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# Detection of Enteroviruses in Water Samples from Yopougon, Côte d'Ivoire by Cell Culture and Polymerase Chain Reaction

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**Abstract** The objective of this study was to compare sensitivities of enterovirus isolation from wastewater in different cell lines as well as to compare the sensitivity and specificity of isolation in cell culture with direct detection by reverse transcription polymerase chain reaction (RT-PCR). Sixty-eight samples of wastewaters were collected between September 2008 and January 2009 in Yopougon, Abidjan. Enteroviruses were concentrated according to World Health Organization recommendations. Viruses were inoculated into various cell lines while direct RT-PCR was performed on water concentrates. The buffalo green monkey kidney cell line was the most sensitive with 58.8 % of viral isolation. This was followed by the rhabdomyosarcoma cell line with sensitivity of 51.6 %, with human epidermoid carcinoma cell line showing sensitivity of 50 % and fibroblastic cells derived from transgenic mice LTK-1 (L20B) cell showing 23.50 % sensitivity. However, a lower specificity of 2.9 % was observed with the L20B cell line. 44.1 % of the samples were positive by direct RT-PCR detection while 51.47 % samples were positive by using RT-PCR on infected cell cultures. No difference in percentage positivity was observed using RT-PCR on infected tissue culture isolates or using RT-PCR directly on wastewater samples.

## Introduction

Enteroviruses are the cause of many diseases of great public health importance and varying severity. These viruses, excreted in the feces of infected persons, can be found in wastewater (Lee et al. 2004). Since the 1988 launch of the global polio eradication initiative through vaccination, significant progress has been recorded in the reduction of acute flaccid paralysis caused by poliovirus (PV), a member of the enteroviruses. The incidence of poliomyelitis has been reduced by over 99 % between 1988 and 2008 (WHO 2010).

The Pasteur Institute is part of a worldwide network of laboratories involved in the surveillance of PV under the auspices of the World Health Organization. Twelve institutes of the International Network of Pasteur Institutes are actively involved in the PV surveillance and enterovirus research. In Côte d'Ivoire, surveillance and detection of wild (W) PV in children under 15 years of age with acute flaccid paralysis (AFP) is carried out in the inter-country WHO polio laboratory of the Pasteur Institute. In 2008–2009, Côte d'Ivoire was affected by a WPV1 outbreak affecting West Africa (and which was recently stopped) (CDC 2008). Also in 2011, WPV3 caused another outbreak in low Sassandra in the southwest of Côte

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d'Ivoire. The detection of these recent cases of WPV1 and WPV3 between 2008 and 2011 highlights the risks of inadequate monitoring to the effort undertaken to eradicate polio worldwide (WHO 2010; CDC 2012). The environmental monitoring of enteroviruses can serve as an early warning system and is of an increasing importance in the polio eradication initiative. Suitable methods for the study of viral contaminants from a sample of wastewater are based either on the detection of infectious virus in cell culture or detection of viral genome by RT-PCR. Comparing the sensitivity of both methods, RT-PCR was reported to be 10–1,000 times more sensitive (Rajtar et al. 2008). In fact an infectious virus detected by cell culture has a complete genome. Conversely, detection by RT-PCR is feasible even if the genome is incomplete or if the capsid around the genome is damaged (Afssa 2005). WHO has, therefore, recommended that research be carried out to improve methods for the detection of PV in environment (Grabow et al. 1999). Moreover, the selection of a sensitive cell line for the determination of infectious viruses in water is important because it decreases the cost and effort required by avoiding the use of multiple cell lines.

This study was undertaken to compare the ability of various cell lines to detect PV and non-poliovirus enterovirus (NPEV) circulating in the population at Yopougon using direct RT-PCR detection and virus isolation as the assessment tool.

## Materials and Methods

### Area of Study

The study area extended from north to south of Yopougon in Abidjan-Côte d'Ivoire as described previously (Momou et al. 2012). All the selected wastewater plants covered areas that had poor sanitation, were densely populated, and had a population of low socioeconomic status individuals, covering 5,89,500 people.

### Sampling Strategy

Sixty-eight wastewater samples were collected along the flow channel leading to Azito in Yopougon during the months of September, October, November, December 2008 and January 2009. Wastewater samples were collected once or twice per week from each site. Samples were collected in the morning after 6.00 a.m. One liter each of wastewater was collected in a sterile Pyrex glass and transported at 4 °C to the Virus Unit of the Nervous System, Department of Virus Epidemic at the Institute Pasteur in Côte d'Ivoire within 1 h after collection (Momou et al. 2012). Adequate personal safety precautions were taken,

and all materials used for sample collection were decontaminated by autoclaving.

### Treatment of Samples

Wastewater samples were concentrated with the two-phase separation protocol (Dextran T40 and polyethylene glycol 6000) recommended by WHO (2003), as follows. 500 mL wastewater samples were centrifuged for 10 min at $4,000 \times g$ and 4 °C. The supernatant and pellet were collected into separate containers, and the pellet was held at 4 °C. The supernatant was neutralized to pH 7.0–7.5 with 1.0 N sodium hydroxide. The volume of supernatant was measured. To every 0.5 L of supernatant, 39.5 mL of 22 % (weight per volume) dextran, 287 mL (weight per volume) of polyethylene glycol 6,000, and 35 mL of a 5 normal concentration of sodium chloride solution were added. The solution was mixed, kept at 4 °C for 1 h, and agitated constantly. The mixture was then poured into a sterile separation funnel and left to stand overnight at 4 °C. In the following day, the entire lower phase and the interface were collected into a sterile tube (usually 10–20 mL of liquid) to which the pellet that had been held at 4 °C following centrifugation of the original sample was then added. The pellet was resuspended, and the mixture was extracted with 20 % (volume to volume) chloroform by vigorous shaking for 20 min at room temperature. The mixture was then centrifuged for 10 min at $4,000 \times g$ and 4 °C. The supernatant was collected and added to a solution of antibiotics (penicillin G and to the final concentrations of 100 IU/mL and 100 mg/mL, respectively). The aqueous phase was collected into aliquots, which were stored at −80 °C.

### Virus Isolation

The 68 wastewater concentrates were inoculated onto confluent monolayers of RD cell line, HEP-2 cells, BGM and L20B cell lines (cells adhered to a density of $5 \times 10^5$ cells per vial). These cells were maintained in minimum essential medium on medium (MEM) containing 5–10 % fetal calf serum (Mendelsohn et al. 1989; Pipkin et al. 1993; Momou et al. 2012).

Approximately 500 μL volume of the undiluted or $10^{-1}$ dilution of each concentrate was used to inoculate three 25 cm$^2$ culture flasks containing freshly confluent monolayers of cells covered by 4.5 mL of MEM 2 %.

Culture flasks were incubated at 36 °C for 5 days and examined using an inverted microscope for cytopathic effects (CPE) characteristic of EV. The appearance of CPE was recorded as CPE (1+ to 4+) to indicate the percentage of cells affected (1+ to upto 25 %, 2+ to 25–50 %, 3+ to 50–75% and 4+ to 100 %).

Cells showing no CPE at first inoculation were further passaged in the same cell line. Cells showing no CPE at the first inoculation were freeze–thawed. Approximately 0.5 mL volume of culture fluids was then transferred to flasks containing fresh monolayers. Culture flasks were incubated at 36 °C and observed for 5 days. Suspensions form samples showing CPE in HEP-2c and RD lines were re-inoculated onto BGM lines and L20B, and those showing CPE in BGM and L20B were re-passaged in corresponding cell lines (Momou et al. 2012). It is now known that a small percentage of PV isolates does not to grow well in L20B cells on the first passage, and may not produce recognizable CPE. They do, however, grow in RD cells, and on passage in L20B cells these isolates produce recognizable CPE. It is important, therefore, that in order not to miss any PV all samples positive in RD, HEP-2c, and BGM cells but negative in L20B cells should be passaged in L20B cells. Sometimes, two passages are necessary for NPEV isolation on RD, HEP-2c, and BGM cells lines.

### Characterization of PV by Micro-neutralization and Intratypic Differentiation (ITD)

Isolates from L20B cell lines were tested by neutralization with a mixture of anti-PV immune serum from the National Institute of Public Health and the Environment in the Netherlands (RIVM) as described in the polio laboratory manual to determine the serotype of PV. Identified strains were then tested by RT-PCR for ITD differentiation (WHO 2004).

### Reverse Transcription Polymerase Chain Reaction (RT-PCR)

#### RT-PCR Performed from Suspensions of Cells with CPE

For the PCR procedures, RNA extraction was performed with modified guanidine thiocyanate extraction method to extract viral RNA from 200 μL of cell culture supernatant from inoculated flasks (Rashmi et al. 2005). We used high-grade phenol:chloroform 5:1 (Sigma) for the extraction. We eluted the viral RNA in 50 μL of nuclease-free distilled water and either used it immediately in molecular assays or stored it at −80 °C.

All positive samples were tested by RT-PCR using degenerate and non-degenerate primers using kits provided by the Centers for Disease Control and Prevention, Atlanta, GA. These consisted of Pan-enterovirus (Pan-EV) and Pan-poliovirus (PV-Pan) primers (WHO 2004). The amplification products were placed in individual wells of 10 % polyacrylamide gel (Bio-Rad) and subjected to electrophoresis at 20 mA per gel for approximately 35 min. The PCR products (amplicons) were visualized after staining in 1 mg/mL ethidium bromide for 15 min (WHO 2004).

#### Direct RT-PCR on Water Concentrates

Viral RNA extraction was performed as described by Zoll et al. (1992). DNA synthesis was performed in a final volume of 10.5 μL using SuperScript II reverse transcriptase (Invitrogen, Cergy-Pontoise, France); the reaction mixture contained 5 μL of purified viral RNA, 2 μL of 5× First-Strand Buffer, 0.01 M dithiothreitol (1 μL), 100 ng of the random primers heptaN (1 μL), 10 pmol of each dNTP (1 μL of a 10 mM mixture), and 100 U of enzyme (0.5 μL). The RT reaction mixture was then incubated at 25 °C for 10 min, 42 °C for 45 min, and 95 °C for 5 min.

cDNAs were then used as templates for amplification in PCR carried out in a final volume of 50 μL that included 5 μL of 10× PCR Buffer, 200 μM of each dNTP, 50 pmol (2.5 μL) of each primer, 2 μL of cDNA, and 2.5 U of *HotStartTaq* DNA polymerase (Qiagen, Courtaboeuf, France). The thermocycler profile was 15 min at 95 °C followed by 30 cycles of 30 s at 95 °C, 30 s at 45 °C, and 2 min at 60 °C (Bessaud et al. 2008). 114-bp-sized bands of the PCR-amplified products were visualized under UV illumination on 10 % polyacrylamide gel after ethidium bromide staining (WHO 2004; Saeed et al. 2007).

### Statistical Analysis

The Fisher's test using software R version 2.15.0 was used for the comparison of proportions. Differences in enterovirus isolation proportion in BGM, HEP-2c, and RD cell lines, with virus detection directly from the water specimen and in infected cultures by RT-PCR performed after culture were tested using Fisher's exact test ($p$ value = 0.05).

## Results

### Sensitivity and Positivity on Cell Lines

Results obtained in this study showed that viruses were more frequently isolated from wastewater in BGM cell line (58.8 %) than in RD (51.47 %) cell and HEP-2c (50 %) (Table 1). Virus isolation in BGM cell line showed that sensitivity to EV infection was 50 %, followed by RD with sensitivity of 48.5 %, HEP-2c with sensitivity of 44.1 %, and L20B cell line with the lowest sensitivity of 2.9 %. Suspected PV was isolated in only 23.5 % of the cell cultures (Table 1). Comparison of proportions by Fisher's exact test using the R software revealed no significant difference between proportions (BGM, RD, and HEP-2c) ($p$ value >0.05).

**Table 1** Sensitivity and specificity of cell culture versus RT-PCR

| Sample No. | Cytopathology[a] | | | | RT-PCR[b] | | | | |
| | L20B | RD | HEP-2c | BGM | Cell lysate of positives sample | | | | Samples concentrate |
| | | | | | L20B | RD | HEP-2c | BGM | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 1 | 2+ | 0 | 0 | 2+ | 0 | / | / | 0 | 0 |
| 3 | 0 | 0 | 0 | 2+ | / | / | / | 0 | 0 |
| 4 | 2+ | 2+ | 0 | 3+ | 0 | 0 | / | 0 | 0 |
| 8 | 3+ | 3+ | 0 | 3+ | 0 | + | / | + | 0 |
| 12 | 2+ | 0 | 0 | 2+ | 0 | / | / | 0 | 0 |
| 14 | 3+ | 0 | 3+ | 3+ | 0 | / | 0 | 0 | 0 |
| 15 | 4+ | 0 | 4+ | 4+ | 0 | / | + | + | 0 |
| 16 | 3+ | 0 | 3+ | 0 | 0 | / | 0 | / | 0 |
| 19 | 0 | 3+ | 3+ | 3+ | / | + | + | + | 0 |
| 22 | 0 | 4+ | 4+ | 4+ | / | + | + | + | + |
| 23 | 4+ | 4+ | 0 | 4+ | 0 | + | / | + | + |
| 24 | 0 | 4+ | 0 | 4+ | / | + | / | + | + |
| 25 | 0 | 4+ | 4+ | 4+ | / | + | + | + | + |
| 26 | 0 | 4+ | 4+ | 4+ | / | + | + | + | + |
| 27 | 0 | 4+ | 0 | 4+ | / | + | / | + | + |
| 29 | 0 | 4+ | 0 | 4+ | / | + | / | + | + |
| 30 | 0 | 0 | 4+ | 4+ | / | / | + | + | + |
| 31 | 3+ | 3+ | 3+ | 3+ | + | + | + | + | + |
| 32 | 0 | 4+ | 4+ | 0 | / | + | + | / | + |
| 33 | 0 | 4+ | 4+ | 4+ | / | + | + | + | + |
| 34 | 0 | 4+ | 4+ | 4+ | / | + | + | + | + |
| 35 | 0 | 4+ | 4+ | 4+ | / | + | + | + | + |
| 44 | 0 | 0 | 4+ | 0 | / | / | 0 | / | 0 |
| 45 | 0 | 3+ | 3+ | 4+ | / | + | + | + | + |
| 47 | 2+ | 2+ | 2+ | 4+ | 0 | + | + | + | + |
| 48 | 4+ | 4+ | 4+ | 4+ | + | + | + | + | + |
| 49 | 0 | 3+ | 3+ | 3+ | / | + | + | + | + |
| 50 | 2+ | 3+ | 3+ | 3+ | 0 | 0 | 0 | 0 | 0 |
| 52 | 0 | 4+ | 4+ | 4+ | / | + | + | + | + |
| 53 | 3+ | 3+ | 3+ | 3+ | 0 | + | + | + | + |
| 54 | 3+ | 3+ | 3+ | 3+ | 0 | + | + | + | + |
| 55 | 3+ | 3+ | 3+ | 3+ | 0 | + | + | + | + |
| 56 | 0 | 3+ | 3+ | 3+ | / | + | + | + | 0 |
| 57 | 4+ | 4+ | 4+ | 4+ | 0 | + | + | + | + |
| 58 | 0 | 4+ | 4+ | 4+ | / | + | + | + | + |
| 59 | 0 | 4+ | 4+ | 4+ | / | + | + | + | + |
| 60 | 0 | 4+ | 4+ | 4+ | / | + | + | + | + |
| 61 | 0 | 4+ | 4+ | 4+ | / | + | + | + | + |
| 62 | 0 | 4+ | 4+ | 4+ | / | + | + | + | + |
| 63 | 0 | 4+ | 4+ | 4+ | / | + | + | + | + |
| 64 | 0 | 4+ | 4+ | 4+ | / | + | + | + | + |
| 65 | 0 | 4+ | 4+ | 4+ | / | + | + | + | 0 |
| 66 | 0 | 4+ | 4+ | 4+ | / | + | + | + | + |

**Table 1** continued

| Sample No. | Cytopathology[a] | | | | RT-PCR[b] | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Cell lysate of positives sample | | | | Samples concentrate |
| | L20B | RD | HEP-2c | BGM | L20B | RD | HEP-2c | BGM | |
| Total | 16/68 (23.5 %) | 35/68 (51.4 %) | 34/68 (50 %) | 40/68 (58.8 %) | 2/68 (2.9 %) | 33/68 (48.5 %) | 30/68 (44.1 %) | 34/68 (50 %) | 30/68 (44.1 %) |

An evaluation of the ability of cell lines (BGM, RD, HEP-2c, and L20B) and RT-PCR to detect enterovirus
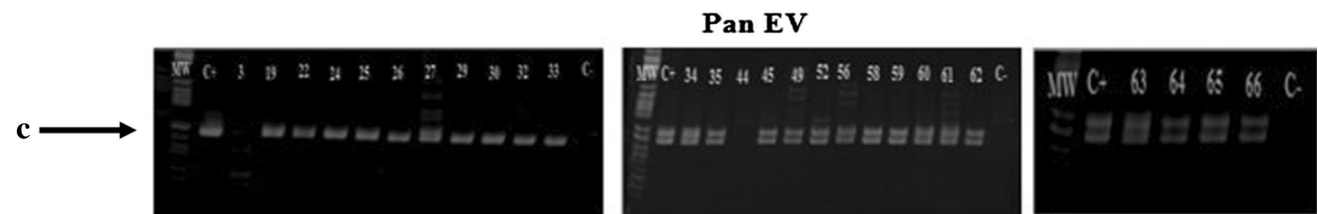
+ positive, 0 negative

[a] Sensitivities of total culturable virus isolation in BGM, RD, and HEP-2c

[b] Sensitivity and specificity of isolation in cell culture compared to direct detection by RT-PCR
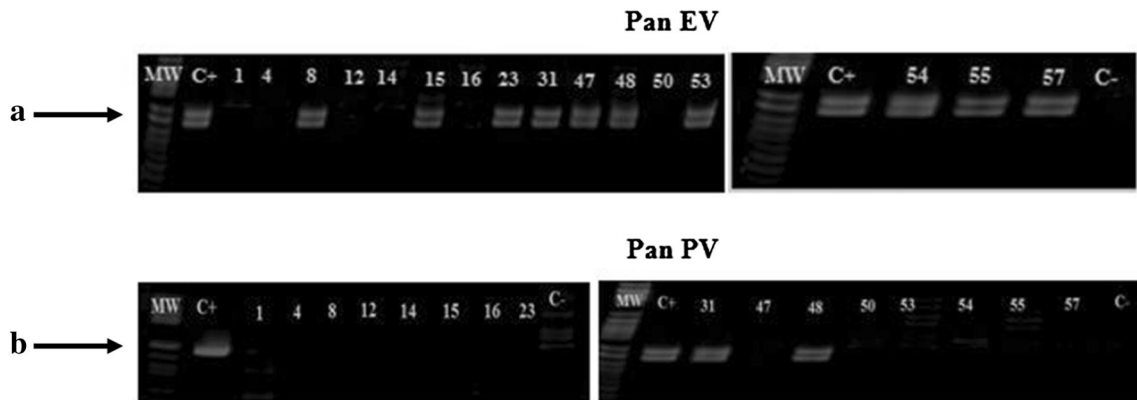


**Fig. 1** RT-PCR analysis of virus strains isolated on L20B cell lines. *Band a* amplicon of 114 bp, *band b* amplicon of 79 bp size. *MW* molecular weight marker, C+ positive control, C− negative control



**Fig. 2** RT-PCR analysis of virus strains isolated on other cell lines than L20B. *Band c* amplicon of 114 bp. *MW* molecular weight marker, C+ positive control, C− negative control

Positivity rate increased with passage levels; thus at zero passage, 14 viruses were isolated, while 28 viruses were isolated after the first passage and 35 viruses were isolated at the second passage.

### RT-PCR After Isolation

Results of PanPolio RT-PCR/PV (PanPV) performed on the samples showed a lower PV isolation rate of 2.9 % PV. All the remaining isolates were NPEV virus and other non-EV whose CPE suggested the presence of reovirus and adenovirus. These were, however, not confirmed by molecular testing and antigenic identification (the primers

used in the PCR will not detect reovirus or adenovirus). From 16 isolates in L20B cell lines, two cultures were positive for PV, and the remaining were NPEV and non-EV (Table 1; Fig. 1). In contrast, the cell lines HEP-2c, BGM, and RD also supported cytopathic growth of several non-PV. From 27 isolates identified, 25 were NPEV positive and two were non-EV positive (3.44) (Table 1; Fig. 2). The cultures in flasks 3 and 44 showed enlarged, rounded cells in tightly associated grape-like cluster, characteristic of adenovirus (Lipson et al. 1993; Clarke 2007). In parallel, the cultures in flasks 1, 4, 12, 14, 16, and 50 had a non-specific-looking granular appearance with progressive degeneration and detaching of monolayer characteristic of

Fig. 3 Intratypic differentiation of polioviruses (ITD). **a** Two samples identified as polioviruses (31, 48) by the amplicon of 79 bp [band (*a*)]. The absence of amplicon *b* shows that they are not serotype 1. **b** Two samples identified as polioviruses (31, 48) by the amplicon of 79 bp [band (*a*)]. The absence of amplicon *d* shows that they are not serotype 3. *MW* molecular weight marker, C+ positive control, C− negative control
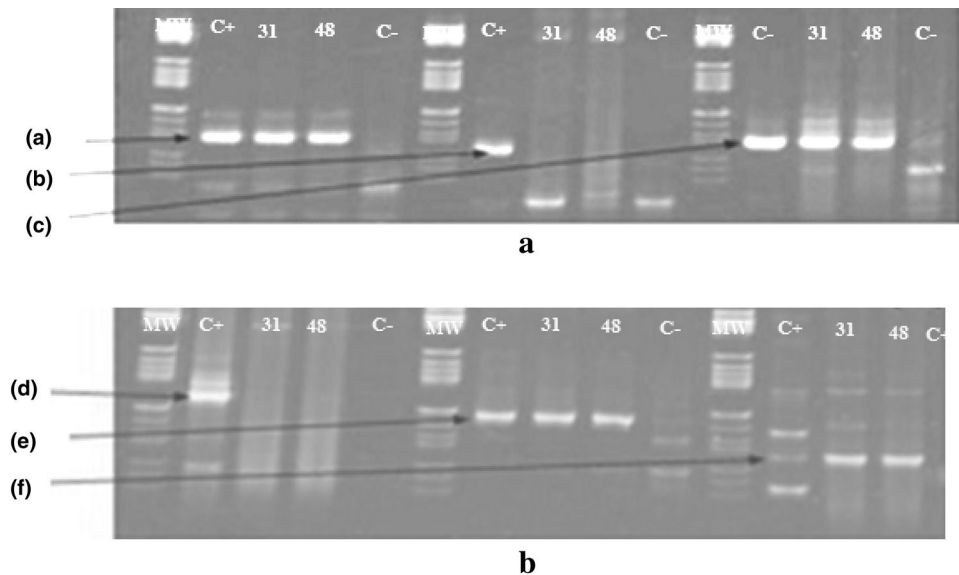


reovirus (Clarke 2007). These CPEs are different from that produced by EV, which produces round, highly retractile cells in loose clusters or dispersed throughout the monolayer (WHO 2004; Clarke 2007).

The intratypic differentiation test and RT-PCR performed on the two polio isolates identified the viruses as Sabin-like type 2 (SL PV2) (Fig. 3a, b). The L20B mouse cell lines express the gene for the human cellular receptor (CD 155) for PVs.

Direct RT-PCR

Sixty-eight samples were analyzed by direct RT-PCR for detection of EV. Overall, 30(44.1 %) were positive for EV (Table 1). Results showed no significant difference between direct RT-PCR and RT-PCR performed after culture. In fact, comparison of proportion by Fisher's exact test revealed no significant difference between RT-PCR of all lysates of positive samples (BGM. RD and HEP-2 cells) and direct RT-PCR (*p* value >0.05) (Table 1).

Discussion

We have used virus isolation in various cell lines and RT-PCR to detect EV from wastewater in the Yopougon area in Cote d'Ivoire, and also determined the sensitivity and specificity of the viruses to the different cell lines used. Our results showed that more viruses were isolated in both the BGM and the RD cell lines. There was, however, no statistically significant difference in the isolation rate (*p* value >0.05) (Table 1). In an earlier study, Muscillo and coworkers, while comparing cell lines, concluded that

BGM cell lines were more susceptible to EV infection than HEP2 cell lines (Muscillo et al. 1997). The results obtained in our study do not appear to support the conclusions of Muscillo et al. (1997). The difference between our observation and that of Muscillo and coworkers may be due to the limited number of samples collected during this study.

The L20B, a transgenic mice cell line to which the PV receptor site CD155 had been genetically engineered, exhibited the highest PV specificity (Hovi and Stenvik 1994). This result is in agreement with the findings of Hovit and Stenvik who found that L20B cell lines exhibited high specificity for PV in clinical samples. In an earlier study, only PV produced CPE on L20B, while adenovirus, NPEV, and reovirus did not (Nadkarni and Deshpande 2003). A similar study conducted by Grabow and coworkers on wastewater reached the same conclusion (Grabow et al. 1999; Zurbriggen et al. 2008). Previous studies about surveillance of viruses in the sewage of Jones Island WWTP in the MMSD reported that of the viruses isolated, 68 % were reoviruses, 28 % were EV, and 4 % were adenoviruses (Rodríguez et al. 2008).

It is obvious from this study and previous studies that non-PV enteroviruses are able to produce CPE in L20B cell lines. However, the type of CPE produced in this cell line is quite different in appearance from those observed with EV. Adenovirus and Reovirus have been widely identified by their CPE. The results obtained in this study appear to support the findings of Leland and Ginocchio (2007) who observed the same characteristic CPE appearance on adenovirus- and reovirus-inoculated cell cultures.

A limited number of non-polio enteroviruses, specifically some strains of Coxsakie A viruses, can also grow on L20B cell lines producing the characteristic EV CPE

(Pipkin et al. 1993). Indeed, a study in India, where the L20B cell lines have been widely used to identify NPEV showed that a small number of clinical strains other than PV were responsible for CPE in L20B (Nadkarni and Deshpande 2003).

According to Pipkin et al. L20B cells are sensitive to animal EV that are non-typable by neutralization with human antisera. L20B cells are ideal for isolation of PV from human stools; however, other enteric viruses from animal stool from water environment can also be isolated (Pipkin et al. 1993).

The detection of PV in the wastewater at Yopougon community reflects the irregular presence of SL–PV-shedding individuals who enter from an OPV setting. This, in turn, emphasizes the necessary of uninterrupted child vaccination in order to ensure sustained high head immunity.

In this study, we observed the same positivity rate using RT-PCR on infected tissue culture as using RT-PCR directly on wastewater samples. This is in agreement with the study of Hassine et al. who used direct RT-PCR to test 93 environmental samples of concentrated wastewater in the region of Monastir using the technique described by Gerba and Goyal and found 35.13 % positives (Hassine et al. 2010). This value was lower than that obtained in our study, revealing a high level of viral pollution from wastewater in Yopougon. The reason for this might have been due to the fact that some of these viruses did not produce CPE the first 5 days after the first inoculation. CPEs were only observed after the first and second passages.

RNA extraction from concentrated environmental samples is often difficult because several inhibitory substances are present in environmental samples which interfere with RT-PCR. Such inhibition, due to metals, humic acids, and other organic matter, has been reported previously (Abbaszadegan et al. 1993; Reynolds et al. 1997). Previous studies have shown that the effects of inhibitors could be removed by dilution (Abbaszadegan et al. 1993). In this study, water concentrates were diluted at a dilution of $10^{-1}$ to remove any effect of inhibitors. According to Greening et al. (2002), dilution of samples with cell culture medium reduced the effect of inhibitory and toxic substances present in environmental samples on both culture cells (BGM and HEP-2c) and in PCR-based assays, but this practice also reduced the overall amount of sample being test.

PCR generally requires 10–50 µL of concentrate whereas 200–500 µL is required for the culture (Shieh et al. 1997; Ma et al. 1995). Currently, the conventional method for the detection of enteroviruses in water is expensive and time consuming. RT-PCR provides an alternative rapid and high sensitive method for the detection of sensitivity but this is reduced by PCR inhibitory substances and low sample volume (Shieh et al. 1997; Ma et al. 1995). Virus isolation, however, remains essential for both diagnosis and epidemiological studies. Typing of isolates and differentiation of vaccine and wild viruses are needed to determine the nature of outbreaks.

## Conclusion

In this study, we could not confirm the generally observed high sensitivity to BGM cell line reported in previous studies, as compared to the other three cell lines. The use of RT-PCR for the direct detection of the 5UTR of EV in wastewater is generally faster, more sensitive, and less expensive than RT-PCR on isolates. In the context of polio eradication, surveillance of EV should be strengthened by monitoring the movement of EV in the environment as recommended by WHO. This monitoring should especially include analysis of viral wastewater to identify residual foci of wild PVs.

## References

Abbaszadegan, M., Huber, M. S., Gerba, C. P., & Pepper, I. L. (1993). Detection of enteroviruses in groundwater with the polymerase chain reaction. *Applied and Environmental Microbiology, 59*(5), 1318–1324.

Afssa (2005) Virus transmissibles a l'homme par voie orale. Comment interpréter la présence du génome viral dans les matrices alimentaires et en milieu hydrique en terme d'infectiosité potentielle. Doc. p. 62.

Bessaud, M., Jegouic, S., Joffret, M. L., Barge, C., Balanant, J., Gouandjika-Vasilache, I., et al. (2008). Characterization of the genome of human enteroviruses: Design of generic primers for amplification and sequencing of different regions of the viral genome. *Journal of Virological Methods, 149*(2), 277–284.

CDC. (2008). Progress toward interruption of wild poliovirus transmission-worldwide, January 2007–April 2008. *Morbidity and Mortality Weekly Report, 57*, 489–494.

CDC. (2012). Assessments of risks to the global polio eradication initiative (GPEI) strategic plan 2010–2012, Doc CDC, p. 20.

Clarke, L. (2007). Viruses, rickettsiae, chlamydiae, and mycoplasmas. *Essential procedures for clinical microbiology* (pp. 533–550). Washington DC: ASM.

Grabow, W. O. K., Botma, K. L., De Villiers, J. C., Clay, C. G., & Erasmus, B. (1999). Assessment of cell culture and polymerase chain reaction procedures for the detection of polioviruses in wastewater. *Bulletin of the World Health Organization, 77*(12), 973–980.

Greening, G. E., Hewitt, J., & Lewis, G. D. (2002). Evaluation of integrated cell culture-PCR (C-PCR) for virological analysis of environmental samples. *Journal of Applied Microbiology, 93*(5), 745–750.

Hassine, M., Sdiri, K., Riabi, S., Beji, A., Aouni, Z., & Aouni, M. (2010). Detection of enteric viruses in wastewater of Monastir region by RT-PCR method. *La Tunisie Médicale, 88*(2), 70.

Hovi, T., & Stenvik, M. (1994). Selective isolation of poliovirus in recombinant murine cell line expressing the human poliovirus receptor gene. *Journal of Clinical Microbiology, 32*(5), 1366–1368.

Lee, C., Lee, S. H., Han, E., & Kim, S. J. (2004). Use of cell culture-PCR assay based on combination of A549 and BGMK cell lines and molecular identification as a tool to monitor infectious adenoviruses and enteroviruses in river water. *Applied and Environmental Microbiology, 70*(11), 6695–6705.

Leland, D. S., & Ginocchio, C. C. (2007). Role of cell culture for virus detection in the age of technology. *Clinical Microbiology Reviews, 20*(1), 49–78.

Lipson, S. M., Poshni, I. A., Ashley, R. L., Grady, L. J., Ciamician, Z., & Teichberg, S. (1993). Presumptive identification of common adenovirus serotypes by the development of differential cytopathic effects in the human lung carcinoma (A549) cell culture. *FEMS Microbiology Letters, 113*(2), 175–182.

Ma, J. F., Gerba, C. P., & Pepper, I. L. (1995). Increased sensitivity of poliovirus detection in tap water concentrates by reverse transcriptase-polymerase chain reaction. *Journal of Virological Methods, 55*(3), 295–302.

Mendelsohn, C. L., Wimmer, E., & Racaniello, V. R. (1989). Cellular receptor for poliovirus: Molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. *Cell, 56*(5), 855–865.

Momou, K. J., Akoua-Koffi, C., Akré, D. S., Adjogoua, E. V., Tiéoulou, L., & Dosso, M. (2012). Detection of enteroviruses in urban wastewater in Yopougon, Abidjan. *Pathologie-biologie, 60*(3), e21–e26.

Muscillo, M., Carducci, A., La Rosa, G., Cantiani, L., & Marianelli, C. (1997). Enteric virus detection in Adriatic seawater by cell culture, polymerase chain reaction and polyacrylamide gel electrophoresis. *Water Research, 31*(8), 1980–1984.

Nadkarni, S. S., & Deshpande, J. M. (2003). Recombinant murine L20B cell line supports multiplication of group A coxsackieviruses. *Journal of Medical Virology, 70*(1), 81–85.

Pipkin, P. A., Wood, D. J., Racaniello, V. R., & Minor, P. D. (1993). Characterisation of L cells expressing the human poliovirus receptor for the specific detection of polioviruses in vitro. *Journal of Virological Methods, 41*(3), 333–340.

Rajtar, B., Majek, M., Polanski, L., & Polz-Dacewicz, M. (2008). Enteroviruses in water environment—A potential threat to public health. *Annals of Agricultural and Environmental Medicine, 15*(2), 199–203.

Rashmi, C., Alka, S., Tapas, D., & Dhole, T. N. (2005). Rapid detection of sewage sample polioviruses by integrated cell culture polymerase chain reaction. *Archives of Environmental and Occupational Health, 158*(8), 807–815.

Reynolds, K. S., Gerba, C. P., & Pepper, I. L. (1997). Rapid PCR-based monitoring of infectious enteroviruses in drinking water. *Water Science and Technology, 35*(11), 423–427.

Rodríguez, R. A., Gundy, P. M., & Gerba, C. P. (2008). Comparison of BGM and PLC/PRC/5 cell lines for total culturable viral assay of treated sewage. *Applied and Environmental Microbiology, 74*(9), 2583–2587.

Saeed, M., Zaidi, S. Z., Naeem, A., Masroor, M., Sharif, S., Shaukat, S., et al. (2007). Epidemiology and clinical findings associated with enteroviral acute flaccid paralysis in Pakistan. *BMC Infectious Diseases, 7*(1), 6.

Shieh, Y. S., Baric, R. S., & Sobsey, M. D. (1997). Detection of low levels of enteric viruses in metropolitan and airplane sewage. *Applied and Environmental Microbiology, 63*(11), 4401–4407.

WHO. (2003). Guidelines for environmental surveillance of poliovirus circulation WHO/V&B/03.03 ORIGINAL.ENGLISH Vaccines and Biologicals World Health Organization.

WHO. (2004). Polio laboratory manuel under revision. Available on the internet at http://www.who.int/vaccines/en/poliolab/Polio Manuel%20version_1May01.pdf.

WHO. (2010). Flambées consécutives à l'importation du poliovirus sauvage dans les régions africaine, européenne et d'Asie du sud-est de l'OMS REH, 85, 445–452.

Zoll, G. J., Melchers, W. J., Kopecka, H., Jambroes, G., Van der Poel, H. J., & Galama, J. M. (1992). General primer-mediated polymerase chain reaction for detection of enteroviruses: Application for diagnostic routine and persistent infections. *Journal of Clinical Microbiology, 30*(1), 160–165.

Zurbriggen, S., Tobler, K., Abril, C., Diedrich, S., Ackermann, M., Pallansch, M. A., et al. (2008). Isolation of sabin-like polioviruses from wastewater in a country using inactivated polio vaccine. *Applied and Environmental Microbiology, 74*(18), 5608–5614.