MICROBIAL ANALYSIS OF DOMESTIC CISTERN WATER IN THE U.S. VIRGIN ISLANDS

by

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ABSTRACT

A significant proportion of the water supply in the U.S. Virgin Islands comes from the rainfall which is stored in cisterns. Since cistern water is not part of the public distribution system, it is not covered by the Safe Drinking Water Act (Public Law 93-523). The objective of this study was to determine whether cistern water supplies pose a potential health hazard to their users.

The cistern water supplies were studied to assess the types of heterotrophic bacteria, algae, and protozoa that may be present. The bacterial study was directed towards identifying those organisms capable of causing disease in water supplies. Total coliform, fecal streptococcus and *Salmonella* sp. were enumerated. The presence of coliform bacteria, fecal streptococcus and *Salmonella* sp. in most of the cistern water supplies suggest a potential health problem.

Algae and protozoa were found in several cistern waters, which indicated a high level of microbial diversity. These complex ecosystems suggest a continuous input of carbon and energy sources into the cisterns. The pollution of these cistern water supplies seem to be of extreme significance, which has been overlooked by those involved in ascertaining the microbial quality of all drinking water in the U.S. Virgin Islands.

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INTRODUCTION

The U.S. Virgin Islands are part of the Lesser Antilles of the West Indies, which separate the Caribbean Sea from the Atlantic Ocean. The average rainfall in the U.S. Virgin Islands is almost 44 inches and accounts for a significant portion of the water supply when it is stored in cisterns, which are underground reservoirs for storing rainwater. The cistern water supply, therefore, is a major contributor to the total available water supply.

While community[1] and non-community[2] water systems must meet the published standards in the provisions of the Safe Drinking Water Act (Public Law 93-523), cistern water supplies are not covered by this Act of Congress. Public Law 93-523 provides the regulations and standards by which all public drinking water supplies are judged. These cistern water supplies, therefore, are not required to be chlorinated, which would provide adequate disinfection, and are not required to be monitored for microbiological contaminants.

This project was undertaken to determine whether these small water collection systems pose any potential adverse health problems to those who use this type of water supply. The objectives of this study were to determine (a) the types of heterotrophic bacteria present, (b) the quantity of microalgae and protozoa and

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[1] Those which serve at least 15 service connections used by year-round residents or regularly serve at least 25 year-round residents.

[2] A public water system that is not a community water system.

(c) the presence of pathogenic microorganisms.

Water quality studies usually include measurements and evaluations based upon aesthetics (odor, taste, color, and viscosity), Biochemical Oxygen Demand (B.O.D.), Dissolved Oxygen Content (D.O.), free carbon dioxide, dissolved solids, and mineralogic analyses. All of these parameters may be influenced by the presence of bacteria, algae and protozoa. Algae are the most significant form of planktonic life to a water quality survey and are an important link in the food chain and can readily affect the aesthetic qualities of water as well as the D.O. and carbon dioxide levels.

Coliform bacteria, inhabitants of the intestinal tract of warm-blooded animals, have been traditionally used as indicators of water quality. It is assumed that the possibility of the presence of pathogens increases with the pollution by feces and urine. Other than coliforms, the most useful indicator organisms for fecal contamination are strains of *Streptococcus faecalis*. The total coliform group and fecal streptococci are considered excellent indicators of water polution, because those bacteria are always present in the normal intestinal tract of humans and other warm-blooked animals and are eliminated in large numbers in fecal wastes.

CISTERNS STUDIES

On the island of St. Thomas, four cistern systems were selected for the present study. Three of the cisterns were in residential homes and one of the cisterns supplied the College of the Virgin Islands. None of the cisterns had been chemically treated in any way.

Six cisterns were selected for study on the island of St. Croix. The characteristics of these cisterns are shown in Table 1.

PROCEDURE

The cisterns were sampled by two methods. A collection container was placed on a weighted line and lowered into the cistern. When removed from the cistern, it was immediately sealed. Temperature readings were taken at the same time. When it was possible to reach a submerged portion of the cistern wall, a second sample was taken. This sample consisted of material and organisms associated with the surface of the walls. It was gathered either by swabbing with a dacron swab or by using a large pipet.

Within a few hours after the water had been removed from th cistern, it was examined. Studies of the algae, protozoa and any microinvertebrates were done on living samples using standard microscopical techniques.

Bacterial analyses were conducted in accordance with the analytical recommendations set forth in "Standard Methods for the Examination of Water and Wastewater," (American Public Health Association, 1975). The standard sample used in the membrane filter procedure was 100ml. The standard sample used in the five-tube most probable number (MPN) procedure (fermentation tube method) was five times the standard portion.

The standard plate count, multiple tube fermentation

TABLE 1

DESCRIPTION OF SOME CISTERN STUDIED

Site	Cistern volume (thousand gals.)	Cistern Age	Cistern Construction	Roof Description	Overhanging Vegetation
St. Croix					
West Indies lab	50	10 years	masonry	Fresh latex paint	No
Bidels	27.5		stone		Yes
Hamil	22.5	about 20 years	poured masonry	Silicone or neoprene paint within 5 years	No
Staple	22.5	about 20 years	poured masonry	Silicone paint sealed with "Anvil"	Yes
Vatch		less than 1 year	poured masonry	Newly painted	No
St. Thomas					
Bovoni	38	15 years	concrete	Painted galva-nize	No
Smith Bay	19	10 years	concrete	Painted June 1980	Yes
College of the Virgin Islands			concrete	Galvanized	Yes
Hoff		12 years		"Permacoat" 1 year ago	No

technique and membrane filter technique for coliform bacteria, for Streptococcus sp. and Salmonella sp. were all done according to the standard methods.

RESULTS AND DISCUSSION

The total bacterial count (Table 2) provided a means of determining the density of aerobic and facultative anaerobic heterotrophic bacteria in the cistern waters. This was an empirical measurement since no single growth medium could satisfy the physiological requirements of all the bacteria in the water sample. Therefore, the actual number of bacteria was probably higher than the actual number of viable bacteria counted.

Many different genera of bacteria were isolated. These genera included Pseudomonas, Flavobacterium, Proteus, Bacillus, Achromobacter, and Serratia. Many of these organisms are considered secondary pathogens, can grow in low nutrient waters, and many suppress the detection of coliform bacteria.

The high density of bacteria in some of the cistern waters (Table 2) suggested an undesirable deterioration of the water quality. This monitoring of bacterial density seemed important in light of the fact that chlorine was not used.

This bacteriological measurement can be used as an indicator of nutrient input into the system. While there was no maximum level for the general bacterial population covered by the Safe Drinking Water Act, it has been generally recommended that drinking water should contain fewer than 5×10^4 bacteria per 100ml.

TABLE 2

TOTAL BACTERIAL COUNT

Bacteria/100ml

Site	Dec.1979	Feb.1980	July 1980	Oct.1980
St. Croix				
West Indies Lab	2.5×10^3	4.1×10^4	6.1×10^5	2.0×10^2
Easter	3.4×10^4	4.7×10^5	N.D.	N.D.
Bidels	4.0×10^4	1.5×10^3	8.2×10^5	$>1.0 \times 10^4$
Hamil	5.7×10^6	2.5×10^4	9.2×10^7	$>2.0 \times 10^2$
Staple	6.0×10^5	1.6×10^5	6.4×10^6	TNTC
Vatch	N.D.	4.7×10^4	5.2×10^6	$>1.0 \times 10^3$
St. Thomas				
Bovoni	8.7×10^3	2.0×10^4	5.8×10^6	$>1.0 \times 10^3$
Smith Bay	7.3×10^5	1.5×10^5	2.4×10^7	$>1.0 \times 10^4$
College of the Virgin Islands	7.0×10^3	7.8×10^4	7.3×10^6	$>1.0 \times 10^3$
Hoff	5.6×10^2	4.0×10^4	4.0×10^6	6.0×10^3

N.D. - Not Done
TNTC - Too Numerous to Count

All counts were done using the standard plate count described in the 14th Edition of Standard Methods for Examination of Water and Wastewater.

of water. In this respect many of the cistern waters were greater than this contaminant level.

For routine examination of most potable water supplies which are usually disinfected with chlorine, the presence or absence of coliform bacteria has been used as a measure of the water quality. These bacteria are always present in the normal intestinal tract of man and other warm-blooded animals and eliminated in large numbers in fecal wastes. Therefore, the absence of total coliform bacteria is used as an indicator of a bacteriologically safe water.

The coliform group has included all aerobic and facultative anaerobic Gram-negative non-sporeforming rod-shaped bacteria that ferment lactose with gas formation within 48 hours at 35°C. Water of good quality for drinking will be less than 20 per 100ml, very good to excellent 1 per 100 ml. Most of the cistern water samples could be classified as non-potable based upon their total coliform count (Table 3). Analyses of the total coliform group through the use of the IMViC test (indole, methyl red, voges-proskauer and citrate) showed that most of the coliform group isolated were of the Klebsiella-Aerobacter type bacteria. This suggested that these cistern waters had been contaminated for a considerable length of time and had not been chlorinated.

The normal habitat of fecal streptococci is the intestines of man and animals, therefore, these organisms can also be used as an indicator of fecal pollution. The data in Table 4, shows that many of the cisterns had become contaminated with fecal streptococci as the study progressed. Some of the cistern waters showed the presence of coliform bacteria, but not the presence of fecal

TABLE 3

TOTAL COLIFORM COUNT

Coliform/100ml

Site	Dec.1979	Feb. 1980	July 1980	Oct. 1980
St. Croix				
West Indies Lab	1.6×10^2	4.3×10^2	4.6×10^3	5.2×10^3
Easter	2.3×10^2	7.3×10^1	N.D.	N.D.
Bidels	2.3×19^2	4.3×10^2	1.1×10^5	$>1.0 \times 10^3$
Hamil	2.3×10^2	0	2.4×10^2	1.5×10^2
Staple	1.6×10^2	9.3×10^2	1.1×10^5	9.0×10^2
Vatch	N.D.	N.D.	1.1×10^5	$>2.0 \times 10^3$
St. Thomas				
Bovoni	9.1×10^1	2.1×10^2	0	$>2.0 \times 10^3$
Smith Bay	7.5×10^2	2.1×10^2	0	1.0×10^3
College of the Virgin Islands	2.3×10^2	N.D.	0	$>2.0 \times 10^3$
Hoff	1.6×10^2	0	1.1×10^5	8.75×10^3

N.D. - Not Done

TABLE 4

TOTAL STREPTOCOCCI COUNT

Fecal Strep/100ml

Site	Feb. 1980	July 1980	Oct. 1980
St. Croix			
West Indies Lab	0	0	4.0×10^2
Easter	7.3×10^1	N.D.	N.D.
Bidels	0	0	5.0×10^5
Hamil	0	0	2.8×10^2
Stable	$<1 \times 10^3$	$<1 \times 10^3$	1.2×10^2
Vatch	$<1 \times 10^3$	$<1 \times 10^3$	2.0×10^5
St. Thomas			
Bovoni	3.6×10^1	$<1 \times 10^3$	4.0×10^2
Smith Bay	0	$<1 \times 10^5$	5.0×10^5
College of the Virgin Islands	N.D.	$<1 \times 10^3$	5.0×10^5
Hoff	0	$<1 \times 10^3$	7.5×10^2

N.D. - Not Done

streptococci. This may well be due to the long survival time of coliform bacteria (Klebsiella sp. and Aerobacter sp.) However, whenever fecal streptococci were detected in cistern waters, coliform bacteria were also found to be present.

The presence of coliform bacteria and fecal streptococci in cistern waters was only an indicator of fecal pollution and suggested the possible presence of pathogenic bacteria. Table 5 shows the data in which Salmonella sp. were enumerated by the membrane filter technique using bismuth-sulfite medium. Again most of the cistern waters showed the presence of Salmonella spp. in great numbers. Many of the Salmonella sp. have long survival times and some can cause a variety of diseases in humans.

The scraping from the surfaces of cisterns which contained coliform bacteria also showed the presence of the same coliform organisms. The cisterns seemed to have organisms colonizing the surfaces which probably were being released into the water. These cisterns would never be devoid of coliform bacteria unless they were chlorinated.

The high level of coliform bacteria, fecal streptococci and Salmonella spp. in many of the cisterns suggested the possible presence of pathogens in these waters. In addition to Salmonella spp., these waters also contained species of Proteus, Shigella, Serratia, and Pseudomonas. These are secondary pathogens which means that they will only initiate an infection if a person has been exposed to a primary pathogen, which has caused a disease.

The source for the pollution of these cisterns is still uncertain. However, the colonization of the surfaces by coliform

TABLE 5

TOTAL SALMONELLA SP. COUNT

Salmonella sp./100ml

Site	Feb. 1980	July 1980	Oct. 1980
St. Croix			
West Indies Lab	+	1.9×10^3	1.0×10^4
Easter	-	N.D.	N.D.
Bidels	+	1.7×10^4	2.0×10^2
Hamil	+	9.6×10^3	1.6×10^5
Staple	+	6.6×10^4	8.0×10^2
Vatch	N.D.	6.6×10^4	8.0×10^5
St. Thomas			
Bovoni	+	N.D.	4.0×10^5
Smith Bay	-	N.D.	8.0×10^5
College of the Virgin Islands	+	6.0×10^1	9.3×10^4
Hoff	-	4.2×10^4	3.3×10^4

N.D. - Not Done
\+ - Present in Significant numbers
\- - Not Present

and other microorganisms would suggest that these organisms may have been introduced into the cisterns at one time and were being sustained by a continuous input of energy sources from the external environment.

The biological communities found within the cisterns ranged from solely bacterial to those containing bacteria, protozoa, invertebrates, and frogs. (See Tables 6-9). Presumably, an environment's ability to support a community structure is regulated by the energy input to the system. Since these habitats are devoid of light, the only energy sources available to the system would most likely be preformed organic materials collecting from the exterior. This material could be in the form of higher vegetation. This contention was borne out by the observation that a cistern with some wood construction in it (Smith Bay) regularly supported an invertebrate community. These higher communities pose no health hazards and may actually serve to keep the bacterial populations in check.

As to the source of the organisms themselves, there were two theoretical possibilities. The first is that when food supplies got scarce, the organisms died. When water along with the organic materials re-entered the cistern, new organisms were introduced. The second possibility was that when food became scarce, the organisms re-entered a dormant state and were reactivated when food was introduced. Of course, a combination of the two possibilities may occur.

Two observations were noteworthy - the cisterns did allow for reproduction of organisms contained within them. This included

TABLE 6

ALGAE AND PROTOZOA

December 1979

Site	Source	Depth (ft)	Temp. (^{o}C)	Cells/100ml	Wall Scrapings	Notes on Condition
St. Croix						
West Indies Lab	cistern	full	26	$5x10^{3}$ ciliates $3.5x10^{4}$ zoo-flagellates	N.D.	recently chlorinated
	tap			0		
Easter	cistern	full	27.5	0	N.D.	clear
Bidels	cistern	full	26.5	$1.5x10^{4}$ zoo-flagellates	attached ciliates (pertitrichs)	floating debris, Cuban frogs, plant & insect remains
Hamil	cistern	full	26.5	0	0	clear
Staple	cistern	full	26	0	0	clear
St. Thomas						
Bovoni	cistern	full	27	0	0	clear
Smith Bay	cistern	full	25	0	ciliates (Gymnostomes) & flagellates	organisms concentrated about decaying timber in cistern
College of the Virgin Islands	cistern	full	29	$1x10^{4}$ zoo-flagellates	N.D.	clear
Hoff	tap			0	N.D.	

N.D. - Not Done

TABLE 7

ALGAE AND PROTOZOA

February 1980

Site	Source	Depth (ft)	Temp. (°C)	H_2O Sample Cells/100ml	Wall Scrapings	Notes on Condition
St. Croix						
FDU	Cistern	7	27	$3.3x10^3$ zooflagellates (e.g. Monas and Bodo)	N.D.	frogs and some debris
Hamil	cistern	7	26	0	N.D.	leaf pieces, surface film
Staple	cistern	2	27	0	N.D.	Cuban frogs, debris
Bidels	cistern	4½	26.5	0	N.D.	some debris on surface
Easter	tap	N.D.	27	0	N.D.	-
Vatch	cistern	1½	26.5	$2.5x10^3$ cilates Chilodonella predominant	N.D.	fine "sand" on surface. New.
St. Thomas						
Bovoni	cistern	full	26.5	0	zooflagellates water mold	insect debris
College of the Virgin Islands	cistern		28	0	N.D.	
Smith Bay	cistern	N.D.	N.D.	0	N.D.	
Hoff	tap	N.D.	N.D.	0	N.D.	
Lerkenlund	tap	N.D.	N.D.	0	N.D.	

N.D. - Not Done

TABLE 8

ALGAE AND PROTOZOA

July 1980

Site	Source	Depth (ft)	Temp. (°C)	Cells/100ml	Wall Scrapings	Notes on Condition
St. Croix						
West Indies Lab	cistern	6	30	0	N.D.	frogs, but generally clean
Bidels	cistern	6	29	0	N.D.	frogs, insect larvae, fibrous material on surface
Staple	cistern	2	28.5	$2x10^3$ ciliates	N.D.	clear; clean
Hamil	cistern	7	27.5	0	N.D.	scum on surface, frogs
Vatch	cistern	1	29	0	N.D.	clean, some milliped fragments
St. Thomas						
College of the Virgin Islands	cistern	15	30	0	N.D.	clean
Hoff	tap		29	0	N.D.	
Bovoni	cistern	7	28.5	0	N.D.	clean
Smith Bay	cistern	8	27	$6x10^3$ flagellates $2x10^3$ ciliates	high #s of flagellates, ciliates, rotifers	decaying timbers produce microscopic wood fragments, H_2O molds

N.D. - Not Done

TABLE 9

ALGAE AND PROTOZOA

October 1980

Site	Source	Depth (ft)	Temp. (°C)	Cells/100ml	Wall Scrapings	Notes on Condition
St. Croix						
West Indies Lab	cistern	full	28	$3.3x10^3$ zoo-flagellates		water very milky in color
Bidels	cistern	full	28	$1.2x10^4$ zoo-flagellates $1.0x10^4$ ciliates	flagellates e.g. Bodo, Monos ciliates, e.g. Halteria, Mesodinium	clear, but some debris; frogs
Staple	cistern	4	28.5	0	N.D.	clear
Hamil	cistern	full	28	0	N.D.	clear but insect fragments & many tadpoles
Vatch	cistern	4	28	0	N.D.	very clean
St. Thomas						
College of the Virgin Islands	cistern	5	29	0	N.D.	clean
Hoff	tap	-	28	0	-	-
Bovoni	cistern	N.D.	N.D.	$1.7x10^3$ flagellates	N.D.	particulate matter, insect fragments
Smith Bay	cistern	N.D.	N.D.	0	flagellates, ciliates, rotifers & nematodes associated with wood	N.D.

N.D. - Not Done

higher forms such as frogs. This would suggest that reintroduction from the exterior was not necessary. Another observation was that the communities tend to be somewhat constant. This would also suggest an isolated population. Nevertheless, the air, soil, water, and overhanging vegetation are filled with spores and cysts so it is only logical to conclude that organisms must also be entering the cistern.

Whether the active organisms are coming from the exterior or dormant forms from within, it must be remembered that they are dependent on an energy source and that the ability of a cistern to purify water is based on energy source depletion.

The results do indicate that these cisterns when not chlorinated can support a complex ecosystem. The presence of indicator organisms such as coliform and Streptococcus sp., along with a variety of algae and protozoa, suggests that cistern waters should be continuously chlorinated and should be covered by the Safe Drinking Water Act.

The data indicate that the cisterns are being contaminated with organisms of fecal origin. Frequently this would indicate the possible presence of pathogens, although none has been definitely confirmed. Nevertheless, a definite potential health problem is present.

SUMMARY

1. Cisterns on the U.S. Virgin Islands have been shown to support large diverse biological populations, including bacteria, algae, protozoa, and invertebrates.

2. Some of these microorganisms are potential human pathogens.

3. Cistern waters should be continually monitored for microbial quality unless chlorination is routinely carried out.

4. Cistern water should comply to the standard set forth in the Clean Water Act of 1975.

REFERENCES

American Public Health Association (APHA), Standard Methods for the Examination of Water and Wastewater, (14th ed.) Washington, D.C. 1975.

Smith, H.H., "Sequential Use of Reclaimed Wastewater Destined for Aquifer Recharge," Caribbean Research Institute, St. Thomas, 1979.