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Viruses in Sewage Waters during and after a Poliomyelitis Outbreak and Subsequent Nationwide Oral Poliovirus Vaccination Campaign in Finland

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During an outbreak of paralytic poliomyelitis in Finland in 1984 and 1985 the widespread circulation of the causative wild-type serotype 3 poliovirus in the population was documented by demonstrating the virus in sewage water specimens in 13 different locations in the greater Helsinki district and in 13 other cities or towns all over the country. After the nationwide campaign with oral poliovirus vaccine in 1985, poliovirus serotypes 2 and 3 were readily isolated from sewage waters for up to 2 months, whereas type 1 poliovirus seemed to disappear from the sewage more rapidly. All of these isolates were temperature sensitive and therefore most likely vaccine related. The efficacy of the vaccination campaign in regard to elimination of the epidemic type 3 strain was evaluated by a follow-up study on viruses in sewage waters continued for 12 months through the subsequent expected season of poliomyelitis. Several types of enteroviruses, including five vaccine-related poliovirus strains, were identified in the 72 virus-positive specimens out of 93 studied. No wild-type polioviruses were found, indicating the success of the campaign.

An outbreak of poliomyelitis was discovered in Finland in 1984 (3) after 20 years without a single reported case and no evidence for indigenous circulation of polioviruses in the population (5). Although only 10 patients developed clinical disease due to poliovirus infection, at least 100,000 persons were estimated to have been excretors of the causative wild-type serotype 3 poliovirus. Apart from direct demonstration of the virus in fecal specimens of healthy excretors, both children and adults, this estimation was also based on sewage water analyses for cytopathic viruses (3). The outbreak was ceased by launching an extensive vaccination campaign comprised of an extra dose of the regular trivalent inactivated poliovirus vaccine to children less than 18 years old and a dose of trivalent oral poliomyelitis vaccine to the entire population (3). Before this campaign the oral poliomyelitis vaccine had never been used in general vaccinations in Finland.

In the 1960s and 1970s the regular sewage screening for cytopathic viruses revealed different enteroviruses and adenoviruses in most specimens but no polioviruses (6). Therefore, no screening of sewage waters took place in Helsinki between 1982 and the outbreak. During the outbreak the regular screening was resumed in Helsinki and some other locations. In this paper we describe in detail the observations on the sewage water specimens during the outbreak in 1984 and 1985, the nationwide vaccination campaign, and the subsequent follow-up period.

MATERIALS AND METHODS

Sewage water specimens. Sewage water specimens in Helsinki were collected from the influent of four independent sewage clearing plants; in some cases a specimen was also taken from the effluent. In all other cities and towns the specimens were taken from the sewage network, from sites presumably containing mainly household sewage from a given district. The specimens arrived in the laboratory within a few hours from the greater Helsinki district and not later than 1 day after collection from the other parts of the country.

Specimen concentration. The sewage water specimens were concentrated about 100-fold by a modification of the two-phase method described earlier (1). Thirty grams of 20% dextran T40 (Pharmacia Fine Chemicals, Uppsala, Sweden) in distilled water, 200.4 g of 30% polyethylene glycol 6000 (Pharmacopea Nordica, Lääketukku Oy, Helsinki, Finland), and 24 ml 5 M NaCl were mixed and filled up to 600 ml with the sewage specimen. The pH was adjusted to 7 to 8 with 1 M NaOH, and the mixture was vigorously agitated for 1 h at room temperature in a horizontal shaker at 260 rpm. The mixture was then transferred to a separation funnel and left overnight at 4°C. After separation the small bottom phase (2 to 3 ml) and a few milliliters of the interphase were harvested separately, extracted with chloroform, and inoculated into cell cultures.

Assay for cytopathogenic agents. Samples (0.5 ml) of chloroform-extracted concentrates of sewage specimens were inoculated into 50-ml plastic flasks (Nunc, Copenhagen, Denmark) with confluent cultures of continuous monkey kidney cell lines (Vero and GMK) and primary human amnion cells. After 1 h of adsorption at 37°C the inoculum was carefully removed, and 5 ml of maintenance medium (Eagle minimal essential medium supplemented with 2.5% fetal calf serum, 100 U of penicillin, and 0.1 mg of streptomycin per ml) was added. Incubation was continued at 37°C, and the maintenance medium was changed every 5 to 7 days. When no cytopathogenic effect was observed within 3 weeks, the cells were mechanically detached and subjected to a blind passage.

Virus identification. Cytopathogenic agents were passaged once in susceptible cells and identified by neutralization with type-specific antisera. All poliovirus isolates were tested for temperature sensitivity by titration at 39.5 and 36.5°C. A titer difference greater than 2 log units indicated unequivocal temperature sensitivity. An attempt to identify the relationship of all the type 3 isolates and the two last type 2 isolates to the corresponding vaccine strain was made by using a

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TABLE 1. Distribution of the epidemic poliovirus type 3 in sewage water specimens in the greater Helsinki district

City/site	Distribution of virus[a]				
	December 1984			January 1985	
	3	10	17	3	15/16
Helsinki					
Laajasalo		NT			− (CB2)
Lauttasaari		+			NT
Kyläsaari		+			NT
Munkkisaari		+			−
Viikki		+			− (CB5)
Vantaa					
Niittytie	−		+		+ (CB2)
Osmankäämintie	NT		+		+
Rekola	+		+		−(CB2)
Vaarala	+		+ (Ad2)		−(CB2)
Vapaala	+		− (CB2)		+
Espoo					
Center				+	
Kauklahti				+	
Lintuvaara				− (Ad2)	
Muurala				+ (CB2)	
Westend				+	

[a] +, Poliovirus type 3 isolated; −, no poliovirus strains isolated. Viruses other than poliovirus are shown within parentheses: Ad, adenovirus; CB, coxsackievirus group B; NT, not tested.

standard microneutralization assay and monoclonal antibodies that were known to recognize vaccine-related strains (2) (gifts from G. C. Schild, London).

RESULTS

Observations during the outbreak. The first two documented clinical cases of the outbreak, both diagnosed retrospectively by using serological tests, had the onset of the disease in late summer 1984 (4). No screening of sewage waters for viruses was being carried out at that time. Only after isolation of the epidemic type 3 poliovirus strain from several contacts of the index case, in late November 1984, was a decision made to resume sewage screening for viruses. Three of the four first independent specimens from different parts of Vantaa, the home city of the person with the index case, were found to be positive for poliovirus type 3 (Table 1). During the subsequent weeks the screening was extended to four other sites in the greater Helsinki district; all these specimens revealed poliovirus type 3 (Table 1). Some of the specimens were also tested without prior concentration and yet they revealed poliovirus type 3 (data not shown).

Since clinical cases and healthy virus excretors were also found elsewhere in the country (3), an attempt was made to evaluate the extent of virus spreading in the population by analyzing sewage water specimens collected all over the country. Sewage specimens were collected in 18 cities or towns during the first half of January 1985. Poliovirus type 3 was isolated from 11 independent sites outside the greater Helsinki district (Fig. 1). Two other specimens from additional independent locations, collected on 30 January and 4 February 1985, respectively, also contained poliovirus type 3.

The frequency of poliovirus in the sewage specimens collected in the Helsinki district in mid-January 1985 was less than that in December 1984, and some of the specimens

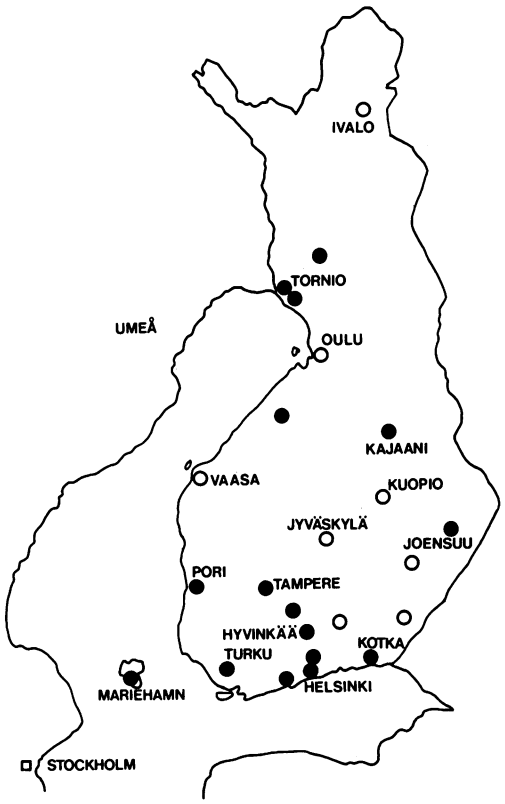

FIG. 1. Widespread distribution of poliovirus type 3/Finland/84-85 during the outbreak. Symbols: ●, locations with type 3 poliovirus in the sewage; ○, sewage specimen in early January 1985 was negative for poliovirus.

yielded group B coxsackieviruses type B instead (Table 1). Specimens collected from a site draining the village of the index case also showed this change. The frequency of poliovirus isolations from the fecal specimens from the village decreased concomitantly (Table 2). It is notable that in December 1984 through January 1985 all children 18 years or younger throughout the country were given an extra dose of trivalent inactivated poliovirus vaccine (3). This may have

TABLE 2. Excretion of epidemic poliovirus type 3 by inhabitants of a village and appearance of virus in local sewage

Year	Week	No. of persons screened	No. of poliovirus type 3 excretors	% Positive	Poliovirus in sewage draining the village[a]
1984	45	72	25	35	NT
	46	144	35	24	NT
	47	115	32	28	NT
	48	43	14	33	NT
	49	32	6	19	+
	50	52	9	17	NT
	51	24	4	17	+
	52	14	0	0	NT
1985	1	24	0	0	NT
	2	15	0	0	NT
	3	4	0	0	−
	4	9	0	0	NT

[a] See footnote *a* of Table 1.

contributed to the cessation of circulation of the epidemic type 3 poliovirus strain.

Nationwide live poliovaccine campaign and subsequent follow-up period. A dose of trivalent oral poliovirus vaccine was recommended to the whole population (with minor exceptions) of Finland between 10 February and 15 March 1985. A coverage of about 94% was reached, according to numeric vaccination recordings carried out at each vaccination site. For sewage water studies, two independent locations in Helsinki were sampled weekly, and two other sites on a monthly basis from February to October 1985, and the specimens were assayed for cytopathic viruses. Polioviruses were readily isolated in most of the specimens collected during the campaign and the 2 subsequent months (Table 3). The two harvested fractions of each sewage specimen were assayed separately, and in some cases different viruses were identified in the two fractions. The last specimen in Helsinki with documented poliovirus content was collected in late June 1985, about 3 1/2 months after ceasing the campaign. Poliovirus type 1 was isolated from the sewage almost exclusively only during the campaign, whereas both type 2 and type 3 isolates were also present in the later positive specimens (Table 3). All poliovirus isolates were found to be temperature sensitive, suggesting vaccine virus origin. Type 3 isolates were also tested for antigenic relationship to the Sabin 3 strain by using a monoclonal antibody. Interestingly, only 15 of 28 tested strains were neutralized with this antibody.

TABLE 3. Disappearance of polioviruses from sewage waters in Helsinki after the oral poliovirus vaccine campaign in 1985

Month	Day	Presence of virus in location[a]:				Isolation of serotype[b]:		
		A	B	C	D	1	2	3
March	7	+	+	NT	NT	+	+	+
	14	+	+	+	+	+	+	+
	21	NT	+	NT	+	+		+
	28	+	+	NT	NT		+	+
April	3	+	+	NT	NT		+	+
	11	+	+	+	+		+	+
	18	NT	+	NT	+		+	+
	25	+	+	NT	NT		+	+
May	2	+	+	NT	NT		+	+
	9	−	+	+	+		+	+
	16	+	−	NT	NT	+		
	23	−	−	NT	NT			
	30	+	+	NT	NT		+	+
June	6	−	−	NT	NT			
	13	−	−	NT	NT			
	19	−	−	NT	NT			
	26	+	−	NT	NT			+
July	4	−	−	−	−			
	11	−	−	NT	NT			
	18	−	−	NT	NT			
	25	−	−	NT	NT			
August	1	−	−	−	−			
	8	−	−	NT	NT			
	15	−	−	NT	NT			
	22	−	−	NT	NT			
	29	−	−	NT	NT			

[a] A, Herttoniemi; B, Viikki; C, Lauttasaari; D, Munkkisaari. See footnote *a* of Table 1.

[b] +, Serotype indicated was isolated from at least one of the tested specimens.

TABLE 4. Viruses identified in sewage specimens subsequent to the nationwide oral poliovirus vaccine campaign in Finland in 1985

Location[a]	Viruses identified in sewage[b]				
	May	July	September	October	November
Ivalo	P2, P3	CB4	E11, Ad3	NT	CB4
Joensuu	NT	NT	NT	NT	CB5
Jyväskylä	P3	P2, P3	CB4	NT	E6
Kajaani	P2, P3	P2	P3	E25	E19
Kotka	P2, P3	CB1	+	NT	−
Tampere	P3	−	E11	NT	Ad2
Turku	CB1	+	E22	NT	CB4
Vaasa	P3	CB5	CB5	NT	−

[a] See Fig. 1.

[b] Ad, Adenovirus; CB, Coxsackievirus group B; E, Echovirus; P, Poliovirus, +, unidentified nonpoliovirus, −, no virus isolated; NT, not tested.

In late May 1985 polioviruses of type 2 or 3 were found in six of the seven tested sites in other parts of the country. Two locations were poliovirus positive in July, and one was poliovirus positive even in September 1985 (Table 4). Poliovirus isolations from the July and September specimens were confirmed by reisolation, and the strains were tested for antigenic properties with neutralizing monoclonal antibodies by us and with absorbed hyperimmune sera (enzyme immunoassay by A. van Wezel, Bilthoven, the Netherlands [personal communication]) and were found to be vaccine related according to both tests.

Sewage specimens from Helsinki collected continuously with a fortnight to 1-month interval have been negative for polioviruses for 2 years now. Likewise, specimens collected in four other locations in Finland in June through October 1986 were negative for polioviruses. However, other enteric viruses have been frequently isolated from the specimens (Tables 4 and 5).

DISCUSSION

Our observations once again demonstrate the well-known fact that polioviruses circulating widely in the population can be readily isolated from sewage waters. Sewage water analysis also turned out to be a useful tool in evaluating the efficacy of a nationwide vaccination campaign with oral poliovirus vaccine. Polioviruses were isolated from the sewage still more than 2 months after the vaccination campaign ended. Occasional specimens were found to contain poliovirus even several months after the campaign. The latter isolates were found to be antigenically related to the attenuated Sabin strains used in the oral vaccine. Thus, they presumably have either replicated for a relatively long period in humans, perhaps infecting several successive hosts, or survived in the sewage network. Polioviruses, like other enteroviruses, are known to be capable of retaining infectivity for several months in natural waters (8).

Since several enteric viruses may circulate simultaneously in human populations, sewage water specimens are often likely to contain a mixture of different viruses. The standard isolation procedure may enrich preferentially one component of a putative virus mixture. However, we are using a set of cell cultures specifically aimed to reveal polioviruses. According to several years of experience in this laboratory (Lapinleimu and Stenvik, personal communication), rapidly growing nonpolio enteroviruses in sewage specimens do not usually replicate well in each of the three cell types used by us, whereas polioviruses do. Therefore, we believe that with

TABLE 5. Results of follow-up sewage waters in Helsinki for possible recurrence of wild-type polioviruses after the trivalent oral poliomyelitis vaccine campaign

Virus and serotype	Isolation of virus[a]															
	1985											1986				
	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5
Poliovirus																
1	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
2	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
3	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
Coxsackievirus																
A9	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
B1	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−
B2	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−
B3	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	+
B4	−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	+
B5	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−
B6	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
Echovirus																
6	−	−	−	−	−	−	−	+	+	+	−	+	−	−	−	−
7	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
11	−	−	−	−	−	+	−	+	−	+	−	−	−	−	−	+
20	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
Adenovirus																
1	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
2	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+
5	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−

[a] Months are numbered 1 through 12. +, Isolation of the indicated serotype at least in one of the several specimens tested during the month; −, all tested specimens during the month indicated negative for the serotype. Apart from the viruses shown above, one untyped adenovirus was isolated in June 1985, and two untyped, nonpolio cytopathogenic agents were isolated in July 1985.

this set of cell cultures we do not often miss polioviruses even when they are present as a minority in a putative mixture.

It was interesting to note that type 1 polioviruses were isolated only exceptionally after the vaccination campaign was ended, suggesting a relatively short excretion time of this serotype. This is in agreement with our studies on vaccine virus excretion in selected groups of vaccinees (7, 9). The excretion times for type 2 and type 3 polioviruses were similar and longer than that for type 1 in the two above studies. In this study we found both type 2 and type 3 polioviruses in sewage waters still later than 2 months after the vaccination campaign was ended. Since both the prevalence and the mean levels of neutralizing antibodies were known to be remarkably higher for type 2 poliovirus than for type 3 poliovirus in the Finnish population (5; K. Lapinleimu and M. Stenvik, unpublished data), one might have expected a longer excretion time for the type 3 vaccine virus. It is possible that the widespread circulation of the epidemic type 3 poliovirus resulted in a booster effect and thus influenced the excretion time of the attenuated type 3 poliovirus during the subsequent vaccination campaign.

No definitely wild-type polioviruses were isolated from the 139 sewage specimens studied after the vaccination campaign, reflecting the success in ending the outbreak. Of course, we cannot exclude the existence of small amounts of wild polioviruses in specimens containing relatively high concentrations of vaccine viruses or other enteroviruses. Although it is known that some attenuated type 3 poliovirus strains are not recognized by the monoclonal antibody used (G. C. Schild, personal communication), the fact that only 15 of 28 of the present sewage isolates were neutralized by the monoclonal antibody makes it difficult to conclude when, exactly, in early 1985 the circulation of the epidemic type 3 virus ceased.

The frequency of enterovirus infections in Finland and of enterovirus isolations from the sewage reaches annual peak values in the autumn and then decreases to a minimum in the winter and early spring (6). Accordingly, circulation of the epidemic type 3 poliovirus was rapidly decreasing in December 1984 through January 1985, before the oral poliomyelitis vaccine campaign was started, among the inhabitants of a village in the Helsinki district. In certain other parts of the country, however, the wild-type serotype 3 polioviruses were isolated from the sewage still in late January and early February 1985. The timing of the nationwide oral vaccination campaign to the natural minimum of the circulation frequency of the epidemic strain may have had a role in the successful eradication of the outbreak.

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