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Analysis of Bacteriological Quality of Drinking Water Samples from Cherthala Taluk, Kerala, India

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Abstract: Microbial flora of water plays a major role in determining quality of potable water. In the present study a comparative analysis is carried out on the microbial population of drinking water samples from wells and municipal water collected from eight locations of Cherthala taluk, Kerala state, for the presence of coliforms, *Vibrio cholerae* and *Salmonella typhi* in two different seasons, i.e., summer and monsoon. The pH of the water samples collected ranged from 6.3 to 6.8. The results showed that in summer, 87.5% of the total samples were positive for *E.coli*, 87.5% positive for *Salmonella typhi* and 25% positive for *Vibrio cholerae* among tap water samples, and in well water 62.5% of the total samples were positive for *E.coli*, 37.5% positive for *Salmonella typhi* and 12.5% positive for *Vibrio cholerae.* In contrast, during monsoon, among well water samples only 37.5% of the total samples were positive for *E.coli*, 12.5% positive for *Salmonella typhi* and *Vibrio cholerae* were absent. Among tap water samples in monsoon only 12.5% of the total samples were positive for *E.coli*, 25% positive for *Salmonella typhi* and *Vibrio cholerae* were absent.

Key words: *Vibrio cholerae, Salmonella typhi,* most probable number, coliforms, heterophilic plate count.

Introduction

Water has been of great importance to human beings and other organisms of the environment for sustenance of life and maintaining the balance of the nature, hence water is the "life blood of the earth" (Krishna Ram et al., 2009). As a result of urbanization and industrialization, ground water has been polluted and this can lead to diseases like typhoid, jaundice, cholera, etc. Apart from industrial and agricultural purposes, ground water is explored in rural areas for drinking, especially in those areas where other sources of water like dam and river or a canal is not available. During the last decade, it was observed that the ground water got polluted drastically because of increased human activities (Jameel, 1998). Consequently number of cases of water borne diseases have been seen which are causes for many health hazards (Desai, 1995; and WHO 1999). Over 3 million deaths per year is attributed to water borne diseases, especially among infants and young children in poor communities in Africa, Asia and South America (Anon, 1997). So, basic monitoring on water quality has been necessitated to observe the demand and pollution level of ground water.

As per World Health Organisation (1993) standards, drinking water should not contain any microorganisms known to be pathogenic or any bacteria indicative of faecal pollution. Hence a factor of prime importance is the microbial flora in water. Detection of faecal indicator bacteria in drinking water provides a very sensitive method of quality assessment and it is not possible to examine water for every possible pathogen that might be present. A good number of water analysis experiments

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are regularly conducted by different groups of chemists and biologists across the country (Mishra and Bhatt, 2008).

The presence of Enterococci is thus an indication of contamination of drinking water. *E. coli* can cause many gastrointestinal diseases. Presence of *Vibrio cholerea* can result in cholera which can spread through contaminated drinking water. *Salmonella typhi*, causative organism of typhoid fever, is also a contaminant in potable water. Substantial evidence has been provided showing that *Vibrio* species are natural inhabitants of marine aquatic environments in both temperate and tropical regions, with most infections acquired by exposure to such environments or to foods derived from them (Kelly et al., 1991; Oliver and Kaper, 1997).

The present study is a comparison of the microbial population of drinking water samples collected from different localities of Cherthala taluk in Alappuzha district of Kerala, in two different seasons – monsoon and summer. Cherthala has been chosen as the area under study due to various reasons. Cherthala is an area with many backwaters. It is an area where there is a high prevalence of water borne and vector borne diseases.

Materials and Methods

Collection of Samples

Drinking water samples were collected from eight wells and eight taps in Cherthala taluk. The sites were Poochakkal, Chakkarakulam, Kuthiathode, Alappuzha, Velliyakulam, Maruthorvattom, Cherthala town and Thanneermukkom in Cherthala taluk, Alappuzha district, Kerala state, India.

Water samples were drawn in sterile 250 ml Erlenmeyer flasks at normal atmospheric temperature and transported to the lab by standard methods as mentioned in APHA 1998. Wells were the sources of ground water and tap water collected from water supplied by the municipality or panchayat. The pH of the water samples were also checked at the time of collection.

Heterophilic Plate Count

Heterophilic plate count (HPC) can provide an indication of general population in the system. Samples to be analysed for quantitative bacterial analysis were plated on Glucose Tryptone (GT) Agar (APHA 1998) and the total plate count was performed of colonies developed after incubation at 37 °C for 24 hours.

Detection of Most Probable Number

The most probable number (MPN) is a suitable and widely used method to determine the microbial quality of water. Qualitative analysis was carried out by multiple tube fermentation technique (APHA 1998) for members of the coliform group. Coliforms were detected by presumptive inoculation into tubes of Lactose broth and their incubation at 37 °C for 48 hours. The positive tubes were sub-cultured into Eosin Methylene Blue (EMB) agar for confirmation. MPN of coliforms were found in terms of index/100 ml by using standard tubes. All the results were compared with the standard MPN chart and the results were expressed as the total number of coliform/ml of the water.

Detection of Presence of *Salmonella typhi* and *Vibrio cholerae*

For the isolation of *Salmonella typhi,* one ml of water sample was inoculated into 10 ml of selenite enrichment broth and incubated at 37°C for 12–18 hours and swabs from the selenite broth were streaked on Bismuth Sulphite (BS) Agar which is widely used for the isolation of *S. typhi* and other *Salmonella* sp. from food, faeces, urine, sewage, and other infectious materials.

Before plating into nutrient medium water samples suspected to contain *Vibrio cholerae* were enriched by adding 100 ml water sample in 200 ml of double strength alkaline peptone water (pH 8.6) at 37 °C for 24 hours and swabs from the alkaline peptone water was streaked on selective plating media, Thiosulphate Citrate Bile salt Sucrose (TCBS) agar and further incubated at 37 °C for 24–48 hours.

Results and Discussion

The MPN values of all the water samples were found out using multiple tube tests. The MPN values were found to decrease in monsoon compared to summer for certain tap water samples (Figure 1). But, in two samples the values were drastically increased during monsoon. The water sample from Maruthorvattom showed only MPN value of 3 in summer while it was reduced to 0 in monsoon. This may be due to the high efficiency of chlorination.

Comparative study of MPN values of tap water in summer and monsoon revealed that in monsoon the MPN values were high, except in Maruthorvattom. All the MPN values exceed the WHO standards and this may be due to the mixing of faecal matter with the waterways and also attributed to improper disinfection. All the eight well water samples showed high MPN values except in samples collected from Velliyakulam and Kuthiathode (Figure 3). According to the Central Pollution Control Board, India, the total number of coliforms shall not

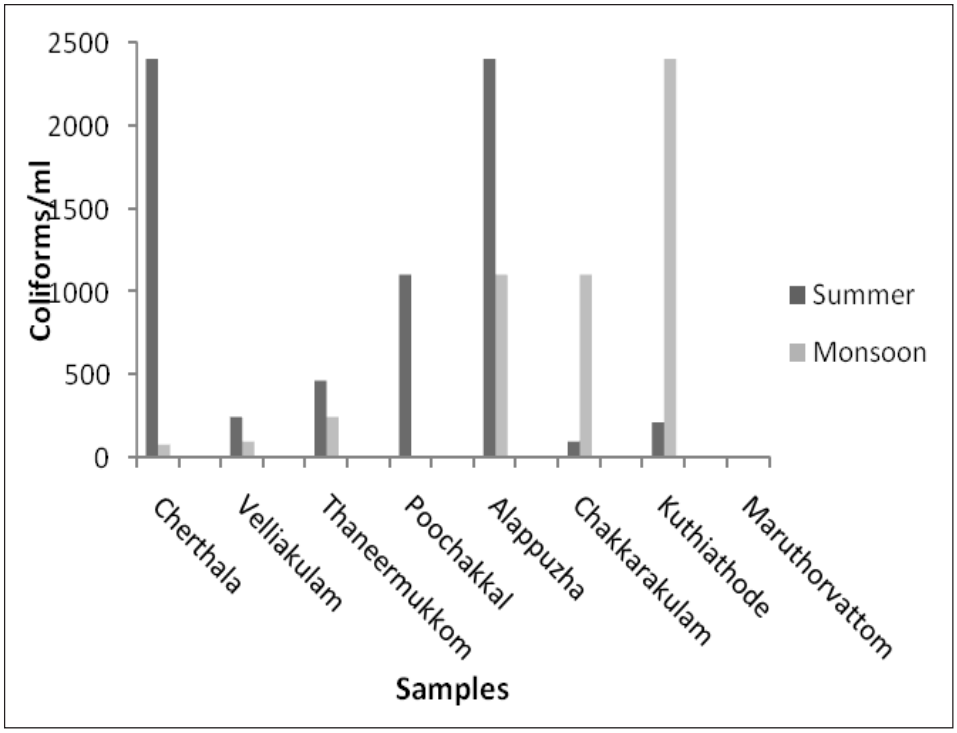

Figure 1: MPN values of tap water samples in two seasons.

exceed 50/100 ml in untreated or non-disinfected drinking water. Hence most of the samples were proved not efficient as potable water. In all other places the MPN values were above the WHO standards and it needs immediate attention of the authorities. The microbial flora remained constant in most well water samples in both seasons. But one sample showed decrease in MPN value.

The pH of tap water samples were also in the range of 6.4 to 6.8 (Figures 2 and 4) and the pH range of well water samples were 6.3 to 6.7 (Figure 4) in both summer and monsoon. The pH values were as per the accepted standards.

The samples, positive for MPN tubes plated on Eosin Methylene Blue (EMB) agar showed a green metallic sheen, which confirmed the presence of *E. coli*. Fresh samples spread on BS agar and TCBS agar showed brown (Figure 5) and yellow colonies (Figure 6) respectively. This clearly indicated the presence of *S. typhi* and *V. cholerae.* In the well water samples of summer, all except one showed positive result. 87.5% were positive for *E. coli*, 87.5% positive for *Salmonella* and 25% were positive for *Vibrio*, which is evident from Table 1. As in the case of *V. cholerae*, 106 organisms or more are typically needed to cause cholera, so that it is unlikely that persons bathing or involved in other recreational water activities would ingest vibrios in numbers high enough to cause gastrointestinal disease (LeChevallier and Au, 2002).

The heterophilic plate count varied significantly during summer and monsoon (Table 1). All the values were above WHO standards and the water is unfit for drinking. The HPC was very high in Maruthorvattom, Kuthiathode and Thaneermukkom in both tap and well water samples. This may be due to high anthropogenic activities. HPC can provide an indication of the level of the general population in the system and is considered as a good general indicator of overall water quality (Obire et al., 2005).

In summer, in well water *E. coli* and *S. typhi* were present in all places except in Velliyakulam and Kuthiathode (Table 2). *V. cholerae* was found only in samples from Alappuzha and Chakkarakulam. Data presented in Table 3 shows that in tap water samples of summer 62.5% of samples were tested positive for *E. coli*, 37.5% of samples were positive for *Salmonella* and 12.5% were positive for *Vibrio*.

In tap water samples *E. coli* was present only in five places. *Salmonella typhi* was present only in three places and *V. cholerae* was present in one place. In tap water samples the number of positive samples was less compared to well water samples. This was mainly due to chlorination. From Table 4, it is clear that in well water

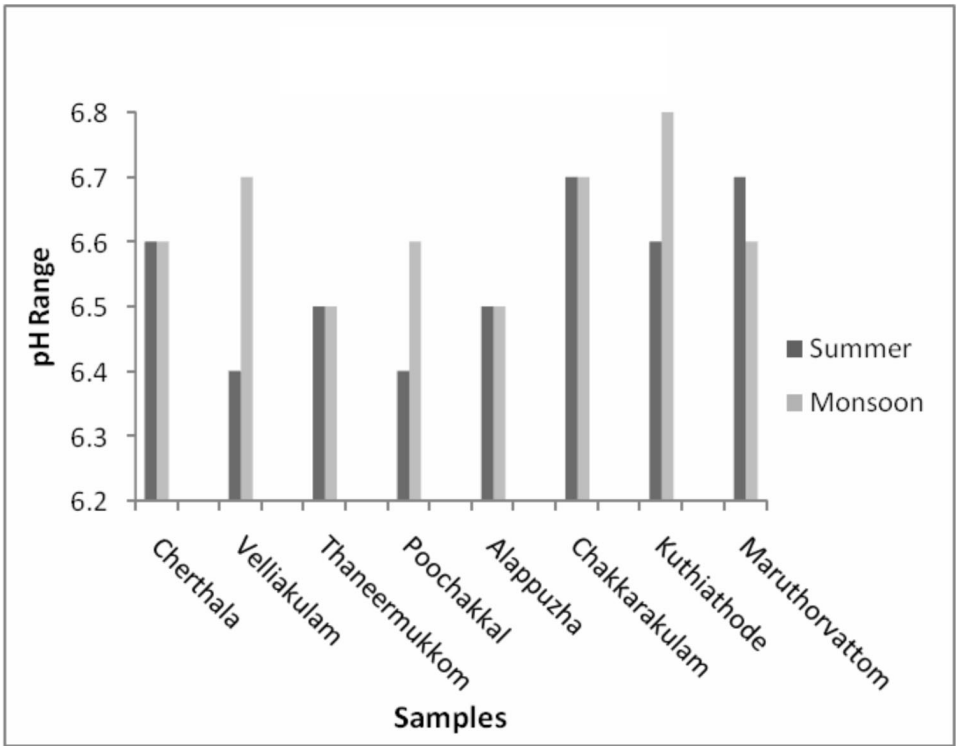

Figure 2: pH of tap water samples.

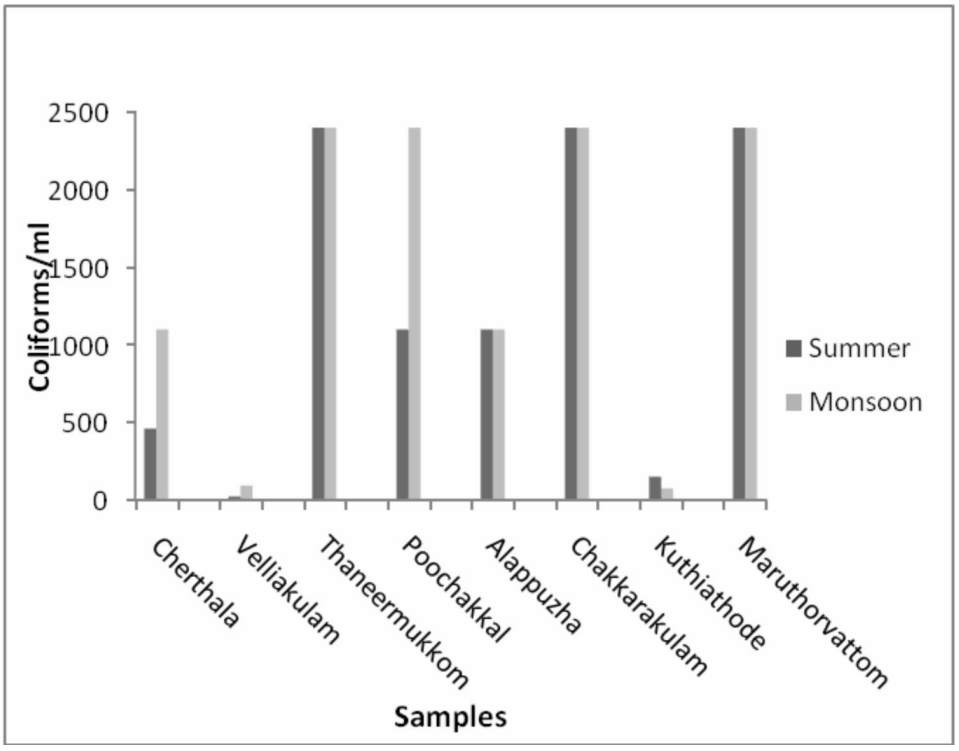

Figure 3: MPN values of well water samples in two seasons.

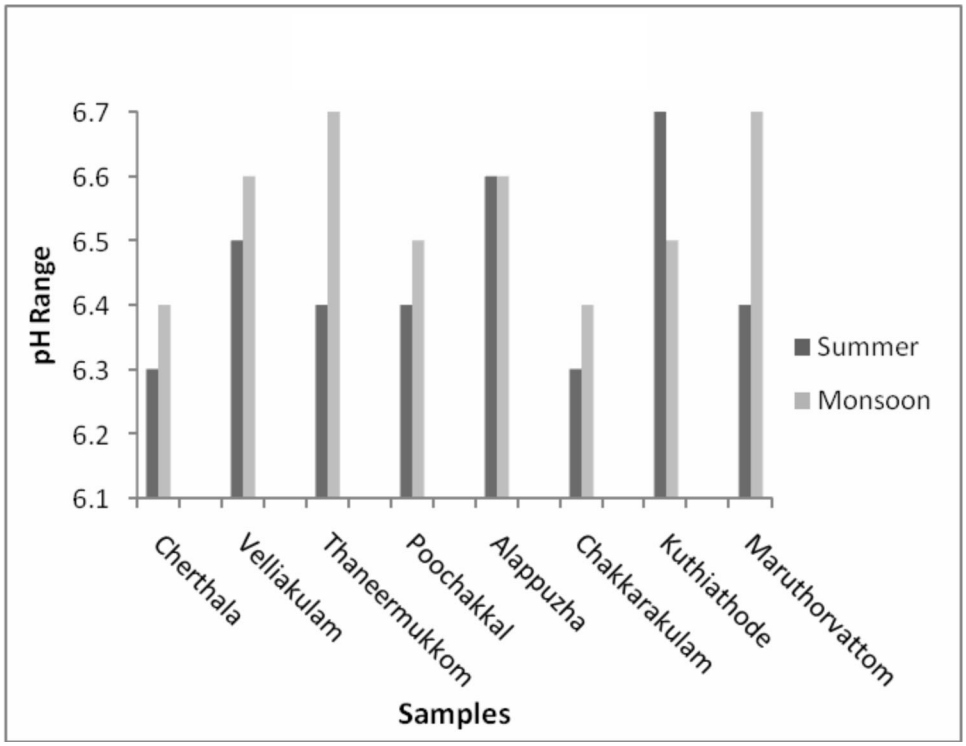

Figure 4: pH of well water samples.

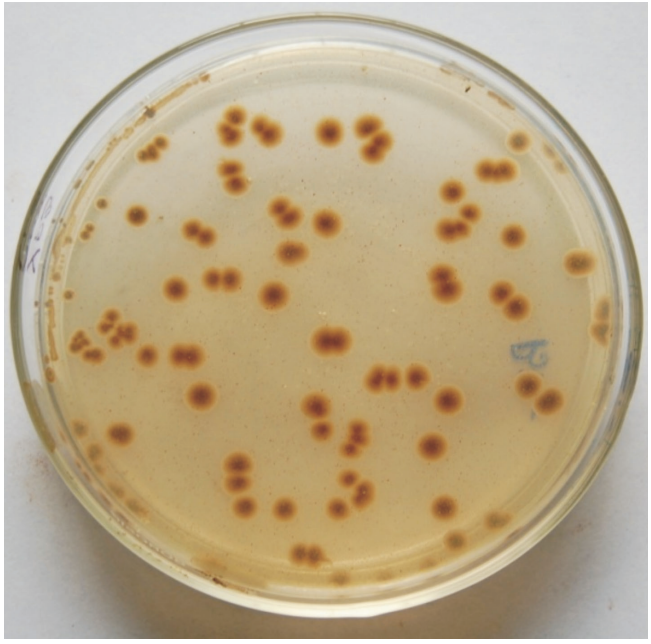

Figure 5: Growth of *Salmonella typhi* on BS agar.

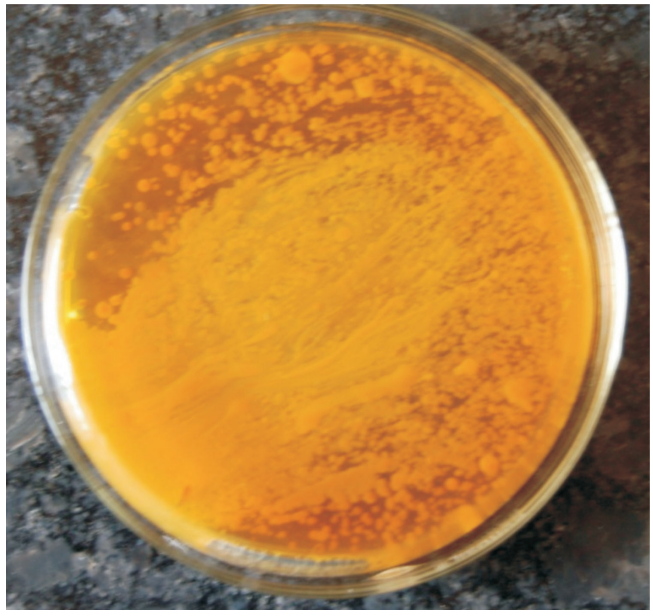

Figure 6: Growth of *Vibrio cholerae* on TCBS agar.

Table 1: Heterophilic Plate Count

Place	*HPC of samples (cfu/ml)*			
	Summer		*Monsoon*	
	Well water	*Tap water*	*Well water*	*Tap water*
Cherthala	1.23×10^2	5.2×10^1	1.53×10^2	7.8×10^1
Velliyakulam	9.6×10^1	1.5×10^2	1.32×10^2	1.42×10^2
Thaneermukkom	8.2×10^2	1.7×10^2	9.08×10^2	2.1×10^2
Poochakkal	3.3×10^1	2.05×10^2	7.5×10^1	1.82×10^2
Alappuzha	8.5×10^1	4.3×10^2	1.4×10^2	3.82×10^2
Chakkarakulam	1.29×10^2	3.3×10^1	1.6×10^2	4.3×10^1
Kuthiathode	2.56×10^2	3.2×10^2	5.75×10^2	4.22×10^2
Maruthorvattom	1.54×10^2	1.45×10^3	4.38×10^2	5.15×10^2

Table 2: Presence (+) or absence (-) of microbes in well water samples in summer

Place	*E. coli*	*S. typhi*	*V. cholerae*
Cherthala	+	+	-
Velliyakulam	-	+	-
Thaneermukkom	+	+	-
Poochakkal	+	+	-
Alappuzha	+	+	+
Chakkarakulam	+	+	+
Kuthiathode	+	-	-
Maruthorvattom	+	+	-

samples of monsoon, 37.5% of samples were tested positive for *E. coli*, 12.5% of samples were positive for *Salmonella* and *Vibrios* were absent. Table 5 shows that in tap water samples of summer, 12.5% of samples were tested positive for *E. coli*, 25% of samples were positive for *S. typhi* and V. *cholera* was absent.

During the monsoon, the presence of *E. coli* was found only in three places, *Salmonella typhi* was found only in Poochakkal area and *Vibrio cholerae* was not found in all places (Table 5). These values showed that there is an abrupt decrease in the microbial flora in water samples during monsoon. This may be due to the fact that the water resources get diluted during the heavy rains.

Microbial faecal contaminant indicators are *E. coli*, Clostridia, Streptococci, and Enterococci that could be of human and non-human origin (Binnie et al., 2002). Several developing countries have embarked on programmes to endeavour to reduce contamination of rural water sources by water borne diseases (Ganoza et al., 2005). Typical methods normally advised for inactivating microbes for rural water treatment at such levels are disinfection treatment (use of hypochlorite) and boiling (use of heat) that have some efficiency on microbial content reduction (LeChevallier and Au, 2002).

Enteric pathogens cannot normally multiply in water hence water is not its mode of transmission to humans.

Table 3: Presence (+) or absence (-) of specific microbes in tap water samples in summer

Place	*E. coli*	*S. typhi*	*V. cholerae*
Cherthala	-	+	-
Velliyakulam	-	-	-
Thaneermukkom	+	-	-
Poochakkal	+	+	+
Alappuzha	+	+	-
Chakkarakulam	+	-	-
Kuthiathode	+	-	-
Maruthorvattom	-	-	-

Table 4: Presence (+) or absence (-) of specific microbes in well water samples in monsoon

Place	*E. coli*	*S. typhi*	*V. cholerae*
Cherthala	-	-	-
Velliyakulam	-	-	-
Thaneermukkom	-	-	-
Poochakkal	+	+	-
Alappuzha	+	-	-
Chakkarakulam	+	-	-
Kuthiathode	-	-	-
Maruthorvattom	-	-	-

Table 5: Presence (+) or absence (-) of specific microbes in tap water samples in monsoon

Place	*E. coli*	*S. typhi*	*V. cholerae*
Cherthala	-	-	-
Velliyakulam	-	-	-
Thaneermukkom	-	-	-
Poochakkal	+	+	-
Alappuzha	-	-	-
Chakkarakulam	-	-	-
Kuthiathode	-	-	-
Maruthorvattom	-	+	-

The infective dose of microbes may vary in people whose body defence mechanisms are low due to any other infections. Very old people and patients undergoing immunosuppressive therapy would also be at risk. Water polluted by bacteria when permitted to contaminate food would lead to the multiplication of pathogens to very large doses (Zividazi et al., 2007). To conclude, the municipal and panchayat authorities have to keep a constant vigil and take necessary precautions to keep the presence of *E. coli* and other pathogenic microbial forms under check. Conducting awareness campaigns regarding the spread of diseases and safe use of drinking water should create necessary awareness among the people. Consistent and periodical examination of drinking water samples and disinfection process should be done if necessary on a case-by-case basis to prevent the spread of pathogenic microbes.

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