Occurrence of Enteroviruses in Community Swimming Pools

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Abstract: Municipal swimming pools and wading pools were examined for the presence of human enteric viruses using a portable virus concentrator at the site to concentrate viruses from 100-gallon to 500-gallon samples. Ten of 14 samples contained viruses; three of these were positive for virus in the presence of residual free chlorine. Enteroviruses were isolated from two pools which exceeded the 0.4 ppm free residual chlorine standard. This study appears to be supportive of recent evidence that indicates a higher incidence of enterovirus infection among bathers. All seven wading pool samples contained virus. Coxsackieviruses B3 and B4, poliovirus 1, and echovirus 7 were isolated. Total coliform bacteria were not adequate indicators of the presence of virus, as six of the samples were positive for virus but negative for coliforms. Total plate counts appeared to provide a better indication of the sanitary quality of the pool water, but viruses could still be detected in samples that met currently recommended bacterial levels. It is possible that swimming and wading pools may serve as a means of transmission of enteroviral disease, especially in children, during summer months. (*Am J Public Health* 1981; 71:1026–1030.)

Introduction

Recreational swimming is enjoyed by millions of people each year. However, this enjoyment is often reduced as a result of infection and illness, particularly ear infections (otitis externa) caused by bacteria such as *Pseudomonas*,[1,2] but also impetigo,[3] shigellosis,[4] and primary meningoencephalitis.[5,6] Although long suspected, transmission of viral infections in swimming pools[7–10] has been difficult to prove. Only recently has direct evidence for transmission of viral infections by swimming pools become available.

D'Angelo, *et al*,[11] established, both by epidemiological methods and by isolation of the virus from the water, that pharyngoconjunctival fever caused by adenovirus type 4 can be transmitted via swimming pools. Furthermore, recent workers[12–14] found a statistically significant relationship between swimming and gastrointestinal illness.

Early studies[11,15–20] (Table 1) sporadically detected enteroviruses in pools, but the methodology was not quantitative and epidemiological evidence was lacking. In addition, since chlorination is believed to disinfect pools adequately, this subject has not been investigated extensively. However, factors such as the well-known resistance of enteroviruses to chlorine, equipment failure, and lapses in efficient operating procedures could lead to transmission of viruses between swimmers in pools and in other swimming areas.[12,13,21,22] The purpose of our study was to survey several swimming pools in a municipal area to determine the frequency with which human enteroviruses could be detected in filtered, chlorinated swimming pool water using recently improved methods.

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Materials and Methods

Sample Source

Swimming pools surveyed in Houston, Texas were chosen because of their proximity to the laboratory and were located in both low-income and middle-income neighborhoods. All samples but one were run on weekdays at the pool site during the hot summer of 1980. The pools were all municipal and open to the public free of charge. The pools ranged in age from 10 to 35 years and differed in design. All were equipped with gas chlorinators and sand and gravel filters. Samples were collected between the hours of 10 am to 1 pm and 2 to 4 pm. The pools close daily from 1 to 2 pm.

Virus Concentration Methodology

Method A—100 gallons of swimming pool water were pumped into a 250-gallon plastic container and simultaneously dechlorinated with excess sodium thiosulfate. The pH of the water was adjusted to 3.5 with HCl. $AlCl_3$ (0.001 M final concentration) was added, and the water was pumped through a portable virus concentrator equipped with a pleated 0.25-μm fiber glass filter as previously described.[23,24] The mean efficiency of this method was 46 per cent (range 19–73 per cent), as determined in tests using poliovirus type 1 (LSc2ab) seeded into swimming pool water collected in 200-gallon containers.[23]

Method B—Water was dechlorinated by in-line injection of sodium thiosulfate using a Dema-203 or 204B injector[25] and was pumped through a charge-modified 1MDS Zeta-plus

TABLE 1—Previous Reports of Viruses Isolated from Swimming and Wading Pools

Pool Type*	Location	Viruses Isolated	Residual Chlorine (mg/l) Free	Residual Chlorine (mg/l) Total	Reference
S	Canada	parainfluenza 1	0.4	NR†	15
W	Canada	coxsackie B1	0	NR	16
W	New York	echo 3, 11	0	NR	17
S	Israel	echo 6, 7; coxsackie B6	NR‡	NR‡	18
S	Georgia	adeno 4	0	0.5	11
S	Germany	coxsackie B3	NR	0.3–0.5§	19
W	Soviet Union	coxsackie B1	NR	NR	20

*S = swimming pool; W = wading pool.
†NR = not reported.
‡Three of six pools had residual chlorine.
§Applied dose.

cartridge filter (AMF CUNO, Meriden, CT) without pH adjustment or salt addition; 100–500 gallons were processed in this manner. The mean efficiency of this method was 38 per cent (range 18–82 per cent).

Virus Elution and Reconcentration

The filters were eluted with 1 liter of 3 per cent beef extract at pH 10; the eluates were then neutralized and reconcentrated by an organic flocculation technique.[26] Briefly, the pH was adjusted to 3.5 with 0.05 M glycine, pH 2, followed by centrifugation for 5 min at 1500 rpm. The pellet was resuspended in 0.05 M glycine, pH 11.0, to raise the pH to 9.5 and the suspension was centrifuged for 30 min at 2500 rpm. The supernatant was retained, adjusted to neutral pH, and treated with antibiotics. The final volume was 10–20 ml.

Isolation and Identification of Viruses and Bacteria

Samples were assayed on BGM (buffalo green monkey) and MA104 cells by plaque assay and cytopathic effects (CPE) as previously described.[27] Isolates were identified by the LBM antiserum pools and use of specific antisera.[28]

Total and fecal coliforms, fecal streptococci, staphylococci, and total plate colonies were enumerated by membrane filter method[29] using either Millipore Growth Culture Media (Millipore Corp., Bedford, MA) or Nutrient Pad Kits (Nalge, Rochester, NY).

Measurements of Additional Factors

Free and total residual chlorine were measured with a DPD field kit (Hach Chemical, Ames, IA). Turbidity of the water samples was measured by a Hach portable turbidimeter. Other parameters measured included the bather load (number of adults and children in the pool at the time of sampling), air and water temperatures, and pH.

Results

Fourteen samples were collected and processed. Ten (71 per cent) were positive for virus at concentrations of 0.2 to 65 plaque-forming units (PFU) per 100 liters. Virus isolates included coxsackievirus B3 and B4, poliovirus 1 and echovirus 7.

Total coliform bacteria were detected in five of 14 samples. Total plate counts ranged from 1 to >300 per 100 ml. No fecal coliforms were detected. Fecal streptococci were detected in three of five samples, and staphylococci were detected in three of three samples.

In five samples, viruses were detected in the absence of total coliforms. In three samples viruses were detected in the presence of free chlorine and the absence of total coliforms. The results are summarized in Tables 2 and 3.

Non-microbiological factors (Table 3) included free and total chlorine residuals, which varied from 0 to 2.2 mg/liter and from 0 to 2.5 mg/liter, respectively. Only four of 14 met the recommended 0.4 mg/liter residual.[30] The pH values ranged from 6.3 to 8.5; 7.2–8.2 is the recommended value, but only six of the 14 samples were within these limits. Turbidity, as chlorine, showed a wide variance from a low of 1.5 to a high of 29. The high value was due to breakdown of circulation equipment; however, the pool was allowed to remain open. Turbidity and bather load values are listed in Table 3 along with the chlorine residuals and pH.

Discussion

The isolation of viruses from wading pools using older, less efficient methods of virus concentration has been reported (Table 1), particularly when chlorination was found to be inadequate. On the other hand, the isolation of viruses from full-sized chlorinated pools until recently was of suspected but unproven health significance. While previously pools could only be implicated in virus transmission, D'Angelo, *et al,*[11] clearly established this means of transmission for adenoviruses. Furthermore, other work by D'Alessio, *et al,*[14] showed that the odds ratio of enterovirus isolation from beach swimmers was more than three times greater than that from nonswimmers. Swimming in inadequately treated water appears to present a definite health risk.

In the present study, using the most advanced methods available, a high frequency of virus isolation (10 of 14

TABLE 2—Isolation of Viruses and Bacteria from Swimming Pools

Pool	Bacteria/100 ml: Total Coliforms	Bacteria/100 ml: Total Plate Count	Virus Concentration (PFU/100 liters)	Virus Identification	Filter type
2–1	0	1	1.7	echo 7, polio 1	F*
2–2	0	1	—	—	F
3	0	TN†	CPE‡	polio 1, echo 7	F
4–1W§	0	7	1.8	coxsackie B3	F
4–2W	0	7	5.8	coxsackie B3	Z
5	0	4	CPE	echo 7	F
6	0	5	—	—	F
8–1W	TN	TN	65.0	coxsackie B3	F
8–2W	TN	TN	37.4	coxsackie B3	Z
9–1W	TN	TN	8.5	polio 1	F
9–2W	TN	TN	8.8	polio 1	Z
13	0	36	0.2	coxsackie B4	Z
14	0	28	—	—	Z
15W	1	TN	—	—	Z

*F = Filterite 0.25NP; Z = Zeta-plus 1MDS.
†TN = too numerous to count (>300).
‡CPE = Virus detected by CPE only.
§W = Wading pool.

samples, or 71 per cent) was reported indicating the common occurrence of enteroviruses in swimming pools. The methods used in this study were largely designed for the isolation of enteroviruses, and it is highly probable that other enteric viruses were also present in the swimming pools. When considered with the above-mentioned epidemiological studies,[11–13] swimming pools may well play a role not only in the transmission of enterovirus infection but also in the transmission of viruses of the rotavirus and Norwalk groups.

The source of viruses is obviously swimmers and, in wading pools, almost exclusively children. Viruses can enter the pool through fecal material and nasal and pharyngeal secretions. Their survival is then dependent on a number of factors as discussed by Cabelli[12,31] and McLean,[15] including their number, disinfection (chlorination), presence of solids, dispersal, sunlight, filtration, pH, temperature, and the inherent resistance of the viruses themselves.[32–34]

Chlorination is the primary method of disinfection of swimming pools. The effectiveness of chlorine in pools is dependent upon the dose and form of chlorine present, free chlorine being more effective than the combined forms.[32] It has been recommended[30] that 0.4 ppm free residual chlorine be maintained. The data given here indicate that recommended levels are not always adequate and certainly are not always maintained. Furthermore, viruses were detected in two samples which exceeded the 0.4 ppm free residual chlorine standard. Besides dose, the presence of interfering compounds such as urine, ammonia, oils, dirt, and suspend-

TABLE 3—Pool Data

Pool	Approximate Pool Volume (gallons)	No. Adults	No. Children	Residual Chlorine (mg/l): Free Chlorine	Residual Chlorine (mg/l): Total Chlorine	pH	Turbidity (NTU)
2–1	6,000	0	13	0.0	0.0	6.3	2.2
2–2	165,000	10	80	0.3	0.3	6.6	2.2
3	310,000	10	30	0.2	0.2	7.4	2.0
4–1W*	6,700	6	35	0.0	0.2	7.2	16.0
4–2W	6,700	6	35	0.0	0.2	7.2	16.0
5	112,000	1	49	0.9	1.0	6.3	4.4
6	185,000	2	54	1.1	1.2	6.9	2.0
8–1W	6,000	3	12	ND	0.5	6.7	4.3
8–2W	6,000	3	12	ND	0.5	6.7	9.5
9–1W	6,700	4	30	0.0	0.2	7.3	29.0
9–2W	6,700	4	30	0.0	0.2	7.3	29.0
13	165,000	4	26	2.2	2.5	7.0	4.3
14	310,000	0	12	0.9	1.2	8.5	1.5
15W	6,000	2	12	0.5	0.7	7.9	11.0

*W = wading pool.
ND = no data.

ed solids also adversely affect disinfection by reacting with chlorine to form the less active combined forms such as chloramines. Furthermore, in the presence of organic solids viruses are protected to some degree from disinfection by chlorine.[35,36] Thus, the standards for swimming pool disinfection may have to be re-examined.

Limited dispersal of viruses in pools was demonstrated in an early study by McLean, *et al,*[33] to be due to rapid inactivation by halogens in the water. In that study, virus was detectable for only 20–60 seconds after injection from a moving raft, indicating that inactivation is rapid when the dose of halogen is adequate, and the pool is clean and free of swimmers. Our data indicate that viruses can survive low levels of chlorination in actively used pools, where the turbidity was often high.

The pH and temperature ranges at swimming pools usually are such that viruses could survive for long periods. Each factor plays a role in the ability of chlorine to inactivate viruses—pH by affecting the type of chlorine present, and temperature by affecting chlorine loss to the atmosphere. Sunlight is detrimental to virus survival, especially in shallow pools. However, its effect would be mitigated by the turbidity of the water, which increases with bather load.

Bacteria are used as indicators of swimming pool water quality. Current recommendations set limits of "not more than 15 per cent of the samples covering any considerable period of time shall either (a) contain more than 200 bacteria per milliliter, as determined by the standard (35°C) agar plate count, or (b) show positive (confirmed test) for coliform organisms in any of the five 10-milliliter portions of a sample or more than 1.0 coliform organisms per 50 ml."[30] Five of 14 samples failed to meet these standards. Total coliforms failed as indicators of viral pollution in all but the most polluted samples. Staphylococci were not tested for enough cases to comment on their usefulness as an indicator as has been suggested.[37] Since viruses are more resistant to chlorination than coliform bacteria, our findings are not surprising, and similar results have been reported in other waters.[38]

Cabelli[12] suggested that free chlorine residuals are of primary importance for monitoring water quality in pools and that if bacteria samples are to be taken, standard plate counts are recommended rather than coliform testing. The present study supports this view.

Standards have been proposed for viral quality of recreational water. Melnick[39] recommended that a limit of 1 infectious unit of virus per 10 gallons of recreational water be considered. This standard was exceeded in at least five of our samples. Because of the lack of epidemiologic data, such a standard is arbitrary and reflects limitations of current detection methodology for enteric viruses in water rather than disease risk.

As mentioned above, other workers have detected viruses in pools. This has not been thought to be a problem, because adequate chlorination was believed to inactivate the viruses. However, the present data indicate that the currently recommended levels of chlorine are not always adequate. A recent paper has recommended levels of 0.8 mg/liter,[40] but this remains to be proven. Additionally, a major cause of low chlorine levels is poor operating procedure and faulty equipment. The concern over chlorine levels which produce eye irritation is common. Purposely maintaining a low level has been responsible for at least one outbreak.[8]

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Three Late-Breaking Events for APHA Annual Meeting in Los Angeles

Harvard Public Health Alumni Association Banquet
Monday, November 2, 1981
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Tour of Ethel Percey Andrus Gerontology Center
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Tuesday, November 3, 1981, 2–5 pm

The visit will consist of a 25-minute introductory film on the Gerontology Center, followed by several presentations on the different divisions of the Center, and current research related to aging. Time will be allotted for questions and answers.

Pre-registration required before October 15th. Send name and address to Patricia J. Bush, Chairperson, Pharmacy Services Committee, DCFM, Georgetown University School of Medicine, Washington, DC 20007. Information on bus service between the LA Convention Center and USC will be sent to all registrants. Limit: 200

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