VIRUSES IN RIVER WATER AND
HEALTH RISK ASSESSMENT

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ABSTRACT
The construction of the Marchfeldkanalsystem (Austria) creates irreversible
environmental changes which could have an impact on environmental health.
Virological investigations of the river Danube have been carried out as a
part of the environmental impact assessment procedure. The repeated
detection of viruses in 61% of all water samples with a mean level of 0.762
MPN/l suggests that a permanent viral contamination of Danube is occurring
and that the self-purifying capacity of the river is relatively inefficient.
Strains of some virus families not hitherto detected in water (toga-(bunya)
viridae) could be found. Provided the proposed treatment facilities are as
effective as to remove the virus load to an extent of 99.99 % the
Marchfeldkanalsystem project will not endanger public health.

KEYWORDS
Danube, Marchfeld, water supply project, virus levels, virus groups (toga–
(bunya)viridae), health hazard assessment.

INTRODUCTION AND STUDY DESIGN

Health hazard assessment is becoming one of the most powerful tools in the
planning of water management projects which have environmental consequences.
It is necessary to foresee conflicts and try to avoid them before they occur.
The construction of the Marchfeldkanal and the creation of the Marchfeldkanal-
system is a multipurpose economic project. During the planning phase an
environmental impact assessment has been done. The factors which have been
taken into consideration were the contents of pollutants in the Danube water
and their seasonal variation.
The virological study was performed by the Institute of General and Community
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from the Errichtungsgesellschaft Marchfeldkanal, Vienna (Austria) from
The Marchfeld covers an area of 1000 km² (640 km² is arable land) and has
around 225 000 inhabitants. It is a part of the Alps–Carpathian mountain range
which had subsided at various fault lines and was subsequently filled with
sediments of tertiary and quaternary formations. The depth of this main ground-
water bearing formation is 10 m on average. Since the beginning of this
century a steadily lowering of the groundwater level (on average 5 cm per year) has been observed partly influenced by the regulation of the Danube in the last century. The risk of steppe formation is now evident because the source of fresh water for irrigation purposes reached its functional maximum point within the last decades. The continuing development of agriculture now depends on the conveyance of water into the Marchfeld region. Therefore a water supply project was scheduled in 1983. It is committed to a policy of increasing use of Danube water. It starts at river-km 1938 with a canal (length 18 km) carrying on average 15 m$^3$/s Danube water to an irrigation site in the northwestern part of the Marchfeld where 250 l/s is to be percolated. The remaining water flows via natural streams and another canal back to the Danube at river-km 1880. This study deals with the site from which water for the canal will be drawn. The Danube's flow varied between 1200 and 2700 m$^3$/s, while the mean flow velocity was 2 to 3 m/s.

Water pollution monitoring

A total of 52 samples were taken for virus analysis, 26 from the middle of the river bed and 26 from the left shore. Sampling took place each second week. 10 l of water were collected from a 60 cm depth and concentrated to 2 ml by a two-stage method (Walter and Rüdiger, 1981) using adsorption of viruses to aluminium hydroxide and dissolving the flocs in citric acid buffer (1 M / pH 7.4). The material from the first concentration stage was frozen at -30 degrees and sent to Dresden by train. Then the samples were further reduced in volume by centrifugation (100 000 g, 4 hours) and checked for the presence of infectious viruses by using Fl-cells applying the Most Probable Number Method (MPN) (Chang, 1958). Virus subgroups were determined by serum neutralisation. The presence of rotavirus- and HAV-antigen was investigated by means of the Elisa technique. Examinations were made with the water concentrates, the un­typable and the slow growing viruses by using transmission electron microscopy (TEM).

RESULTS

Virus load

Viruses were detected in 61.5 % of samples (32/52). The virus levels ranged between 0.153 and 2.542 MPN/l but there was one exception in June 1987 when levels as high as 14.14 MPN/l could be demonstrated. The overall mean of only the positive results was $\bar{x} = 1.238$ MPN/l, $s = 2.506$ MPN/l. The mean of all results was $\bar{x} = 0.762$, $s = 2.046$.

![Seasonal variation of the virus contamination](image-url)
Seasonal variation of the virus contamination at both sites is presented in fig. 1. It can be stated that there is a distinct but not statistically significant difference between the isolation frequency and the virus levels at both sampling sites. Higher levels of contamination were found at the middle of the river. The data on virus levels and on the flow-through of the Danube have been used to evaluate the virus load of the river.

Applying this formula:

\[
\text{Virus load} = \text{Virus level} \times \frac{\text{MPN}}{\text{s}} \times \text{Flow} \times \frac{m^3}{s}.
\]

A mean daily virus load in the Danube has been calculated for the entire observation period:

\[
\text{Mean load} = 0.762 \times 1743.8 \times 8.64 \times 10^7 \text{MPN} = 11.5 \times 10^{10} \text{MPN}.
\]

Viral excretion is generally limited to approximately 10% of any population at any given time. Calculation based on per capita daily discharge of viruses results in an estimated virus input in the Danube from the 17.6 Mio people living in the catchment area upstream of river km 1938.

Applying this formula:

\[
\text{Estimated virus input} = \frac{\text{MPN}}{\text{d}} = \text{number of inhabitants} \times 0.1 \times \frac{150}{\text{d}} \text{MPN} / \text{l} \times 10,000 \text{MPN/l} \text{(mean virus level of sewage)}
\]

\[
= 17.6 \times 10^6 \times 0.1 \times \frac{150}{\text{d}} \times 10,000 \text{MPN/l} = 264.0 \times 10^{10} \text{MPN/d}.
\]

When drawing a parallel between this general estimated input and the load at km 1938 there is a remarkably low self-purifying capacity within the order of 96%. This should necessitate an investigation of the impact of the dams associated with the hydroelectric power plants existing in the upstream sector of the Danube.

The contamination levels were subject to statistically significant seasonal variations. Based on the isolation frequency, summer was the low (37.5%) and winter the high (100%) risk season.

**Virus types**

Virus detected by cell culture technique. The 114 isolated strains have been recovered over the entire incubation period of 50 days. In fig. 2 the time before the first CPE occurred is plotted against the frequency of isolation.
Comparing the results of three other rivers investigated with the same method it could be stated that the Danube is characterized by a very distinct virus population as far as the cell culture adaptation time is concerned. We split up the 114 strains into two groups, a fast growing (≤26 days) and a slowly growing (≥26 days) group (tab. 1). In the fast growing group all but four viruses could be classified easily. With one exception they belong to the picornaviridae family. Their overall replication pattern was rather homogeneous with a mean replication time of 2 to 3 days. In the slow growing group up to now only 43% could be classified. In this group also members of the picornaviridae family rank first. The next most common viruses detected have been reoviridae, then two toga-(bunya)viridae and at least one adenovirus and one hitherto unclassifiable 70 nm isometric virus.

The members of reo- and toga-(bunya)viridae families show a prolonged replication time of 5 to 8 days. Given that picornaviridae are the environmentally most resistant group we have found a relatively higher proportion of sensitive viruses such as the enveloped toga-(bunya)viruses than in other river studies. Some arthropod-transmitted members of these families cause acute encephalitis and meningitis in human beings which is endemic in that area of Austria. Some other members cause bovine diarrhea and hog cholera (pestivirus) which also were prevalent in the river basin during the investigation period.

### Table 1 Viruses from Danube

<table>
<thead>
<tr>
<th>Virus family</th>
<th>Method of classification</th>
<th>Growth pattern (≤26 days)</th>
<th>Growth pattern (≥26 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>not determined</td>
<td>TEM</td>
<td>4</td>
<td>36</td>
</tr>
<tr>
<td>picornaviridae</td>
<td>TEM</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>echo (B1-group)</td>
<td>TEM</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>echo (6,7,11,30 group)</td>
<td>TEM</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>enterovirus mixture</td>
<td>TEM</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>reoviridae</td>
<td>TEM</td>
<td>–</td>
<td>6</td>
</tr>
<tr>
<td>toga-(bunya)viridae</td>
<td>TEM</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>adenoviridae</td>
<td>TEM</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>70 nm isometric virus</td>
<td>(TEM)</td>
<td>–</td>
<td>1</td>
</tr>
</tbody>
</table>

*Serotyping

Virus detected by Elisa. The HAV-test with the water concentrates was done by using a test kit developed by Alvermann and Müller (1985). 4 out of 42 concentrates examined have been considered positive, based on a positive/negative ratio of -2. The positive samples were from 18.11.1986, 15.6.1987, 14.7.1987, 28.7.1987. Monitoring the incidence of hepatitis A in two regions of the upstream catchment area "Oberösterreich" and "Niederösterreich" revealed a peak from September until November 1986. During the entire observation period there was a certain level of hepatitis A infections in the river basin. The sample collected in November 1986 during the decline of the outbreak demonstrated with a 3.7 ratio the highest concentration of HAV-antigen in our water samples. The rotavirus test was carried out on concentrates by using the ROTAZYME II Diagnostic Kit (Abbott Laboratories, North Chicago). No rotavirus antigen could be detected.
Viruses in river water

Aggregates of small isometric virus particles of 20-24, 25 and 30 nm in diameter could be detected in three samples (fig. 3). In all samples tailed phages could be found.

Bacteriological investigations. The determination of total bacteria E. coli and enteroccoci were done by Jaksoh (1988) at river km 1933. The results were used to calculate mean values for the observation period: total bacteria = 1795, s = 2482; E. coli = 1701, s = 1347; enteroccoci = 37.5, s = 32.7. Consideration should be given to the fact that the only extreme value of total bacteria (> 11 000) coincided with the extreme value of viruses (14.14 MPN). The findings confirmed the expectation that the water of the Danube is bearing a low to moderate sewage load.

DISCUSSION

A review of the circumstances surrounding the Marchfeldkanal project indicates the need for taking into consideration the virological water quality of the Danube. The isolation frequency was with 61.5 % positive results unexpectedly high. But when compared with four other fast flowing rivers investigated with the same method, virus isolation frequency from the Danube was next to lowest, (Hahn et al., 1988; 83 %; Walter et al., 1985; 82.3 %; Guyer, 1988; 73.6 %; Kante und Klauwitter, 1988; 56.8 %). Much more emphasis should be placed on the virus levels. The average mean was 0.762 MPN/l. The transmission of water borne diseases of viral origin and the relative importance attached to this risk must be decided by taking into account the local situation: 250 l/s water will be percolated in order to cover up the groundwater deficiencies. In this way Marchfeld groundwater would receive an daily input of at least 16.5 x 10^6 MPN. For drinking purposes only 5.7 % of this water will be used. This corresponds to an overall exposure dose of 94 x 10^4 MPN/day.

For some virus infections it has been assumed that there is a finite probability of 2 to 10 infectious particles of a given virus type initiating an infection in a vulnerable person. Taking this into account there might result a finite infectious risk for all inhabitants. But considering the type of canal, the planned treatment facilities (oxidation pond and a high standard percolation area) and the groundwater storage time of 10 to 12 months an eliminating efficiency as high as 99.99 % could be expected. The remaining virus dose of 94 MPN/day could infect at most 9 to 47 vulnerable persons a day. Provided 0.5 % of them develop a clinical disease then 17 to 86 episodes per year could occur. This risk seems acceptable, but it should be judged against the background of the number of normally occurring virus diseases in the Marchfeld.

86 % of the water is used for irrigation. It can transmit viruses via aerosols, contaminated food and groundwater. Due to a lack of baseline data for health risk assessment we adhere to the Arizona Standard that no more than one infectious virus particle should be detected in a 40 l sample (Sproul, 1983). If the treatment plants work well this would be easily reached.

Considering the complex nature of the environmental changes caused by this project and in order to avoid any non-acceptable health risk we strongly support the integration of an optimized treatment technology in the MFS with virus eliminating efficiencies as high as 99.99 - 99.999 % and propose a pilot study of the final treatment procedure by using a model in reduced scale before the ultimate decisions are made.

REFERENCES


