WATERBORNE PATHOGENS

OF THE U.S. VIRGIN ISLANDS

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ABSTRACT

A series of water quality analyses were carried out during 1985 on drinking water sources in the U.S. Virgin Islands. The object of the study was to determine which human pathogens might be present, the implications of their presence, and the value of current Environmental Protection Agency indicators for predicting the presence of pathogens.

Water samples were taken from seven cisterns, four wells, and five places along the public water line (16 total). A four liter sample was taken at each site and returned immediately to the lab for processing. Each sample was checked for total and fecal coliform using both multiple tube and membrane filter methods approved by the American Public Health Association and U.S. Environmental Protection Agency. They were cultured, where applicable, on standard enrichment broth and on selective media. Individual colonies were removed and cultured on eight differentiating media as well as on the API strips (Analytab Products, Plainview, N.Y.: 20 tests). Controls were run simultaneously using: Blanks, (no inoculum) and pure cultures of eight species from the American Type Culture Collection (ATCC).

The results of these 1800 separate tests were collated and compared for consistency, occurrence of pathogens, and reliability of prediction from the coliform tests. Thirty-

two species of bacteria, twenty of them pathogens, were found. This analysis showed that:

1. All of the 16 sources had human pathogenic bacteria present. Fifteen of the 16 sources also had fecal streptococcus present while 9 of the contaminated sources would pass current coliform EPA water quality standards.
2. Use of a "pre-enrichment" technique is vital to get a true estimate of the less vigorous pathogens and injured coliforms.
3. Current EPA indicators used for contaminated drinking or groundwater are, at least in the V.I., useless for predicting the presence or absence of pathogens.

Further comparison of these results with earlier work on ground and cistern water indicates that the problem may be more severe and ubiquitous than previously thought.

Recommendations are made for future management, detection studies, and control.

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INTRODUCTION

The U.S. Virgin Islands are a group of three major islands, and 27 smaller islands and cays clustered around the 18th parallel. The islands are about 1100 miles southeast of Miami (Figure 1). Only the three larger islands of St. Thomas, St. John and St. Croix support any significant permanent populations. In 1984, approximately 140,000 permanent residents occupied the 60 odd square miles of land. In addition over 1 million persons a year visit the islands. These visitors comprise a large potential vector pool which could transmit pathogens widely. Located in the trade-winds zone the islands have a high humidity but low rainfall. Annual rainfall varies from 20 inches to 70 inches per year in different parts of the islands. With this rainfall and population demand, water becomes a major commodity. The 4-5 million gallons of public water per day required comes from three sources: rainwater catchment, groundwater, and sea water desalination. Of these sources only the desalinated public water is routinely treated prior to distribution. Even so, due to an antiquated, leaky distribution system, (see Figure 2) the public water is liable to contamination before reaching the public. All of the water sources are cross

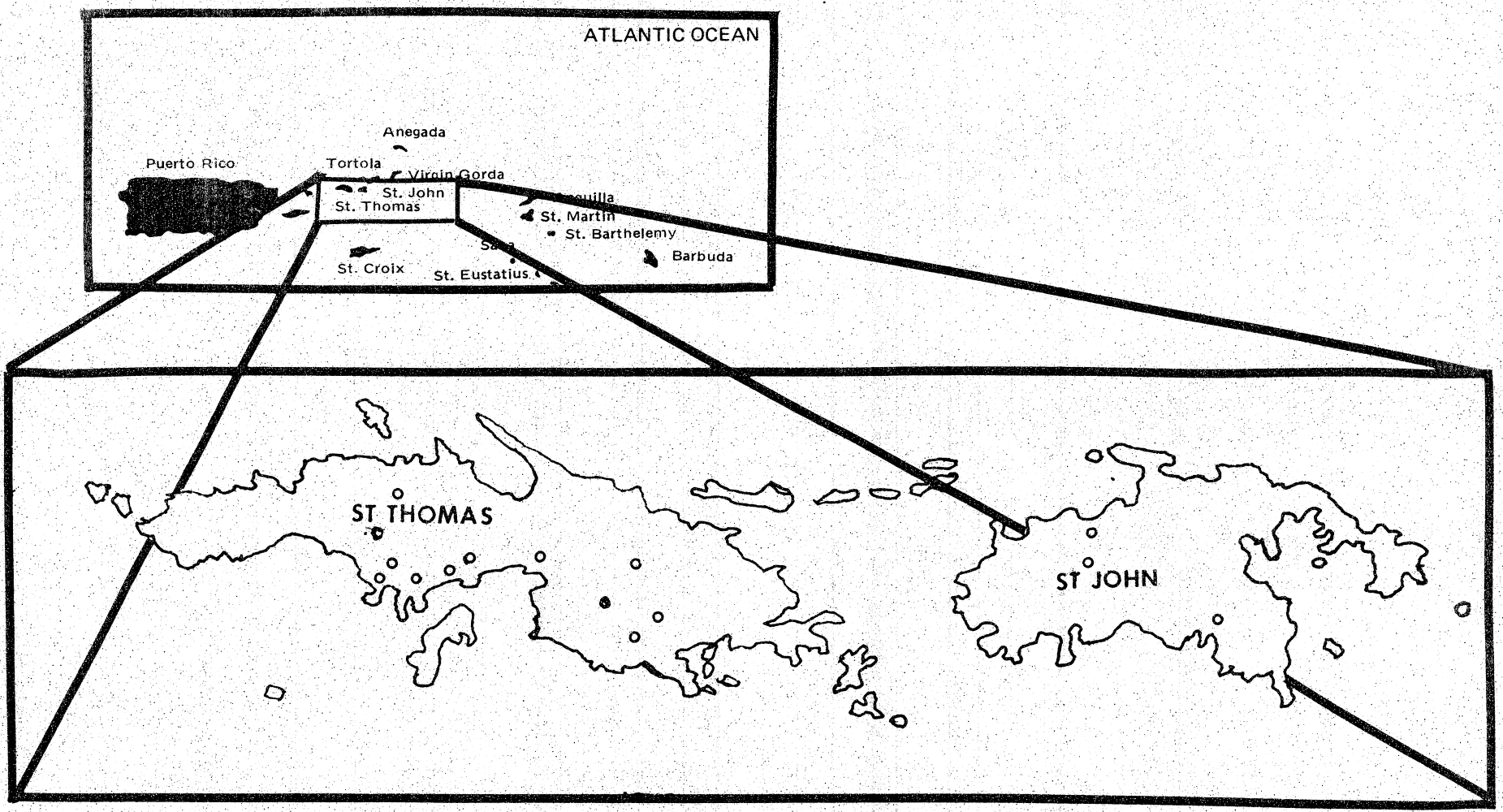


Figure 1. Geographic Location Map for the Virgin Islands.

connected via unregulated commercial water hauling trucks, which carry water from wells or the public water supply to cisterns in homes, businesses, and public buildings without cleaning the trucks.

The objectives of this research are to: 1) Determine presence or absence of common human pathogens in the various water sources of the V.I., (2) to determine whether their presence has any implications, (3) to evaluate whether the use of Environmental Protection Agency standards concerning coliforms is adequate for the various sources of water in the V.I..

METHODS AND MATERIALS

Water samples from each source (Figure 2) were taken in four liter sterile bottles. These bottles were kept at ambient temperature, transported directly to the lab, and analysis begun immediately. An aliquot was taken from the sample for conductance, turbidity and pH analysis. Each sample was filtered through HA and HC membranes (Millipore, Mass.) in 5-10 and 50 ml (duplicate) volumes. Where applicable, membranes were preincubated on pads with enrichment broths; for 2-3 hours on laurel tryptose broth for total coliforms; or 4 hours on phenyl red lactose broth at 35°C before being transferred to selective growth media, then reincubated at an appropriate temperature (Canoy and Knudsen, 1983). Quality control tests were run by: (A) uninoculated media for sterility, (B) using American Type Culture Collection (ATCC) pure cultures with test samples, and (C) running simultaneous biochemical tests on commercial media and on Analytical Profile Index (API-20E, 20S and Staph-Ident) I.D. kits. Anomalous tests were run again and also were queried via the API computer.

The API 20E System is a standardized, miniaturized version of conventional procedures for the identification of Enterobacteriaceae and other Gram-negative bacteria. It is a ready-to-use, microtube (Bartoli, et al, 1972) system designed for the performance of 23 standard biochemical tests from isolated colonies of bacteria on plating medium. Used in conjunction with the API Profile Recognition System (Robertson, et al, 1976), it is intended to enable laboratory personnel to identify members of the family Enterobacteriaceae and other Gram-negative bacteria accurately and easily. The API system has procedures for same day and 18-24 hour identification of Enterobacteriaceae as well as 18-24 or 36-48 hour identification of other Gram-negative bacteria. The biochemical identification of Salmonella arizona, Shigella, Escherichia coli, as well as Vibrio cholerae should be considered a presumptive identification and confirmed serologically.

Specimen Collection and Processing

Samples were collected at 0700-0800 at the stations shown in Figure 2, transported to the laboratory immediately after collection, and processed beginning at 0900. Care was used in proper selection of adequate plating media and the conditions of incubation. For the API 20E, blood agar

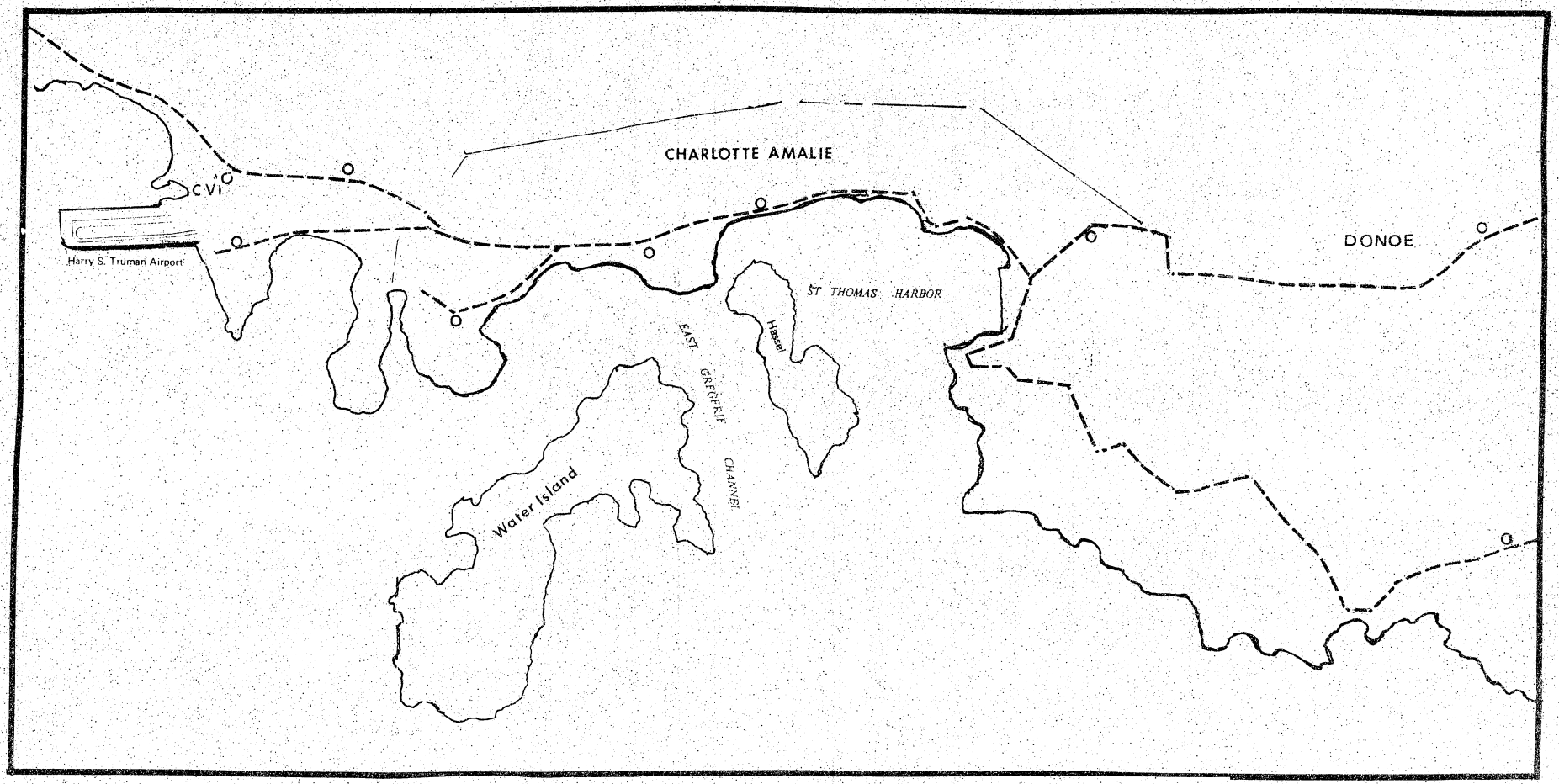


Figure 2. Diagram of the Main Trunk Distrubution System & Sample Stations

plates or MacConkey's agar were streaked for initial enrichment and isolation of colonies to run through the biochemical tests. The API system is standard for medical microbiology.

Chemical and Physical Principles

The API 20E System consists of microtubes containing dehydrated substrates. These substrates are reconstituted by adding a bacterial suspension, incubated so that the organisms react with the contents of the tubes, and read when the various indicator systems are affected by the metabolites or added reagents, generally after 18-24 hours incubation at 35-37°C.

Components

The procedure for each of the tubes are given in Appendix B. The ONPG tube contains an ingredient that functions as an internal indicator. The ADH, LDC , ODC and URE tubes contain phenol red as the indicator. The CIT, GLU, MAN, INO, SOR, RHA, SAC, MEL, AMY and ARA tubes contain charcoal and the H S tube contains iron salts as indicators. The TDA, IND and VP tubes contain no indicator. All of the tubes contain buffers, and all tubes, with the exception of the CIT, and URE tubes, contain peptone. All of these components retain their reactivity for 18 months.

Identification of Organisms

Identification of the organism can be made either with the aid of API differential charts, or by using the computer Profile Recognition System.

When differential charts are used to determine the identity of cultures, the aggregate reactions given by the strains should be considered as a whole. Even when this is done, however, the conclusions reached may differ from laboratory to laboratory.

For rapid identification of species and biotype levels, the API Profile Recognition System (PRS), Computer Service and Analytical Profile Indexes are available. A data base compiled from strains of the collections of several institutions as well as type and neotype cultures allows very precise statistical calculation and thus the identification of less commonly occurring microorganisms.

Quality Control

Biochemicals incorporated into the API 20E System are quality controlled by standard testing procedures prior to their usage in the system. Each lot is controlled with stock cultures for sensitivity and specificity.

Because of the use of lyophilized cultures for quality control procedures, several transfers were made prior to the inoculation.

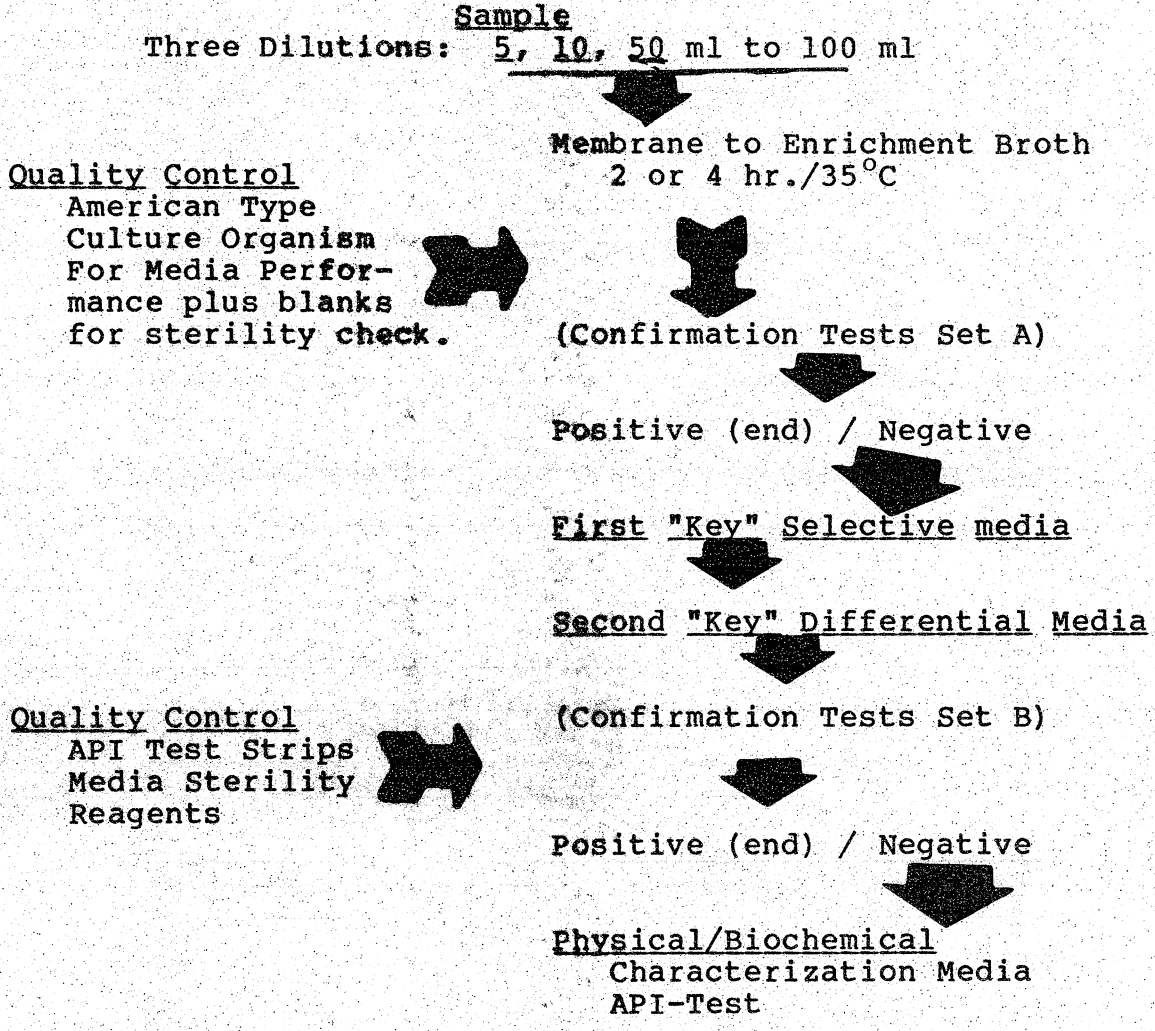
API recommends that quality control be done using the 18-24 hour procedure. Same day results for the recommended ATCC cultures are available from Technical Services, API for:

1.	Klebsiella pneumoniae	ATCC 13883
2.	Enterobacter cloacae	ATCC 13047
3.	Proteus vulgaris	ATCC 13315
4.	Pseudomonas aeruginosa	ATCC 10145

API differential charts have been derived from type and neotype cultures and strains from various collections. The percentages obtained on API may differ from percentages appearing in published material based on macromethods. For example, the urease test found on API is a Ferguson (1948) formulation which is less sensitive than Christensen's formulation which is used widely in existing macrotechniques. Reactions recorded as delayed positive with macromethods generally are shown as negative on the API chart for Enterobacteriaceae since this chart is based on results obtained exactly after 5 or within 18-24 hours of incubation depending on the coordinate parallel standard tests being carried on at the same time.

Figure 3.

Generalized Flow Diagram of Microbiological Samples.



The first quality control effort is made by setting aside 1% of the containers with uninoculated media, which is watched for contamination. A second quality assurance measure involved inoculation of each media with pure culture bacteria from the American Type Culture Collection (ATCC) (Table 1) and observing their relative reactions. The final confirmation was a comparison of Analytical Profile Index (API) test strips with standard (APHA, 1984) characterization medias.

Table 1.

Quality Control Confirmation: Type Cultures Used

Difco Bactrol Disk, (Lot # 1628-32-2 and Lot # 1656-32-7)

ATCC # 8100 - *Serratia marcescens*

ATCC # 13315 - *Proteus vulgaris*

ATCC # 27853 - *Pseudomonas aeruginosa*

ATCC # 23355 - *Enterobacter cloacae*

ATCC # 13048 - *Enterobacter aerogens*

ATCC # 25922 - *Escherichia coli*

ATCC # 13883 - *Klebsiella pneumonia*

ATCC # 8090 - *Citrobacter freundii*

ATCC # 19606 - *Acinetobacter calcoaceticus*; var. *anitratus*

ATCC # 14028 - *Salmonella typhimurium*

ATCC # 19615 - *Streptococcus pyogenes*

ATCC # 25923 - *Staphylococcus aureus*

ATCC # 12228 - *Staphylococcus epidermitis*

Table 2.

Media Used In Isolation and Identification

---

mEndo-Agar-LES
mFC-Agar
mStaphylococcus Broth
mBrilliant Green Broth
mBismuth Sulfite Broth
mTetrathionate Broth Base
Phenol Red Lactose Broth
Plate Count Agar
Brain-Heart Infusion Broth
Brain-Heart Infusion Agar
Brain-Heart Infusion Agar w/5% blood
Nutrient Broth
Simmons Citrate Agar
MR-VP Medium
Tryptone
Urea Disk
Lauryl Tryptose Phosphate Broth
Brilliant Green Bile Broth 2%
EC Broth
Oxidation-Fermentation Glucose Medium
Levine EMB Agar
Bismuth Sulfite Agar
S/S Agar
Pseudomonas Isolation Agar
Motility GI Medium
Rabbit Plasma w/EDTA
XLD Agar
Sellers Agar
Hectoen Agar
MacConkey Agar
Triple Sugar Iron Agar
Yersinia Selective Agar
DNase Test Agar w/Methyl Green
Decarboxylase Base (Moeller) w/Lysine - Arginine & Ornithine
Nutrient Agar
KCN Broth Base
Phenylalamine Agar
Bile Esculin Azide Agar
KF-Streptococcus-Broth<KF-Streptococcus Agar
Phenol Red Lactose Broth w/Rhamonose
Phenol Red Lactose Broth w/Melizitose
E. Coli OK Antiserum Poly A, Lot 2677-47 (Difco)
E. Coli OK Antiserum Poly B, Lot 2731-47 (Difco)

RESULTS

As can be seen from Tables 3 A - C there is a wide variety of bacteria in Virgin Islands water. Furthermore certain bacteria, Klebsiella, Streptococcus, and Acinetobacter species were found in every sample regardless of origin.

Of 16 samples in this study, 8 were positive for the human fecal indicator organism Escherichia coli, and only 3 of the 8 showed fecal coliforms were in numbers above EPA guidelines. Of these eight samples seven showed Standard Plate Counts in excess of 1 million (1×10^6) per 100 ml. Presence of E. coli however, would have failed to predict the occurence of pathogens in three sources (Samples 261,265,268) while total coliform and total plate counts did. No discernable pattern exists for relating E. coli to anything other than general enteric bacteria. Fecal streptococcus however occurred in 15 of 16 samples and could be related to both the number of pathogens and the Standard Plate count (See Table 3A).

The largest number of pathogens were found in the St. Thomas potable water distribution system, increasing in number directly proportional to the distance from the holding tank. (See Figure 2.)

Table 3A.

Source and Identification of Waterborne Bacteria from the Virgin Islands.

Location/Sample Number	Source	Organism
#252	Cistern residential	Streptococcus faecalis
		S. faecium (Group D.)
		Escherichia coli,
		E. Coli, (H_2S+)
		Acinetobacter calcoaceticus var. anitratus
		Kluyvera sp.
		Enterobacter agglomerans (alt. Erwinia)
		Aeromonas hydrophilia
#253	Cistern residential	Streptococcus faecalis
		Flavobacterium meningosepticium
		Pseudomonas fluorescens
#254	Cistern, Public Housing	Streptococcus faecalis
		S. faecium (Group D)
		Escherichia coli
		Acinetobacter colcoaceticus var. anitratus
		Pseudomonas aeruginosa
		Citrobacter freundii (H_2S+)
		Enterobacter cloacae
		Morganella morganii
		Proteus mirabilis
		Staphylococcus haemolyticus

Table 3A Con't

Location/Sample Number	Source	Organism
#255	Cistern, Public Housing	Streptococcus faecalis
		Enterobacter cloacae
		Aeromonas hydrophilia
		Enterobacter agglomerans
		Pseudomonas sp. (fluorecent group)
		Escherichia hermanii (CDC Group II)
		Proteus vulgaris
		P. mirabilis
		Staphylococcus simulans
		S. saprophyticus
#256	Cistern residential	Streptococcus faecalis
		Klebsiella pneumonia
		Escherichia coli (Poly "B" pos.)
		E. coli ("A" & "B" neg.)
		Enterobacter cloacae
		E. sakazaki
		Escherichia hermanii (CDC Group II)
		Pseudomonas aeruginosa
		Acinetobacter calcoaceticus var. anitratus
#257	Cistern residential	Streptococcus faecalis
		Klebsiella pneumonia

Table 3A Con't

Location/Sample Number	Source	Organism
		Enterobacter cloacae
		Pseudomonas sp.
		Pseudomonas aeruginosa
		Escherichia coli (poly "A" pos./"B" neg.)
		Flavobacterium breve (non-sachrolytic)
		Acinetobacter calcoaceticu var. Lwoffi
#258	Cistern residential	Streptococcus faecalis
		Enterobacter cloacae
		Citrobacter sp.
		Klebsiella terrigana
		Pseudomona aeruginosa
		Staphyloccocus simulans
		S. saprophyticus
#259	Cistern residential	Streptococcus faecalis
		Acinetobacter calcoaceticus
		Flavobacterium meningosepticum
		Staphylococcus hemolyticus
#261	Potable Water (VI Intern. Rev.)	Streptococcus faecalis

Table 3A Con't

Location/Sample Number	Source	Organism
#261 Con't	Potable Water	Klebsiella terrigana
		Escherichia coli (Poly A neg/ Poly B neg)
		Enterobacter cloacae
		E. sakazakii
		E. agglomerans
		Klebsiella planticola
		Vibro fluvialis
		Providencia rettgerii
		Klebsiella oxytoca
		Kluyvera sp.
		Enterobacter aerogens
		Aeromona hydrophylia
		Serratia marcescens
		Acinetobacter calcocaceticus var. anitratus
		Pseudomonas putida
		Staphylococcus hemolyticus
#262	Potable Water System Subbase	Streptococcus faecalis
		Klebsiella pneumonia
		K. planticola
		K. terrigana
		Acinetobacter calcoaceticus var. Lwoffi

Table 3A Con't

Location/Sample Number	Source	Organism
#263	Well (CRI)	Streptococcus faecalis
		S. faecium
		Citrobacter freundii (H_2S+; Indole -)
		Streptococcus avium
#266	Well (Private)	Streptococcus faecalis
		Escherichia coli (H_2S+) ("Poly A" ++)
		E. coli ("Poly B" +)
		Klebsiella planticola
		Enterobacter cloacae
		Aeromonas hydrophilia
		Staphylococcus saprophyticus
#267	Potable Water System W. Veteran's Drive (Public School)	Streptococcus faecalis
		Acinetobacter calicoaceticus var. anitratus
		A. calicoaceticus var. Lwoffi
		Enterobacter cloacae
		E. sakazakii
		Klebsiella pneumonia
		Flavobacterium odoratum
		Chromobacterium typhiflavorum

Table 3A Con't

Location/Sample Number	Source	Organism
#267 Cont'd		Pseudomonas aeruginosa
		Staphylococcus xylosus
#268	St. Thomas Airport (Drinking fountain)	Streptococcus faecalis
		Acinetobacter calcoaceticus var. Lwoffi
		Pseudomonas cepacia
		P. fluorescens
		CDC Group IVE - (possibly) Alcaligenes group
		Klebsiella pneumonia
		Enterobacter sakazakii
		E. agglomerans
		Staphylococcus hyicus
		S. sciuri

A study of the occurrence and etiology of waterborne diseases in the U.S. (Table 4) shows only those diseases causing gastrointestinal illness and ignores known respiratory, skin, and genitourinary tuberculosis, Streptococcus infections, etc.. Still this record indicates that 55% of gastroenteritis were of "unknown" origin. From a public health standpoint the implications are amazing. Well over half the true potential for waterborne diseases is in a "blank" data area.

DISCUSSION

Organisms Of Concern

Waterborne Respiratory Pathogens (See Table 6 for Etiology)

Respiratory diseases are not generally considered to be waterborne but in fact many are spread by microscopic droplets (foamites) from fountains, showers, sprinklers, or "wet" air conditioners. The most dramatic of these diseases recently has been Legionnaires' Disease which by a social/environmental fluke achieved national attention. Other non-pneumococcal pneumonias include Klebsiella, Staphylococcus, and Streptococcus as well as various gram negative Enterobacter, Proteus, Serratia, and Pseudomonas. These are opportunistic pathogens which are seldom epidemic and generally strike the very young and old and the already ill or immuno-compromised individual.

Ninety four percent of the drinking water tested in the V.I. was found to be contaminated with Streptococcus bacteria which can cause respiratory infections. Furthermore, 15 of 16 samples had one or more other species of bacteria implicated in respiratory disorders. The species involved are from the genera Acinetobacter, Aeromonas, Enterobacter, Flavobacterium, Klebsiella, Moraxella, Pseudomonas, Staphylococcus, and Streptococcus (Tables 3A-C & 5). The most common genera, occuring in 75% of the

samples, were pathogenic Enterobacter, Klebsiella, Streptococcus, and Pseudomonas, Acinetobacter spp. Eight of the 16 samples included a study on the presence of Legionella spp.; of these 63% were positive for Legionella pneumophilia (Table 3B).

Legionnaires' disease was named for the first recognized epidemic which began with an American Legion Convention in 1976. In retrospect it is now known that epidemics have occurred back at least as far as 1947.

Of the established 22 species of Legionella the organism L. pneumophila is the major pathogen. Probably common environmental sources of infection are sprays, showers, and cooling towers. It has been found in a variety of waters in Puerto Rico and the Virgin Islands.

The causative organism initially found in the V.I. in cistern water (Schlech, et al, 1985) and reconfirmed by Hazen, Knudsen, and Canoy (1985), has now been isolated from drinking water supplies. Five serogroups of L. pneumophila were identified in this study and more extensive sampling could possibly bring the number to ten or more.

The greatest health risk is not from drinking water but from showers, humidifiers, nebulizers, or inhalation therapy machines. The high risk groups are infants, aged, or medically compromised persons.

Table 3B.

Water Borne *Legionella* Species From the Virgin Islands

ID	*L. pneumophilia* Serogroup 1	*L. pneumophilia* Serogroup 2	*L. pneumophilia* Serogroup 3	*L. pneumophilia* Serogroup 4	*L. pneumophilia* Serogroup 5
252A*	$6.3 \times 10^{2}_{2}$	$1.6 \times 10^{2}_{2}$	$2.3 \times 10^{2}_{2}$	$2.1 \times 10^{1}_{2}$	$2.3 \times 10^{2}_{2}$
252B**	$2.1 \times 10^{1}_{2}$	$1.9 \times 10^{2}_{2}$	$1.9 \times 10^{2}_{2}$	$2.2 \times 10^{2}_{2}$	$2.0 \times 10^{2}_{2}$
256A	$2.4 \times 10^{1}_{1}$	$6.7 \times 10^{1}_{1}$	$3.2 \times 10^{1}_{1}$	$3.2 \times 10^{1}_{1}$	$3.8 \times 10^{1}_{1}$
251A	$<1 \times 10^{2}_{2}$	$<1 \times 10^{2}_{2}$	$<2 \times 10^{2}_{2}$	$<1 \times 10^{2}_{2}$	$<1 \times 10^{2}_{2}$
250A	$7.4 \times 10^{2}_{2}$	$5.5 \times 10^{2}_{2}$	$4.1 \times 10^{2}_{2}$	$2.1 \times 10^{2}_{2}$	$3.7 \times 10^{2}_{2}$
257A	$1.1 \times 10^{2}_{1}$	$1.3 \times 10^{2}_{1}$	$4.6 \times 10^{2}_{1}$	$4.0 \times 10^{2}_{1}$	$4.0 \times 10^{2}_{1}$
249A	$<1 \times 10^{1}_{1}$	$<1 \times 10^{1}_{1}$	$<1 \times 10^{1}_{1}$	$<1 \times 10^{1}_{1}$	$<1 \times 10^{1}_{1}$
253A	$<1 \times 10$	$<1 \times 10$	$<1 \times 10$	$<1 \times 10$	$<1 \times 10$

* Ambient temperature sample source
** Hot water tank (residential) sample source
NB All data per one ml sample

Table 3C.

A Comparison of Two Indicators of Contamination
With Actual Occurrence of Pathogens

ID	A Fecal Coliform (MPN)	B Fecal Streptococcus (MPN)	C Standard Plate Count	D No. of Pathogenic Species
252	1.6×10^4	2.6×10^3	1.1×10^6	*~~0~~ 6
253	3.3×10^1	3.5×10^2	1.4×10^5	*~~0~~ 4
254	4.9×10^1	2.4×10^2	2.0×10^6	*~~0~~ 8
255	7.9×10^1	1.1×10^3	1.4×10^6	*~~0~~ 8
256	1.6×10^3	1.3×10^2	4.0×10^6	*~~0~~ 8
257	0.2×10^1	4.6×10^1	3.9×10^4	*~~0~~ 7
258	0.2×10^1	-0-	6.5×10^4	~~0~~ 4
259	-0-	-0-	4.1×10^4	~~0~~ 3
261	-0-	5.4×10^2	7.1×10^1	! 13
262	-0-	-0-	0.7×10^6	! 2
263	-0-	5.4×10^2	2.6×10^1	*~~Ø~~ 3
264	-0-	-0-	2.8×10^2	! 2
265	-0-	0.7×10^1	6.6×10^3	! 10
266	0.8×10^1	1.0×10^1	1.2×10^2	~~Ø~~ 3
267	0.9×10^1	2.0×10^3	3.7×10^3	!~~Ø~~ (Mix) 9
268	0.2×10^1	5.2×10^1	2.5×10^3	! 11

* Samples which proved 1 million (1×10^6) bacteria or more per 100 ml
! Samples from public potable water
~~0~~ Samples from roof catchment cistern resovoir
~~Ø~~ Samples from wells
NB All figures listed in Column A,B, & C are per 100 ml sample volume

Table 4.

Etiology of Waterborne Disease Outbreaks in the United States, 1971-1979

	Outbreaks (Percent)
Acute Gastrointestinal Illness	55
Chemical Poisoning	11
Giardiasis	11
Shigellosis	8
Hepatitis A	6
Salmonellosis	3
Viral Gastroenteritis	2
Typhoid	2
Toxigenic *E. coli* Gastroenteritis	1
Campylobacter Gastroenteritis	1
	100

*From: US EPA, 1983. "Assessment of Microbiology and Turbidity Standards for Drinking Water". Proc. of Workshop - (NTIS A EPA 570-9-83-001).

Table 5 . Classification of Water Borne Streptococci from the V.I. and the Diseases They May Cause

Lancefield Group	Species	Hemolysis	Cellular Products	Extracellular Products	Clinical Diseases
A	S. pyogenes	Beta	Hyaluronic acid capsule Group A carbohydrate M protein	Streptolysins Hyaluronidase. Streptokinase Leukocidin Erythrogenic toxic	Pharyngitis-tonsilitis Skin infections (impetigo) Erysipelas Scarlet Fever Postpartum endometritis Rheumatic fever Glomerulonephritis Endocarditis
B	S. agalactiae	Beta	Group B carbohydrate	Streptolysins	Neonatal sepsis and meningitis Postpartum endometritis
C	S. equisimilis	Beta	Group C. carbohydrate	Streptolysins	Impetigo-Pharyngitis
D	Enterococci (S. faecalis)* Non-enterococci (S. bovis)*	Gamma	Group D carbohydrate-amino acid complex		Endocarditis Urinary tract infections
K	S. salivarius	Alpha	Group K carbohydrate	Glucan-like substance	Endocarditis Dental caries
H	S. sanguis	Alpha	Group H carbohydrate	Glucan-like substance	Endocarditis Dental caries
Not grouped	S. mitis	Alpha			Endocarditis Dental caries
Not grouped	S. mutans	Alpha		Glucan	Dental caries Endocarditis

* Other species of GROUP D streptococci occur that may be alpha, beta, or gamma hemolytic

Table 6.

Etiology of V.I. Waterborne Bacterial Diseases
Acquired Through the Respiratory Tract

Clinical Disease	Causative Organism	Other Possible Entry Routes
Upper Respiratory Infections		
Pharyngitis	Streptococcus (group A)	
Laryngitis (children)	Streptococcus pneumoniae Streptococcus (group A) Staphylococcus aureus enteric bacilli Gram-negative	
Bacterial Pneumonias		
Other bacterial pneumonias	Streptococcus pyogenes Staphyloccus aureus Klebsiella pneumoniae	
Legionnaires' disease	Legionella pneumonphila	Unknown

Table 6 Con't

Clinical Disease	Causative Organism	Possible Entry Routes
Streptococcal Diseases		
A. Strep (sore) throat	Streptococcus pyogenes	
B. Scarlet fever	Streptococcus pyogenes	
C. Sequelae of group "A" strep infections:	"	
Rheumatic Fever	"	
Acute hemorrhagic glomerulonephritis	"	
Tuberculosis		
"Atypical " Tuberculosis		
Mycobacterium spp. (not M. tuberculosis)	"Atypical" mycobacteria	

Waterborne Gastrointestinal Pathogens
(See Table 7. for etiology)

Salmonella (Salmonellosis)

The genus Salmonella occurs in the feces of man and a number of birds and animals. It is normally spread via water or food contamination. The organism prefers nutrient rich media and temperatures of less than 40°C. While there are several hundred serotypes known only some 20-30 are common and of those only about 13-15 normally cause disease in humans. The "Typhoid Fever" organism S. typhi is the only species disease specific for man.

Salmonellosis (Including typhoid)

At one time over 1800 serotypes of Salmonella were considered separate species. Now this has been reduced to about 200 and probably soon this will reduce to one or two "serologically highly diverse" species. While only S. typhi is specific for many humans, perhaps most of the others, are pathogenic to some degree.

Gastroenteritis is the generic term for Salmonella food poisioning. Various Salmonella species may be involved and the source is usually water or food. In many cases contamination comes from a non-symptomatic person ("the carrier") or from the animal which produced the food, as often occurs with eggs. Salmonellids were found in a well on St. Croix during a previous study (Canoy, Knudsen

Table 7.

Etiology of V.I. Waterborne Bacteria
Acquired Through the Alimentary Tract

Clinical Disease	Causitive Organism	Other Possible Entry Routes
Acute Gastrointestinal Infections		
Salmonellosis	Salmonella spp. (many sterotypes)	
Shigellosis (bacillary dysentery)	Shigella spp.	
Gastroenteritis		
	Enteropathogenic E. coli	
	Campylobacter spp.	Sexual Placental Water
	Yersinia enterocolitica	Respiratory? Water?
	Bacillus cereus	
Epidemic diarrhea nurseries	Enteropathogenic Escherichia coli	
Diseases Caused by Ingestion of Preformed Toxin		
Staphylococcal intoxication	Staphylococcus aureus enterotoxin	

Table 7.

Clinical Disease	Causative Organism	Other Possible Entry Routes
Infectious Systematic Diseases		
Typhoid Fever	*Salmonella typhi*	
Salmonellosis	*Salmonella* spp.	
Salmonella septicemia	*Salmonella cholerae-esuis*	

and Garcia, 1985) but were not definitely identified during this study of water in the Virgin Islands. Due to the generally high number of Salmonella necessary to cause an infection and the probability of being "masked" by other bacteria, a lack of disease or colony isolates does not necessarily mean they are absent.

The genus Salmonella was isolated from a cistern in St. Thomas in 1983 (Canoy and Knudsen, 1983). Shortly thereafter a few isolated cases of typhoid occurred on St. Croix. A study of wells in the V.I. in 1984 confirmed Salmonella spp. from two wells on St. John and one on St. Croix. In 1985, the 3rd largest U.S. outbreak of typhoid occurred on St. Croix totaling 66 cases hospitalized.

Considering the low level of Salmonella, even in known contaminated areas, detection is not easy in any case. To find positive identification in four sites was in itself cause for some concern. No attempt was made to identify the organisms to species, which would have required serology and/or immuno-fluoro techniques presently beyond the laboratory. Shigellosis is an intestinal disease of varying severity ranging from simple dysentary to cholera-like symptoms. Shigella species are responsible for about 30% of the "summer dysentery" outbreaks in the U.S. In the U.S. and Europe Shigella related fatalities

are only about one percent. In tropical areas with poor health care, fatalities in epidemics may reach twenty percent.

Salmonella and Shigella spp. are not detectable using the standard indicator tests. Only lactose fermenting bacteria are detected in standard water tests; and except for a few genera these are non-pathogenic.

Escherichia coli, Enteropathogenic varieties (acute gastroentritis)

E. coli is a normal inhabitant of the human colon. As such it has become the standard indicator organism for human fecal pollution. Many scientists now criticize this on the basis that, "E. coli is not a pathogen". In actuality about 0.5 - 1.0% of the U.S. children under 5 years of age and 2.2 - 3.8% of the children in developing countries are stricken with E. coli gastroenteritis (WHO annual report, 1978). The symptoms of the disease are a profuse watery diarrhea, with little mucous or blood, nausea, prostration and dehydration. The organism apparently excretes an enterotoxin and also penetrates the colonic epithelia. With the advent of modern antibiotics and intervenous feeding, the disease in the developed countries has become only an unpleasant nuisance. In developing

countries, and in poverty areas of the U.S. it is a serious, often fatal, disease of young children.

The isolation of hydrogen sulfide (H_2S) producing E.coli from wells (Canoy, Knudsen, Garcia, 1985) and from public drinking water (this study) indicates a potential pathogenic E. coli. Due to the biochemistry of this variety it is not detected on standard confirmed fecal coliform tests. The presence of these bacteria in the water is, especially for infants and medically compromised patients, of concern.

Waterborne Disease and Environmental Health Considerations

Potential dispersal routes for waterborne pathogens include:

Human vectors

Potable Water Distribution System

Water Transport Trucks

Sewage

Animal vectors

Rain/Wind

Human vectors (as opposed to general sewage) include persons who handle food or water or those who deliberately spit or excrete into and around cisterns and wells. This category also includes persons in charge of wells, cisterns, trucks, and barges who do not clean or close them properly.

At this time (1985) there is no satisfactory estimate as to the importance of this vector. In a recent typhoid epidemic, statements were made, but not substantiated, that food handlers were the source. Other reports said that feces deposited around or in the cisterns were the source or blamed cross contamination between water and sewer pipes. The importance of this vector needs to be addressed even though it is very time consuming.

The potable water distribution system has been blamed often for allowing cross-contamination with sewage. Again there is no good estimate of the magnitude of this problem. However in the present study six samples were taken along the distribution line. The closest sample to the storage tanks was number 262 in Subbase and had two (2) potential pathogens. After this point one pipe goes to Veteran's Drive West and one to Veteran's Drive East and the town of Charlotte Amalie. The Veteran's Drive West sample (267) had nine (9) pathogens, the East Veteran's Drive (264) had two (2) pathogens and (261) east of town (towards end of line) had thirteen (13) pathogens. Another sample, on a branch of the system, was from the airport and had eleven (11) potential pathogens.

From the limited number of samples it appears that in fact the farther from the storage and treatment site, the

greater numbers of total bacteria and potential pathogens. This would imply some sort of cross contamination. As critical as this factor is, a great deal of importance should be placed on determining the locale of contamination. This could be done in 100 samples using both bacterial tests at various points in the lines, and an extremely fluorescent dye such as Rhodamine B injected into the sewage lines. It is possible that area treatment equipment should be installed or other precautions taken.

Water transport trucks are used to deliver drinking water to areas not served by the public distribution system. Some of these trucks are owned or leased by the Housing Authority or Public Works, however, most are private contractors. They all have a potential for contamination from polluted wells, public cisterns, or illegal withdrawals from the distribution system. Since there is no control on servicing, cleansing or monitoring, these trucks are potential hazards. The owners will not permit sampling by private researchers and so any evaluative study will have to have government backing. Furthermore, because of the unique environment, any simple coliform test would be meaningless. The tests required would be a Total Plate Count plus some other indicator, perhaps fecal Streptococcus, Clostridium, Aeromonas, or Pseudomonas.

Sewage contamination has often been blamed for lowered water quality in cisterns, wells, and the public system. The type and number of bacteria found in this series of studies (Rhineheart, et al, 1983; Canoy, Knudsen and Garcia, 1985) confirm sewage contamination but do not pinpoint a single source of contamination. There are three major potential sources; cross contamination between water and sewage pipes, surface and subsurface entry from septic tanks, and runoff carrying surface wastes. No factorial study has ever been done to evaluate these three routes.

Animal vectors include cattle, pets, wild birds, rats, mongooses, and reptiles or amphibians. These animals deposit feces around poorly sealed wells and cracked cisterns. At times not only the feces of smaller animals but smaller animals themselves may enter directly into open cisterns or wells.

These animals carry pathogenic Salmonellas, Streptococcus and atypical tuberculosis as well as a variety of Aeromonas and Pseudomonas.

Rain and wind play an unknown role in carrying pathogens. Flooding and soil percolation of rain can carry pathogens into wells and cisterns. Road dust is constantly being swirled up into the air to be added to water foamites from wastes and sewage aerators as well as

dust borne on upper winds from as far away as Saharan Africa. This mixture can then be rained out on water catchment surfaces locally.

CONCLUSIONS

This research has shown that the private and public potable water systems in the Virgin Islands are frequently contaminated with potentially hazardous bacteria. In the present study, and two preceeding efforts; 125 cisterns, 26 wells, and 6 public water outlets were tested. Even though the tests were not exhaustive for all possible pathogens only two wells, four cisterns, and no public water outlets were found to be free of the present EPA standard indicator bacteria. The implications of this in terms of basic human health and high risk to infants, elderly, and medically compromised persons are dramatic. Special monitoring and prophylaxis for the high risk groups are a critical need. Furthermore the large influx of tourists and foreign immigrants in the V.I. complicate the water pathogen situation by the introduction of organisms and by providing a large mobile host reservoir because those travelers are from many areas where typhoid, cholera, yellow fever, and other "exotic" pathogens still are active. When introduced here, transients going to the U.S. mainland or Europe could easily transfer the disease before

it is even identified like the controlled outbreaks of typhoid in 1984-85. This would be nothing short of an economic, as well as a public health, crisis.

The majority of the pathogens found cause one of three general maladies: 1) Skin or wound septicemia, 2) Respiratory and Genito-urinary tract infections (particularly of women), 3) Gastro-intestinal infections. Less common are the atypical bacterial pneumonias and tuberculosis. These bacteria are not generally (but can become) primary pathogens and so usually become critical in already weakened hosts or cause chronic low level debilitating diseases.

Current water standards in the V.I. are those developed in the U.S. for a different geography, population, and water system. Neither the tests, monitoring, or indicator organisms appear appropriate to the local situation. These are particularly questionable in view of the diverse sources of water, dissolved organics, heat, turbidity, and storage times of water. Studies are required to provide data to develop more appropriate tests and to recommend to the lawmakers more appropriate local regulations for water systems including wells, trucks, and public cisterns. Strict regulation of water distribution and treatment, septic tanks, and sewage is required.

In addition to the physical measures called for, a massive educational effort is necessary on the part of schools, government, and civic organizations to create an informed and active public relative to water quality.

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Appendix A.

Classification of Medically Important Waterborne Bacteria from the U.S. Virgin Islands

Table 8. Classification of Medically Important Water Borne Bacteria Found in the V.I.

Classification		Disease
A. Group: Gram-negative rods, facultatively anaerobic		
Family I.ENTEROBACTERIACEAE		
Motile or nonmotile; differentiate fermentations and antigenic properties; some commensalistic in intestinal tract with pathogenic potential for extraintestinal tissues; some are pathogenic parasites acquired through alimentary route		
Genus I:	Escherichia E. coli	Epidemic diarrhea of infants; urinary tract and wound infections; "turista"
Genus IV:	Salmonella	
	S. typhi	Typhoid Fever
	S. enteritidis	Gastroenteritis, para typhoid, and enteric fevers (many) varieties of this species may cause these diseases)
	S. cholera-suis	Septicemia, multiple abcesses

Table 8 Con't

Classification	Disease
A. Group: Gram-negative rods, strictly aerobic	
Family I.PSEUDOMONADACEAE	
Genus I: *Pseudomonas*	
P. aeruginosa	Burn, wound and systematic infections
B. Group: Gram-negative rods, facultatively anaerobic	
Family I. Enterobacteriaceae	
Motile or nonmotile differentiated by carbohydrate fermentations and antigenic properties; some commensalistic in intestinal tract with pathogenic potential for extra-intestinal tissues; some are pathogenic parasites acquired through alimentary route	
Genus I: *Escherichia*	
E. coli	Epidemic diarrhea of infants; urinary tract and wound infections; "turista"
Genus IV: *Salmonella*	
S. typhi	Typhoid fever
S. enteritidis	Gastroenteritis, paratyphoid, and enteric fevers (many varieties of this species may cause these diseases)
S. cholera-suis	Septicemia, multiple abscesses
Genus V: *Shigella S.*	
S. boydi, flexneri, *S. sonnei*	Severe diarrheic disease
Genus VI: *Klebsiella*	
K. pneumoniae	Pneumonia Urinary tract infections

Table 8 Con't

Classification	Disease
Genus VII: *Enterobacter* *E. cloaçae* *E. aerogenes* *E. agglomerans* (see *Erwina* Genus XII in family Enterobacteriaceae)	Normal intestinal tract, may cause urinary tract infections Has caused systematic infection when introduced in contaminated solutions injected intravenously
Genus IX: *Serratia* *S. marcescens*	Urinary and respiratory tract infections
Genus X: *Proteus* *P. vulgaris* *P. mirablis*	Urinary tract and wound infections; infant diarrhea
Genus XI: *Yersinia* *Y. enterocolitica*	Intestinal tract of animals and humans, may cause diarrhea, mesenteric lymphadenitis, abscesses, meningitis
Genus XII: *Erwina* *E. herbicola* (a plant pathogen) also classified as *Enterobacter agglomerans*	Has caused systemic infection when introduced in contaminated solutions injected intravenously.
Family II.VIBRIONACEAE	
Genus I: *Vibrio* (curved rods)	Marine environment, seafoods, may cause acute enteritis
Genus II: *Aeromonas*	Associated with systemic and intestinal infections
Genera of Uncertain Family	
Genus *Legionella* *L. pneumophila*	Legionnaire's disease (mild to severe pneumonia)

Table 8 Con't

Classification	Disease
C. Group: Gram-positive cocci, aerobic and/or facultatively anaerobic, some strictly anaerobic	
Family I.MICROCOCCACEAE Arranged in clusters or packets, saprophytic or parasitic, identified by coagulase, hemolysin, and pigment production	
Genus II: Staphylococcus	
S. aureus	Skin abscesses, impetigo wound infections, pneumonia and other systemic infections, enterotoxin-producing strains cause food poisoning, exfoliative toxin causes "scalded skin" syndrome infants, other toxins cause toxic shock syndrome
S. epidermidis	Normal commensal of skin but may cause local or systemic infections if introduced by contaminated sutures or instruments
S. saprophyticus	Associated with urinary tract infection in young women

Table 8 Con't

Classification	Disease
Family II. STREPTOCOCCACEAE Changing cocci, commensalistic and/or pathogenic	
Genus I: Streptococcus	
S. pneumoniae	Pneumonia, other systemic infections
S. pyogenes (usually beta-hemolytic)	Scarlet fever, sore throat, erysipelas, impetigo, postpartum endometritis, wound and burn infections, and other serious diseases
S. faecalis ("enterococci")	Normal intestinal tract, sometimes cause respiratory and urinary tract infections, infective endocarditis
S. bovis	Intestinal tract of cows, and other animals, causes human endocarditis
S. agalactiae	Mastitis in cows, in humans, neonatal septicima and meningitis (infected mothers may also have serious systematic disease)
Viridans group (alpha hemolytic species that produce greening of blood agar); includes following species:	Normal respiratory tract; chronic mucosal infections; infective endocarditis
S. mitis	Normal respiratory tract; may cause endocarditis, dental caries
S. salvarius	Same as above
S. mutans	Major cause of dental caries; may cause endocarditis
S. equismilis	Impetigo, pharyngitis