy that water quality is declining as a result of pollution from various organic and inorganic substances (2). In addition to poor sanitation and hygiene, deteriorating water quality poses microbiological risks due to microbial contamination, which accounts for 85% of all water-borne illnesses (2). Because groundwater serves as the primary source of drinking water for most homes, its quality is crucial for human consumption (4,5). However, given the recurrent outbreak of water-borne illnesses in recent times, contamination is becoming more and more obvious (4). Through leaking sewage or direct discharge following human activities like urban, industrial, and agricultural activities, contaminated groundwater may be released into the environment (4). It has been reported that 54% of water-borne diseases were directly linked to untreated groundwater., Enteric bacteria such as *Salmonella spp* and *Shigella spp* were associated with high prevalence during these outbreaks (6).

Significant sources of groundwater pollution come from poultry farms (7). Poultry litter penetrates the groundwater by runoff, diffusion, or flooding during transfer and is reportedly colonized by pathogens, antimicrobials, and antibiotic-resistant genes (7,8). The enteric bacteria are rampant colonizers of poultry litter, for instance, *Salmonella spp* with similar serotypes implicated in human infections have been reportedly detected in poultry litter and are found to be highly dominant (8). The infiltration of the naturally uncontaminated groundwater by poultry litters and its constituents consequentially results in microbial pollution by enteric pathogens capable of causing waterborne-related disease outbreaks (9). Currently, there is a paucity of information on the microbial safety of groundwater near poultry farms in this locality; the purpose of this study is therefore to investigate the existence of enteric bacteria in groundwater in the environment of poultry farms in this area. The outcome of this study could raise possible health hazards that may be associated with the use of polluted groundwater in this locality. Information gathered could also aid water health policy on groundwater near poultry farms in the local government area.

MATERIAL AND METHODS

Study Design

The study design followed a purposive sampling technique. Groundwater samples were collected from wells in close proximity to poultry establishments.

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Study area

The samples were obtained from five different wells within a 30m-60m distance used by poultry establishments located in the four rural communities (Ago, Oru, Awa, Ijebu-Igbo) in the Ijebu North Local Government, Ogun State Nigeria.

Sample Collection

A probability systematic sampling technique was employed in this study during the sample collection procedure. Five (5) groundwater samples were taken from the wells once a week for five (5) weeks to make up a total of twenty-five samples (25). Two hundred (200ml) of the samples were aseptically collected in sterile sampling bottles using plastic fetchers at various depths ranging from 50 to 98 meters for each sample. The bottles were labeled with full details of the source, date, and numbers and were immediately brought to the lab for bacteriological analysis. To investigate the bacteriological relationship with groundwater samples, soil samples from the poultry farm environments were also taken.

Table 1: Sample Area Codes

Sample area	Code
Ago – Iwoye A	AGWA
Ago- Iwoye B	AGWB
Awa	AWA
Ijebu-Igbo	IJB
Oru	ORU

Physicochemical analysis

The physicochemical parameters were established using the APHA(1998) procedure, according to Atlas and Bartha (10). The parameters determined in this study include temperature in degrees Celsius, pH, conductivity, calcium, and phosphorus concentration. At the location of sample collection, samples' temperature and pH were measured using thermometers and veneer pH meters, respectively.

Bacteriological Analysis

The dilution plating method was used to analyze the soil and water samples in this study. In the dilution plating method, samples are serially diluted before being cultured using a pour plate technique which supports the growth of distinct colonies. The water samples underwent five serial dilutions, resulting in dilutions ranging from 10^{-1} to 10^{5}. For soil samples, 1g of soil sample was firstly submerged in 10 ml of distilled water, before performing the subsequent five serial dilutions to produce dilutions 10^{-1} to 10^{-5}. Then, using a pour-plate technique, an aliquot of 0.1 ml of 10^{-5} from each water and soil sample was grown on Nutrient Agar, Salmonella-Shigella Agar, and Eosin Methylene Blue Agar. The plates were incubated, then further sub-cultured to produce pure cultures, after which colonial characteristics, gram reactions, and biochemical tests like the catalase test, citrate utilization test, urease test, and indole test were used to characterize and identify the organisms as described in the Bergey's Determinative Bacteriology Manual (11). Following sub culturing, each water sample collected from the different study areas was examined weekly for the total viable count. Both the soil and the water samples' total viable counts of bacteria were reported. Where applicable, mean, standard deviation, chi-square, and p-values for significance were reported.

Results

Physicochemical Analysis

The physicochemical characteristics of the water samples were immediately assessed after each sample collection each week, and the mean was calculated in the conclusion of sampling. The depth of each groundwater sample were recorded to be 68m for AGWA, 50 for AGWB, 73m for AWA, 98m for IJB, and 61m for ORU. The mean±SD temperature of water samples from the study areas were 28.6°C±0.37, 28.6°C±0.23, 28.1°C±0.68, 28.2°C±0.14, 28.8°C±0.24 for AGWA, AGWB, AWA, IJB and ORU sample areas, respectively. The highest mean pH was observed to be 6.46±0.11 from ORU while the lowest was observed to be 6.1±0.55 from AWA. The highest and lowest mean±SD conductivities were reported to be 201.8±78.18 and 109.6±14.67 from AGWA and AWA respectively. The mean±SD dissolved solids mg/L of water samples ranged from 22±277.29 from AGWA to 61±27.46 from AWA. While the mean phosphate and calcium concentrations ranged from 6.98±2.21 to 4.81±2.59 mg/L and 0.86±0.44 to 0.70±0.46 mg/L respectively (Table 2).

Table 2: Physicochemical analysis of water samples

Sample	Week	Temp °C	pH	Cond (S/M)	TDS (mg/L)	Phos (mg/L)	Cal (mg/L)
AGWA	1	28.1	6.2	0153	0723	9.12	0.96
	2	28.8	6.5	0147	0069	4.19	0.90
	3	28.7	6.2	0237	0112	3.41	0.54
	4	29.1	6.2	0324	0115	4.92	1.49
	5	28.5	6.4	0148	0123	2.39	0.30
MEAN±SD		**28.6±0.37**	**6.3±0.14**	**201.8±78.18**	**228±277.29**	**4.81±2.59**	**0.84±0.45**
AGWB	1	28.4	6.5	0201	0063	9.0	0.90
	2	28.5	6.2	0106	0060	8.6	0.54
	3	28.6	6.6	0206	0071	8.0	1.40

	4	29.0	6.2	0104	0724	4.0	0.31
	5	28.6	6.2	0132	0047	5.3	0.96
MEAN±SD		**28.6±0.23**	**6.3±0.20**	**149.8±50.28**	**193±296.96**	**6.98±2.21**	**0.82±0.42**
AWA	1	26.9	6.7	0132	0062	5.50	0.91
	2	28.2	6.6	0091	0043	6.30	0.99
	3	28.5	5.5	0106	0050	4.19	0.50
	4	28.6	5.8	0109	0042	7.01	1.49
	5	28.2	5.7	0110	0108	3.80	0.39
MEAN±SD		**28.1±0.68**	**6.1±0.55**	**109.6±14.67**	**61±27.46**	**5.36±1.36**	**0.86±0.44**
IJB	1	28.0	6.5	0200	0104	5.00	0.90
	2	28.1	6.2	0210	0113	4.19	0.54
	3	28.3	6.0	0153	0069	4.10	1.47
	4	28.3	6.2	0148	0124	7.00	0.34
	5	28.3	6.0	0146	0120	7.40	0.96
MEAN±SD		**28.2±0.14**	**6.18±0.21**	**171.4±30.18**	**106±22.03**	**5.54±1.56**	**0.84±0.43**
ORU	1	28.9	6.6	0147	0132	6.13	0.54
	2	29.1	6.3	0200	0113	6.90	1.48
	3	28.6	6.5	0207	0106	7.10	0.33
	4	28.5	6.5	0201	0120	6.25	0.46
	5	28.8	6.4	0205	0115	6.80	0.67
MEAN±SD		**28.8±0.24**	**6.46±0.11**	**192±25.31**	**117.2±9.68**	**6.64±0.42**	**0.70±0.46**

Key: Temp – Temperature, Cond- Conductivity, Cal- Calcium, Phos, Phosphate, TDS-Total Dissolved Solids.

Bacteriological analysis

Total Viable Count

The highest mean total viable count observed for water samples was 4.94 x10^5 cfu/ml while the lowest was 3.02 x10^5 cfu/ml. While the total viable count for soil samples was observed once and the highest were 10.2 x10^5 cfu/ml and the lowest 8.5 x10^5 cfu/ml. The total viable count of the soil samples was generally higher than that of the groundwater samples. Statistical analysis revealed the chi-square value to be 20.00 while the p-value (0.202) was found to not be statistically significant (Table 3)

Table 3: Total Viable Count of bacteria isolates from water and soil samples

Sample	Soil (x10^5 cfu/ml)	Groundwater samples						Chi-square	p-value
		Week 1 (x10^5 cfu/ml)	Week 2 (x10^5 cfu/ml)	Week 3 (x10^5 cfu/ml)	Week 4 (x10^5 cfu/ml)	Week 5 (x10^5 cfu/ml)	Mean±SD (x10^5 cfu/ml)		
AGWA	9.1	4.8	3.0	2.5	2.8	2.0	3.02±1.06	20.00	0.2202
AGWB	8.5	3.9	4.0	2.9	3.8	3.8	3.68±0.44		
AWA	10.2	7.8	6.7	3.0	4.3	2.9	4.94±2.21		
IJB	9.2	3.1	3.5	3.1	3.8	4.0	3.50±0.41		
ORU	9.9	4.2	3.1	3.2	3.5	3.3	3.46±0.44		

Isolated Bacteria Species

The bacteria isolates obtained from weekly collected water samples across study areas were *Escherichia coli, Klebsiella spp, Pseudomonas spp, Shigella spp, Salmonella spp, Staphylococcus spp* (Table 4). *Salmonella spp* was the most occurring bacteria in both groundwater and soil samples obtained while *Staphylococcus spp* was the least occurring bacteria. The Chi -square value was 15.00 while the p-value (0.2414) was not statistically significant (Table 5).

Table 4: Bacteria Isolates obtained from groundwater and soil samples

Sample area	Soil	Groundwater
AGWA	*Salmonella spp, Escherichia coli*	*Staphylococcus spp, Salmonella spp, Escherichia coli, Klebsiella spp*
AGWB	*Staphylococcus spp, Salmonella spp, Shigella,*	*Salmonella spp, Shigella, Klebsiella spp, Escherichia coli,*
AWA	*Shigella spp, Salmonella spp, Escherichia coli,*	*Klebsiella spp, Shigella spp, Salmonella spp, Escherichia coli, Pseudomonas spp*
IJB	*Shigella spp, Salmonella spp, Pseudomonas spp,*	*Escherichia coli, Shigella spp, Salmonella spp, Pseudomonas spp,*
ORU	*Salmonella spp, Klebsiella spp, Shigella spp,*	*Salmonella spp, Pseudomonas spp, Escherichia coli, Shigella spp,*

Table 5: Percentage Occurrence of Bacteria Isolates

Isolates	Groundwater (%)	Soil (%)	Chi-square	p-value
Escherichia coli	27	24	15.00	0.2414
Klebsiella spp	9	5		
Salmonella spp	38	47		
Shigella spp	22	19		
Staphylococcus spp	4	5		

Discussion

For drinking and other domestic uses, groundwater is a perfect source of potable, safe water (cite). However, the presence of poultry nearby may have an impact on the quality of the groundwater through direct human activity-related contamination with poultry waste or indirect environmental contamination via wind, soil, or other means (cite). The results demonstrate that the water sample temperatures observed in this study are higher than the 25°C drinking water standard, although this variation may be caused by differences in groundwater levels, soil types, or weather conditions (12). However, similar findings were reported by Taiwo et al(2011) in a study conducted in Abeokuta, which is part of a different local government in the same state (13). All of the water sample pH readings were found to be slightly acidic and out of the WHO-recommended pH range of 6.5 to 8.5 (14). This is in line with the findings of Owamah et al.(2020), who also noted acidic pH values for groundwater samples in Niger Delta area ranging from 4.82 to 6.50 (15). Even though the mean highest pH reading was 6.5, the slightly acidic readings may have been caused by the discharge of poultry litter into the environment or the water, which may have changed the nature of the groundwater. Lower pH readings may have been caused by H^+ sensitive biochemical reactions that were catalyzed by ammonia toxicity, soluble metal ions, and chlorine disinfection efficiency. Since both showed similar trends across all sample areas and were typically lower than the WHO guidelines (MPL of 1000 mg/L), the conductivity and total dissolved solids obtained from the groundwater samples were proportional (16). These characteristics can be used to distinguish one another and are connected to the water's hardness; higher total dissolved solids signify a rise in the water's hardness. The findings in this study show that phosphate levels are above the 0.1 mg/L WHO permissible limit, which has an impact on the taste of groundwater used for domestic purposes (17). High phosphate levels consequently cause uncontrolled growth of plants and algae in groundwater, rendering it unfit for human consumption. The calcium level was also reported to be much lower than the 200 mg/L WHO maximum limit (14).

In all study areas, soil samples were reported to contain an enteric bacteria family. However, this is consistent with a previous study by Omoya et al. from 2016 that found a high microbial load of bacteria in poultry soil (18). It is noteworthy that this occurrence may be caused by human activity in the environment as well as contamination of the soil with poultry litter, such as feed, droppings, and bird feathers. A seasonal investigation of the microbial contamination of feces and soil by Traswinska et al.(2016) revealed results that were similar to those found in this study's soil samples, which had a high total viable count of bacteria (19). Although the population of bacteria in the soil could be significantly influenced by climatic factors, contamination rates, and soil quality. In line with earlier studies like Oluyemi et al (2016), and Hubbard, et al (2020), where *Salmonella* spp. were the most reported bacterial species in poultry soil samples, soil samples from all study areas were found to contain *Salmonella* spp (18,20). The significant presence of *Salmonella* spp may be directly related to the substantial amounts of poultry litter that have been deposited in the soil.

Notably, the presence of bacterial contamination is one of the main causes of the study's deteriorated water quality. The main contaminants of the groundwater samples obtained for this study were enteric bacteria; these results were consistent with those of Al-Saffawi et al.(2020), who investigated the quality of groundwater used to hydrate livestock and poultry (21). When comparing the limits, the minimum total viable count obtained in this study significantly exceeds the WHO drinking water guidelines (14). The groundwater's vulnerability to a contaminated environment is a significant factor, as was further demonstrated by a prior study by Wang et al. (2017) (22). In all study areas, *Salmonella* spp. was the most prevalent bacteria contaminating the groundwater; Hubbard et al.(2020) also found similar findings after a thorough investigation. Despite the fact that Wang et al. (2017) findings are in direct opposition to ours, Escherichia coli was the dominant enteric bacteria in that study. However, it is important to note that enteric bacteria are significant groundwater contaminants close to poultry farms when taking into account both this study and earlier studies. The current study’s discovery of enteric bacteria from the same species in both samples from various study areas supports the idea that soil may act as a source of contamination. Enteric bacteria have reportedly been shown to persist long after inoculation and seep into the soil to reach groundwater sources (23,24).

Conclusion

The results of this study unequivocally demonstrate that enteric bacteria are significant environmental contaminants of groundwater sources in poultry farm environments. It was also proven that the soil surrounding the groundwater close to the poultry farms might act as a transporter for microbial contaminants. The potential risk of exposing to these enteric bacteria could include diarrhea and other digestive disorders, because both humans and animals use this groundwater for drinking and other domestic purposes. Therefore, a safe treatment method can be used to prevent the contamination of groundwater near poultry farms. In order to stop enteric bacteria from seeping through the soil, an impermeable floor layer can also be applied. Public health regulation of water sources used by poultry farms could also prove to be advantageous.

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