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Evaluation of Antibiotic-resistant Bacteria and Physicochemical Parameters in Groundwater, Impacted by Dairy Farms in Hatillo, Puerto Rico

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Abstract:

The dairy industry produces many contaminants. This industry constantly uses a water resource in its processes, which carries these contaminants through the soil to groundwater supplies. Dairy cows excrete a significant fraction of antibiotics, posing a substantial risk that active or unchanged metabolites into the environment and leach to groundwater. The aim of this study is to determine if the dairy industry represents a source of bacterial contamination to groundwater, analyze the resistance to antibiotics in the identified bacteria and evaluate the risk to which nearby communities are exposed when they use them as drinking water. Samples were taken from three wells revealing antibiotic-resistant autochthonous bacteria. The physicochemical parameters were analyzed. Eight different types of bacteria were identified, *Bacillus* sp., *Micrococcus* sp., *Acinetobacter baumannii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *E. coli*, *Pseudomonas aeruginosa*, and *Pseudomonas putida*. Ammonium and nitrate in wells A and B exceeded limits, turbidity in wells A and C exceeded limits. However, the pH and temperature were within limits. Dairy farms can be a source of contamination to groundwater. Improvements in manure management are recommended using more profitable technologies in the transformation of waste from dairy industries. These contaminants can migrate through the soil and reach groundwater supplies, which can contribute to potential impact on public health and the environment.



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INTRODUCTION

The mobility of microorganisms in groundwater represents a serious problem for public health (Ashraf *et al.*, 2019; Saleem *et al.*, 2020; Lambert *et al.*, 2021). There are numerous sources of microorganisms that affect groundwater such as: runoff from agricultural industries, oxidation ponds, wastewater irrigation of pastures, and malfunctioning septic tanks (Fatima *et al.*, 2021; Khan *et al.*, 2021). Referring to runoff from agricultural industries, surface waters that carry waste from livestock, such as: food, pasture, feces, fertilizers, fungicides, pesticides, among others (Morgan, 2003; Lambert *et al.*, 2021).

These dairy industries produce a considerable amount of waste, which is stored in oxidation ponds, used as fertilizers, through spraying methods or falls to the ground as part of the daily activities of the industry (Christou *et al.*, 2017; Du and Liu, 2012).

The oxidation pond is the place where the waste is stored and used as fertilizer through spray methods or falls to the ground as part of the daily activities of the industry (Da'ana *et al.*, 2021; Du and Liu, 2012). However, this can be a source of contamination due to the significant presence of microorganisms. The irrigation of pastures with used water increases the risk of the presence of microorganisms in groundwater, including their resistance to antimicrobial treatments (Franklin *et al.*, 2016; Du and Liu, 2012; Morgan 2003). The groundwater resource represents an important fraction of drinking water and the processes in water treatment plants are often ineffective for the removal or inactivation of infectious organisms (Franklin *et al.*, 2016; Morgan, 2003).

We can point out that veterinary antibiotics are widely used in the treatment of diseases and to protect the health of livestock animals (Ashraf and Iqbal, 2021; Hasan, 2021; Kivits *et al.*, 2018). However, this type of drug is poorly absorbed into the animal system resulting in a large percentage of the parental compound or its metabolites being excreted (Ezzariari *et al.*, 2018). The continued use of veterinary

antibiotics is believed to contribute to the spread of antibiotic-resistant bacteria, a global threat to public health (Ashraf *et al.*, 2020; Iqbal and Ashraf, 2018; Iqbal and Iqbal, 2020; Saleem *et al.*, 2018). Although the use of manure as part of the livestock industry processes is common, there is a limited amount of information about the impacts on microorganisms in groundwater (Kümmerer, 2009).

The presence of unmetabolized antibiotics and/or their by-products in the environment has been shown to have quantitative and qualitative effects on pathogenic and non-pathogenic organisms. This will result in the development and selection of resistant and multiresistant strains through a process known as horizontal gene transfer. Which in turn produces a clonal spread of those bacteria that are now resistant (Kümmerer, 2003; Wellington *et al.*, 2013; Williams-Nguyen *et al.*, 2016).

A major human health concern is associated with antibiotics and antibiotic-resistant bacteria in drinking water (Iqbal *et al.*, 2016; Urooj *et al.*, 2018). This health threat is dual, i.e. (1) a potential direct toxic effect of the antibiotic and (2) the accumulation and proliferation of specific types of resistance that can potentially lead to treatment failure (Williams-Nguyen *et al.*, 2016). Holmes *et al.* (2007) speculate that concentrations of antibiotics in localized water bodies in developing regions are typically low and therefore direct toxic effects to humans are unlikely. On the contrary, chronic low-dose antibiotic exposure can result in the proliferation of antibiotic-resistant bacteria and/or antibiotic-resistance genes, potentially resulting in treatment failure when bacterial infections occur regardless of the source of the pathogen (Wellington *et al.*, 2013; Fernández *et al.*, 2015).

In microbiological testing, identified infectious organisms are usually subjected to various tests to determine their degree of resistance to various anti-infective substances to avoid the administration of ineffective treatments. The results of the test will establish a classification based on the activity of the individual drugs against the isolated organism. This classification is based on three terms: susceptible,

intermediate, or resistant. Data of this type are used to form a picture of the degree of resistance of each drug in the general population. The latter is in turn an important criterion in the selection of antibiotics for the empirical treatment of infection diseases (Rello and Díaz, 2003). For the purposes of simplifying the evaluation of the degree of resistance, a standardized scheme based on thresholds has been introduced in which the degree of effectiveness of the drug is characterized as susceptible, intermediate or resistance according to the MIC value (Gould, 1998, Kiem and Schentag, 2006).

According to the International Organization for Standardization (Rodloff *et al.*, 2008), these terms are defined as follows:

- Susceptible (S): the bacterial strain is said to be susceptible to a given antibiotic, when it is inhibited in vitro by a concentration of the drug that is associated with a high probability of therapeutical success.
- Intermediate (I): The sensitivity of a bacterial strain to a given antibiotic is said to be intermediate when it is inhibited in vitro by a concentration of that drug that is associated with an uncertain therapeutic effect.
- Resistant (R): A bacterial strain is said to be resistant to a given antibiotic when it is inhibited in vitro by a concentration of this drug that is associated with a high probability of therapeutical failure.

The CDC (2013) points out that gastroenteric infections caused by antibiotic-resistant pathogens are associated with increased rates of morbidity and mortality, resulting in higher health care costs. Of particular concern is the proliferation of antibiotic resistance among vulnerable populations (<5 years, >65 years), among whom bacterial infections and subsequent complications are more prevalent (Parry and Palmer, 2000).

Lapworth *et al.* (2012) predicts that the number of emerging contaminants with defined drinking

water standards, environmental quality standards, groundwater values, and/or monitoring requirements will increase substantially in the coming years. This will also likely occur with antibiotics, leading to improve our understanding of current spatio-temporal patterns, and sampling patterns should be prioritized to inform future groundwater research and monitoring strategies.

The objective of this study is to identify and quantify bacteria in groundwater of water wells located in dairy farms of Hatillo, Puerto Rico, and also to evaluate the possible impact of this industry on the environmental and human health.

MATERIAL AND METHODS

Water samples were taken once a month for 15 months in each of the water wells of the selected dairy farms. The sampling points are identified with the letters A, B, C in the municipality of Hatillo (Figures 1, 2 & 3). Simultaneously, measurements were also taken for the following physicochemical parameters: pH, turbidity, ammonium, nitrate, and temperature, using the Lab Quest Vernier equipment.



Fig. 1. (Water well A)



Fig. 2. (Water well B)



Fig. 3. (Water well C)

Microbiological analysis was performed through the Vitek 2 instrument, bacteria were identified by gender, species, and quantity (Biomérieux University, 2017). The instrument also provided results about susceptibility to antibiotics and the detection of microbial resistance mechanisms (Hackman *et al.*, 2013; Maran *et al.*, 2016).

For the statistical analysis of the physicochemical parameters the Kruskal-Wallis statistical test was used, which is a non-parametrical method that allows us to know if there are differences in the distributions of the variables in the study (Holmes and Huber, 2019). Spearman's non-parametric correlation coefficient test was also used. This test was used to evaluate data as evidence of an association between two quantitative variables of interest (Van Enden, 2019). In the same way, the statistical model of linear regression was used that allowed us to investigate the relationships between different quantitative variables of interest, through a line that best adapts to the trend of the data (LeBlanc, 2004).

Study area

The municipality of Hatillo in Puerto Rico has depended on agriculture, especially livestock, this being the fundamental basis of its economy. Milk production per square mile in this municipality exceeds that of any other municipality dedicated to the same activity (Guzmán, 2014).

The northwestern region of the island is geographically dominated by limestone plains and its alluvial soils are the most excellent for agricultural activity (Guzmán, 2014). The dairy industry benefits from the plains, which facilitate the movement of cattle and prevent soils from becoming excessively erodible or saturated during the rainy season. In addition, the northern karst zone has the largest aquifer on the island and provides groundwater for animal and human consumption. However, many of these qualities also make the area attractive for urban expansion (Fosberg *et al.*, 2014).

The area that constitutes the limestone rock in Puerto Rico covers approximately 27.5 percent of the island's surface. It is subdivided into the northern limestone zone, the southern zone, and what is known as dispersed limestone. All the limestone areas mentioned have karstic characteristics. The part of the limestone zone to the north contains the most impressive karst topography and is called the karst belt. This area covers 142,544 ha, 65% of the northern limestone zone. The northern limestone zone has the largest freshwater aquifer, the largest expanse of mature forest, and the largest coastal wetlands, estuaries, and underground cavern systems in Puerto Rico. Figure (4) shows the karst zone in Puerto Rico and Figure (5) shows the location of the water wells investigated in this study. The karst belt is highly diverse, and its heterogeneous topography concentrated in such a limited area, making it unique in the world (Lugo *et al.*, 2004).

Lugo *et al.* (2004) also indicates that the northern limestone zone supplies 22% of the fresh water extracted by public entities on the Island. 78% of the water extracted in the northern limestone zone is groundwater and an

average of 340,000 of the population uses this water.

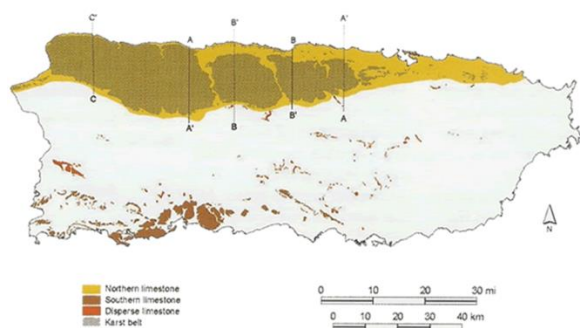


Fig. 4. Karst zone of Puerto Rico (Monroe, 1976)



Fig. 5. Water wells location (Google maps 2022)

The karst belt of Puerto Rico currently represents one of the least intervened karst habitats in the Caribbean area. However, the limestone region in general is vulnerable to

human activities, including the cutting of vegetation, the paving of forest areas, the desiccation and filling of wetlands, the conversion and transformation of land uses, the overexploitation of aquifers and groundwater pollution (Ozgul and Dindaroğlu, 2021).

RESULTS AND DISCUSSION

This investigation lasted fifteen months, starting from February 2018 and ended in April 2019. The microbiological analysis identified eight bacteria, two of these gram-positive, *Bacillus sp.* *Micrococcus sp.* (Table 1) and six gram-negative, *Acinetobacter baumannii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *E. coli*, *Pseudomonas aeruginosa*, and *Pseudomonas fluorescens/putida* (Table 2). These only showed growth in water well A, for 7 months (February, March, April, May, June, November 2018, and January 2019) of the investigation.

It should be noted that the number of bacteria identified was distributed by one per month, both in the dry and wet seasons, except for *Micrococcus sp.* which had a growth during the months of March 2018 (dry season) and November 2018 (rainy season). A higher number of gram-positive bacteria was identified in the wet season, unlike gram-negative bacteria, which showed their highest growth in the dry season (Jury *et al.*, 2007; Torres *et al.*, 2014).

Table 1. Gram-positive bacteria identification and description.

Waterwell A		
Gram-positive Bacteria	Description	Reference
<i>Micrococcus sp.</i>	<i>Micrococcus</i> are considered harmless saprophytes that generally and consistently inhabit the skin of large mammalian populations in greater numbers than other sources.	(Kocur <i>et al.</i> , 1992; Musa and Abdelgadir, 2014)
<i>Bacillus sp.</i>	The various species of the genus <i>Bacillus</i> are known as opportunistic pathogens. They have a wide range of physiological lifestyle. This heterogeneity, ecology and genetics make it difficult to categorize the genus or generalize about it.	(Logan, 1988; Gillespie and Hawkey, 2006; Dworkin <i>et al.</i> , 2006)

Table 2. Gram-negative bacteria identification and description.

Waterwell A		
Gram-negative Bacteria	Description	Reference
<i>Acinetobacter baumannii</i> cop.	<i>Acinetobacter</i> are often considered ubiquitous in nature since they can be recovered from almost all soil and water samples. Upregulation of the organism's innate resistance mechanisms, together with the acquisition of foreign determinants, has played a crucial role in the express route the organism has taken to become a multidrug-resistant pathogen.	(Gallego and Towner, 2001; Towner, 1996; Howard <i>et al.</i> , 2012)
<i>Enterobacter aerogenes</i> , <i>Enterobacter cloacae</i>	<i>Enterobacter</i> species are found in the natural environment in habitats such as water, sewage, vegetables, and soil. These organisms are increasingly found causing various infections. Two well-known species, <i>Enterobacter aerogenes</i> and <i>Enterobacter cloacae</i> , have acquired nonclinical significance as opportunistic bacteria that have emerged as pathogens.	(Davin-Regli and Pagès, 2015)
<i>Escherichia coli</i>	<i>Escherichia coli</i> is a common inhabitant of gastrointestinal tract of humans and animals. It can be found secondarily in soil and water because of fecal contamination. Healthy cattle that have been colonized, along with other ruminants, are the most significant animals that act as reservoirs harboring <i>E. coli</i> .	(Bermudez and Hazan, 1988; Munns <i>et al.</i> , 2015)
<i>Pseudomonas aeruginosa</i> , <i>Pseudomonas fluorescens/putida</i>	The genus <i>Pseudomonas</i> is known for its metabolic versatility and genetic plasticity. Strains of <i>Pseudomonas</i> species are often resistant to antibiotics, disinfectants, detergents, heavy metals, and inorganic solvents. The genus is also notorious for species that are opportunistic pathogens, as well as plant pathogens that significantly impact agricultural and economic well-being globally.	(Schroth <i>et al.</i> , 1992; Van der Krooij, 1977; Ivanova <i>et al.</i> , 2002; Bossis <i>et al.</i> , 2000; Scales <i>et al.</i> , 2014)

Resistance patterns in gram-positive bacteria were identified in *Bacillus* sp. with resistance to ceftriaxone and susceptibility to ampicillin in the β -lactam group. Aminoglycosides, fluoroquinolones, and tetracyclines showed resistance, but susceptibility to sulfonamides.

Micrococcus sp. showed resistance to ceftriaxone and susceptibility to ampicillin in the β -lactam group. It also showed resistance to fluoroquinolones, and susceptibility to aminoglycosides, sulfonamides, and tetracyclines (Table 3).

Table 3. Antibiotic resistance patterns identified in Gram-positive bacteria.

Water well A	Antibiotics used					
	β-lactam		AMG	FQs	Sulfonamides	TET
	AMP	CRO	GEN	CIP	SXT	TET
Gram-positive Bacteria						
<i>Bacillus</i> sp.	S	R	R	R	S	R
<i>Micrococcus</i> sp.	S	R	S	R	S	S

The gram-negative bacteria were more variable in resistance than the gram-positive ones. For gram-negative bacteria in β -lactam antibiotics, *Acinetobacter baumannii* cop., *Enterobacter cloacae* and *E. coli* showed the highest

resistance. *Enterobacter aerogenes* and *Pseudomonas aeruginosa* had the least resistance. The least susceptibility was identified in *Pseudomonas fluorescens/putida*. We can highlight that *E. coli* had a pattern of resistance

and susceptibility to all β -lactam antibiotics (Table 4).

Resistance to aminoglycoside antibiotics was not identified in gram-negative bacteria. The highest susceptibility was identified in *E. coli*, *P. aeruginosa* and *P. fluorescens/putida*. The

lowest susceptibility was identified in *Acinetobacter baumannii cop.* and *Enterobacter aerogenes*. *Enterobacter cloacae* showed intermediate susceptibility to gentamicin (Table 5).

Table 4. Patterns of resistance to β -lactam antibiotics in gram-negative bacteria.

Water well A														
Gram-negative Bacteria	β -lactam													
	SAM	AMP	AMC	CRO	CAZ	CTX	FEP	CFZ	CXM	ETP	IPM	MEM	TZP	TIM
<i>A. baumannii cop.</i>	S	R	R	S	S			R			S		S	
<i>E. aerogenes</i>			R	S	S	S	S				S	S		
<i>E. cloacae</i>	R	R		S	S		S		R	S	S	S	S	
<i>E. coli</i>	S	R	R	S	S	S	S	S	S	S	R	S	S	S
<i>P. aeruginosa</i>					S		R				S	S	S	S
<i>P. fluorescens/putida</i>				S	S	S	S				S	S	S	

Table 5. Patterns of resistance to aminoglycoside antibiotics in gram-negative bacteria.

Water well A	AMK	Aminoglycosides	TOB
Gram-negative Bacteria		GEN	
<i>A. baumannii cop.</i>		S	S
<i>E. aerogenes</i>	S	S	
<i>E. cloacae</i>	S	I	
<i>E. coli</i>	S	S	S
<i>P. aeruginosa</i>	S	S	S
<i>P. fluorescens/putida</i>	S	S	S

The highest susceptibility to fluoroquinolones in gram-negative bacteria was identified in *Acinetobacter baumannii cop.*, *E. coli*, *Pseudomonas fluorescens/putida* and *Pseudomonas aeruginosa*, the latter also being resistance to ciprofloxacin. *Enterobacter aerogenes* and *Enterobacter cloacae* showed the least susceptibility (Table 6).

Resistance patterns for nitrofurantoin, sulfonamides and tetracycline, *Acinetobacter baumannii cop.* was resistant to nitrofurantoin and susceptible to sulfonamide. *Enterobacter aerogenes* showed resistance to sulfonamide and susceptibility to tetracycline. *E. coli* was susceptible to sulfonamide and intermediate to tetracycline. There was no pattern of resistance and susceptibility for *Enterobacter cloacae* and

Pseudomonas aeruginosa to nitrofurantoin, sulfonamide and tetracycline (Table 7).

Table 6. Patterns of resistance to fluoroquinolone antibiotics in gram-negative bacteria.

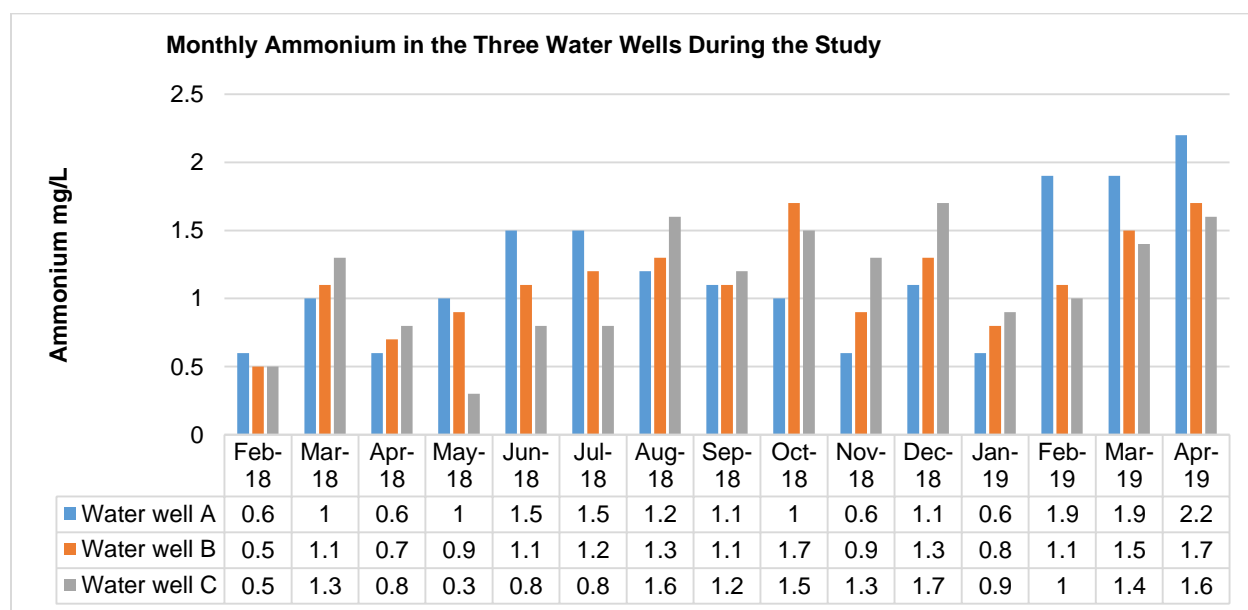
Water well A		
Gram-negative Bacteria	Fluoroquinolones	
	CIP	LVX
<i>A. baumannii cop.</i>	S	S
<i>E. aerogenes</i>	S	
<i>E. cloacae</i>	S	
<i>E. coli</i>	S	S
<i>P. aeruginosa</i>	R	S
<i>P. fluorescens/putida</i>	S	S

Table 7. Patterns of resistance to the antibiotics nitrofurantoin, sulfonamides and tetracyclines in gram-negative bacteria.

Water well A			
Gram-negative Bacteria	Nitrofurant	Sulfonamides	TET
	NIT	SXT	TET
<i>A. baumannii</i> cop.	R	S	
<i>E. aerogenes</i>		R	S
<i>E. cloacae</i>			
<i>E. coli</i>		S	I
<i>P. aeruginosa</i>			
<i>P. fluorescens/putida</i>		S	S

The months with the highest ammonium were April 2019 (2.2 mg/L), followed by February (1.9 mg/L), March (1.9 mg/L). The highest ammonium was found in the water well A with 2.2 mg/L in April 2019. It should be noted that

the results obtained in the months of February and March 2019 also come from the water well A. Similarly, the lowest ammonium was found during the month of February (0.5 mg/L) and May 2018 (0.3 mg/L). The lowest ammonium was found in water well C with 0.3 mg/L during the month of May 2018. All water wells during the months of the study exceeded the established parametric value of 0.3 mg/L, except for the water well C, on the date indicated above. Water well A was the one presenting the highest ammonium values during all the months of the study. Elevated ammonium levels can arise from intensive agriculture and then captured by water sources. Therefore, ammonium is an indicator of possible bacterial contamination of wastewater and animal waste (Figure 6).

**Fig. 6.** Monthly ammonium concentration during the study in the three water wells.

Ammonia in water supplies comes from agricultural and industrial processes, as well as disinfection with chloramines. Elevated ammonium levels can arise from intensive agriculture on uptake in the water source. Therefore, ammonium is an indicator of possible bacterial contamination of wastewater and animal waste. Ammonia by itself does not constitute a health risk, but the parametric value

of 0.30 mg/L serve as a valuable indicator of the source of the contamination (EPA, 2015).

The highest nitrate during the months of the investigation was April 2018 (168.8 mg/L) and April 2019 (182.9mg/L). The highest nitrate was found in the water well A with 182.9 mg/L in April 2019. The lowest nitrate was found during the month of February 2018. The lowest nitrate was

found in the water well B with 9.6 mg/L on the date before mentioned. All the water wells exceeded the established parametric value of 50 mg/L during the month of September, November, December 2018, and January, February, March, and April 2019. All water wells have a lower record of nitrates, compared to the established parameters during the month of

February, March, May and August 2018. The water well A was the one that presented the highest nitrate values consistently during eleven months of investigation. Most of the nitrate found in natural waters comes from organic, such as sewage discharges and inorganic sources, such as chemical fertilizers (Figure 7).

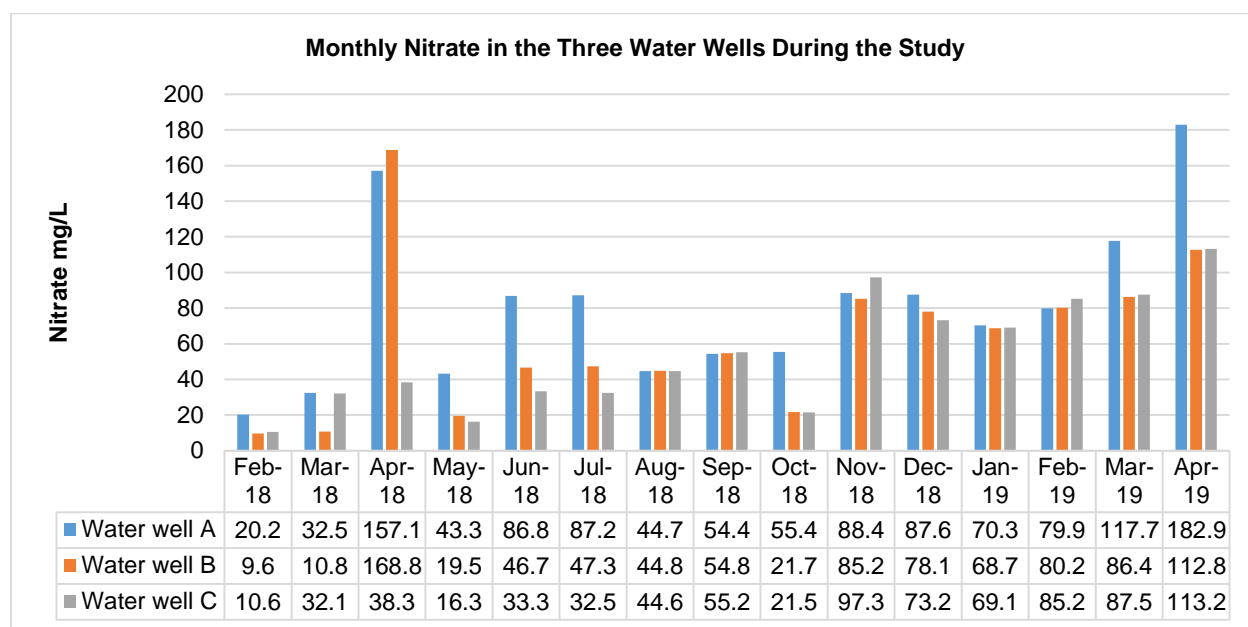


Fig. 7. Monthly nitrate concentration during the study in the three water wells.

Nitrate by itself is not directly a toxic, but it is a health hazard due to its conversion to nitrite which reacts with blood hemoglobin to cause methemoglobinemia (Titov and Petrenko, 2005; Richard *et al.*, 2014). The established parametric value is 50 mg/L, although it is dangerous for infants above 11 mg/L (EPA, 2015).

The months with the highest pH were July (8.68) and December 2018 (8.71). The water well C showed highest pH with 8.71 in December 2018. While the lowest pH was found during the months of February (7.1) and March 2018 (7.1). The water wells that showed the lowest pH were A and C with 7.1 during the before mentioned months. The pH results for all water wells during

the 15 months of the study were within established water quality parameters. The pH values guide the behavior of other important parameters of the quality of the water. Changes in soil pH correlated with three factors: 1) type of soil cover, 2) soil texture, and 3) irrigation periods (Figure 8).

The pH scale ranges from 0 (very acidic) to 14 (very alkaline). The natural pH range of freshwater extends from around 4.5 for turbid waters, to more than 10.0 in waters where there is intense photosynthetic activity produced by algae. However, the most frequently found range is 6.5-8.0. The parameters for water quality established in pH units are ≥ 6.5 and ≤ 9.5 (EPA, 2015).

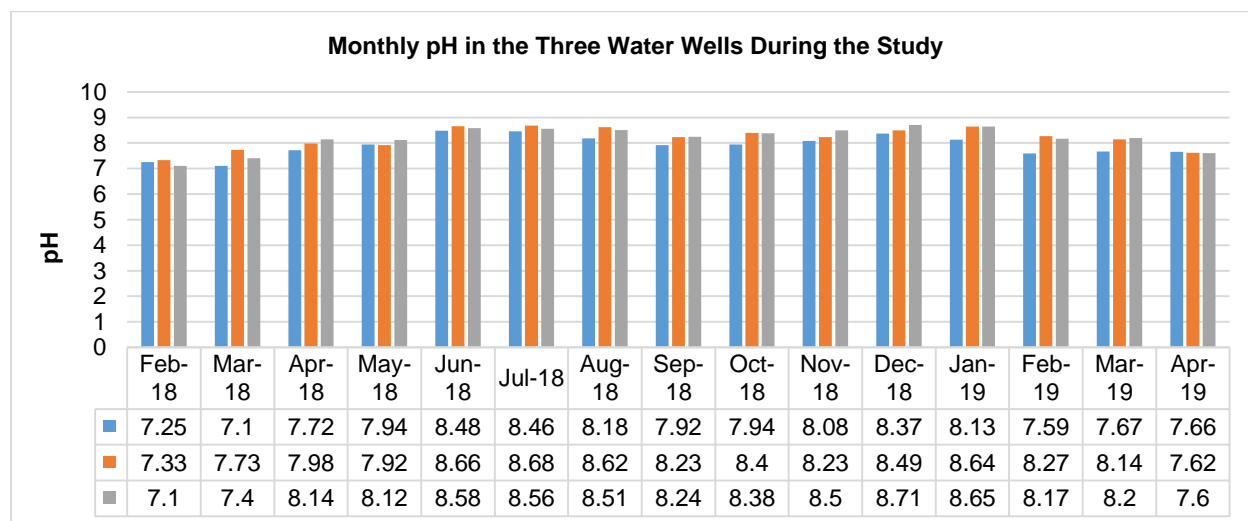


Fig. 8. Monthly pH of ground water during the study in the three water wells.

The highest temperatures were recorded in March (32.1 °C), June (29.6 °C), July (29.6 °C) and September 2018 (29.7 °C). The water well A presented the highest temperature with 32.1 °C in March 2018. The lowest temperatures were found during the months of December 2018 (23 °C), March (22.1 °C) and April 2019 (24.5 °C). The water well A showed the lowest temperature with 22.1 °C during the months of March 2019.

All the water wells presented a uniform behavior and were within the temperature standards for water quality. Temperature is a critical factor in the survival of pathogens. It's possible adverse effects on humans are directly relates to its interaction with various environmental media, such as manure, soil sediments, surface, and groundwater (Figure 9).

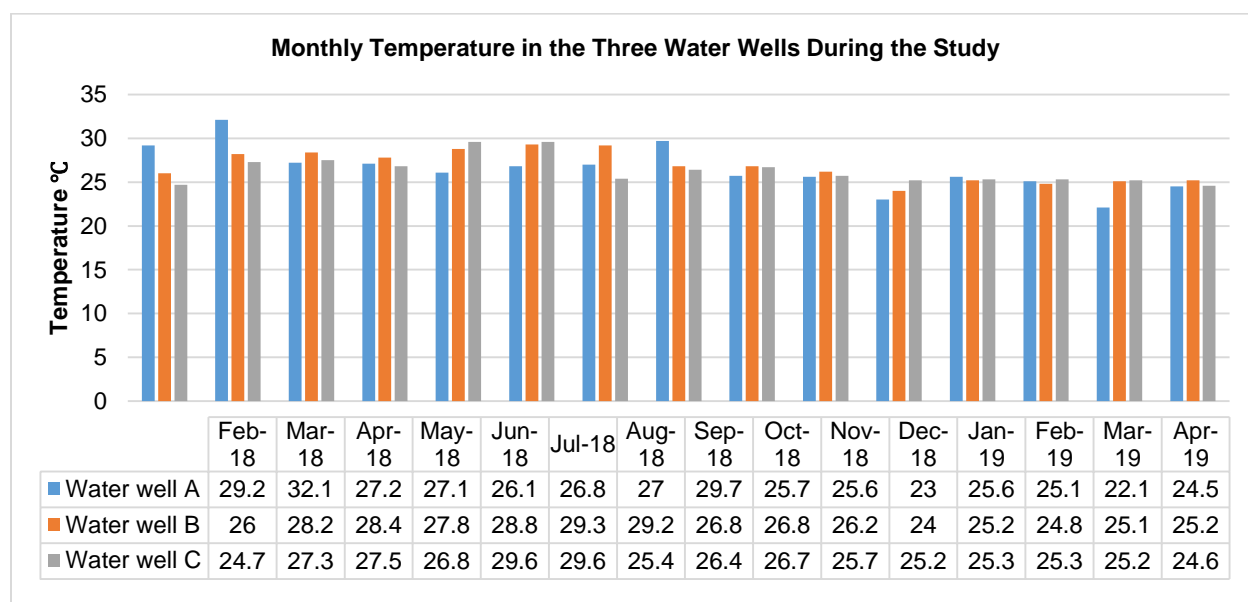


Fig. 9. Monthly temperature of ground water during the study in the three water wells.

Temperature is a critical factor in pathogen survival, with cold temperatures generally allowing longer survival times. The increase in temperatures on the contrary accelerates death (Walker *et al.*, 2001; Rogers and Haines, 2005). The extend of this effect varies by organism, but survival in manure is generally markedly decreased at temperatures above 20 °C to 30 °C compared to pathogen survival at cold temperatures (1 °C to 9 °C) (Rogers and Haines, 2005). This dependence of survival times on temperature results in seasonal trends (Venglovsky *et al.*, 2009; Howard *et al.*, 2017). Established water quality temperature criteria are 32.2 °C (EPA, 1988).

The months with the highest turbidity were May (64.7 NTU) and October 2018 (109.7 NTU).

Water well A showed the highest turbidity with 109.7 NTU in October 2018. The lowest turbidity was found during the month of December 2018 (4.2 NTU). Water well B showed the lowest turbidity at 4.2 NTU during the above mentioned month. Water well B (10.8 NTU) had the lowest turbidity for the month of April 2019. The turbidity results for all water wells during the 15 months of the study, excluding water well B (4.2 NTU) in the month of December 2018, were above the established water quality parameters. Turbidity in groundwater can originate from soil material, but also from rapid inflow of surface water runoff or surface percolation. Turbidity can be correlated with microbiological contamination in water sources (Figure 10).

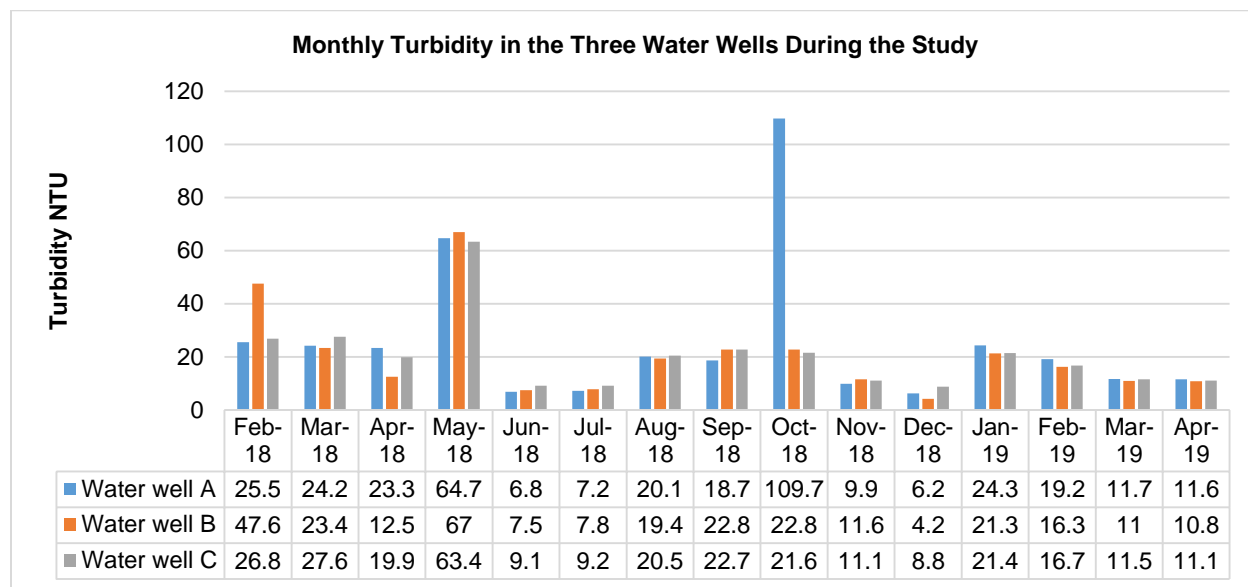


Fig. 10. Monthly turbidity of ground water during the studying the three water wells.

Despite its limitations, turbidity has been considered relevant enough to be included in the drinking water regulations of many development countries. Regulations in the U.S. covering turbidity have been amended and revised since the Surface Water Treatment Rule of 1989,

which states that filtered water supplies are now required to conduct turbidity monitoring at each filter at 15 minutes intervals. The rules specify that the combined filter effluent turbidity must be ≤ 0.3 NTU in at least 95% of the measurements

take each month and no individual measurement must exceed 1NTU (EPA, 2015).

The statistical analysis was done to have more data which help in making better corrective decisions. It is very important to know where these significant differences are found and if there are trends that allow us to determine the best action to follow when deciding the most appropriate corrective actions.

The results of the Kruskal-Wallis test for ammonium were not significant ($N=45$), $p=0.993$. This p value, if compared to a significance of 0.05 ($p=0.993 \geq 0.05$), indicates that there is not enough evidence to infer that there is a significant difference in the mg/L of ammonium between the three wells of the dairy farms. Through this study it is understood that the three wells have the same results, exceeding the parameters established by the EPA. Corrective filtration actions are needed in case these waters are used as drinking water.

The results of the Kruskal-Wallis test for nitrate were not significant, ($N=45$), $p=0.2101$. This p value, if compared to a significance of 0.05 ($p=0.2101 \geq 0.05$), indicates that there is not enough evidence to infer that there is a significant difference in the mg/L of nitrate between the three wells of the dairy farms in the town of Hatillo. Through this study is found that the three wells have the same results, exceeding the parameters established by the EPA during seven months of the 15 that the investigation lasted. Corrective filtration actions are needed in case these waters are used as drinking water.

The results of the Kruskal-Wallis test for pH were not significant, ($N=45$), $p=0.0519$. This p value, if compared to a significance of 0.05 ($p=0.0519 \geq 0.05$), indicates that there is not enough evidence to infer that there is a significant difference in pH between the three wells of the dairy farms in the town of Hatillo. The results of the three water wells do not exceed the parameters established by the EPA. No filtration corrective actions will be required in this regard.

The results of the Kruskal-Wallis test for temperature were not significant, ($N=45$), $p=$

0.8518. This p value, if compared to a significance of 0.05 ($p=0.8528 \geq 0.05$), indicates that there is not enough evidence to infer that there is a significant difference in temperature ($^{\circ}\text{C}$) between the three wells of the dairy farms. The results of the three water wells do not exceed the parameters established by EPA. No filtration corrective actions will be required in this regard.

The results of the Kruskal-Wallis test for turbidity were not significant, ($N=45$), $p=0.9101$. This p value, if compared to a significance of 0.05 ($p=0.9101 \geq 0.05$), indicates that there is not enough evidence to infer that there is a significant difference in turbidity (NTU) between the three wells of the dairy farms in the town of Hatillo. The results of the three water wells exceed the established parameters by EPA. Corrective filtration and disinfection actions are needed in case these waters are used as drinking water.

Through the results, a moderately negative correlation could be found in the value of r_s (-0.4082). There is a 1% probability that there is no correlation between turbidity and ammonium $p=0.01$ (99% level of statistical significance).

The risk factors that can contribute to the spread of infectious diseases through the consumption of groundwaters without the proper filtration processes are very high (Close *et al.*, 2008). The communities served by these water wells are responsible for complying with federal and state regulations to prevent contagious diseases. Most residents are not properly educated about the risk to their health of using this untreated water. For this reason, these people are more vulnerable to diseases that can be caused not only by bacteria but also by any other contaminant that is carried from the surface to groundwater supplies (Donham *et al.*, 2007).

There is a series of possible health risk for the communities that reside in the vicinity of the dairy industries, as well as the potential health effects of using untreated agricultural well water as drinking water. Community health impacts identified in the literature included: zoonotic diseases, pathogen-related risk, antimicrobial

resistance genes, respiratory symptoms, and health risk in children, the elderly and immunocompromised patients (Grout *et al.*, 2020).

Since resistance genes are frequently found on mobile genetic elements, they can move between pathogens, as well as between nonpathogenic and pathogenic commensal bacteria. Therefore, the issue of resistance must be considered beyond the veterinary profession and specific pathogens (Aitken *et al.*, 2016). Indeed, there is increasing evidence that the resistance genes identified in human bacterial pathogens were originally acquired from non-pathogenic indigenous environmental bacteria through horizontal gene exchange (Martinez, 2012).

Resistance genes can spread rapidly between bacteria and sometimes to unrelated genera (Call *et al.*, 2008). Even if an ingested bacterium resides in the gut for only a short time, it will have the ability to transfer its resistance genes to the resident microbiota, which in turn can serve as a reservoir of resistance genes for pathogenic bacteria. The bias toward gene sharing raises concerns about the possible spread of antimicrobial resistance determinants from commensal organisms in animals and humans to human pathogens (Chen *et al.*, 2016; Hashempour-Baltork *et al.*, 2019; Van den Bogaard and Stobberingh, 2000; Zhang *et al.*, 2019).

Therefore, the epidemiology of antimicrobial resistance goes beyond the limits of veterinary and human medicine. The complexity of the movement of microorganisms and horizontal gene transfer involved in the epidemiology of resistance at the global level is difficult to understand (Van den Bogaard and Stobberingh, 2000; Hashempour-Baltork *et al.*, 2019).

It is of great importance that diagnostic laboratories comply with standards to provide reliable and reproducible susceptibilities data for physicians, veterinarians, and other health professionals. It must be recognized that most studies of antimicrobial resistance in veterinary pathogens are not based on a representative

sample of pathogen populations, but rather on laboratory diagnostic representations, so these reports may overestimate the prevalence of resistance in veterinary pathogens. Consequently, better designed studies are needed to assess the real situation of resistance to veterinary pathogens at all levels, locally, internationally, and globally (Giguère *et al.*, 2013).

CONCLUSION

The results of this study indicate that the groundwaters underlying the dairy facilities are susceptible to contamination by pathogenic bacteria, which also show resistance to various veterinary antibiotics. Resistant pathogenic bacteria can also migrate through the karst soil, directly affecting groundwater, which are extracted by the water wells of the dairy industries.

This study demonstrated that dairy farms can be a source of contamination by bacteria resistant to antibiotics. They can move through the soil and reach groundwater supplies, contributing to a potential impact on the environment. It was identified that proper manure management practices can reduce the spread of antibiotic-resistant bacteria through various environmental pathways until reaching the groundwater. In this study it was also found that there is no temporal variability in the quantification of bacteria in groundwater. This is because the pollution generated by the dairy industry is constant and therefore the increase or reduction of risk is generally difficult to quantify. It was also possible to show that the communities adjacent to the dairies that use these waters from wells as drinking water are more vulnerable to diseases. These can not only be caused by bacteria but also by any other contaminant that is carried from the surface to groundwater supplies. The risk factors that can contribute to the spread of infectious diseases through the consumption of this type of water without the proper filtration process are very high.

RECOMMENDATIONS

It is recommended to improve education on antimicrobial resistance and prudent use of antimicrobials, from students to doctors and veterinarians, to ensure the appropriate use of antimicrobials. Better education will facilitate making optimal treatment decisions and encourage better communication to counter pressure from owners or growers to dispense antimicrobials in situations where antimicrobial therapy is not prescribed. Education related to antimicrobial stewardship requires acceptance that antimicrobial stewardship is relevant. Wider acceptance of the role of veterinary medicine in antimicrobial resistance is clearly needed to facilitate the development of good management practices. Fortunately, there are veterinary organizations that have embraced a proactive approach in support of good antimicrobial stewardship. Although general awareness about antimicrobial resistance and concerns about antimicrobial overuse is increasing in the population, health professionals face a lack of knowledge about these issues that will lead to inappropriate use of antimicrobials. The general and inappropriate use of antimicrobials could be reduced, using better management initiatives, and encouraging greater education, both at the professional level and that of clients.

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CONFLICT OF INTEREST

The author declares that this article content has no conflict of interest.

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