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Bacteriological contamination in the karstic aquifer of Yucatán, Mexico

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RESUMEN

El acuífero kárstico de Yucatán es al mismo tiempo la única fuente de agua para abastecimiento y receptor de las aguas de desecho. El uso y consumo de esta agua sin algún tratamiento ocasiona en los habitantes algunos problemas de salud como son la diarrea, gastroenteritis, infecciones del tracto urinario y septicemia. El objetivo de este estudio fue determinar el grado de contaminación bacteriológica en el agua subterránea de una región rural que carece de condiciones de saneamiento. La técnica empleada para la determinación de las densidades de organismos coliformes totales y fecales fue la de fermentación en tubos múltiples; además, se identificaron cinco géneros de la familia de enterobacterias por medio de reacciones bioquímicas. Los resultados mostraron que el comportamiento de las densidades de los organismos coliformes totales y fecales, tuvieron el mismo patrón de comportamiento que las precipitaciones pluviales durante el período en estudio y que dichas densidades excedían los límites máximos permisibles por la Norma Oficial Mexicana. La media geométrica para las densidades de organismos coliformes fecales estuvieron en el rango de 7320-12989 NMP/100 ml. Los géneros de la familia de Enterobacterias que mostraron los porcentajes de frecuencia más elevados fueron la Escherichia, Serratia y Enterobacter y son los responsables de algunos cuadros clínicos de gastroenteritis. Por lo tanto, esos géneros deberían ser monitoreados de manera rutinaria en el agua de consumo.

PALABRAS CLAVE: Coliformes totales, familia de enterobacterias, Yucatán, acuífero kárstico.

ABSTRACT

The karstic aquifer of Yucatán serves as the only source of drinking water and the direct recipient of all sewage generated throughout the peninsula. Gastroenteritis, diarrhea, urinary tract infections and septicemia occur among the inhabitants of the rural areas as a result of usage and consumption of untreated ground water. In a rural region without sanitary facilities, multiple-tube fermentation technique was used for the quantification of total and faecal coliform. Qualitative isolation and identification by biochemical reactions was used to identify five tribes of the enterobacteriaceae family. We find that the bacteriological behavior of total and faecal coliform closely follow the precipitation pattern and that their concentrations exceeded the limit established by the Mexican Drinking Water Norm, as it ranged between 7,320 and 12,989 MPN/100 ml. *Escherichia, Serratia* and *Enterobacter* showed the higher percentage frequency occurrence values.

KEYWORDS: Coliform, enterobacteriaceae, karst aquifer, Yucatán, Mexico.

INTRODUCTION

Most of the inhabitants from rural areas of developing countries lack adequate sanitary systems and are exposed to diseases due to the presence of pathogenic bacteria in ground water. The primary objective of monitoring drinking water is to protect the health of the community by preventing the spread of waterborne diseases. Drinking water supplies can easily become contaminated with coliform and pathogenic bacteria in karstic terrains if care is not taken to treat and dispose human and animal wastes properly. More importantly, the detection of any organisms not previously observed or a marked increase in the number of a specific pathogen in the source water may provide an early indication of possible health risks (Singh and McFeters, 1992).

Routine examination of water for pathogenic bacteria is expensive and time consuming, and no single procedure is available that can be used to isolate and identify individual pathogens. There is a growing body of evidence that suggests that coliform indicators may not be entirely adequate to judge the safety of water (Olivieri, 1982). For tropical waters, a number of studies have shown poor correlation between bacterial pathogens and coliform indicators. *Escherichia coli* has been shown to have high survivability in tropical environments. Thus, coliform indicators may be unacceptably conservative for estimation of human health risk by faecally transmitted pathogens (Hazen *et al.*, 1987). Several other studies have shown that bacterial species other than *Escherichia coli* are often present in high numbers in warm tropical environments. No alternative to the total and faecal determination has been yet adopted.

The isolation of faecal coliforms, especially *Escherichia coli*, is of particular health significance as these organisms have been generally reported as a causative agent of gastroenteritis in humans. *Salmonella* spp. and *Klebsiella pneumoniae* have been identified in ground water. These species can cause a variety of human diseases. This finding may imply human epidemiological hazards (Ogbondeminu, 1994), if the ground water is used as the source of water. Thus, the presence in drinking water supplies of particular pathogens may serve to alert health authorities with regard to a possible epidemic outbreak. Cholera, dysentery, paratyphoid fever, infectious hepatitis, gastroenteritis and septicemia are among the diseases which are transmitted via faecal contamination of ground water. This is particularly severe in areas where defecation occurs in the open and organic wastes near public water supplies. Many such diseases are common to warm or tropical developing countries (Ogbondeminu, 1994) because the optimum temperature for the reproduction of bacteria is around 37°C (Williams, 1980).

The presence of pathogens in ground water is particularly acute in tropical karstic environments where high permeability of the rocks and the lack of topsoils permit the rapid growth and dissemination of the pathogenic bacterias in ground water. In Yucatán, more than 40% of deaths of children under six years old were attributable to gastrointestinal disease caused by pathogens transported by ground water (Dohering and Buttler, 1974). Casares (1983) determined a correlation coefficient of 0.81 between gastroenteritis diseases, average annual precipitation and maximum temperatures. Thus, the rainy season (May to October) is also known as the “diarrhea season” (Marín and Perry, 1994).

In Mérida, Yucatán, Mexico our study carried out from July 1991 to April 1993 showed that the absence of a city-wide system of sewers and the disposal of the majority of the urban wastewater by septic tanks, soakaways and cesspits provides the main pollution source and the principal ground water quality concern. Faecal coliforms were commonly present in samples from dugwells and to a lesser extent in water supply wells. The faecal coliform counts fluctuate seasonally, with the lowest values observed in the drier season from January to April. The presence of faecal coliform at concentration in excess of 1000/100 ml is a cause of great concern (BGS *et al.*, 1995).

Outside of the major cities, domestic water is obtained from shallow hand-dug wells; depth to water table ranges from 5.0 to 6.5 m. These wells have an approximate diameter of one meter and are finished 0.5 to 1.0 m below the water table. Because they are hand-dug, they tend to be located at the lowest point of the property. Many inhabitants do not use latrines or other sanitary facilities. Common practices include defecation inmediately outside of the living quarters (typically, less than ten meters away from their drinking water supply). Also, it is common to find animals within the property, such as pigs, chicken, dogs, etc.

In this area, limestone bedrock is typically exposed on the surface. The upper part consists of a hard impermeable caliche layer with cracks that allow for rapid infiltration. Underneath this layer there is an unsaturated zone of eight meters approximately, below which there is a freshwater lens with an approximate thickness of 45 meters underlain by salt water (Marín and Perry, 1994; Steinich and Marín, 1996). As a result much of the combined human and animal waste produced in these communities flows directly into the aquifer. Pathogens present in fresh organic matter are able to migrate over short time spans into the ground water.

Our study area is located just north of the city of Mérida (Figure 1). Included are four small towns with fewer than 2000 inhabitants each. Fifteen years ago, only major cities had water supply wells. In the rest of the state, the drinking water still comes from shallow, hand-dug wells. The ground water samples were taken from villages which lacked any sort of sanitary facilities, and such is still the case today. The only difference between 1983 and today is that the drinking water is now supplied from the wells that feed the city of Mérida. However, because of the cost for this service, the hand-dug wells are still commonly used. The hydraulic properties of the aquifer are poorly known due to a well developed karst system (Marín and Perry, 1994). The regional hydraulic gradient is low, on the order of 7-10 mm/km. Ground water flow direction in the vicinity of Mérida is south to north (Marín, 1990). Normally, the rainfall season is from May to October; however, rains may start as early as April, or as late as June, with an average annual precipitation of 1200 mm. The average annual temperature is 26.3° (INEGI, 1992).

The objectives of this study were (1) to determine whether total and faecal coliforms can be correlated with precipitation in a tropical karstic environment; (2) to determine whether selective pathogens were present in the ground water; (3) to compare the presence of total and faecal coliforms in both an urban environment with sanitary facilities and a rural environment that lacks these facilities.

METHODS

The Facultad de Ingeniería has had an operating metereological station from 1980 to the present. Average monthly temperatures were analyzed to determine the range, minimum, maximum, average, and standard deviation for the study period (FIUADY, 1983). A biological monitoring network was implemented. Ground water samples were analyzed for the presence of total and faecal coliforms, as well as the following organisms*: Escherichia, Shigella, Edwardsiella, Salmonella, Arizona, Citrobacter, Klebsiella, Enterobacter, Pectobacterium, Serratia, Proteus, and Providencia*.

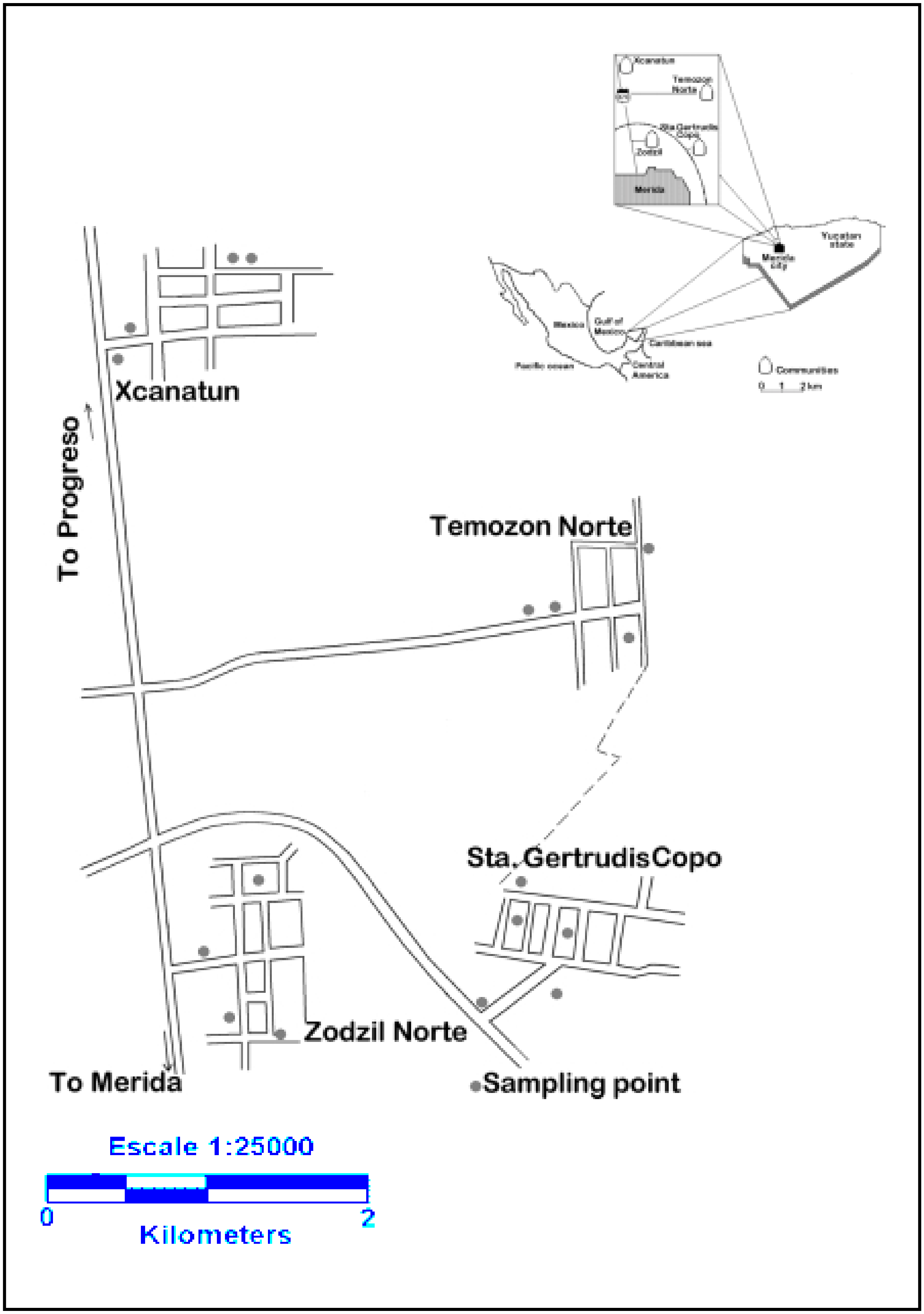

Fig. 1. The study area and the sampling sites.

Two hundred and seven samples of ground water were collected from 17 shallow wells (Figure 2) each month during 1983. Ground water samples were taken directly from the wells by means of a sterilized bottle fitted with a weight on the base, and careful attention was taken to avoid contaminating the sample by any surface scum. Each sample was sealed, labeled and iced (4°C) and transported to the laboratory for bacteriological analysis. The most probable number (MPN) of coliforms per 100 ml from ground water samples was determined by the multiple tube fermentation technique. For total coliforms, selective lactose broth fermentation tubes were incubated at 35°C for 24 h. An incubation temperature of 44.5°C (±0.5°C) was employed for faecal coliforms. In both cases, tubes showing gas production within 24 h. were considered positive (APHA, AWWA,WPCF, 1980). Twelve genera from the *enterobacteriaceae* family (Table 1) were identified using an array of biochemical tests (Edwards and Ewing, 1973).

RESULTS AND DISCUSSION

The average monthly temperature for 1983 is given in

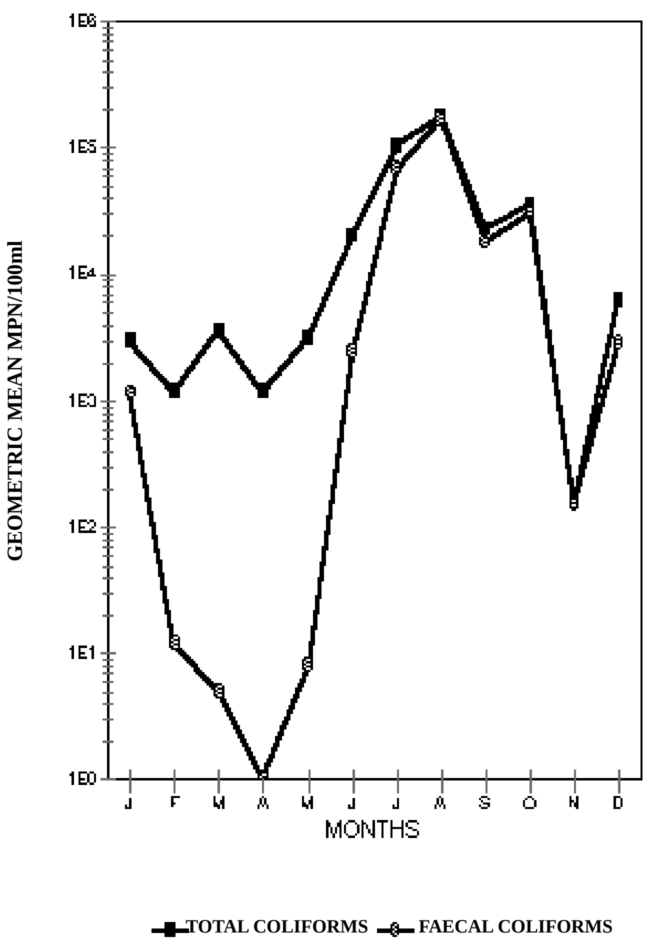

Fig. 2. Seasonal fluctuations of geometric mean total and faecal coliform densities at all sampling sites.

Table 1. Basic statistics were also determined for this data set and are shown in Table 1. The average monthly temperature for 1983 is 33.4° centigrades. Thus, the temperatures recorded in the Mérida region for 1983 support the growth of bacteria (Williams, 1980).

The results for total and faecal coliform densities were expressed in terms of the Most Probable Number (MPN per 100 ml) for each ground water sample and the geometric mean for each month was calculated (Table 2). The ground water in the study area was characterized by total coliform counts which ranged from 1.57 x 10^2 per 100 ml to 1.71 x 10^5 per 100 ml and the faecal coliforms varied from not detectable to 1.71 x 10^5 per 100 ml (Figure 2). Figure 3 shows an interesting temporal pattern that supports the hypothesis presented by other authors (Doehring and Butler, 1974; Marín, 1990; Marín and Perry, 1974): as precipitation increases, so does the presence of fecal coliforms in the ground water. As it begins to rain, human and animal excrement is washed into the low sections of the terrain (which also happen to be where the hand-dug wells are located). Since the aquifer has a high hydraulic conductivity, the infiltrating water containing the untreated organic waste, quickly infiltrates into the aquifer through cracks, fissures, etc.

The values reported in this study well exceed the permissible limits according to the Mexican Drinking Water Norm which allows for two MPN per 100 ml for total coliforms and “no detectable” MPN per 100 ml for faecal coliform (NOM, 1994). The presence of large counts of both total and faecal coliforms in the ground water supply of rural Yucatán lacking sanitary facilities is critical. These communities now have drinking water supplied from the city of Mérida, but the lack of sanitary facilities remain.

Table 1

Average monthly temperatures for Mérida for 1983. Units are degrees centigrade

Month	Temperature		
January	27.60	Max	37.9
February	29.00	Min	27.60
March	31.40	Average	33.35
April	34.00	Std Dev	2.50
May	37.90		
June	36.40		
July	34.20		
August	34.80		
September	34.20		
October	32.90		
November	31.60		
December	30.50		

Table 2

Ewing's classification of enteric microorganisms.

Tribe	Genera
I. Escherichiae	Escherichia
	Shigella
II. Edwardsiellae	Edwardsiella
III. Salmonellae	Salmonella
	Arizona
	Citrobacter
IV. Klebsiellae	Klebsiella
	Enterobacter
	Pectobacterium
	Serratia
V. Proteae	Proteus
	Providencia

From Smith (1977).

The bacteria speciation studies were conducted at the genera and species level, as follows:

Tribe I. *Escherichiae. Escherichia coli*, was found throughout the study period, and their percentage varied from 40 to 97. These bacteria are the most common cause of pyelonephritis and urinary tract infections and an important cause of epidemic diarrhea for newborn infants (Smith, 1977). *Shigella* (Figure 2, No.2) was only detected in October (6%) and suggests a casual incidence. All species of these pathogenic bacteria can cause dysentery, especially *the Shigella dysenteriae* specie (Smith, 1977).

Tribe II. *Edwardsielleae. Edwardsiella genera* had an average incidence of 23% for the study period except for January and April. Biochemical characteristics are similar to *Salmonella* genera and some strains can cause gastroenteritis in humans (Smith, 1977).

Tribe III. *Salmonellae. Salmonella* genera (Figure 2, No. 4) were detected in the range of: 0-40%, with the higher values during February through August. The occurrence of *Salmonella*, which can cause a variety of human diseases such as typhoid, is particularly worrisome. *Arizona* was detected 10% for the months of May through October. *Citrobacter* genus (Figure 2, No. 5) occurrence varied from 18 to 80% and was present in all ground water samples during the period. These pathogenic bacterias have similar biochemical characteristics as *Salmonella* and may be responsible for some cases of gastroenteritis or septicemia (Smith, 1977).

Tribe IV. *Klebsiellae. Klebsiella* and *Pectobacterium* genera were not found. *Klebsiella* could be masked by *Enterobacter* and *Serratia* due to their biochemical similar behavior (Smith, 1977). *Enterobacter* and *Serratia* (Figure 2, Nos. 7 and 8) were found in all ground water samples during the study period with the following frequency occurrence: 22 to 84% and 30 to 95%, respectively. Some strains of *Enterobacter aerogenes* can cause urinary tract infections and septicemia, and *Serratia marcescens* specie can cause septicemia (Smith, 1977).

Tribe V. *Proteae. Proteus* genera (Figure 2, No. 9) were found throughout the study period except in December. Their presence was observed in a frequency range from 6 to 84%. *Providence* (Figure 2, No. 10) was found during the months of May through July. Some strains of this genus can cause urinary tract infections, as well as diarrhea and septicemia (Smith, 1977).

The bacterial speciation studies show that all twelve species tested for were present in the ground water. Species which cause the greatest concern are *Escherichia*, *Shigella*, and *Salmonella* because they are responsible for all types of gastrointestinal diseases.

For comparison with an urban environment where sanitary facilities exist, we used the data from BGS *et al.* (1995) for Mérida. Marín (1990) has shown that ground water flow in the vicinity of Mérida is from south to north. We plotted the total coliform MPN count for Mérida (from the BGS *et al.*, 1995 study) together with our data. The origin was taken at Mérida. Figure 4 shows that the geometric means of the MPN per 100 ml counts of the faecal coliforms in the study area are orders of magnitude higher than those of Mérida (413 against 7320, 12 018, and 12 989). Further downstream the MPN increases. This may be due to the local production of total coliforms plus the contribution from the upgradient area. The lack of sanitary facilities for the rural communites is a tremendous source of contamination. One suggestion that may help significantly to reduce faecal coliform concentrations is building sanitary outhouses, which consist of digging a shallow hole, and using lime to eliminate bacteria.

CONCLUSIONS

Total and faecal coliforms in the ground water of rural Yucatán show a close relationship, supporting the hypothesis that the coliforms contaminate the ground water by infiltration. The highest MPN per 100 ml counts of total and faecal coliforms were found in a rural environment that lacked sanitary facilities in 1983. Piped water is now supplied from Mérida, but no sanitary facilities are available.

Three species of pathogens that have been closely linked with serious gastrointestinal diseases were identified in

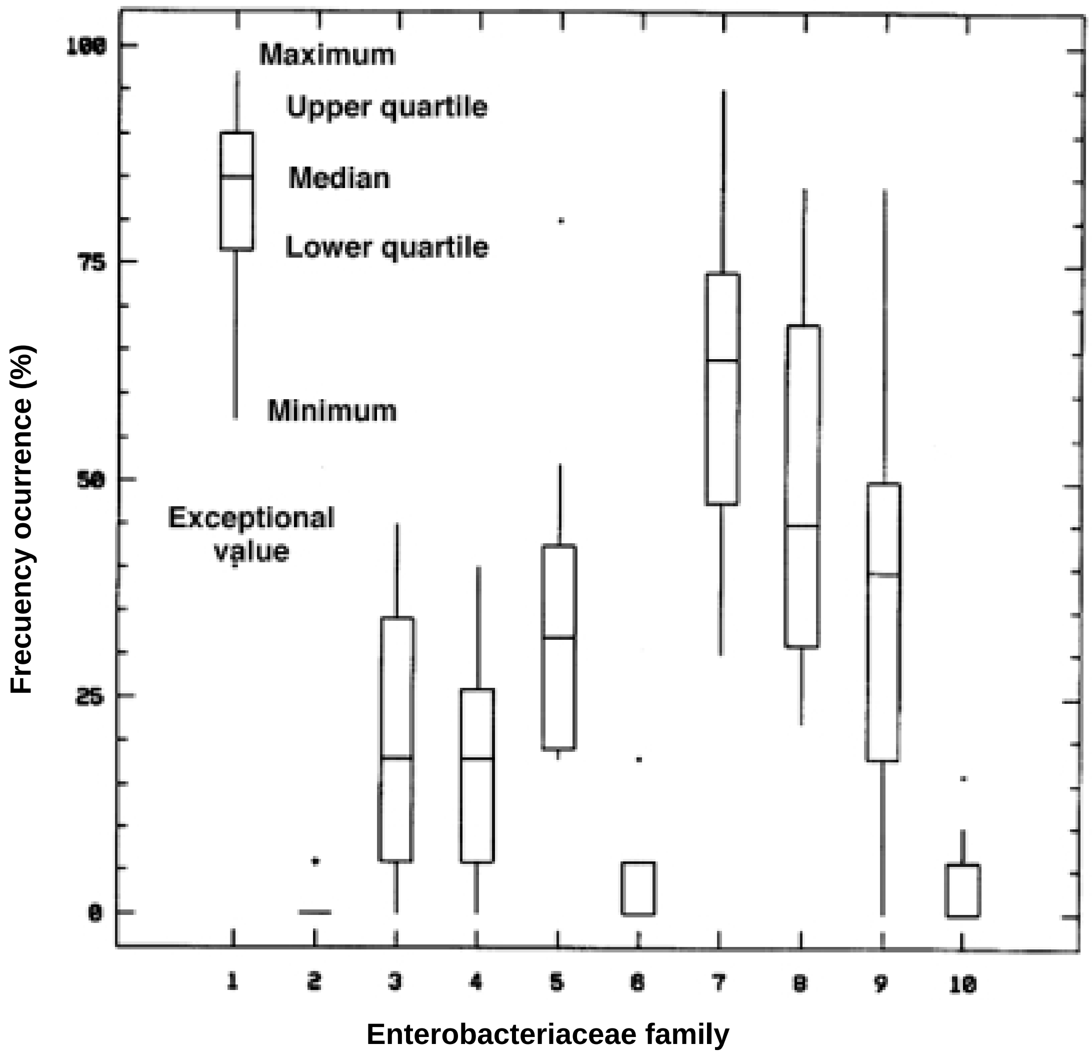

Fig. 3. Percentage frequencies of the Enterobacteriaceae family.

ground water just north of Mérida. These are *Escherichia, Shigella, and Salmonella*. These bacteria endanger the rural population, and particularly, the young (i.e. children less than five years old). The high coliform bacteria densities and the faecal component suggest that animal and human wastes form a significant portion of domestic pollution which is leaching directly to water table in karstic regions.

A comparison between the sources from Mérida, a city with sanitary facilites, and a rural environment suggests that the presence of these pathogens can be eradicated with proper sanitary facilities. The conditions described for the study area are still common throughout the state of Yucatán, and many inhabitants continue to be at risk from pathogens found in the ground water, particularly during the rainy season.

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