

1 **Comparative study of enteric viruses, coliphages and**
2 **indicator bacteria for evaluating water quality in a tropical,**
3 **high-altitude system**

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21

1 **Abstract**

2 **Background**

3 Bacteria used as indicators for pathogenic microorganisms in water are not considered
4 adequate as enteric virus indicators. Surface water from a tropical high-altitude
5 system located in Mexico City that receives rainwater, treated and non-treated
6 wastewater used for irrigation, and groundwater used for drinking, was studied.

7 **Methods**

8 The presence of enterovirus, rotavirus, astrovirus, coliphage, coliform bacteria, and
9 enterococci was determined during annual cycles in 2001 and 2002. Enteric viruses in
10 concentrated water samples were detected by reverse transcriptase-polymerase chain
11 reaction (RT-PCR). Coliphages were detected using the double agar layer method.
12 Bacteria analyses of the water samples were carried out by membrane filtration.

13 **Results**

14 The presence of viruses and bacteria in the water used for irrigation showed no
15 relationship between current bacterial indicator detection and viral presence.
16 Coliphages showed strong association with indicator bacteria and enterovirus, but
17 weak association with other enteric viruses. Enterovirus and rotavirus showed
18 significant seasonal differences in water used for irrigation, although this was not
19 clear for astrovirus.

20 **Conclusions**

21 Coliphages proved to be adequate faecal pollution indicators for the irrigation water
22 studied. Viral presence in this tropical high-altitude system showed a similar trend to
23 data previously reported for temperate zones.

1 **Background**

2 At present, public health concerns remain focused on waterborne diseases,
3 with incidence data in both developed and developing countries making
4 gastroenteritis highly important. A diversity of enteric bacteria and viruses has been
5 associated with outbreaks of waterborne gastroenteritis [1, 2].

6 Since the late 19th century, bacteria have been used as indicators of water
7 quality [3]. Although there are reports concerning the inadequacy of bacteria as
8 microbiological water quality indicators [4], it has been recognized that they are
9 indicators of a broad bacterial group and regular human microbiota [5]. Nevertheless,
10 bacteria alone offer limited information regarding microbiological water quality as
11 they do not reflect the presence of enteric viruses or protozoa [6].

12 The presence of viruses and other pathogens in the environment is a sign of
13 faecal pollution that poses a potential risk to the exposed population, since such
14 pathogens do not constitute normal gastrointestinal flora, and are only excreted by
15 sick individuals [7]. Rotavirus is recognized as being responsible for diarrheal disease
16 in young children with a worldwide mortality rate of 600,000 per year [8]. Astrovirus
17 is also considered one of the most important agents of viral gastroenteritis [9,10], and
18 is ranked second after rotavirus as the major cause of diarrheal disease in young
19 children and adults [11]. While the actual contribution of rotavirus to total incidence
20 of diarrheal disease is between 25% and 52%, astrovirus is much lower being
21 responsible for between 5% and 10% of cases [12].

22 In Mexico, during autumn and winter, which correspond to the cold-dry
23 season, rotavirus has been reported as the main etiologic agent of diarrheal disease in
24 children aged 2 years and under, and as has already been mentioned, it is responsible
25 for approximately 25%–50% of all gastrointestinal cases [13,14]. Epidemiological

1 studies show a seasonal incidence of bacterial diarrheal disease mainly during the
2 summer months that coincides with the warm-rainy season [15,16,17].

3 It is important to consider enteric viruses in water quality studies not only
4 because of their incidence as causal agents for diarrheal disease [13,8], but also due to
5 their characteristics, which allow them to survive in the environment for long periods
6 of time, and tolerate changing environmental conditions [18,4].

7 Although it is not possible to establish a direct relationship between
8 epidemiological and environmental data, it is important to consider microbial water
9 quality in terms of water use. Furthermore, it is vital to assess the potential risk to the
10 exposed population, especially in developing countries, considering that recycled
11 water has been associated with the presence and re-emergence of waterborne diseases
12 worldwide [19].

13 Mexico is one of the main countries that reuse wastewater for irrigation of
14 land used for crop cultivation, an area which has been calculated to be approximately
15 180,000 ha [20]. This practice is likely to increase, and therefore, it would be
16 advisable to assess water quality in terms of both viral composition and load in order
17 to decrease the associated risk to the population. For such assessment it would be
18 necessary to evaluate the most adequate indicator from a public health perspective,
19 whether it is bacterial or viral.

20 The aim of this study was to compare the presence of enterovirus, rotavirus,
21 astrovirus, coliphages and indicator bacteria in a tropical high-altitude system, which
22 supplies the Southern area of Mexico City with water for both irrigation and drinking.

23 **Methods**
24

1 The study area is located in the South of Mexico City. It is a tropical high-
2 altitude aquatic system located at 2240 masl, between 19°02' and 20°12' N and 98°28'
3 and 99°32' W, covering an area of 1,479 km². The average annual temperature is
4 16°C, but the temperature fluctuates greatly during the day with an average maximum
5 of 25°C and minimum of 8°C. The rainy season mainly occurs during the summer and
6 autumn months (May to October), while the rest of the year remains dry.

7 Agriculture and farming remain the main activities in this area with water
8 being pumped from the canal network for surface irrigation. Flowers and vegetables
9 are cultivated in the area with some of the latter being eaten raw. There are some
10 domestic animals, as well as “conservation areas” that have been invaded by squatter
11 settlements, a common practice as part of the urbanization process in developing
12 countries.

13

14 **Water samples**

15 The presence of rotavirus, enterovirus and astrovirus, as well as the abundance
16 of indicator bacteria in the water source and in water used for irrigation was
17 determined from samples obtained during the cold-dry (November to February) and
18 warm-rainy (May to October) seasons in 2001 and 2002. These seasonal categories
19 were defined according to two meteorological parameters: temperature and rainfall
20 [21,22]. Samples from water used for irrigation were obtained from ten sampling
21 points, randomly selected from a regular grid of 250 observation points covering the
22 Xochimilco canal network, which had been set up for previous studies in the area
23 [23]. For viral detection, a 20 L volume was collected at each sampling point for each
24 season per year. Samples for bacteriological analyses were collected at a depth of
25 40cm in 1 L sterile polypropylene flasks.

1 Water source samples were obtained from ten wells randomly selected from
2 the total of 60 wells that form part of the Mexico City water supply system. Samples
3 were taken directly from the wells prior to chlorine disinfection. For each season and
4 year, 1200 L of water was filtered through a 1MDS electropositive filtering cartridge
5 at each well (CUNO, Meriden, CO). Within six hours of sampling, the cartridges were
6 transported cold (4°C) to the laboratory. For bacterial analyses, 1 L samples were
7 taken in sterile polypropylene containers. At each sampling point, pH, temperature
8 and conductivity were measured using a portable YSI 3500 pH-conductivity meter
9 (Yellow Spring, OH) and dissolved oxygen measured with an YSI 51B oxygen meter
10 (Yellow Spring, OH). In the laboratory, the 80 water samples (10 samples from
11 irrigation water and 10 water source samples, both taken each season for two years)
12 were analyzed for the following enteric viruses: enterovirus (EV); rotavirus (RV); and
13 astrovirus (AST); and for indicator organisms including total coliform (TC), faecal
14 coliform (FC), and enterococci (FE), as described below.

15

16 **RNA extraction and cDNA synthesis**

17 Water samples were filtered through electropositive Virosorb 1MDS
18 cartridges (CUNO, Meriden, CO). Once water samples were concentrated to a 30 mL
19 volume, RNA was extracted using a Trizol LS reagent (Invitrogen, Carlsbad, CA) and
20 chloroform. Aliquots of 300 µL of water were mixed with 300 µL of PBS 1X and
21 shaken vigorously five times, leaving the vials on ice for 1 minute between each
22 shaking, and then centrifuged at 12,000 X g for 5 minutes. The upper phase
23 containing RNA was transferred and 500µL of Trizol added, gently mixing for 1
24 minute before replacing on ice. This procedure was repeated five times. Subsequently,
25 100 µL of chloroform was added gently and shaken vigorously five times.

1 Following centrifugation at 12,000 X g for 5 minutes, the upper phase was
2 recovered and incubated with the same volume of isopropanol at 4°C for 30 minutes,
3 and then centrifuged for 15 minutes at 12,000 X g at 4°C. The pellet was washed with
4 1 mL of absolute ethanol and centrifuged for a further 5 minutes at 12,000 X g at 4°C.
5 Finally, the pellet was dried at room temperature and re-suspended in 20 µL RNase
6 free water, and stored at -70°C until reverse transcriptase-polymerase chain reaction
7 (RT-PCR) analysis took place.

8 cDNA synthesis (RT reaction) was performed in a 20 µL reaction volume
9 containing 1 µL of RNA, 1 µL of 5 pM primer, and 9.9 µL nuclease-free water
10 (Invitrogen, Carlsbad, CA) at 70°C for 10 minutes. Subsequently, 8.1 µL of a mix
11 containing 4 µL of 5x first strand buffer [250 mM Tris-HCl (pH 8.3), 375 mM KCl,
12 15 mM MgCl₂], 2 µL 0.1 M DTT, 2 µL 5 mM dNTPs and 20 U Super Script II
13 reverse transcriptase (Invitrogen) was added. The reaction was carried out at 42°C for
14 1 hour and subsequently at 70°C for 15 minutes.

15

16 **cDNA amplification**

17 Polymerase chain reaction (PCR) was carried out in a 25 µL volume with a
18 mix of 17.27 µL nuclease-free water, 2.5 µL 10X buffer [100 mM Tris-HCl (pH 8.3),
19 500 mM KCl, 15 mM MgCl₂, 0.01% w/v gelatin], 1.6 µL 5 mM dNTPs, 1 µL of 25
20 pM of each primer, and 0.625 U of Ampli Taq Polymerase (Roche). The primers used
21 to amplify the conserved region for group A that codes for the VP7 structural protein
22 of rotavirus (RV) were as follows: forward (5-
23 GGCTTTAAAAGAGAGAATTTCCGTCTGG-3) and reverse (5-
24 GATCCTGTTGGCCATCC-3) [24], for enterovirus (EV) the highly conserved region
25 among picornaviruses 5'NCR forward (5-TCCGGCCCCTGAATGCGG-3) and

1 reverse (5-CACCGGATGGCCAATCCAAT-3) [24], and for astrovirus (AST) the
2 conserved region of ORF2 forward (5- GGTGTCACAGGACCAAACC-3) and
3 reverse (5-TTAGTGAGCCACCAGCCATC-3) [25].

4 The amplification conditions included denaturation at 94°C for 1 minute and
5 33 cycles at 94°C for 30 seconds, 50°C for 30 seconds and 72°C for 25 seconds, with a
6 final elongation at 72°C for 7 minutes. Agarose gels were stained with ethidium
7 bromide and examined under ultraviolet light.

8

9 **Bacteriological water analyses**

10 Bacteriological analyses of TC, FC, and FE were carried out according to the
11 membrane filtration method using selective media and following standard procedures
12 [26]. The bacteriological culture media used were m-Endo (BBL), m-FC (BBL) and
13 KF-Streptococcus Agar (BBL) according to manufacturer's instructions for TC, FC
14 and FE respectively. Briefly, 1 L water samples were taken in sterilized
15 polypropylene bottles. The samples were transported to the laboratory under cold
16 conditions (4°C) and processed in the within 6 hours of sampling at the most. When
17 necessary samples were diluted, mainly for irrigation water, while 100 mL of drinking
18 water samples were directly filtered. After filtration through a 0.45 µm nitrocellulose
19 membrane (Millipore), the media plates were incubated at 36° for 24 h for TC, at
20 44.5°C for 24 h for FC and at 36°C for 48 h for FE.

21

22 **Detection of Coliphages**

23 Coliphages were detected from concentrated water samples using *Escherichia*
24 *coli* K12 Hfr (ATCC) as the host bacterium according to the double layer agar
25 method. Briefly, 5 mL of Trypticase peptone semisolid agar (1%) containing 500 µL

1 of K12 in exponential growth phase and 500 μ L of concentrated water sample were
2 poured onto Trypticase peptone solid agar. Plates were incubated at 37°C for 18 h and
3 the coliphage plaques counted.

4

5 **Statistical analysis**

6 Data were analyzed using a generalized linear model approach [27,28]. First,
7 differences between years, between seasons and between seasons within each year
8 (year x season interaction) were tested for the presence of viruses and the abundance
9 of indicator bacteria. For viruses, the response variable (presence/absence) was
10 assumed to follow a Bernoulli distribution; the abundance of bacteria was assumed to
11 follow a Poisson distribution. A full factorial model with Year (2001 vs. 2002) and
12 Season (cold-dry vs. warm-rainy) factors was attached to each response variable
13 (presence of rotavirus, enterovirus and astrovirus; abundance of TC, FC, and FE)
14 separately. Statistical significance was determined by the change in deviance that its
15 deletion from the model produced, which approximately follows a Chi-square
16 distribution [27].

17 Second, in order to examine the relationship between the presence of viruses
18 or the abundance of bacteria with the physicochemical water variables recorded, log-
19 linear (for bacteria abundance) or logit (for viral presence), separate models were
20 attached to each response variable (abundance of TC, FC, and FE; presence of
21 rotavirus, enterovirus and astrovirus) with each environmental variable (temperature,
22 conductivity, pH and dissolved oxygen concentration) allowing these models to be
23 considered as predictors. For viral models, the abundance of indicator bacteria (i.e.,
24 TC, FC and FE) was also included as a predictor. In each case, the significance of
25 predictor variables in their relationship to the response variables was identified in the

1 same way as before, *i.e.* considering the change in deviance that its deletion from the
2 model produced.

3 A logistic regression analysis was applied to the virus, coliphages and bacteria
4 presence/absence data in order to investigate their association.

5 **Results**

6 **Water used for irrigation**

7 Deviance analysis resulting from the generalized linear model approach
8 showed no significant differences between years, between seasons, nor between
9 seasons within each year in terms of AST presence. However, there were significant
10 differences between seasons when the presence of both EV and RV were considered.
11 The presence of these pathogens was significantly more frequent during the cold-dry
12 season (0.75 and 0.35, respectively) than in the warm-rainy season (0.10 and 0.05,
13 respectively) (Figure 1).

14 The presence of AST and RV showed no significant relationship with any of
15 the environmental variables recorded (pH, temperature, conductivity and dissolved
16 oxygen concentration) or with the abundance of bacterial indicators. By contrast, EV
17 presence was significantly related to temperature but not to the abundance of any of
18 the bacterial indicators.

19 As for bacterial indicators, all samples were positive for the three bacterial
20 groups, indicating continuous faecal contamination of the water used for irrigation.
21 Deviance analysis showed no significant differences between years, between seasons,
22 or between seasons within each year when the abundance of faecal coliform (FC) or
23 enterococci (FE) were considered. However, there was a significant relationship
24 between the abundance of these bacterial groups and pH, when the water pH
25 increased, bacterial abundance decreased. By contrast, there were significant

1 differences in the abundance of TC between years and between seasons. TC was
2 significantly more abundant in 2001 than in 2002, and slightly higher during the dry
3 season than during the rainy season (Figure 2). However, the abundance of TC was
4 not significantly related to any of the environmental variables (pH, temperature,
5 conductivity and dissolved oxygen concentration).

6 Table 1 shows the association between enteric virus, coliphages and indicator
7 bacteria when detected in irrigation water. This association is clear as reflected by the
8 *p-value*. Positive samples for coliphages coincided more frequently with the positive
9 samples for indicator bacteria than EV, or the other enteric viruses.

10

11 **Water used for drinking**

12 No viruses were detected in the drinking water wells prior to chlorination.
13 According to the physicochemical parameters: pH; temperature; dissolved oxygen
14 concentration; and conductivity; there were no differences in prevailing conditions for
15 each sampling station. This demonstrated that there was no seasonal difference in the
16 conditions pertaining to these sources of drinking water.

17 Indicator bacteria were detected in the pre-chlorinated drinking water samples.
18 Results of TC, FC, and FE presence (Table 2) show that FE were most frequently
19 isolated and most abundant, with 5 positive samples in 2001 and 13 positive samples
20 in 2002. In terms of FE abundance, deviance analysis showed no significant
21 difference between years, between seasons or between seasons within each year.

22 Similarly, no significant relationship was found between the abundance of FE and any
23 of the environmental variables (pH, temperature, conductivity and dissolved oxygen
24 concentration). There were significant differences between years and between seasons
25 in both TC and FC abundance. These bacteria were significantly more abundant in

1 2002 than in 2001, and showed a higher presence during the dry season than the rainy
2 season. TC and FC abundance were also significantly related to variation in
3 conductivity.

4

5 **Physicochemical parameters**

6 The physicochemical parameters recorded were temperature, conductivity, pH,
7 and dissolved oxygen. Average values for each season, year, and water type are
8 shown in Table 3.

9 When water used for irrigation was considered, the two-way variance analysis
10 showed no significant differences between seasons or between years in terms of
11 average pH or average dissolved oxygen concentration. The averages used in the
12 analyses were mean averages. By contrast, average temperature during the cold-dry
13 season was significantly ($p < 0.001$) lower than during the warm-rainy season.

14 Significant variations between seasons within each year in terms of average
15 conductivity were also found; in 2001 there were no significant differences between
16 seasons, but in 2002, average conductivity was significantly higher during the cold-
17 dry season suggesting that there was a variation between years for some parameters.
18 For water source samples, analyses showed no significant variations in terms of
19 average temperature. By contrast, there were significant differences between years
20 ($p < 0.001$) and between seasons ($p < 0.01$) in terms of average pH. Significant
21 variations between seasons within each year for average dissolved oxygen
22 concentration were also found. In 2001, average conductivity was significantly higher
23 during the warm-rainy season, whereas in 2002 there were no significant differences
24 between seasons. Finally, average conductivity was significantly higher ($p = 0.011$)
25 during the cold-dry seasons.

1 **Discussion**

2 The Mexico City area, where the study was carried out has been classified as a
3 tropical highland [29], with an altitude of 2240 masl and climatic conditions, with a
4 cold-dry autumn and winter followed by a warm-dry spring and a warm-wet summer.
5 The current study used two basic meteorological parameters to define the seasonal
6 categories (cold-dry and warm-rainy); temperature and rainfall as reported by the
7 official National Meteorological System (Sistema Meteorológico Nacional) [21,22].

8 Treated wastewater represents the most important input to the aquatic system;
9 a process that does not consider virus elimination. There are also raw wastewater
10 discharges directly from households (2,015 houses with about 4.5 persons) that lack
11 sewer system [30] and it is estimated that 2,116 kg of faeces enter the aquatic system
12 daily. Due to the continuous faeces contribution and enteric virus detection
13 predominantly in the cold-dry season, a seasonal pattern regarding viral presence is
14 suggested. On the other hand, studies of diarrheal disease caused by rotavirus and
15 astrovirus in young children from Southern Mexico City [14,31] showed higher rates
16 in the autumn and winter months [14]. This increased incidence reflects the findings
17 of the current study in which viral presence is higher in the cold-dry season.

18 Unfortunately in Mexico, the National Epidemiological Surveillance System
19 [32] does not report epidemiological information that would indicate the actual
20 number of viral gastroenteritis cases, nor their seasonal behaviour. This is despite the
21 fact that gastrointestinal disease at a local level (Mexico City) is among the 20 main
22 causes of hospitalization [33].

23 During the cold-dry season the average low temperature was 4°C with an
24 average precipitation of less than 10 mm [21,22], which had an effect on the presence
25 of both bacteria and enteric viruses. This is related to lower water levels, higher
26 concentration of organic matter [23], and lower temperature [34], the latter favoring

1 the presence of enteric viruses [35]. These measurements of temperature and
2 precipitation support the current study for EV and RV, which showed a higher
3 presence during the cold-dry season. Conversely, AST was present in only 10% of the
4 samples. The variation between the frequencies of different viruses can be associated
5 with structural virus characteristics, in that the RV capsid presents three protein
6 layers, as compared with only one for AST. The higher frequency of EV could be
7 related to the massive polio vaccination campaigns that are carried out in Mexico
8 three times a year for <5 year old children. In previous studies, polio vaccine was
9 isolated from both wastewater and river water 2 or 3 months following the vaccination
10 campaign [36,37,38]. Other studies are needed to ensure that the EV detected in the
11 water samples used for irrigation corresponds to the vaccine type.

12 In the warm-rainy season, the temperature can reach an average of 24°C
13 [21,22], while rainfall can reach 1,500 mm. At the beginning of the rainy season, two
14 natural processes are evident in the canal system: soil washing and water dilution.
15 These promote an increase in bacterial density and counts in water, while towards the
16 middle of the rainy season, bacterial density decreases due to dilution. Although
17 warm-rainy temperature favors bacterial growth, enteric viruses could be damaged by
18 rising temperatures, as proved previously, when EV and RV were studied in fresh
19 water at 22°C and 20°C [39,40]. EV, RV and AST were practically absent during the
20 warm-rainy season in both years.

21 The rainfall, plus a significant increase in temperature compared with that of
22 the cold-dry season, contributes to the presence of these viruses in the water used for
23 irrigation from this tropical high-altitude area. Additionally, solar radiation, especially
24 UVB (320-280 nm), has recently been reported as an important parameter that affects

1 viral presence and infectivity [41,42], another environmental parameter that should be
2 included in future studies.

3 It is important to point out that TC is a group that includes enteric and non-enteric
4 bacteria [43], and the lower TC counts could be related to interference of non-
5 coliform bacteria that inhibit coliform bacteria growth, as has been shown by
6 Burlingame *et al.* [44], when m-Endo medium was used. Moreover, FC cultivated in
7 m-FC medium at 44.5°C has been reported to promote non-*E. coli* thermophilic
8 growth [45], which can produce a FC overestimation or a false positive reading. The
9 culture media used are those recommended by Standard Methods [26] and also
10 correspond to the official Mexican methods [46] for the enumeration of TC and FC in
11 water samples. However, the use of other methods to measure indicator bacteria that
12 show more specific results, mainly for water from tropical and subtropical areas [45],
13 is recommended for subsequent studies.

14 The results obtained in this study showed that coliphages can be used as
15 indicators of faecal contamination in reused water, in a complementary role to
16 indicator bacteria. On the other hand, they are useful as indicators of the presence of
17 enteric viruses, due to the clear relationship shown with EV and similar physical
18 characteristics, as well as resistance to wastewater treatment. Therefore, coliphages
19 have been shown to be complementary or equivalent to other indicators.

20 Mexico is considered to be a leading country in terms of wastewater recycling
21 [20]. This practice does not appear to be on the wane and it is envisaged that more
22 land will make use of wastewater for irrigation in the future. Mexican regulations
23 [47,48,49] and World Health Organization guidelines for irrigation [50] consider \leq
24 1000 CFU/100mL coliform bacteria as an acceptable limit for the irrigation of land
25 that is used to grow crops. However, according to the results discussed here, this limit

1 has been exceeded in the study area. The enteric virus and bacterial survival on
2 vegetable surfaces [51], constitutes a serious health risk for agricultural workers, as
3 well as for consumers [52].

4 Enteric viruses were not detected in the water sources during the seasons and
5 years studied. Nevertheless, the importance of this area as a source of drinking water
6 makes it vital to monitor viral presence regularly. Coliphages may provide adequate
7 viral indicators representing the large group of EV, but further evaluation is required
8 before they can be used for this purpose; tests that were not performed as part of this
9 study due to time and financial constraints.

10 Indicator bacteria detected in water sources did not show any seasonal trends.
11 The higher FE frequency suggests that these could be better bacterial contamination
12 indicators as compared with TC and FC levels in the region. The water extraction
13 wells are located in the transition area, where sedimentary soil composition is known
14 to favor water infiltration. This can affect groundwater quality because the sewage
15 system is insufficient for the growing population of these areas, and does not exist at
16 all in squatters settlements. Sewer breakages, which are frequent occurrences, could
17 explain the presence of indicator bacteria in the drinking water sources, as well as
18 being important for viral contamination. Groundwater as a source for drinking water
19 presents more stable conditions as compared with surface water; non-solar irradiation
20 and relatively low temperatures are favorable for enteric viral presence and infectivity
21 [18]. In these circumstances, preventive actions should be taken.

22

1 **Conclusions**

- 2 1. Enterovirus, rotavirus, astrovirus, total coliform, faecal coliform, enterococci
3 and coliphages considered in this study were present in water to be used for
4 irrigation.
- 5 2. The abundant presence of indicator bacteria and enteric viruses in irrigation
6 water proves a continuous raw residual water supply to the aquatic system.
- 7 3. Viral presence in irrigation water, for the specific tropical highland system
8 under study, is similar to that previously reported for temperate zones during
9 colder months.
- 10 4. The detection of indicator bacteria in the sources of drinking water shows the
11 contribution of faecal matter in the aquifer and reinforces the need for an
12 adequate disinfection process in order to ensure good water quality in the
13 public supply system.
- 14 5. Analysis to identify the presence of coliphages as indicators of faecal
15 contamination is recommended. These should be considered as
16 complementary to bacterial indicators, and to reflect the general survival
17 conditions of enteric viruses. The fact that coliphages are tolerant to
18 wastewater treatment makes them suitable indicators for the evaluation of
19 recycled water to be used for irrigation and recreational purposes.
- 20 6. This low-cost strategy of using microbial indicators to confirm water quality
21 for drinking and irrigation is attractive and advisable for low income countries,
22 with returns being found in public health benefits.

23 **Competing interests**

24 The authors declare that they have no competing interests.

1 **Authors' contributions**

2 ACE and MMH conceived and designed this study, performing some preliminary
3 assays. ACE contributed to the acquisition of field and experimental data, and carried
4 out the analytical work. ACE initiated data interpretation, and drafted and revised the
5 manuscript. MMH and CFA offered analytical suggestions, assisted with the
6 interpretation of results, and made critical revisions to the manuscript suggesting
7 details for the final draft. SSC contributed to statistical analyses and interpretation of
8 data. All authors read and approved the final manuscript before submission.

9

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19

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8

9 **Figures**

10 **Figure 1 - Percentage of RT-PCR positive samples**

11 Rotavirus (RV), enterovirus (EV) and astrovirus (AST) in water used for irrigation
12 over annual cycles (2001 and 2002). The Astrovirus genome was not detected either
13 of the two warm-rainy seasons, and the genome rotavirus was not detected during the
14 2002 warm-rainy.

15

16 **Figure 2 - Indicator bacteria in irrigation water.**

17 Counts of total coliforms (TC), faecal coliforms (FC) and enterococci (FE) in water
18 used for irrigation over two seasons each year. Bacterial counts were determined by
19 filtration membrane method and standard culture media.

1 **Tables**

2

3 **Table 1 – Association of indicator**

4 Association of enteric viruses, bacteria and coliphage detection in irrigation water.

5

6 **Table 2 - Indicator bacteria in the water source.**

7 Total samples for year and season, and their total counts (CFU/100mL) for TC (total
8 coliforms), FC (faecal coliforms) and FE (enterococci).

9

10 **Table 3 - Physicochemical parameters.**

11 Average physicochemical parameters registered in water source samples and samples
12 of water used for irrigation.

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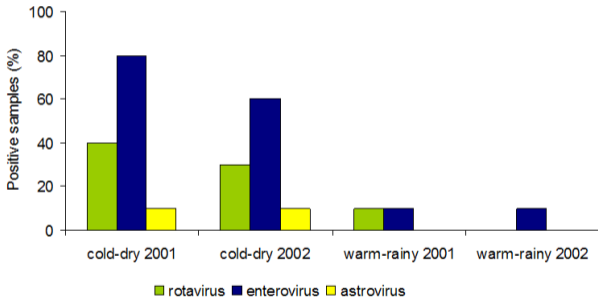


Figure 1

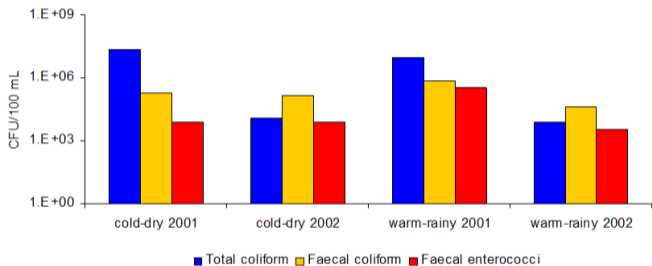


Figure 2

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