Assessment of cell culture and polymerase chain reaction procedures for the detection of polioviruses in wastewater

W.O.K. Grabow,[1] K.L. Botma,[2] J.C. de Villiers,[3] C.G. Clay,[4] & B. Erasmus[3]

WHO considers that environmental surveillance for wild-type polioviruses is potentially important for surveillance for acute flaccid paralysis as a means of confirming eradication of poliomyelitis. The present study investigated methods for detecting polioviruses in a variety of water environments in South Africa. Most polioviruses were isolated on L20B mouse cells, which, however, were not selective: 16 reoviruses and 8 enteroviruses, apparently animal strains, were also isolated on these cells. Vaccine strains of polioviruses were isolated from surface waters during and shortly after two rounds of mass vaccination of children in an informal settlement where there was no sewerage. The results demonstrated the feasibility of poliovirus surveillance in such settlements. It was also evident that neither poliovirus vaccine strains nor other viruses were likely to interfere significantly with the detection of wild-type polioviruses. Optimal isolation of polioviruses was accomplished by parallel inoculation of L20B mouse cells and at least the PLC/PRF/5 human liver and buffalo green monkey (BGM) kidney cell lines. Analysis of cell cultures using the polymerase chain reaction revealed that 319 test samples contained at least 263 human enteroviruses that failed to produce a cytopathogenic effect. This type of analysis thus significantly increased the sensitivity of enterovirus detection.

Keywords: environmental monitoring; evaluation studies, polioviruses, isolation and purification; polymerase chain reaction; water microbiology; water pollution analysis; sewage analysis.

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Introduction

Significant progress has been made in WHO's programme for the eradication of poliomyelitis by the year 2000 (*1*). However, poliovirus remains endemic in some areas, including parts of sub-Saharan Africa. For example, over 600 cases were reported in Angola during March and April 1999 (ProMED mail, 30 April 1999). The strategy for poliomyelitis eradication includes routine immunization, surveillance for acute flaccid paralysis, and mopping-up activities. The final stage, in which eradication is certified, requires surveillance of clinical cases of acute flaccid paralysis in order to obtain evidence of the absence of wild-type polioviruses. It is considered that environmental monitoring, mainly involving the screening of wastewater, can provide additional evidence of the circulation or absence of wild-type polioviruses (*1*). The ratio of subclinical infections to cases of acute flaccid paralysis may exceed 1000:1. Poliovirus excretors occur at a frequency of 1 per 2000 persons (*1, 2*). Furthermore, it has been observed in many parts of the world that the screening of wastewater is a more sensitive tool for the detection of wild-type polioviruses circulating in communities than is surveillance for cases of acute flaccid paralysis. Furthermore, wild-type polioviruses have frequently been isolated from wastewater in the absence of cases of acute flaccid paralysis (*1–7*). Environmental surveillance may not only confirm the elimination of wild-type polioviruses but may also prove useful for monitoring the success of vaccination and detecting the reintroduction of wild-type strains into communities previously considered free of poliomyelitis (*8, 9*).

Various techniques are being used for the environmental surveillance of polioviruses, but there is little information on their efficiency and reliability. WHO has therefore recommended that research be carried out to improve methods for the detection of polioviruses in the environment (*10*) and has established a working group on the formulation of standard procedures for the environmental surveillance of these viruses (*1*, *2*).

The present study deals with an evaluation and optimization of techniques for the detection of polioviruses in wastewater in South Africa. The L20B

[1] Professor and Head, Department of Medical Virology, University of Pretoria, PO Box 2034, Pretoria 0001, South Africa. Correspondence should be addressed to this author.

[2] Postgraduate Student, Department of Medical Virology, University of Pretoria, Pretoria, South Africa.

[3] Senior Research Assistant, Department of Medical Virology, University of Pretoria, Pretoria, South Africa.

[4] Senior Lecturer, Department of Medical Virology, University of Pretoria, Pretoria, South Africa.

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mouse cell line (*11–13*), the PLC/PRF/5 human liver cell line (PLC) (*14*), the buffalo green monkey kidney cell line (BGM), and primary vervet monkey kidney cells (PVK) (*15, 16*) were compared for the isolation of polioviruses. These cells were selected on the basis of evidence on their susceptibility to enteric viruses (*14–19*). L20B mouse cells carry the receptor site for polioviruses, and this makes them selectively susceptible to polioviruses in the presence of human enteric viruses (*11*). Techniques involving the polymerase chain reaction (PCR) were used to detect enteroviruses in water samples and suspensions of cell cultures with or without cytopathogenic effect (CPE). A glass wool adsorption–elution procedure was used to recover viruses from water samples (*20*). The study was carried out on environmental waters containing a variety of viruses at a range of concentrations. Samples were collected from wastewater in a community of low socioeconomic status before, during, and after two rounds of mass immunization of children with oral poliovirus vaccine (OPV), from wastewater in an abattoir, and from river and dam water polluted with domestic and animal waste.

Materials and methods

PLC (PLC/PRF/5 cell line, ATCC Code CRL 8024, passage 85–100), BGM (BGM cell line, BioWhittaker Catalogue No. 71-176B, passage 80-95), L20B (L20B cell line, passage 50–65, kindly supplied by Dr David J. Wood, National Institute for Biological Standards and Control, UK) and PVK cells were cultured by established procedures (*14, 19*). These essentially consist of cultivation in Eagle's minimum essential medium (MEM) with Earle's salt solution and antibiotics (penicillin and streptomycin) supplemented with appropriate concentrations of fetal calf serum for the cultivation or maintenance of cell cultures.

Test samples were collected at frequent intervals, generally once or twice a week between 15 January 1996 and 14 October 1997. One series of 80 samples was collected over 38 days between 15 July 1996 and 15 December 1996 in an informal settlement near Pretoria of approximately 15 000 people of low socioeconomic status. The samples were collected from small roadside streams or patches of shallow surface water formed by diffuse effluents including domestic waste discharges and seepage from pit latrines. Occasionally these sources were supplemented by surface run-off after showers of rain. The sanitary facilities in the settlement consisted predominantly of pit and bucket latrines. Samples were collected at six points at regular intervals from 14 days before to 88 days after two rounds of a mass campaign of immunization with OPV in which approximately 5800 children were immunized over two 5-day periods. A series of 79 samples was collected from the Klip river (sampling point K19) near Johannesburg on 79 days between 15 January 1996 and 4 August 1997. This river carries substantial loads of human and animal wastes. A similar number of samples was obtained from sluice-gate water (sampling point A18) of the Vaal dam, near Vereeniging, on the same days. The Vaal dam carried relatively low levels of faecal pollution. A total of 81 samples were collected from various points in a multiple-barrier system for the reclamation of water at the City Deep Abattoir (where predominantly cattle and sheep were slaughtered) in Johannesburg on 28 days between 20 February 1996 and 14 October 1997. The 10-litre samples were collected aseptically in sterile containers, transported at ambient temperature to the laboratory, and processed within 2 hours of collection. Viruses were recovered from 9 litres of these samples using a glass wool adsorption–elution procedure (Rantigny-725, Isover Saint-Gobain, Les Miroirs, France) with a recovery efficiency of 60–95%, depending primarily on the turbidity and the suspended solids in the samples (*20*); 100 ml of glycine/beef extract buffer (pH 9.5) was used to elute viruses from 10 g of glass wool in columns. This volume of extract buffer proved to be the optimum required for the efficient recovery of viruses. After neutralization using sodium hydroxide (1 mol/l) the eluates were decontaminated with chloroform (10% v/v for 30 min) and 1 ml was inoculated into each of three 25-ml flasks with confluent monolayers of PLC, BGM, L20B or PVK cells. Tenfold dilutions of decontaminated original water samples were also inoculated into 24-well multidish microtitration plates (Nunc, Roskilde, Denmark) with the same cell cultures for most-probable-number determination of viral titres. Cells were incubated in a humidified 5% carbon dioxide atmosphere at 37 °C, passaged twice at 7-day intervals and observed daily for CPE. A reverse transcription PCR involving a set of nested primers (*21*) was used to detect enterovirus-specific RNA in homogenates of the final passage of each of the three flasks for each of the four cell cultures inoculated with every sample. Since PCR technology of appropriate specificity and sensitivity was not in place initially, only the most recent of some sampling sets were tested for enteroviruses. Cross-contamination in PCR tests was eliminated by using accreditation-specified procedures, performed by separate laboratories in our department, for steps such as RNA extraction from test material, the preparation of reaction mixtures, PCR amplification, nested PCR procedures and gel electrophoresis, as well as by the enzymatic destruction of potential PCR contamination products using uracil DNA glycosylase (*21*).

Isolates of cytopathogenic viruses were typed by inoculating cell culture tubes with cover slips for staining and characterization of viruses according to CPE (*16*). Isolates were further typed by cell culture neutralization tests in which commercial standard Lim Benyesh-Melnick pools of antisera (Statens Seruminstitut, Copenhagen, Denmark) were used. Dr Claudia Chezzi (National Institute for Virology, Johannesburg, South Africa) used PCR techniques

(*22*) to confirm the serological typing of poliovirus isolates and distinguish between vaccine strains and wild strains.

Faecal coliform bacteria (M-FC agar) and enterococci (M-Enterococcus agar) were determined using standard membrane filtration techniques, and somatic and F-RNA coliphages were determined using standard double agar layer plaque assays (*20*).

Results

Vaccine strains of polioviruses were isolated only from environmental waters at the informal settlement (Table 1). The viruses were obtained from samples collected during the week of both rounds of immunization with OPV, up to 17 days after the first round and 44 days after the second. Wild-type polioviruses were never isolated from any samples. A total of 9 of the 20 polioviruses (45%) were isolated on L20B cells, 5 (25%) on BGM cells, 4 (20%) on PLC cells and 2 (10%) on PVK cells (Table 2). Of these isolates, 13 (65%) were Sabin vaccine type 3 strains, 4 (20%) were type 1 strains, and 3 (15%) were type 2 strains. Apart from the polioviruses, the water samples from the settlement yielded four coxsackie B viruses, one untypable enterovirus, and 45 reoviruses. Viruses were isolated on PLC, BGM, PVK and L20B cells from 20 (25%), 10 (13%), 28 (35%) and 12 (15%) of the 80 samples collected in the informal settlement respectively (Table 1). Although more polioviruses were isolated on L20B cells than on other cells, the L20B cells were not selective because 16 reoviruses and 8 untypable enteroviruses were also isolated on them. From the 319 samples analysed, cell culture isolation by CPE yielded a total of 58 enteroviruses (18%), of which 20 were polioviruses, 4 were coxsackie B viruses and 34 were untypable enteroviruses. All five polioviruses isolated on BGM cells were from samples that yielded negative results on L20B cells, as were three of the four polioviruses isolated on PLC cells and one of the two isolated on PVK cells. Six of the nine polioviruses isolated on L20B cells were from samples that yielded negative results for the other three cell types.

From the same 319 samples, 150 (47%) reoviruses were isolated, representing the great majority of isolates from all sampling sites except water from the Vaal dam (Table 1). The CPE of reoviruses typically appeared later than that of other viruses on all cell cultures. Reoviruses were predominantly isolated on PVK cells, which yielded 71 isolates from the 319 samples. The 81 samples of abattoir wastewater yielded 27 isolates of untypable enteroviruses (33%), the highest frequency of these viruses in the water environments investigated. The CPE of untypable enteroviruses isolated from abattoir wastewater was not typical of that of human enteroviruses. Only four coxsackie B viruses were isolated, all from water in the informal settlement. The only isolate of an adenovirus (type undetermined, but not type 40 or 41) was obtained from the river water.

The highest incidence of cytopathogenic viruses was in settlement water, where 70 isolates were obtained from 80 samples (88%) (Table 1). Abattoir wastewater, river water and dam water yielded 70 isolates from 81 samples (86%), 66 from

Table 1. **Detection of enteric viruses by cytopathogenic effect (CPE) and enteroviruses by polymerase chain reaction (PCR) in four cell cultures inoculated with concentrates of various environmental waters**

Samples and viruses	Viruses isolated and enteroviruses detected on each cell culture			
	PLC/PRF/5	BGM	PVK	L20B
Settlement				
CPE/samples[a]	20/80 (25%)	10/80 (13%)	28/80 (35%)	12/80 (15%)
Poliovirus Sabin 1	1 (1%)	1 (1%)	0 (0%)	2 (3%)
Poliovirus Sabin 2	1 (1%)	1 (1%)	0 (0%)	1 (1%)
Poliovirus Sabin 3	2 (3%)	3 (4%)	2 (3%)	6 (8%)
Coxsackievirus B	3 (4%)	0 (0%)	1 (1%)	0 (0%)
Enterovirus untypable	0 (0%)	1 (1%)	0 (0%)	0 (0%)
Reovirus	13 (16%)	4 (5%)	25 (31%)	3 (4%)
PCR enterovirus/samples[b]	35/80 (44%)	37/80 (46%)	42/80 (53%)	44/80 (55%)
River				
CPE/samples[a]	21/79 (27%)	7/79 (9%)	31/79 (39%)	7/79 (9%)
Enterovirus untypable	2 (3%)	1 (1%)	0	0
Adenovirus	1 (1%)	0	0	0
Reovirus	18 (23%)	31 (39%)	7 (9%)	7 (9%)
PCR enterovirus/samples[b]	16/60 (27%)	16/60 (27%)	13/60 (22%)	12/60 (20%)
Dam				
CPE/samples[a]	2/79 (3%)	0/79	0/79	1/79 (1%)
Enterovirus untypable	2 (3%)	0	0	1 (1%)
PCR enterovirus/samples[b]	10/60 (17%)	9/60 (15%)	10/60 (17%)	8/60 (13%
Abattoir				
CPE/samples[a]	14/81 (17%)	22/81 (27%)	21/81 (26%)	13/81 (16%)
Enterovirus untypable	4 (5%)	10 (12%)	6 (7%)	7 (9%)
Reovirus	10 (12%)	12 (15%)	15 (19%)	6 (7%)
PCR enterovirus/samples[b]	8/54 (15%)	13/54 (24%)	9/54 (17%)	3/54 (6%)

[a] No. and percentage of total number of samples from which viruses were isolated by CPE.

[b] No. and percentage of samples in [a] yielding positive PCR results for enteroviruses in tests on cell cultures.

Table 2. **Vaccine strains of polioviruses isolated on four cell cultures from environmental water samples during and after two rounds of mass vaccination of children**

Poliovirus strains	No. of strains isolated on each cell culture				Total isolates
	PLC/PRF/5	BGM	PVK	L20B	
Type 1	1	1	0	2	4 (20)[a]
Type 2	1	1	0	1	3 (15)
Type 3	2	3	2	6	13 (65)
Total	**4 (20)**	**5 (25)**	**2 (10)**	**9 (45)**	**20**

[a] Figures in parentheses are percentages.

Table 3. **Counts of faecal indicator organisms in environmental waters analysed for enteric viruses**

Indicator	Range of counts (per 100 ml)			
	Settlement	River	Dam	Abattoir
Faecal coliforms	670–18 000 000	70–42 000	9–93	0–90 000 000
Enterococci	130–2 100 000	50–1110	30–178	0–280 000
Somatic phages	0–242 000	98–16 400	1–640	0–240 000
F-RNA phages	0–380 000	1–5300	1–10	0–68 000

Table 4. **Detection of enteroviruses in four cell cultures by cytopathogenic effect (CPE) and polymerase chain reaction (PCR)**

Result/ samples	No. for each cell culture			
	PLC/PRF/5	BGM	PVK	L20B
CPE positive/PCR negative (untypable enteroviruses)				
Settlement	1	2	2	0
River	1	0	0	0
Dam	0	0	0	0
Abattoir	2	5	5	5
CPE negative/PCR positive for enteroviruses				
Settlement	31	33	41	39
River	15	16	13	12
Dam	10	9	10	8
Abattoir	6	10	7	3

79 samples (84%), and three from 79 samples (4%) respectively. However, the populations of viruses isolated from the different sources and the numbers of viruses isolated on cell cultures varied substantially. The highest rate of isolation on cell cultures was on PVK cells, which yielded 31 isolates, all of reoviruses, from 79 samples (39%) of river water (Table 1).

Most-probable-number calculations of multidish plate titrations on water samples collected at the settlement yielded highest counts of 8900 cytopathogenic viruses per litre on PLC cells and 6900 per litre on L20B cells. The counts of indicator organism reveal a wide range in levels of faecal pollution for the waters analysed: the highest levels occurred in water at the settlement and in the abattoir; moderate levels occurred in the river water; and low levels were found in the dam water (Table 3). There was no meaningful correlation between levels of faecal pollution and those of viruses.

PCR tests on 12 cell culture homogenates (three for each of four cell cultures, including those with CPE) from each sample indicated the presence of enteroviruses in 64 of the 80 samples of settlement water analysed (80%), 21 of the 60 river water samples (35%), 14 of the 60 samples of dam water (23%), and 20 of the 54 abattoir wastewater samples (37%) (Table 1). Positive PCR results for enteroviruses were obtained with 263 cell cultures without CPE (Table 4). These results indicated the presence of 343 enteroviruses in the 319 samples analysed. PCR tests yielded negative results for cell culture preparations with CPE by enteroviruses isolated from the abattoir wastewater which were not typable by neutralizing sera for human enteroviruses (Table 4).

Discussion

The L20B mouse cell line proved the most suitable for the isolation of polioviruses from water environments, yielding 45% of the 20 poliovirus isolates, whereas the BGM monkey kidney cell line, the PLC human liver cell line and the PVK cells yielded 25%, 20% and 10%, respectively (Table 1). Technically, the L20B cell cultures proved as suitable as the other cell cultures for the cultivation of viruses from the waters examined and were therefore suitable for the isolation of polioviruses from wastewater and environmental water. This finding is in agreement with those of two previous studies (*11, 12*) but not with that of Sellwood et al. (*13*), who reported that L20B cell cultures were too sensitive to the toxic elements of concentrated sewage to be of practical use. However, in contrast to suggestions made by Pipkin et al. (*11*) and Hovi & Stenvik (*12*), L20B cells were not selective for polioviruses: 16 reoviruses and 8 enteroviruses not typable by neutralizing sera for human enteroviruses were also isolated from various water environments by CPE on these cells (Table 1). Hovi & Stenvik (*12*) found that reovirus type 2 showed atypical CPE at 14 days and grew when subcultured. The enteroviruses not typable by neutralizing sera for human enteroviruses were predominantly isolated from abattoir wastewater, suggesting that they had an animal origin. This seems to be supported by the CPE of these viruses, which was distinguishable from that of human enteroviruses. L20B cells may therefore be susceptible to animal enteroviruses that are not typable by neutralizing sera for human enteroviruses. Similarly, the reoviruses isolated on L20B cells may be of animal origin. These findings are not surprising, since mouse cells can be expected to be susceptible to animal viruses. This suggests that L20B cells are suitable for the selective isolation of polioviruses from human excreta but that other viruses may also be isolated from environmental waters containing animal excreta.

Differences in the number of polioviruses isolated on various cell cultures may be due to differences in the susceptibility of the cell cultures used. Previous studies have shown that the cell cultures were superior to primary human embryonic fibroblasts, the MA-104 and FRhK-4R fetal rhesus kidney cell lines, and the Caco-2 human colon carcinoma cell line for the isolation of enteric viruses from water environments (*17*). In the present study, PVK cells yielded slightly lower numbers of polioviruses and other enteroviruses than the three cell lines (see Table 1 and 2). These findings indicate that polioviruses are best isolated from wastewater

and environmental waters by using a combination of cell cultures, including at least L20B, BGM and PLC cells. Combinations of cell cultures have previously been used for the isolation of polioviruses from wastewater (*23, 24*). There appear to be no grounds for suspecting that the susceptibility of these cells to wild-type polioviruses differs significantly from that of the vaccine strains studied here. The higher incidence of type 3 among the 20 isolates of vaccine polioviruses (Table 2) accords with earlier findings. Pöyry et al. (*23*) found that type 1 vaccine strains disappeared rapidly from sewage, while type 2 and type 3 were detectable up to 3 months after vaccination campaigns.

There is no indication that the cell culture isolation of vaccine strains of polioviruses was restricted by interference between the viruses themselves, or that these viruses interfered with the isolation of wild-type polioviruses if any were present. In cultures of all samples, including those collected during mass vaccination in the settlement, there was a substantial number of flasks without CPE. These flasks offered an opportunity for infection by poliovirus vaccine or wild-type strains that may have been present in detectable numbers without interference by other viruses (Table 1). For the 24-well multidish plate cultures there was an even greater opportunity for viruses to infect cells without interference. The large numbers of reoviruses present in many samples did not seem to interfere with the isolation of enteroviruses, since their CPE only appeared a few days later than that of enteroviruses. Some reoviruses were probably missed in cell cultures destroyed by enteroviruses before reovirus CPE was detectable. The incidence of cytopathogenic reoviruses reported here is, therefore, an underestimate (*3*). These findings are in agreement with the cell culture isolation of enteric viruses from samples of water collected elsewhere in South Africa (*20*), as well as the isolation of enteric viruses (including wild-type polioviruses and vaccine strains) from wastewaters in other parts of the world (*2–7, 24*). Consequently, concerns about failure to isolate wild-type polioviruses from water environments because of interference by vaccine strains of polioviruses and other enteroviruses do not seem to be justified. The results of this and previous studies indicate that the only time at which vaccine strains could interfere with the cell culture isolation of wild-type polioviruses would be during and shortly after mass administration of OPV — when the circulation of wild-type polioviruses would be least expected. The ability of the cell culture system used here to detect a variety of enteric viruses meets recommendations that methods for environmental surveillance should also be able to detect enteroviruses other than polioviruses (*25*).

The failure to detect poliovirus vaccine strains except during mass vaccination in the settlement (Table 1) may be attributable to the low levels of faecal pollution in many of the samples (Table 3), which were deliberately selected to assess the sensitivity of cell cultures for the isolation of polioviruses. This is supported by the most-probable-number estimates indicating a maximum of 6900–8900 cytopathogenic viruses per litre in settlement water, substantially below the counts of up to 100 000 per litre reported for sewage (*26*). The presence of some vaccine polioviruses would be expected in view of the recommendation for immunization with OPV at birth and at the ages of 6 weeks, 10 weeks, and 14 weeks, as well as 18 months and 5 years. In recent studies on wastewater with higher levels of faecal pollution in areas nearby (*16*), and in other parts of the world (*3, 12, 23, 24*), larger numbers of poliovirus vaccine strains have been reported. The relative incidence of coxsackie viruses recorded here seems lower than previously reported for wastewater (*3, 12, 16, 26*), possibly because of seasonal and epidemiological variations (*3, 23, 27*). There is, however, no evidence that other enteroviruses interfere to any meaningful extent with the isolation of wild-type polioviruses or vaccine polioviruses. If reasons arose for concern about such interference, a number of strategies could be employed for the selective recovery and detection of wild-type polioviruses and vaccine polioviruses. These could include the selective recovery of polioviruses using appropriate antibodies in antigen-capture or affinity chromatography procedures (*18*), the inclusion of neutralizing antibodies directed against interfering viruses (*14*), or incubation at elevated temperatures to select for wild-type polioviruses (*3*).

The glass wool adsorption–elution technique for the recovery of viruses from water appeared successful, as in earlier studies (*20*). The recovery of polioviruses from environmental waters in the settlement during both weeks of mass vaccination of children would seem to be in agreement with earlier findings about the sensitivity of the method. These findings also indicate that polioviruses released by children in an informal settlement without a conventional sewerage system are rapidly detectable in related water environments. In a community with a conventional sewerage system the detection of polioviruses will probably be more sensitive since excreta are mixed and pooled more efficiently and the viruses survive better in the protected sewerage environment than in the shallow patches of open water sampled in this study (*1*). This conclusion is in agreement with previous findings about the sensitivity of wastewater monitoring for polioviruses circulating in communities (*1–7*). The detection of polioviruses in water samples during each 2-week period of mass vaccination of children with OPV illustrates the rapid spread of polioviruses in the environment and suggests that wild polioviruses may spread just as rapidly. This study proves that environmental monitoring for polioviruses is feasible in informal settlements without conventional sewerage systems. Such communities are at highest risk for the spread of wild-type polioviruses and are particularly likely to experience shortcomings

in immunization. Furthermore, surveillance for acute flaccid paralysis is most difficult in these communities.

The absence of a meaningful correlation between indicator organisms and the incidence of viruses in the water environments confirms earlier data on the shortcomings of faecal bacteria and phages as indicators of the virological quality of water (*20*).

The substantial number of samples for which cell cultures had no CPE but positive PCR results for enteroviruses (Tables 1 and 4) accords with previous findings that cytopathogenic viruses represent a minor component of the enteroviruses present in water environments (*28, 29*). Since the PCR tests were carried out on second-passage cell culture preparations, the positive results represent enteroviruses that are at least potentially viable. These may include viruses which, because of damage in the environment or other shortcomings, fail to produce a CPE in the cell cultures used (*29, 30*). Amplification of viral nucleic acid in cell cultures without CPE is well documented for enteric viruses (*28, 31, 32*). Negative results with the PCR for enteroviruses not typable by neutralizing sera for human enteroviruses (Table 4) seem to confirm earlier conclusions that these may be animal viruses and that they are not detectable by PCR primers designed for human enteroviruses. Cell culture amplification in combination with detection by PCR has proved more sensitive for the detection of enteric viruses in water environments than either method alone (*28, 29, 31, 32*), and the results reported here show that this is also the approach of choice for environmental surveillance of polioviruses. ■

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Résumé

Evaluation de la culture cellulaire et de la PCR pour la recherche des poliovirus dans les eaux usées

La surveillance du milieu est un outil sensible de détection des poliovirus sauvages en circulation dans les communautés. Elle peut également servir à surveiller l'efficience des campagnes de vaccination et à déceler la réintroduction des poliovirus sauvages dans des groupes de populations considérés comme indemnes de poliomyélite. Diverses techniques sont utilisées sans que l'on connaisse bien leur sensibilité et leur fiabilité. L'optimisation de ces techniques est essentielle, la poliomyélite restant une maladie endémique dans certaines parties du monde. Pour l'OMS, la surveillance du milieu est un outil potentiellement important de la surveillance de la paralysie flasque aiguë dans la mesure où elle permet de confirmer l'éradication de la poliomyélite, et l'Organisation a désigné un groupe de travail chargé d'examiner les méthodes applicables. La présente étude portait sur la recherche des poliovirus dans divers milieux hydriques en Afrique du Sud. Les travaux antérieurs indiquaient que plusieurs lignées cellulaires, L20B de souris, BGM de rein de singe et PLC/PRF/5 de foie humain, ainsi que les cellules primaires de rein de vervet, sont les types cellulaires les mieux adaptés à l'isolement des poliovirus. Nous avons donc comparé l'aptitude de ces lignées à mettre en évidence les poliovirus et d'autres virus entériques dans divers milieux hydriques, et notamment les eaux usées de surface dans un établissement humain sauvage, avant, pendant et après une campagne de vaccination de masse des enfants contre la poliomyélite, les eaux usées d'un abattoir, une rivière polluée et les eaux de retenue d'un barrage où la pollution était limitée. La plupart des poliovirus ont été isolés avec la lignée murine L20B ; ces cellules n'étaient toutefois pas sélectives : 16 réovirus et 8 entérovirus, des souches animales apparemment, ont également été isolés. Les souches vaccinales de poliovirus ont été isolées dans les eaux de surface de l'établissement humain sauvage, au cours et peu après deux séries de vaccination de masse des enfants. Ces résultats montrent que la surveillance du poliovirus est possible dans ce type d'établissement dépourvu de systèmes d'égouts. On est ainsi parvenu à isoler les virus à partir des échantillons d'eau grâce à une méthode par absorption-élution peu coûteuse et facile à utiliser, utilisant des filtres en laine de verre spéciaux. Il semble que ni les souches vaccinales de poliovirus ni les autres virus entériques ne perturbent la recherche des poliovirus de type sauvage. L'isolement des poliovirus est optimal si l'on inocule en parallèle trois cultures cellulaires, à savoir la lignée L20B murine et aussi les lignées PLC/PRF/5 de foie humain et BGM de rein de singe. C'est avec les cellules primaires du rein de vervet que l'on obtient les meilleurs résultats pour l'isolement des réovirus, mais non pour des entérovirus. La PCR (amplification génique) réalisée sur les cultures cellulaires a mis en évidence la présence d'au moins 263 entérovirus non cytopathogènes parmi les 319 échantillons analysés, prouvant que ces tests sont indispensables pour la recherche des entérovirus.

Resumen

Evaluación de los procedimientos basados en cultivos celulares y en la reacción en cadena de la polimerasa para la detección de poliovirus en las aguas residuales

La vigilancia del medio ambiente es un instrumento sensible para detectar los poliovirus salvajes que circulan en las comunidades. También puede servir para controlar la eficiencia de las campañas de inmunización y detectar la reintroducción de poliovirus salvajes en las comunidades que se consideran exentas de poliomielitis. Son diversas las técnicas utilizadas, pero se dispone de pocos datos en lo que respecta a su sensibilidad y fiabilidad. La optimización de esas técnicas es fundamental, ya que la poliomielitis sigue siendo endémica en algunas partes del mundo. La OMS considera que la vigilancia del medio ambiente puede ser una ayuda importante en la vigilancia de la parálisis fláccida aguda como medio para confirmar la erradicación de la poliomielitis, y ha establecido un grupo de trabajo para investigar los procedimientos correspondientes. En el presente estudio se analizan varios métodos de detección del poliovirus en diversos entornos acuáticos en Sudáfrica. En trabajos anteriores se había observado que las líneas de células de ratón L20B, de células de riñón de mono BGM y de células de hígado humano PLC/PRF/5, así como las células primarias de riñón de mono vervet, eran los tipos celulares más idóneos para aislar los poliovirus. Por consiguiente, comparamos la capacidad de esas células para detectar poliovirus y otros virus entéricos en diversos entornos acuáticos, en particular en las aguas residuales superficiales de un asentamiento marginal antes, durante y después de una campaña de vacunación infantil masiva; en las aguas residuales de un matadero; en las aguas de un río contaminado; y en las de un embalse relativamente poco contaminado. La mayoría de los virus se aislaron en las células de ratón L20B, pero estas células no fueron selectivas: se aislaron también 16 reovirus y 8 enterovirus, al parecer cepas animales. Se aislaron cepas vacunales de poliovirus en las aguas superficiales del asentamiento marginal durante las dos tandas de la campaña de vacunación infantil y poco después. Los resultados muestran que es posible la vigilancia de los poliovirus en los asentamientos marginales que no disponen de un sistema de alcantarillado. Se recogieron sin dificultad los virus en las muestras de agua por un procedimiento cómodo y poco costoso de adsorción-elución en el que se utilizaron filtros especiales de lana de vidrio. No es probable que las cepas vacunales de poliovirus u otros virus entéricos entorpezcan la detección de los poliovirus salvajes. Se logró un aislamiento óptimo de los poliovirus inoculando en paralelo células de ratón L20B y por lo menos también la línea de células de hígado humano PLC/PRF/5 y la línea de células de riñón de mono BGM. Las células primarias de riñón de mono vervet resultaron ser las más eficientes para aislar los retrovirus, pero no así para los enterovirus. Las pruebas basadas en la reacción en cadena de la polimerasa en cultivos celulares revelaron la presencia de al menos 263 enterovirus no citopatógenos en las 319 muestras analizadas, lo cual demuestra que esas pruebas son fundamentales para la detección de ese tipo de enterovirus.

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