# Reviews/Analyses

## Presence of enteric viruses in freshwater and their removal by the conventional drinking water treatment process*

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*A review of results published in English or French between 1980 and 1990 was carried out to determine the levels of indigenous human enteric viruses in untreated surface and subsurface freshwaters, as well as in drinking water that had undergone the complete conventional treatment process. For this purpose, the conventional treatment process was defined as an operation that included coagulation followed by sedimentation, filtration, and disinfection. Also assessed was the stepwise efficiency of the conventional treatment process, as practised at full-scale facilities, for removing indigenous viruses from naturally occurring freshwaters. A list was compiled of statistical correlations relating to the occurrence of indigenous viruses in water.*

Human enteric viruses are frequently found in water sources, including groundwaters (*3*). At least 140 human enteric virus types are known (*11*), among which are the 72 serotypes of enteroviruses, in addition to adenoviruses, reoviruses, rotaviruses, hepatitis virus E, Norwalk virus, calicivirus, and the "small round viruses". These viruses are transmitted by the faecal–oral route, i.e., infection is acquired through the consumption of faecally contaminated materials. Such viruses can represent a serious contaminant if they are present in water that is consumed without first having undergone proper treatment to ensure their removal or inactivation (*4*). Water treatment processes to remove viruses and other microorganisms range from the very simple, such as, on an individual household basis, addition of natural clay to serve as a flocculating agent (*16*), to the complex community projects used by large cities.

The present article reviews the literature published in English or French from 1980 to 1990 on the occurrence of human enteric viruses in raw (untreated) surface and subsurface freshwaters and in water that had undergone "conventional treatment". Here such treatment was defined as a multistage process that included coagulation, followed by sedimentation, filtration, and disinfection. The first three of these processes can be particularly effective because indigenous viruses in natural freshwaters are likely to be adsorbed on particulates (*9*, *22*). Final disinfection then serves to provide an additional measure of protection to the human population being served. Also reviewed was the stepwise efficiency of virus removal at different stages of the conventional drinking water treatment process, as practised by full-scale plants. Although slow sand filtration is not included in the assessment of the conventional treatment process, this technique does deserve mention since it can be an effective means of removing viruses from water. A list is included of those water characteristics that correlated statistically with the incidence of indigenous viruses in water.

### Viruses in raw water

A total of 16 studies were identified that yielded usable data on the presence of indigenous human enteric viruses in naturally occurring freshwaters, and Table 1 summarizes the results of calculations based on the data reported. The disparity in the

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various study designs was, however, too great to allow calculation of the overall mean values for the information shown. Instead, median values were calculated, and these indicate that 47.2% of samples were positive for the presence of indigenous human enteric viruses. The median values for the highest level and the average level of viruses, including all negative samples, were 22.1 and 1.4 infectious units (IU) per litre, respectively. Based on a median value of 1.4 IU per litre for the average level of virus in untreated water, it can be estimated that a person who consumed 2 litres of raw water per day would ingest 1022 viral IU over the course of a year (1.4 IU per litre × 2 × 365). This could clearly represent a

Table 1: **Prevalence of human enteric viruses in naturally occurring freshwaters**

| Water source and location | Viruses investigated | No. of samples examined | % of samples positive for viruses | Level of viral presence (No. of infectious units/l) | | Reference |
|---|---|---|---|---|---|---|
| | | | | Highest level | Average level (includes all negative samples) | |
| *Surface:* | | | | | | |
| Bolivia | Enteroviruses, rotaviruses | 2 | 100.0 | NR[a] | NR | *30* |
| Canada | | | | | | |
| Manitoba | Adenoviruses, enteroviruses | 33 | 3.0 | NR | 0.002 | *27* |
| Quebec | Enteroviruses | 28 | 100.0 | NR | NR | *19* |
| Quebec | Enteroviruses, and morphologically similar others | 66 | 48.5 | NR | NR | *20* |
| Quebec | Enteroviruses | 303 | 45.9 | NR | 11.40 | *23*[b] |
| Czechoslovakia | Enteroviruses | 66 | 48.5 | NR | NR | *28* |
| German Democratic Republic | Adenoviruses, enteroviruses | 1908 | 20.0 | 22.10 | 2.70 | *31* |
| Israel | Enteroviruses | 8 | 12.5 | NR | NR | *17* |
| Mexico | | | | | | |
| Jalisco | Enteroviruses, rotaviruses | 7 | 71.4 | 34.91 | 9.34 | *14* |
| South Africa | Unspecified | 261 | 0.4 | 315.00[c] | 5.18[c] | *8* |
| Spain | Enteroviruses | 28 | 85.7 | 55.00 | 3.06 | *15* |
| USA | | | | | | |
| Michigan | Enteroviruses | 4 | 50.0 | 0.11 | 0.04 | *13* |
| Michigan | Enteroviruses | NR | NR | 0.98 | 0.14 | *29* |
| Missouri | Enteroviruses | NR | NR | 0.06 | 0.01 | *1* |
| *Surface/spring* | | | | | | |
| USA | | | | | | |
| Arizona | Enteroviruses, rotaviruses | 41 | 43.9 | NR | 0.08 | *25* |
| *Groundwater* | | | | | | |
| Bolivia | Enteroviruses, rotaviruses | 4 | 100.0 | NR | NR | *30* |
| Czechoslovakia | Enteroviruses | 9 | 0.0 | NA[d] | NA | *28* |
| Israel | Enteroviruses | 99 | 20.2 | NR | NR | *17* |
| Median value | | — | 47.2 | 22.1 | 1.4 | — |
| No. of values included in the statistic | | — | 16 | 7 | 10 | — |

[a] NR = not reported.
[b] Data from this study (on either porcine enteroviruses or reoviruses presumed to be avian, both of which may have been contaminants from abattoir waste) were not included in the analysis.
[c] The values listed for this study are half those in ref. *8*, where they were presented in the form of 50% endpoints ($TCID_{50}$—tissue culture infectious doses as a 50% endpoint). By dividing the $TCID_{50}$ values by two the results can be compared more directly with those in the other referenced studies, whose data were 100% endpoints (either as plaque-forming units (PFU) or most probable number (MPN)) (*10*).
[d] NA = not applicable, since no viruses were detected in any of the samples examined.

Table 2: **Prevalence of human enteric viruses in conventionally treated drinking water**[a]

| Location | Viruses investigated | No. of samples examined | % of samples positive for viruses | Level of viral presence (No. of infectious units/l) | | Reference |
|---|---|---|---|---|---|---|
| | | | | Highest level | Average level (includes all negative samples) | |
| Bolivia | Enteroviruses, rotaviruses | 5 | 40.0 | NR[b] | NR | *30* |
| Canada | | | | | | |
| Quebec | Enteroviruses, | 31 | 100.0 | NR | NR | *19* |
| Quebec | Enteroviruses | 144 | 7.6 | NR | NR | *21*[c] |
| Quebec | Enteroviruses | 31 | 0 | 0 | 0 | *24* |
| Mexico | | | | | | |
| Jalisco | Enteroviruses, rotaviruses | 18 | 77.8 | 104.48 | 6.95 | *14* |
| South Africa | Unspecified | 146 | 0 | 0 | 0 | *8* |
| USA | | | | | | |
| Michigan | Enteroviruses | 36 | 0 | 0 | 0 | *29* |
| Michigan | Enteroviruses | 4 | 0 | 0 | 0 | *13* |
| Missouri | Enteroviruses | 65 | 0 | 0 | 0 | *1* |
| Median values | | — | 0 | 0 | 0 | — |
| No. of values included in the statistic | | — | 9 | 6 | 6 | — |

[a] Conventional treatment consisted of coagulation, sedimentation, filtration, and post-filtration disinfection using chlorine and/or ozone.
[b] NR = not reported
[c] Data from this study (on either porcine enteroviruses or reoviruses presumed to be avian, both of which may have been contaminants from abattoir wastes) were not included in the analysis.

significant personal health risk. A method for mathematically assessing these risks has been developed by Gerba & Haas (*7*). Two studies addressed the presence of reoviruses in naturally occurring freshwaters (*23,26*); the results are, however, not included in this review because human reoviruses are indistinguishable from those shed by other animals. Data for surface waters described as being heavily impacted by sewage effluent are also not shown in Table 1. Such waters clearly do occur, since sewage is frequently discharged without adequate treatment or disinfection. Thus, freshwater sources may contain levels of virus far higher than those listed in Table 1.

## Virus removal by water treatment

Full conventional drinking water treatment appears to be reasonably effective at removing indigenous enteric viruses. Nine studies examined conventionally treated drinking water for the presence of indigenous human enteric viruses, and Table 2 summarizes the results of calculations based on the reported data. The median percentage of samples that were positive for enteric viruses was 0.0, while the median values for the highest level and the average level of viruses detected were both 0.0 IU per litre. These findings should certainly be encouraging to communities whose drinking water undergoes the conventional treatment process. However. it must also be noted that four of the nine studies found viruses in treated water, including an investigation by Payment (*19*) in which each of 31 samples examined was positive for the presence of human enteric viruses. Furthermore, in one sample of treated drinking water Keswick et al. found > 100 viral IU per litre (*14*). It must be recognized that the efficiency of virus removal may differ with respect to virus type and also that the range of viral sensitivity varies, depending on the detection technique. Thus, some of the water samples that were reported to contain no enteric viruses may well have been positive if they had been examined for additional virus types or using more sensitive techniques. The methodology used for detecting viruses in water has recently been reviewed by Hurst et al. (*11*).

Four of the studies reported sufficient information to allow calculation of the efficacy of virus removal after individual stages of the conventional treatment process (Table 3). In examining studies of the efficiency of virus removal at treatment plants, it is important to note that water samples from different stages of the treatment process are often not collected in the proper sequence, nor is adequ-

Table 3: **Step efficiency for removal of indigenous human enteric viruses and coliphage by conventional full-scale water treatment**[a]

| Location | Viruses | Efficiency of virus removal (%) | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | Prechlorination | Coagulation and sedimentation | Filtration | Coagulation, sedimentation, and filtration[b] | Post-filtration disinfection | Full conventional treatment process[c] | |
| Canada | | | | | | | | |
| Quebec | Enteroviruses | 97.9 | 77.8 | 93.7 | 98.6 | 40.0 | 99.2[d] | *21* |
| Quebec | Enteroviruses | NR[e] | NR | NR | NR | NR | NR[f] | *24* |
| Mexico | | | | | | | | |
| Jalisco | Coliphage (male host) | NA[g] | 64.8 | 81.2 | 93.4 | >20.8[h] | >94.8 | *14* |
| Jalisco | Enteroviruses | NA | −138.9 | 48.3 | −23.5 | 18.1 | −1.1 | *14* |
| Jalisco | Rotaviruses | NA | 10.0 | 90.0 | 91.0 | −57.7 | 85.8 | *14* |
| USA | | | | | | | | |
| Michigan | Coliphage (male host) | NA | 56.0 | 33.5 | 70.5 | >95.8 | >98.8 | *13* |
| Michigan | Coliphage (female host) | NA | 29.0 | 49.7 | 64.3 | >99.0 | >99.9 | *13* |
| Michigan | Enteroviruses | NA | 50.9 | 71.6 | 86.1 | >50 | >93.0 | *29* |
| Michigan | Enteroviruses | NA | 50.0 | 72.7 | 86.3 | >66.7 | >95.4 | *13* |
| Median value | | — | 50.4 | 72.1 | 86.2 | ≥45.0[i] | ≥95.1 | |
| No. of values included in the statistic | | — | 8 | 8 | 8 | 8 | 8 | |
| Median value (negative values eliminated) | | no negative values | 50.9 | no negative values | 86.3 | ≥50.0 | ≥95.4 | |
| No. of values included in the statistic | | | 7 | | 7 | 7 | 7 | |

[a] Conventional water treatment consisted of coagulation (flocculation) followed by sedimentation, filtration, and post-filtration disinfection by addition of chlorine with or without ozone.
[b] Estimated using the following relationship: $CSF = 100 - [(100 - CS) \times (1 - 0.01F)]$ where $CS$ = % of virus removed by coagulation + sedimentation; $F$ = % of virus removed by filtration; $CSF$ = estimated % of virus removed by stepwise coagulation and sedimentation followed by filtration.
[c] Estimated using the following relationship: $T = 100 - [(100 - CS) \times (1 - 0.01F) \times (1 - 0.01D)]$ where $CS$ = % of virus removed by coagulation + sedimentation; $F$ = % of virus removed by filtration: $D$ = % of virus removed by post-filtration disinfection; and $T$ = estimated % of virus removed by conventional treatment, defined here as stepwise treatment by coagulation + sedimentation, filtration, and disinfection. The symbol > associated with some of the values for $D$ was dropped prior to the calculation and reinserted at the value calculated for $T$.
[d] Incorporation of the stepwise efficiency for virus removal by prechlorination increases this value to 99.98%.
[e] NR = not reported.
[f] Efficiency values were provided only for virus removal by a treatment chain consisting of prechlorination followed by conventional treatment; these ranged from >98.2% to >99.7%.
[g] NA = not applicable.
[h] Figures preceded by the > symbol are minimum estimates.
[i] Figures preceded by the ≥ symbol are minimum estimates and indicate that at least one of the numbers ranking in order below the median value had been accompanied by a > symbol.

ate consideration given to treatment process retention times. Thus, they can represent only general estimates of the true situation at a treatment plant. Such sampling schemes occasionally result in an apparent increase in the virus titre for a treatment process. For example, some of the efficiencies in Table 3 are negative, suggesting that a particular stage of treatment produced an increase in the level of enteric viruses. These reported increases are no less accurate than observed decreases of equivalent magnitude. As far as the disinfection process is concerned, there were many reported instances when the level of a virus could be assessed prior to this stage but not afterwards. In such cases the estimated minimum level of virus removed is shown. The median values for the data in Table 3 suggest that the overall efficiency of coagulation and sedimentation at removing viruses was 50.4%. The median values for the efficiency of virus removal during subsequent stages in the treatment sequence

Table 4: **Statistical correlations for indigenous viruses in water**

| Parameter | Statistical test | Coefficient of correlation (*r*-value) | *P*-value | Reference |
|---|---|---|---|---|
| Presence of enteric viruses | | | | |
| $BOD_5$ | NR[a] | NR | <0.05 | *31* |
| High turbidity (>10 NTU)[b] | Corrected $\chi^2$ | NA[c] | <0.05 | *20* |
| Presence of enteroviruses; presence of bacteria detectable by total bacterial count | Spearman's rank-order | NA | <0.05 | *17* |
| Level of enteroviruses; total bacterial count | Pearson's correlation | 0.972 | <0.00001 | *13* |
| Level of coliphage detected using a male host strain of bacteria; level of *Clostridium perfringens* | Pearson's correlation | 0.616 | 0.011 | *13* |
| Level of coliphage detected using a male host strain of bacteria; that of coliphage detected using a female host strain of bacteria | Pearson's correlation | 0.896 | 0.00008 | *13* |
| Log (level of enteric viruses); log (level of faecal streptococci) | Linear regression | −0.26 | ≤0.01 | *6* |
| Log (level of coliphage detected with female host strain of bacteria) | Linear regression | 0.42 | ≤0.01 | *6* |
| Water conductivity | Linear regression | 0.38 | ≤0.01 | *6* |
| Water temperature | Linear regression | −0.64 | ≤0.01 | *6* |
| Turbidity | Linear regression | −0.50 | ≤0.01 | *6* |
| Log (level of coliphage detected with female host strain); log (level of total coliform) | Linear regression | 0.69 | ≤0.001 | *32* |
| log (level of faecal coliform) | Linear regression | 0.62 | ≤0.001 | *32* |

[a] NR = not reported.
[b] NTU = nephelometric turbidity units.
[c] NA = not applicable.

were filtration, 72.1%; and post-filtration disinfection, ≥ 45.0%. The median efficiency for the combination of coagulation and sedimentation followed by filtration was 86.2%. The median value for the entire conventional treatment process, including post-filtration disinfection, was ≥ 95.1%. Exclusion of negative values in calculating the median values did not change any of the results by more than five percentage points (Table 3). Data reported by Payment et al. (*21*, *24*) indicate that carrying out prechlorination prior to the conventional treatment of raw water can greatly increase the overall efficiency of virus removal (99.98% efficiency was calculated for enterovirus removal (*21*)).

## Coliphage

Certain groups of coliphage, bacterial viruses that infect *Escherichia coli,* may be useful as indicators of the efficiency of virus removal by drinking water treatment processes. Coliphage that belong to the genus *Levivirus* (*18*) are of particular importance in this regard because they are morphologically similar to the enteroviruses. The leviviruses infect only male bacteria, i.e., those with sex pili (*2*) that serve as the site of attachment before the virus genome enters the host bacterium. Data on coliphage removal have been included in Table 3, where it is indicated whether the bacterial host strain used for a particular study was male or female. Male host strains are susceptible to infection also by other groups of bacterial viruses that attach to their host bacterium at sites other than the sex pili. Only the latter bacterial viruses can be detected by female bacterial strains (i.e., those that lack sex pili). An excellent review of coliphages in the environment has been prepared by Furuse (*5*). The rod-shaped inoviruses are another group of male-specific bacterial viruses. These are morphologically distinguishable from the icosahedral leviviruses in the electron microscope; however, most of the protocols commonly used for assaying the infectivity of bacterial viruses would probably not be able to distinguish between leviviruses and inoviruses.

## Factors that correlate with viral presence

Table 4 lists water parameters that have been found to correlate with the presence of indigenous viruses in water. Included are numerous chemical, physical, and microbiological characteristics. Two particularly notable factors have been associated with the level of human enteric viruses in freshwaters: seasonal changes in water temperature (viral levels are higher during winter) (*6, 13, 28, 29*); and a "rainy season" effect observed by Keswick et al. (*14*). Water temperature exerts an extremely strong influence on

viral stability, lower temperatures increasing the survival time (*12*). The rainy season effect may arise because at that time of the year freshwater has a very high turbidity which correlates in a statistically significant manner with the presence of indigenous viruses in water (Table 4) and with virus stability in water under laboratory conditions (*12*).


## Résumé

### Élimination des entérovirus présents dans l'eau douce par les procédés classiques de traitement de l'eau de boisson

Dans cette étude sont examinés les résultats publiés entre 1980 et 1990 sur la présence d'entérovirus humains dans les eaux de surface et les eaux souterraines non traitées ainsi que dans l'eau de boisson ayant subi un traitement "classique". Il s'agit d'un procédé en plusieurs temps où se succèdent divertses phases: coagulation, sédimentation, filtration et désinfection. On a également cherché à savoir comment chaque étape du procédé de traitement permet d'éliminer les entérovirus dans les grandes installations. En outre, on a établi une liste des caractéristiques statistiquement corrélées avec la fréquence des virus indigènes dans l'eau.

Seize études ont fourni des données relatives à la présence d'entérovirus humains indigènes dans les eaux douces naturelles. On a ainsi pu calculer les médianes de divers types de données, ce qui a permis d'établir que 47,2% des échantillons renfermaient des entérovirus humains indigènes. Les médianes de la plus forte concentration virale trouvée dans chaque étude et de la concentration virale moyenne, y compris les échantillons négatifs, sont respectivement de 22,1 et 1,4 unités infectieuses par litre.

Neuf études ont porté sur la mise en évidence d'entérovirus humains dans l'eau de boisson traitée de façon classique. La médiane du pourcentage d'échantillons renfermant des virus est de 0,0%, et les médianes de la plus forte concentration virale et de la concentration virale moyenne sont toutes deux de 0,0 unités infectieuses par litre. Deux études font exception; dans la première, chacun des 31 échantillons examinés renfermait des entérovirus humains et dans la seconde, un échantillon renfermait plus de 100 unités virales infectieuses par litre d'eau traitée.

Quatre études ont permis de déterminer la quantité de virus éliminée à chaque étape du processus de traitement courant. Les médianes montrent que coagulation + sédimentation permettent d'éliminer 50,4% des virus. Les médianes d'efficacité des étapes suivantes sont: filtration, 72,1%; désinfection après filtration, >45,0%. Pour l'ensemble du procédé de traitement, y compris la désinfection après filtration, la médiane d'efficacité est >95,1%. Les résultats d'une étude montrent que la chloration de l'eau avant traitement augmente l'efficacité totale du traitement, la faisant passer à 99,98%.

On a pu établir une corrélation entre de nombreux paramètres chimiques, physiques et microbiologiques et la présence de virus indigènes dans l'eau. De plus, la concentration virale dans l'eau est souvent plus élevée en hiver. Les basses températures augmentent le temps de survie des virus, ce qui explique probablement que la concentration d'entérovirus humains dans les eaux douces soit associée aux changements de température saisonniers.


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