

# Long-term study of virus contamination of surface water in the German Democratic Republic

R. WALTER,<sup>1</sup> H.-J. DOBBERKAU,<sup>2</sup> W. BARTELT,<sup>3</sup> W. DIENER,<sup>4</sup> I. HÄRTEL,<sup>5</sup> U. HEINRICH,<sup>6</sup> U. MÜLLER,<sup>7</sup> S. RÜDIGER,<sup>8</sup> & B. STETTNIŠCH<sup>9</sup>

*Between 1 January 1970 and 31 December 1979, a study of the concentration of viruses in surface water was carried out by 4 virological laboratories in different regions of the German Democratic Republic. All these laboratories used the same methods for virus detection.*

*Altogether 1908 samples from 30 sampling points were evaluated. The rate of virus isolation ranged from 8% to 92% with a mean of 20%. There were considerable differences in isolation rate among the sampling points, and the rate for any particular point varied from year to year.*

*The mean value of virus concentration, determined by the most probable number technique, was 2.7 cytopathogenic units (CU) per litre, while the maximum was 22.1 CU/litre. Viruses seen throughout the 10-year investigation included poliovirus, types 1, 2, and 3, echovirus types 6, 11, and 30, and coxsackievirus B 3 and B 5; echovirus 7 and 24, coxsackievirus B 1, and adenovirus 5 were seen occasionally. The results of the study reflected the high level of use of surface waters in the German Democratic Republic.*

*Where water is intended for human use, e.g., as drinking-water or for recreation, reasonable safety measures, such as water treatment and disinfection, should be taken, in order to ensure that the level of viral contamination is within the permissible limits.*

The epidemiological importance of water-borne transmission of viruses has been difficult to assess because of the lack of suitable techniques. The isolation of viruses from water should provide valuable information on their circulation in the environment.

The German Democratic Republic is a highly industrialized country with a high water demand but limited resources and virological quality assessment is most important for water that is used as a source of drinking-water or for recreation.

Between 1 January 1970 and 31 December 1979, virological examinations were carried out on samples of surface water from 30 sampling points (rivers, canals, lakes, and coastal brackish water) in different areas of the German Democratic Republic. The same methods and cell-line (FI) were used each time, in order to obtain comparable information on the distribution and concentration of viruses in surface water. Where the water was intended for human use, specific estimates were made of the health hazard associated with the viral pollution and appropriate recommendations made concerning water treatment.

<sup>1</sup> Head, Research Centre for Water Virology, Research Institute for Hygiene and Microbiology, Berlin, German Democratic Republic. Requests for reprints should be addressed to Forschungsstelle für Wasservirologie, Forschungsinstitut für Hygiene und Mikrobiologie, Wiltbergstrasse 50, 1115 Berlin, German Democratic Republic.

<sup>2</sup> Director, Research Institute for Hygiene and Microbiology, Bad Elster, German Democratic Republic.

<sup>3</sup> Scientist, Department of Virology, District Sanitary Inspectorate and Institute, Rostock, Institute of Hygiene, Greifswald, German Democratic Republic.

<sup>4</sup> Scientist, Research Centre for Water Virology, Research Institute for Hygiene and Microbiology, Berlin, German Democratic Republic.

<sup>5</sup> Scientist, Virology Department, District Sanitary Inspectorate and Institute, Potsdam, German Democratic Republic.

<sup>6</sup> Head, Department of Virology, District Sanitary Inspectorate and Institute, Rostock, Institute of Hygiene, Greifswald, German Democratic Republic.

<sup>7</sup> Head, Department of Virology, District Sanitary Inspectorate and Institute, Suhl, German Democratic Republic.

<sup>8</sup> Scientist, Research Centre for Water Virology, Research Institute for Hygiene and Microbiology, Berlin, German Democratic Republic.

<sup>9</sup> Head, Virology Department, District Sanitary Inspectorate and Institute, Potsdam, German Democratic Republic.

## MATERIALS AND METHODS

### Sampling points

The 30 sampling points were chosen for their hygienic importance, and were situated in recre-

ational waters, drinking-water sources, or sewage contaminated water. The water samples were analysed in one of four virological laboratories in different regions of the German Democratic Republic.

Region I was a congested area with numerous flowing, back-dammed, and stagnant waters. It contained 14 sampling points in rivers, lakes, and canals.

Region II was an agricultural area, with 7 sampling points in rivers and canals.

Region III was on the Baltic coast, and had 8 sampling points in a shallow bay containing brackish water.

Region IV was situated in the south of the country, and consisted mainly of woodland and some agricultural land. There were 2 sampling points which were very similar and which are treated as one point in the analysis in order to obtain a sufficient number of samples for analysis.

The investigation was carried out between 1 January 1970 and 31 December 1979. During this time, only two lakes were investigated continuously; the other points were sampled at irregular intervals.

#### *Virus isolation*

The four virological laboratories used the virus concentration method developed at the Research Centre for Water Virology in Berlin. The water sample (usually 10 litres, but only 5 litres in 1970-71) was taken immediately to the virological laboratory, taking appropriate precautions to avoid any contamination. The water temperature was adjusted to 18-22 °C, and the sample was mixed, with vigorous stirring, with a solution of aluminium sulfate (100 g/litre) to give a ratio of 200 mg of  $\text{Al}_2(\text{SO}_4)_3 \cdot 18 \text{H}_2\text{O}$  per litre of water; the pH was adjusted to about 5.5 with either 0.1 mol/litre NaOH or 0.1 mol/litre HCl. The mixture was left for 2 h at room temperature or overnight at 4 °C, then the clear supernatant was discarded and the settled aluminium hydroxide precipitate was separated out by centrifugation at 3000 g for 20 min. The  $\text{Al}(\text{OH})_3$  pellet was suspended in Hanks' solution to a volume of 30 ml (for a 10 litre sample), and 0.03 g of streptomycin, 30 000 IU of penicillin, and 30 000 IU of nystatin were added. Then, 6 ml of ether were added, and the mixture was shaken for 2 h. The ether was then evaporated (either under normal pressure in a refrigerator overnight or in a vacuum desiccator at room temperature for 1 h) and various dilutions of the suspension were inoculated directly onto monolayers of F1 cells (maintenance medium, lactalbumin hydrolysate) in tubes, according to the most probable number (MPN) method of Chang et al. (3). In the laboratory in region II, a slightly different inoculation method was used from 1976 onwards. There, 2.5-ml aliquots of the  $\text{Al}(\text{OH})_3$  pellet suspension were inoculated onto

monolayers of F1 cells in 5 200-ml flasks (maintenance medium, Eagle's MEM). Both tubes and flasks were incubated for 35-40 days, involving 5-6 passages. The efficiency of recovery of the aluminium sulfate flocculation method ranged from 75% to 95% (15).

#### *Protection against cross-contamination*

In order to ensure the reliability of the results, all tests were carried out either in a special department for environmental virology, e.g., the Research Centre for Water Virology (68% of tests), or in special water laboratories separated from the clinical laboratories.

#### *Analysis*

*Virus isolation rate.* This was defined as the percentage of the samples examined that were virus-positive, and was evaluated for each of the sampling points.

*Concentration of viruses.* In estimating the MPN, the total incubation period was taken into account independently of the day of appearance of cytopathic effects. Tube cultures were evaluated according to the original tables published by Chang et al. (3) and flask cultures by means of a somewhat simpler version derived by one of the present authors using the maximum likelihood method (17). (This technique was based on a single dilution with a multifold parallel design.) In calculating the means, only MPN values greater than zero were considered. In some years the virus content was determined only qualitatively.

*Spectrum of viruses.* The determination of virus strain was performed by means of neutralization tests with rabbit hyperimmune sera on F1 cells.

*Hygienic water quality.* In addition to the virological assays carried out in the regional laboratories, several other criteria of water quality were determined by local water laboratories. These investigations included such factors as coliform titre, total plate count, chemical oxygen consumption (COC), biochemical oxygen demand over 5 days ( $\text{BOD}_5$ ), and levels of nitrites, nitrates, and ammonium. Unfortunately, different techniques were used in the various laboratories, and only the ammonium and  $\text{BOD}_5$  levels (5) were comparable. The ammonium content was known for 18 sampling points (930 values), and  $\text{BOD}_5$  values were determined for 22 sampling points (903 values).

*Statistical tests.* The statistical calculations were done on a small computer, type C 8206 Z. The statistical tests used were Fisher's exact test (16), *F*-test (8), *t*-test (8), and the outlier test according to Thompson's rule (7).

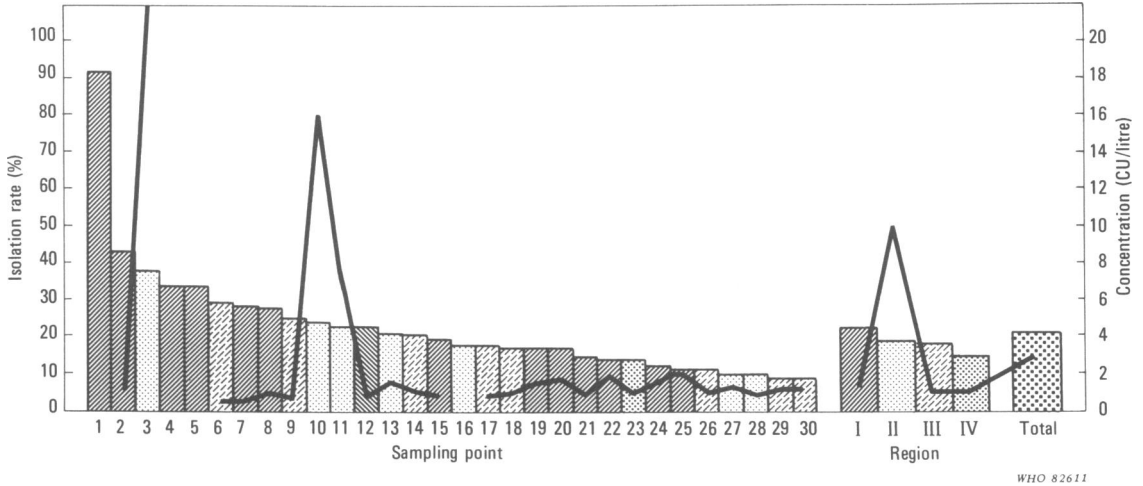


Fig. 1. Virus isolation rate (columns) and virus concentration (line) for the 30 sampling points, 1970–79.

## RESULTS

### *Virus isolation rate*

A total of 1908 samples were analysed. The sampling points were numbered from 1 to 30 in order of decreasing virus isolation rate (Fig. 1). The percentage of samples containing viruses varied from 92% to 8%, with a weighted mean of 20%. Sampling points 1, 2, and 3 (two heavily polluted rivers and a canal) had a significantly higher isolation rate than most of the other points ( $P < 0.05$ ). Sampling points 7, 8, 25, 27, and 28 also showed a significant difference from at least 6 other sampling points ( $P < 0.05$ ); the sample sizes of these points were considerably larger than those of 20 of the remaining 22 points, which had a statistically homogeneous distribution.

The overall virus isolation rate varied considerably during the 10-year investigation (Fig. 2). In 1975, 1976, and 1978, the isolation rates were significantly lower than in most of the other years ( $P < 0.05$ ). In 1973 and 1974, the isolation rates were significantly higher than in 1972, 1977, and 1979 ( $P < 0.05$ ).

Comparing the overall isolation rate with those for sampling points 8 and 20 (which were investigated continuously throughout the 10 years), it can be seen that they showed very similar variations (Fig. 3). These results confirm that the observed differences in the total virus isolation rate were not artefacts caused by changing the sampling points.

### *Virus concentration*

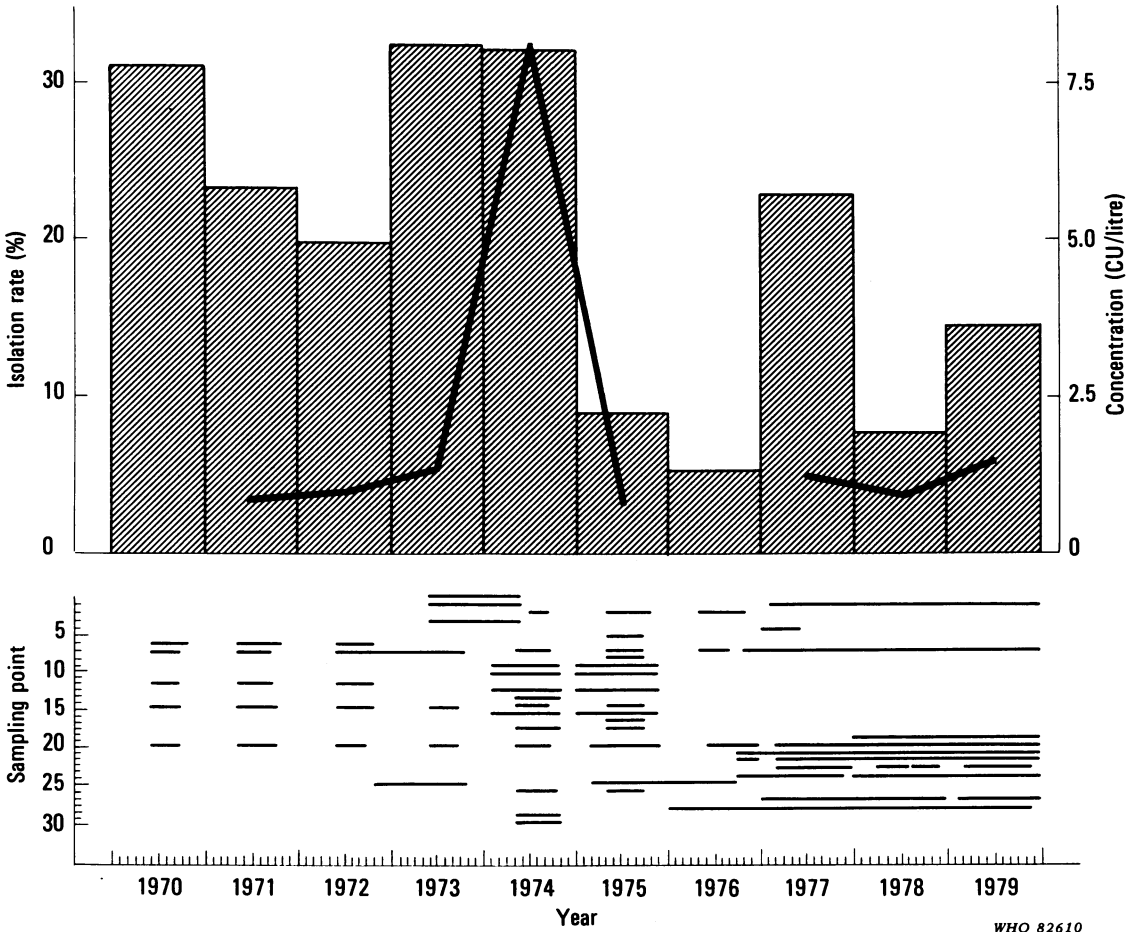
The arithmetic mean of the MPN values was calculated for each sampling point. As shown in Fig. 1

and 2, the virus concentration varied considerably; the highest level determined was 22.1 cytopathogenic units (CU) per litre of surface water, with a mean of 2.7 CU per litre. A direct comparison of the mean value for each sampling point was not possible because of the wide variance. However, the 95% confidence interval of the arithmetic mean for region II did not overlap those for the other regions. The mean virus concentration for all regions rose to 8.11 CU/litre in 1974 (Fig. 2). The 95% confidence interval of this mean value was outside those for all other years.

### *Spectrum of viruses*

The spectrum of viruses found over the years 1970–79 is presented in Fig. 4. Only the viruses isolated 4 or more times are shown on the chart. The changes in the spectrum of viruses over the ten years may be linked to the circulation of viruses in the population and the resistance of the various types in the environment. Some viruses, such as poliovirus type 1, 2, and 3, echovirus type 6, 11, and 30, and coxsackievirus B 3 and 5, were detected throughout most of the period. Echovirus 11, coxsackievirus B 3 and B 5, and echovirus 30 showed a gradual increase in incidence followed by a slow decrease. The dynamics were very different in the cases of coxsackievirus B 1, echovirus 7, and echovirus 24, where the first isolation was immediately followed by a widespread distribution and then complete disappearance.

Adenovirus 5 displayed an intermediate behaviour; it showed a rapid increase to reach a maximum in 1973, followed by a gradual decrease until it disappeared completely in 1979.



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Fig. 2. The upper graph shows the overall virus isolation rate (columns) and virus concentration (line) throughout the 10-year investigation. The lower graph shows the periods during which each point was sampled.

### Water quality

The values obtained for ammonium content and BOD<sub>5</sub> were divided into two groups according to whether the water sample had been virus-positive or virus-negative. Sample size, arithmetic mean, and 95% confidence limits of the mean were calculated for each group. The results of the statistical evaluations provided no evidence for a relationship between the presence of virus and the ammonium content or the BOD<sub>5</sub> (Table 1). However, over the period 1970–77, the BOD<sub>5</sub> showed a significant correlation with the presence of viruses ( $P < 0.05$ ). The existence of such a relationship has been suggested previously (14).

### DISCUSSION

The use of a standardized method permitted comparable, reproducible analysis of water samples over a long period. Disregarding the extreme values, the virus isolation rate ranged from 10% to 30%, indicating that, in general, all the water sources were contaminated by viruses pathogenic to man.

Similar virus isolation rates were found by Prima-vesi (11) in the river Ruhr and by Nestor (9) in the river Somes, whereas a higher level of contamination was found in the river Moskva (68%) and in the river Volga (37%) (1, 10).

Nearly all surface waters of the German Demo-

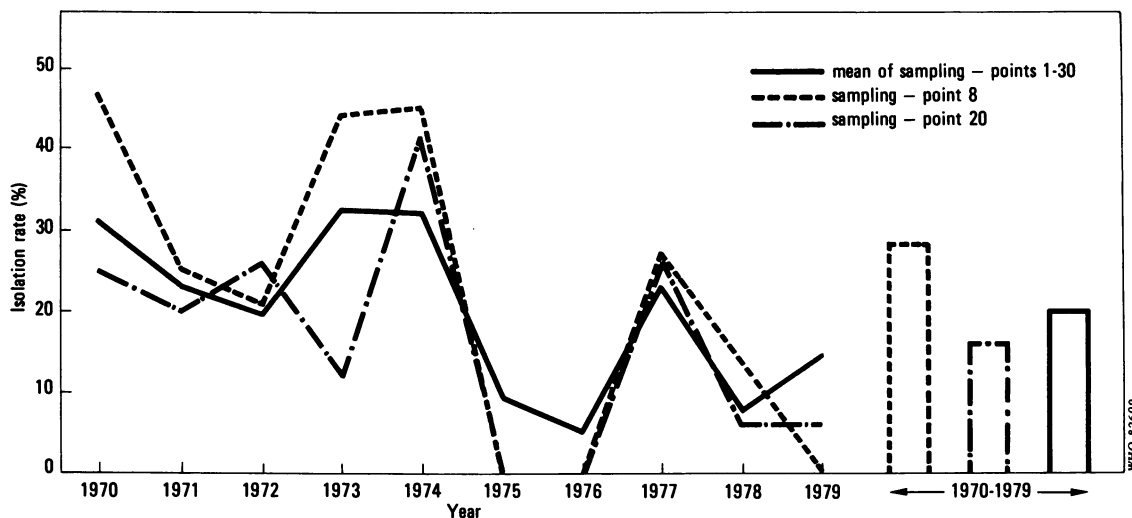


Fig. 3. Comparison of virus isolation rate for sampling points 8 and 20 with the overall rate, 1970-79.

cratic Republic are used by man, but the mean virus concentration of 2.7 CU per litre of water is considerably less than those of 35 CU/litre in the river Houston, reported by Grinstein et al. (4), and of 100 CU/litre in the river Thames, reported by Slade (12). However, at one sampling point, we found a mean concentration of 22 CU/litre.

It is well known that many viruses survive present sewage treatment methods and may persist in surface water for several months. All potable water supplies derived from surface water should be specially treated to guarantee virus-free drinking-water. A high degree of purification can be achieved by slow sand filtration or by flocculation in combination with rapid filtration (13).

Water used for recreation was found to be virus-positive in 20-30% of cases, and may also constitute a health hazard. A high incidence of viral diseases has been associated with swimming and wading, and it has been observed that people who swim in polluted

seawater suffer significantly more often from gastrointestinal illness than people who swim in clean water, or non-swimmers (2).

The types of virus found in water and their variation with time reflected virus excretion by the population in the area and the ability of the viruses to survive under the local conditions. Thus, we isolated some of the more resistant viruses of the coxsackie B group (6) throughout the whole 10-year investigation.

The virus isolation rate showed a rhythm over the years, whereby years with a relatively high isolation rate were followed by years with a low level of viruses. This variation was not caused by changes in the sampling points; it was observed by all 4 laboratories independently and corresponded with the results seen in the area of Moscow by the Institute of Poliomyelitis and Viral Encephalitis of the Academy of Medical Sciences of the USSR (V. A. Kasantseva, personal communication, 1979).

Table 1. Ammonium level and BOD<sub>5</sub> in water samples, according to presence or absence of viruses

Samples	Ammonium level		BOD <sub>5</sub>	
	No. of samples <sup>a</sup>	Mean value	No. of samples <sup>a</sup>	Mean value
Virus-positive	216 (207)	0.68	189 (183)	6.44
Virus-negative	733 (723)	0.75	753 (720)	5.92

<sup>a</sup> The figure in parentheses is the number of samples when outlying values are disregarded. The mean value is based on this figure.

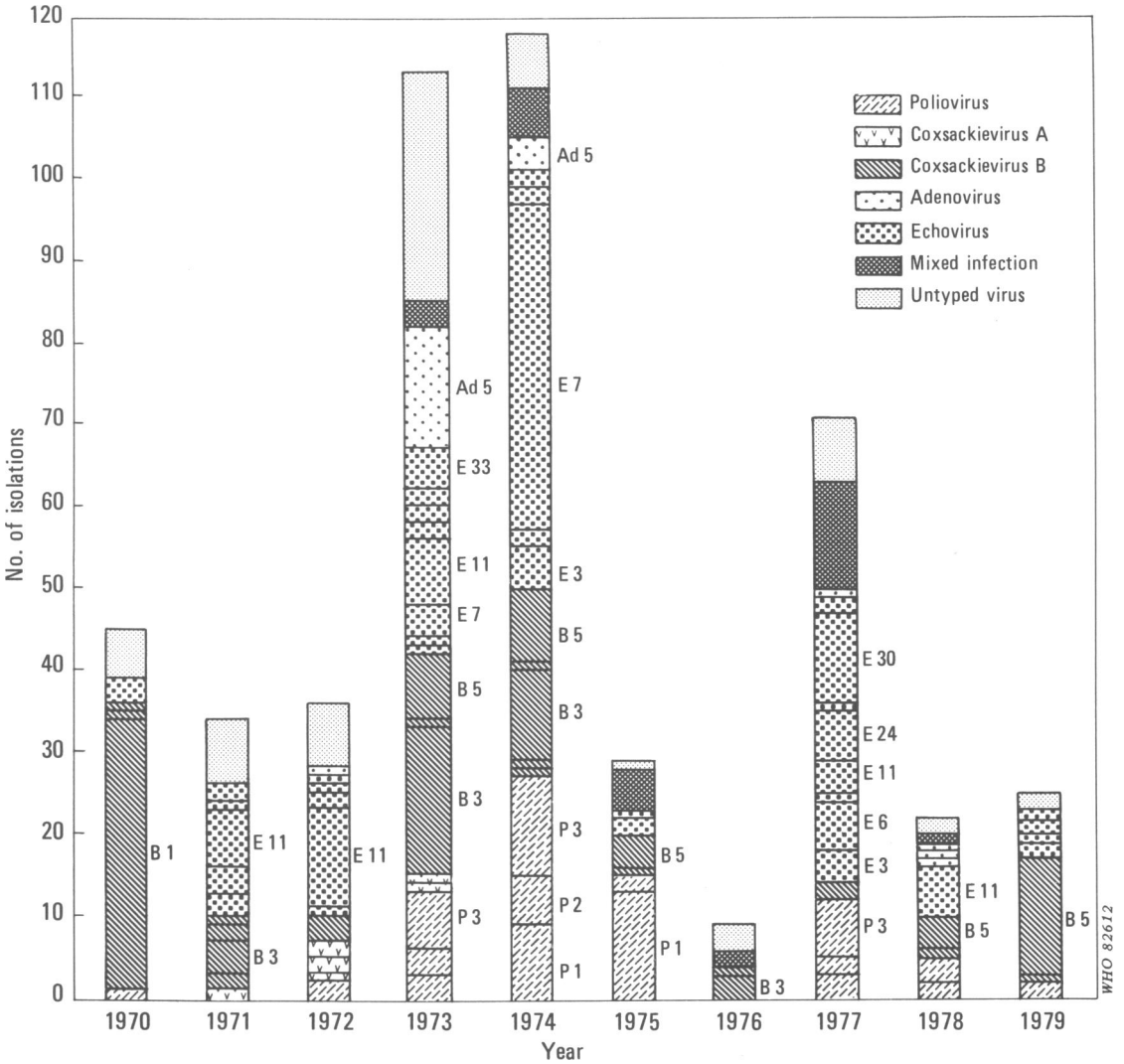


Fig. 4. Spectrum of viruses isolated from water samples, 1970-79.

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## RÉSUMÉ

ÉTUDE À LONG TERME SUR LA CONTAMINATION VIRALE DES EAUX  
DE SURFACE EN RÉPUBLIQUE DÉMOCRATIQUE ALLEMANDE

Au cours de la période allant du 1er janvier 1970 au 31 décembre 1979, quatre laboratoires virologiques de quatre régions différentes de la République démocratique allemande ont procédé à une étude sur la concentration des virus dans les eaux de surface. Tous ces laboratoires utilisaient les mêmes méthodes pour la détection des virus.

Au total, 1908 échantillons provenant de 30 points de prélèvement ont été évalués. Le taux d'isolement des virus allait de 8% à 92%, avec une moyenne de 20%. Il y avait des différences considérables dans les taux d'isolement selon les points d'échantillonnage et, pour chaque point particulier, le taux variait d'une année sur l'autre.

La valeur moyenne de la concentration de virus, déterminée par la technique du nombre le plus probable, était de 2,7

unités cytopathogènes (UC) par litre, alors que le maximum était de 22,1 UC/litre. Les virus observés pendant toute la durée des 10 ans d'étude comprenaient: des poliovirus des types 1, 2 et 3, des echovirus des types 6, 11 et 30 et des coxsackievirus B3 et B5; des echovirus des types 7 et 24, le coxsackievirus B1 et l'adenovirus 5 étaient rencontrés occasionnellement. Les résultats de cette étude témoignent du degré élevé d'utilisation des eaux de surface en République démocratique allemande.

Là où l'eau est destinée à l'usage humain, par exemple comme eau de boisson ou à des fins récréatives, des mesures de sécurité raisonnables, telles que traitement et désinfection appropriés de l'eau, doivent être prises pour garantir contre la contamination.

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