Isolation of apparently wild strains of poliovirus type 1 from sewage in the Ottawa area

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In the first 4 months of 1974, 140 gauze pad samples of sewage collected in the Ottawa area were analysed by the BS-C-1 cell system for the presence of viruses pathogenic for humans. Viruses were isolated from 111 (79%) of the samples. Of the 72 (65%) isolates identified by serology and electron microscopic examination, 56 (78%) were reoviruses and 16 (22%), enteroviruses. The enterovirus isolates included one coxsackievirus B_4, one vaccine strain of poliovirus type 3, nine vaccine strains of poliovirus type 1 and five strains of poliovirus type 1 that proved by serodifferentiation and temperature marker tests to be different from vaccine strains. The fact that these strains were present in the community sewage in readily detectable concentrations at a time when immunity against polioviruses is declining in such communities is a cause for concern.

Durant les 4 premiers mois de 1974, 140 échantillons d'égouts de la région d'Ottawa ont été prélevés sur tampons de gaze et analysés au moyen du système cellulaire BS-C-1 pour la présence de virus pathogènes pour l'homme. Des virus ont été isolés de 111 (79%) échantillons. Des 72 souches isolées et identifiées par méthode sérologique et au microscope électronique, 56 (78%) étaient des réovirus et 16 (22%), des entérovirus. Les entérovirus isolés comprennent un virus coxsakie B_4, une souche vaccinale de virus polio type 3, neuf souches vaccinales de virus polio type 1 et cinq souches de virus polio type 1 qui se sont avérées, par sérodifférenciation et aux épreuves thermiques, différentes des souches vaccinales. Il y a lieu de s'inquiéter que ces souches soient présentes en concentrations décelables dans les égouts communautaires alors que l'immunité contre le virus polio diminue au sein de ces mêmes communautés.

As a result of the extensive vaccination programs initiated in 1955, there has been a great reduction in the number of cases of paralytic poliomyelitis in Canada.[1] This has created a general complacency, which is clearly reflected in the declining immunity against this infection.[2]

Several viruses pathogenic for humans were isolated during an investigation of the virus-eliminating efficacy of primary treatment (sedimentation) and chlorination of sewage. Among the virus isolates were five potentially virulent strains of poliovirus type 1. Although the presence of such strains of poliovirus in sewage was not altogether unexpected, it was none the less alarming in view of the declining immunity against polioviruses in such communities. In this paper we describe the isolation of these viruses and discuss their significance to the health of the community.

Materials and methods

Sewage treatment plants

The samples of sewage were collected from the Green Creek Pollution Control Centre and the Bilberry Creek Sewage Treatment Plant. Both plants are in the Ottawa area and are run by the Regional Municipality of Ottawa–Carleton. They serve populations of 358 000 and 4500, respectively.[3]

The Green Creek plant is the largest sewage treatment facility in the area. Here sewage is subjected to sedimentation followed by chlorination of the effluent, which is discharged into the Ottawa River. This plant treats approximately 2.7×10^8 *l* (60 million imperial gallons) of sewage per day.[3]

The Bilberry Creek plant is situated in Orleans, Ont. This is also a primary treatment plant. It processes about 1.8×10^6 *l* (400 000 imperial gallons) of sewage per day.

Ottawa and the surrounding area are essentially nonindustrial. Therefore the sewage is predominantly of domestic origin.

Sewage sample collection and processing

The gauze pad (Moore swab) technique[4] was used to collect sewage samples during the first 4 months of 1974 (Jan. 22 to Apr. 29). A strip of absorbent cotton 240 x 25 cm was folded several times to form a pad approximately 25 x 10 cm. The pad was inserted into a piece of surgical stockinet 30 x 10 cm. The two ends of the stockinet were then tied with pieces of string. The pad was suspended by a brass chain in the waste water to be sampled for at least 48 hours and for no more than 72 hours, then sent to the laboratory in a sterile plastic bag for immediate processing.

Extraction was performed while the pad was still in the plastic bag. The

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liquid was expressed out of the pad and its pH increased to 8.0 by the addition of 1 N NaOH. The pad was resoaked in this liquid and gently kneaded for about 2 minutes. The liquid was again squeezed out of the pad and collected in a sterile measuring cylinder. Approximately 60 ml of liquid was obtained from each pad.

The pH of the sample was immediately decreased to 7.2 and the liquid was centrifuged for 20 minutes at 750 x *g* to remove the large particulate materials. The supernatant was collected and fetal calf serum added to a concentration of 10%. The sample was passed through a 0.22-μm membrane filter (Millipore Corp., Bedford, Massachusetts) to remove bacterial and fungal contaminants. The presence of serum in the sample avoided virus loss by adsorption to the membrane filter. The filtrate was inoculated into cultures.

Isolation of viruses

BS-C-1 cells[5] (obtained through the courtesy of the virology laboratory of the Children's Hospital of Eastern Ontario, Ottawa) were cultivated in Eagle's minimum essential medium (MEM) supplemented with glutamine and 10% fetal calf serum (Gibco, Grand Island, New York). The cells were maintained in the same medium but with only 2% of the serum.

The filtrate from the processed gauze pad sample was inoculated into monolayers of these cells grown in 25-cm^2 plastic bottles (Falcon Plastics, Los Angeles, California). The cultures were observed periodically over 3 weeks. Material from cultures showing cytopathic effects was passaged in a fresh lot of cells. Material from cultures that were negative at the end of 3 weeks was blind-passaged once: if no cytopathic effect became detectable the sample was considered to be negative for virus.

Identification of virus isolates

Identification of the virus isolates by electron microscopic examination and serology was carried out by Dr. A. Kelen and Mr. Dan McLeod of the bureau of virology, Laboratory Centre for Disease Control (LCDC), Ottawa. Serodifferentiation (McBride[6]) and temperature marker (rct/40)[7] tests were performed on the polioviruses to determine whether they were vaccine strains by Dr. J. Furesz and Mr. R. Armstrong of the bureau of biologics, LCDC, Ottawa, by procedures described elsewhere.[8]

Results

Of the 140 gauze pad samples studied 111 (79%) were found to be positive for virus by our BS-C-1 cell system. Serologic and electron microscopic examination of 72 (65%) of the virus isolates revealed 56 (78%) to be reoviruses and 16 (22%) to be enteroviruses. The enterovirus serotypes included 1 strain of coxsackievirus B_4, 14 strains of poliovirus type 1 and 1 strain of poliovirus type 3.

The poliovirus type 3 isolate proved to be of vaccine origin. Of the 14 poliovirus type 1 isolates 9 were identified as Sabin-type vaccine strains and the remaining 5 as different from vaccine strains and from a virulent 1962 strain on the basis of both serodifferentiation and temperature marker tests. The five nonvaccine strains had been isolated from samples collected during the 2-week period Mar. 22 to Apr. 4; three were from the Bilberry Creek plant and two from the Green Creek plant (Table I).

Discussion

The study that led to the isolation of the nonvaccine strains of poliovirus type 1 from sewage was aimed at assessing the virus-eliminating efficacy of primary treatment and chlorination of sewage in the winter. The presence of potentially wild strains of poliovirus in the community sewage was not unexpected. However, the fact that these five strains were isolated from two sewage treatment plants within a 2-week period was unexpected and possibly alarming. Though additional tests (for example, to demonstrate neurovirulence) could have been carried out to substantiate further the nature of our five isolates, the results of the McBride and temperature marker tests suggest their potential virulence.[9]

Polioviruses isolated from orally vaccinated children often present changes in the d[10] and rct/40[7] characters[10-12] as well as some increase in neurovirulence for monkeys.[11-13] It has also been shown that replication of such viruses in the human intestinal tract results in antigenic drift.[14]

No cases of clinical poliomyelitis were reported in the area during this period. Isolation of nonvaccine strains of poliovirus from feces and sewage during periods when there were few or no cases of clinical poliomyelitis in the community has been reported.[15,16]

The predominance of reoviruses among the virus isolates in this study is compatible with the results of other similar and recent investigations on sewage carried out in Ontario (T.P. Subrahmanyan, Central Public Health Laboratories, Toronto: personal communication, 1976).

In addition to their periodic importation from areas where poliomyelitis is still endemic,[17,18] virulent strains of polioviruses are maintained in the community by multiplication in nonvaccinated persons and perhaps those immunized by Salk vaccine.[19] There is also evidence that polioviruses may have extrahuman reservoirs.[20] The continued free circulation of such potentially dangerous viruses is important in communities where the immunity gap against poliomyelitis is rapidly widening. The lack of maintenance of adequate protection while these viruses are circulating could easily lead to a substantial increase in the number of cases of clinical poliomyelitis.[21,22] The decrease in immunity has already resulted in the reappearance of clinical poliomyelitis after several years of absence in certain areas of North America.[23,24] When unvaccinated adolescents and adults in such areas are infected by polioviruses the disease is generally much more serious.[21] The poliomyelitis outbreak of 1972 in a private school in Connecticut is a case in point.[25]

Another factor that may be important in the maintenance and circulation of nonvaccine polioviruses in the community is the inefficient removal and inactivation of viruses by current waste treatment methods.[26] Large numbers of potentially dangerous viruses are continually being discharged in sewage effluents and sludge into recreational and potable water. The potential of such polluted waters to transmit poliomy-

Table I—Places and dates of collection of gauze pad samples yielding "wild" strains of poliovirus type 1

Gauze pad no.	Place of collection	Collection dates: Pad immersed/pad removed	Marker test results: Antigenic (McBride)	Marker test results: rct/40
103	Bilberry Creek plant	Mar. 22/Mar. 25	Nonvaccine*	+
105	Bilberry Creek plant	Mar. 25/Mar. 27	Nonvaccine*	+
112	Green Creek plant	Mar. 29/Apr. 1	Nonvaccine*	+
114	Bilberry Creek Plant	Apr. 1 /Apr. 4	Nonvaccine*	+
116	Green Creek plant	Apr. 1 /Apr. 4	Nonvaccine*	+

*The strains showed no antigenic relationship with either the Sabin type 1 or a "wild" type 1 strain isolated in 1962 in the Ottawa area.

elitis and other viral infections is becoming increasingly evident.[27,28]

The fact that the nonvaccine strains of poliovirus were readily detected in sewage in the absence of clinical disease in the area indicates the importance of sewage examination for surveillance of enteric viruses. Studies in the Netherlands,[29] Czechoslovakia[30] and Sweden[31] have demonstrated that viruses isolated from clinical specimens and those detected in the sewage of the same community correspond. There is also evidence that certain viruses may become detectable in sewage before clinical disease due to the same infectious agents is evident in the population.[29] Rhodes and colleagues[32] detected polioviruses in sewage 5 weeks before the first cases of the disease appeared in the community.

Results of studies by Chin and colleagues[33] and Horstmann and associates[34] indicated that polioviruses become detectable in sewage only when 0.27 to 0.40% of the population are excreting the viruses. Thus, in a community of 500 000 people 1300 to 2000 persons must excrete the virus before it could be detected in the community sewage. From the data gathered in this study it is not possible to speculate either on the exact source of these viruses or on the numbers of people in the community who may have been excreting the virus at the time the samples were collected. However, the presence of these potentially dangerous strains in sewage in such readily detectable numbers is a cause for concern. Subrahmanyan and colleagues,[24] while investigating two cases of paralytic poliomyelitis that occurred in Ontario in 1969 and 1971, found that 21 nonvaccinated contacts of one of the patients were excreting the same strain of virus.

Conclusion

The presence of apparently wild strains of poliovirus in our communities, as evidenced by their isolation from sewage, further emphasizes the importance of maintaining adequate immunity against polioviruses in the general population and, in particular, in health workers.[17] It also underscores the need for the continuation of surveillance programs for poliomyelitis, as has recently been re-emphasized by the World Health Organization.[35]

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