Assessment of swimmer behaviors on pool water ingestion

Laura M. Suppes, Leif Abrell, Alfred P. Dufour and Kelly A. Reynolds

ABSTRACT

Enteric pathogens in pool water can be unintentionally ingested during swimming, increasing the likelihood of acute gastrointestinal illness (AGI). AGI cases in outbreaks are more likely to submerge heads than non-cases, but an association is unknown since outbreak data are self-reported and prone to bias. In the present study, head submersion frequency and duration were observed and analyzed for associations with pool water ingestion measured using ultra high pressure liquid chromatography – tandem mass spectrometry. Frequency of splashes to the face was also quantified. Reliable tools that assess activities associated with pool water ingestion are needed to identify ingestion risk factors and at-risk populations. Objectives were to determine if the observed activities were associated with ingestion, and to test environmental sensor and videography assessment tools. Greater frequency and duration of head submersion were not associated with ingestion, but frequency of splashes to the face, leisurely swimming, and being ≤18 were. Videography was validated for assessing swimmer head submersion frequency. Results demonstrate ingestion risk factors can be identified using videography and urine analysis techniques. Expanding surveys to include questions on leisure swimming participation and frequency of splashes to the face is recommended to improve exposure assessment during outbreak investigations.

Key words | exposure factor, head submersion, ingestion, pool, recreational water, swimming

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INTRODUCTION

Recreational waterborne illness (RWI) in the United States was at an all-time high between 2007 and 2008 since reporting began in 1978, with 38 states reporting a total of 134 outbreaks (Yoder *et al.* 2008; Hlavsa *et al.* 2011). Ingestion was the most common source of exposure (60.4%), but direct skin contact (18.7%), inhalation (13.4%), mixed exposure routes (4.5%) and other (3.0%) were also identified. Health outcomes ranged from acute gastrointestinal illness (AGI), respiratory ailments, skin infections and irritations, to hospitalization. The causative agents in RWIs are as varied as the exposure route; however several trends are notable: (1) the majority of outbreaks (60.4%; 81/134) resulted in AGI; (2) the majority of outbreaks (86.5%; 116/134) and illnesses (96.5%; 13,480/13,966)

occurred in treated water venues; and (3) most were caused by the protozoa, *Cryptosporidium* spp. (44.8%; 60/134). Other etiological agents of RWIs between 2007 and 2008 include norovirus, *Giardia*, *Leptospira*, *Vibrio* spp., *Campylobacter jejuni*, and *Shigella sonnei*. Swimmer exposure to waterborne enteric pathogens is via the fecal oral route. Accurate assessment of pool water ingestion during swimming activities is important for assessing enteric pathogen infection risk. Swimming pool water ingestion can be measured by comparing cyanuric acid concentrations in swimmer urine with pool water (Briggle *et al.* 1981; Allen *et al.* 1982; Dufour *et al.* 2006).

Cyanuric acid is a chemical added to outdoor pool water to prevent photodegradation rates of chlorine, and

when ingested, is not metabolized by the human body but is completely excreted within 24 h (Andersen 1965; Allen *et al.* 1982). Cyanuric acid is present in household cleaning materials (bleach, cleansers, dishwashing compounds, and sanitizers) and the only known exposure route is ingestion (Allen *et al.* 1982; Cantu *et al.* 2000). Ingestion during swimming is therefore a primary exposure to cyanuric acid.

Reports following AGI outbreaks at swimming pools show cases are more likely to submerge heads than non-cases (Causer *et al.* 2006; Boehmer *et al.* 2009); however, the actual distribution of head submersion duration and frequency among swimmers is unknown, as the majority of outbreak and exposure assessment data are self-reported and can be biased. Further, activities such as lap and leisure swimming may lead to different water ingestion exposures if head submersion frequency and duration differ between activities, or if other micro-activities differ. The objectives of this study were to evaluate risk factors of ingestion and test exposure assessment tools. To achieve this, pool water ingestion was quantitatively measured using ultra high pressure liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS). Head submersion frequency and duration, and number of splashes to the face, were also quantified by videography and environmental sensor methods. The observed activities were analyzed for associations with pool water ingestion. Other activities and risk factors suspected of increasing pool water ingestion were noted during videography to make recommendations for future research. Quantitative activity and exposure information were also separated by age and activity for use in future risk assessments.

METHODS

Measuring pool water ingestion

Four pool sites and 126 swimmers were recruited in Tucson, Arizona between June and September 2012. Free and informed consent of the participants or their legal representatives was obtained using a consent form approved by the University of Arizona Human Subjects Research and Institutional Review Board. Swimmers arriving at each pool were approached by the research team,

provided with study objectives, and asked if they would participate by consenting to video recording, wearing an environmental sensor, and completing a self-administered questionnaire via tablet or home computer after swimming. All swimmers were asked to participate regardless of gender, age or other factors. Participants submitting urine were instructed to swim 45 minutes or more, and swimmers who did not submit urine swam about 1 hour, but were not restricted to a timeframe. Participants submitting urine ($n = 46$) were instructed not to swim 24 h before or after the swimming trial event. Each participant was given a sterile 3 L urine collection container (Fischer Scientific Company, 14-375-248) for a 24 h post-swimming composite sample and instructed to collect urine at each restroom visit for 24 h after exiting the pool. Participants were excluded if sufficient urine volumes based on age were not provided (Dorevitch *et al.* 2011). Urine volumes for age <10 had to exceed 150 mL; 200 mL for 11–16 years; and 300 mL for >17 years. Pool water samples were collected at the beginning of each observed swim 30 cm below the surface using 20 mL containers from four locations within each pool, transferred on ice, and stored at 4 °C. Pool water and urine were processed within 24 h of collection at the University of Arizona Medical Research Building and tested for cyanuric acid within 31 days of collection at the university's Arizona Laboratory for Emerging Contaminants using previously published methods (Cantu *et al.* 2000, 2001a; Smoker & Krynitsky 2008). To preserve samples, 170 µL of pool water or urine was pipetted into a 2 mL microcentrifuge tube. A 10% perchloric acid (ThermoScientific, Waltham, MA, v/v) and 1% metaphosphoric acid (ThermoScientific, w/v) solution was added to each tube. Samples were centrifuged at 13,500 g for 17 min to remove proteins, kept in the dark at 4 °C, and processed within 31 days.

Urine samples were cleaned by solid phase extraction (SPE) with strong anion exchange (SAX) cartridges (SampliQ Silica, 3 mL, 500 mg; Agilent, 5982–2035) on a benchtop SPE manifold (Vac Elute 12 Manifold; Agilent, 5982–9110). The cartridges were conditioned by gravity with 2.5 mL of acetonitrile, followed by 2.5 mL of 5% aqueous ammonium hydroxide. Cartridges were loaded by gravity with a mixture of 1.6 mL of 5% aqueous ammonium hydroxide and 1.2 mL of urine sample followed

by 2.5 mL of acetonitrile. Cartridges were dried under vacuum (−10 psi) for 30 s followed by elution of cyanuric acid with 1 mL of 4% formic acid in acetonitrile. Remaining eluent was collected by a final, short vacuum application before analysis by UHPLC-MS/MS. Pool water samples (without SPE) were diluted 1 : 20 before analysis by UHPLC-MS/MS.

Selected reaction monitoring data were recorded on a Quattro Premier XE triple-quadruple mass spectrometer (Waters Corp., Milford, MA) by electrospray ionization in the negative mode (ESI-); 2.0 µL of pool water and urine extracts were injected into an Acquity UHPLC (Waters Corp., Milford, MA) onto a Hypercarb graphite column (ThermoScientific, Waltham, MA, 50×2.1 mm, 3 µm) in an isocratic mobile phase of 50% (v/v) aqueous acetonitrile at 0.05 mL/min. Each urine and pool water sample was injected twice between blank MilliQ lab water in separate runs. Selected reaction monitoring chromatograms were obtained by monitoring the cyanuric acid transition 128 > 42 in ESI- with capillary, cone, and collision energy voltages of 2.9 kV, 22 V, and 12 V, respectively. The ion source temperature was kept at 120 °C and desolvation gas temperature was set to 250 °C. Nitrogen was used as both cone gas and desolvation gas, and high purity argon was used as the collision gas (9.25×10^{-3} mbar). Cyanuric acid peaks eluting at 4.7 min were integrated and quantified using TargetLynx Application Manager software (Waters Corp., Milford, MA). Standard curves were created from a 20 mg/L stock solution of cyanuric acid that was prepared by dissolving 100% cyanuric acid (MP Biomedical 0520871580) in MilliQ water and filtering through a 0.2 µm cellulose filter. A set of 11 calibration standards of 1.0, 2.0, 4.0, 7.0, 15, 31, 62, 125, 250, 500, and 1,000 µg/L were prepared by serial dilution with 10% methanol in MilliQ purified water. The UHPLC-MS/MS limit of detection (LOD) was defined as having a signal to noise ratio (S/N) greater than 3.0. The method LOD was determined by identifying the lowest concentration recovered from participant urine after blank subtraction.

Quality control measures taken to ensure accuracy and validity of analytical results included MilliQ water blank LC-MS injections, matrix blanks, and positive controls for pool water and urine. Pool water controls included a pool water blank (no cyanuric acid used at the pool) and a

15 µg/L cyanuric acid pool water spike. Pool water quality controls were not passed through SAX cartridges for consistency with pool water sample analysis. Urine control samples were passed through SAX cartridges and included a urine matrix blank, a MilliQ water field blank, a blank sample of deionized water with preservatives, and a 50 µg/L cyanuric acid urine spike. The 50 µg/L urine spike passed through a SAX cartridge was used to estimate percentage recovery of cyanuric acid. The deionized water-preservative control was made at the time urine and pool water were preserved. The MilliQ water field blank was collected by instructing a non-swimmer to add MilliQ water to a urine collection container over 24 h in a volume approximate to the participant's urination volume per-episode. Another non-swimmer provided a 24 h urine sample used for a urine matrix blank control.

Ingestion volumes were adjusted using blank subtraction correction for potential false positive measurements resulting from cyanuric acid carry-over between sample injections. Participants with average cyanuric acid concentrations in urine lower than blanks were considered measurements less than the method LOD. Urine samples with a $S/N \geq 3.0$, in which no cyanuric acid was detected, were also considered measurements lower than the method LOD.

Water ingestion volumes were calculated using cyanuric acid concentrations in urine and pool water (Equation (1)) (Briggle *et al*. 1981; Allen *et al*. 1982; Dufour *et al*. 2006). Urine containers were weighed before and after urine collection to determine gravimetric urine volume. Thus, dilution effects were adjusted by multiplying the ratio of cyanuric acid in urine to pool water by the total volume of urine collected over 24 h, during which all cyanuric acid was excreted and collected in the container.

$$
\begin{aligned}
\text{water ingestion (L)} \\
= \left([\text{cyanuric acid}]_{\text{urine}} \left(\frac{\mu g}{L}\right) \div [\text{cyanuric acid}]_{\text{pool water}} \left(\frac{\mu g}{L}\right) \right) \\
\times \text{urine volume (L)} \quad\quad\quad\quad\quad\quad\quad\quad (1)
\end{aligned}
$$

Videography and questionnaire

Activities of 64/126 swimmers were recorded on videotapes. Swimmers were excluded ($n = 2$) if swimming

off-camera for a portion of the time, not visible at the given camera angle, or not visible because of poor lighting conditions. Each swimmer was equipped with a color-coded headband and associated identification number, and was instructed to state the number into a camera before entering pool water. Two digital cameras with tripods were placed in different locations on pool decks to ensure each swimmer was filmed. After filming, each video was previewed to determine which angle best captured the swimmer's activity, and at what time individual swimmers entered the water. Water entry times of participants on the two videos were recorded. Each swimmer was observed for the entire length of the swim on one video-tape in 10 min consecutive segments by three trained reviewers. Reviewing was limited to 10 minute segments to minimize viewer fatigue (Ferguson *et al.* 2006). Video reviewers were trained by reviewing swimmers with pre-determined head submersion and face-splash quantities. Reviewer results were compared with pre-determined results to ensure variability of <10% (Ferguson *et al.* 2006). Reviewers were instructed to record full-head submersion to test correlations between submersion by videography and environmental sensors. Head submersion frequency and duration were quantified using a smartphone application (Stopwatch, the Official Timer Eflag Corporation), which records time in hours, minutes, seconds, and milliseconds (submersion duration), and the number of times the stopwatch is started (submersion frequency). Splashes of water to the face were tallied by each reviewer. Four of 64 swimmers (6.3%) were viewed twice to estimate method variability. Thirty-five of 64 swimmers were analyzed with paired urine samples. Total time in water was quantified by viewing videos and used to adjust pool water ingestion volumes (mL/h), submersion frequencies (frequency/h), and submersion duration (minutes submerged/h). Age, gender, and type of swimming activity (splashing, playing, diving, wading, sitting, and lap swimming) were determined by a self-administered questionnaire completed post-swim on tablets or at home by computer. The questionnaire can be accessed at www.ghi.arizona.edu. Participants reporting both lap and leisure swimming were categorized into the activity group they spent >50% of the observed swim performing. Activity duration was determined during videography.

Environmental sensors

The temperature-logging environmental sensors worn by all swimmers reviewed by videography ($n = 62$) were developed by attaching i-Button thermometers (Thermochron DS1922L) to the light mounting bracket on modified elastic headbands (1200 Lumens 6 LED 3 Mode Headlamp) (Figure 1). The i-Button is water resistant, has a temperature range from -40 to $85\,^{\circ}$C, is accurate to $\pm 0.5\,^{\circ}$C, and can store up to 8,192 readings logged every 1 to 600 s. Fifteen headbands were color-coded with vinyl tape (Fischer Scientific 19040024) and assigned a number. The i-Buttons were programmed to begin logging a temperature every 3 s with One-wire Viewer Software (Maxim Integrated) a half hour before swimmers entered the water. Up to 15 swimmers per site visit (10 visits) were provided with a color-coded headband and identification number after signing a consent form approved by The University of Arizona Human Subjects Research and Institutional Review Board. Temperatures were uploaded using One-wire Viewer Software for analysis. Measurements lower than the air temperature by $0.5\,^{\circ}$C were considered a head submersion event. Head submersions were identified by declines in the temperature output. Total decline events $\geq 0.5\,^{\circ}$C were counted to quantify head submersion frequency, and the total time (seconds) of each decline event was recorded as the time between head submersion and head emergence and added to the total time of all events to determine head submersion duration. The i-Button was pre-tested in a controlled environment to examine the instrument's ability to record a lower temperature in water.

Figure 1 | Environmental sensor.

Data analysis

STATA Statistics/Data Analysis version 11.0 software (College Station, TX) was used to perform statistical tests. Pearson's correlation and unpaired *t*-tests were applied to determine associations between head submersion durations and frequencies and frequencies of receiving a splash to the face, and pool water ingestion in all swimmers, as well as child, adult, lap, and leisure swimmers. A χ^2 test was used to assess age differences in lap and leisure swimming groups. Swim duration, age, average duration of pool visits throughout the year, and frequency of receiving a splash to the face were included in a stepwise regression model using backwards selection to identify influential variables on pool water ingestion. Videography and environmental sensor methods were analyzed with a paired *t*-test. Associations were considered statistically significant at a p-value <0.05 (95% confidence).

RESULTS

Thirty-eight of 46 (82.6%) urine samples were usable, and 35 of the 38 participants (92.1%) were analyzable on video. The three swimmers not analyzable by video were either swimming off-camera for a portion of the time, not visible at the given camera angle, or not visible because of poor lighting conditions. The eight swimmers with unusable urine samples either did not submit a questionnaire (no data on age or activity for statistical analysis, $n = 7$), or submitted a urine sample below the required volume ($n = 1$). Twenty-nine additional swimmers who did not submit urine were observed on video (total viewed, $n = 64$) (Table 1). Four of 64 participants did not report age or swim activity type (lap or leisure). Ten participants (six children and four adults) reported both lap and leisure swimming activities on the questionnaire. All spent >50% of the observed swim engaging in leisure activities. Ages ranged from 5 to 52 years.

Videography method validation

The average head submersion frequency and standard deviation reported by two video reviewers on the same four swimmers was 134.3 ± 12.4 submersions (8.5% variation

Table 1 | Measured ingestion, videography, and environmental sensor sample sizes by age and activity

Data source	All participants (%)	Adults (%)	Children (%)
All participants	64	26 (40.6)[a]	34 (53.1)[a]
Ingestion	35 (54.7)	20 (57.1)	16 (45.7)
Videography	64 (100)	26 (40.6)[a]	33 (53.1)[a]
Environmental sensors	41 (64.1)	16 (39.0)[a]	22 (56.7)[a]
Ingestion and videography	35 (54.6)	19 (54.3)	16 (45.7)
Ingestion and environmental sensors	24 (37.5)	11 (45.8)	13 (54.2)

[a]Four of 64 participants did not report an age on the questionnaire. All participants with measured ingestion values reported age.

from the mean). Durations between reviewers differed on average ±1 min 19 s (average, 8 min 8 s; 12.8% variation from the mean), and splashes by ±2.1 (average, 5.25; 27.8% variation from the mean). Acceptable inter-observer variability in exposure assessment (variation between reviewers) by videography is <10% (Ferguson *et al*. 2006).

Environmental sensors, videography, and water ingestion

Sixty-four swimmers were recorded on video-tapes, and 35 of 64 had measured ingestion values. Only swimmers with reliable videography results were considered when comparing ingestion and head submersion. Twenty-four participants with measured ingestion values had analyzable environmental sensor data. No associations were found using Pearson's correlation test between pool water ingestion and head submersion frequency or duration measured by videography ($p > 0.3445$ and 0.4570, respectively), but swimmers ingesting more water tended to have fewer head submersions (Figure 2), suggesting greater head submersion frequencies and durations are not driving pool water ingestion as initially hypothesized. Swimmers engaging in less frequent head submersion were suspected to have participated in other micro-activities associated with ingestion not quantified in this research. Based on videography behavior assessment, there was no visible trend between water ingestion and head submersion durations.

Head submersion and duration measured by environmental sensors showed poor accuracy with videography
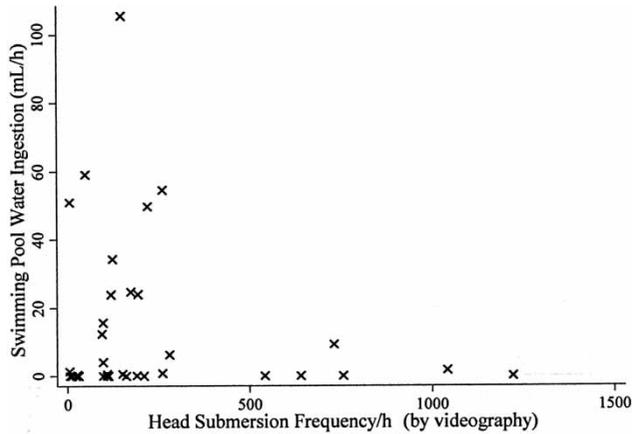
Figure 2 | Swimmer head submersion frequency/h swimming (by videography) and pool water ingestion. Swimmers ingesting pool water tended to have lower head submersion frequencies (95% CI: [−0.086, 0.067]).

measurements when compared using a paired t-test. Submersion frequency was under-estimated by the sensors ($p < 0.000$), and duration was over-estimated ($p < 0.001$) relative to the video analysis estimates. Average submersion frequency from videography was 199.1 submersions/h, and 8.5 submersions/h from environmental sensors. The average submersion duration per hour of swimming was 7 min, 51 s from video data, and 22 min 7 s from environmental sensors (Table 2). Environmental sensor data were therefore not used for identifying ingestion exposure micro-activity factors.

Two distinct types of swimmers were observed during this study, recreational swimmers who engaged in leisure time activities in the water (splashing, playing, diving, wading, sitting) and lap swimmers who engaged in serious, vigorous exercise in the water. The former we will describe as leisure swimmers and the latter as lap swimmers throughout the

text. The most influential parameter identified by stepwise regression on pool water ingestion was frequency of receiving a splash to the face ($R^2 = 0.3281$), which was higher among leisure swimmers (Pearson's correlation test: $p < 0.0003$). The swimmer with the highest ingestion value (105.5 mL/h) was 10 years old and received 33 splashes to the face, which was above average (all swimmers on average received 12.5 splashes to the face; min: 0, max: 47, SD: 12.16). The swimmer with the most splashes to the face (47 splashes) ingested 35.1 mL/h of pool water and was a leisure swimmer. Leisure swimmers were more likely using Pearson's correlation test to have less frequent head submersion ($p < 0.000$), a micro-activity that tended to be associated with higher ingestion rates. A χ^2 test indicated children were more likely to engage in leisure swimming than adults ($p < 0.001$).

Of all participants, 40 reported leisure swimming, defined as splashing, playing, diving, wading, or sitting (26/40 had measurable cyanuric acid), and 20 were lap swimmers (9/20 had measurable cyanuric acid). An unpaired t-test identified that leisure swimmers are more likely to ingest pool water than lap swimmers ($p < 0.024$) (Table 3). Submersion duration did not differ between the groups using an unpaired t-test ($p > 0.510$). The five highest pool water ingestion measurements were all child (≤ 18) leisure swimmers. Adults ingested 22.3 mL/h less than children, which was a significant difference using an unpaired t-test ($p < 0.0046$). All children with measurable cyanuric acid were leisure swimmers. Ten leisure swimmers were >18.

Cyanuric acid analysis

A paired t-test indicated cyanuric acid concentrations between injection runs differed ($p < 0.0420$). When average

Table 2 | Sensor and video analysis method comparison. The environmental sensors over-estimated head submersion duration, and under-estimated head submersion frequency

	Frequency of head submersions/h		Duration of head submersions (min:s/h)	
	Videography	Environmental sensor	Videography	Environmental sensor
Mean	199.1	9.6	7:51	22:07
SD	±256.3	±5.2	±7:42	±17:21
Min	4.5	1.37	0:23	0:23
Max	1,222	22.2	32:38	66:00

Table 3 | Pool water ingestion by activity and age group among video-taped participants

		Ingestion (mL/h)		
Group	n	Mean	SD	Range
All swimmers	35	13.7	24.0	0–105.5
Adults	19	3.5	11.7	0–50.9
Children	16	25.7	29.2	0.9–105.5
Lap swimmers	9	1.6	3.13	0–9.19
Leisure swimmers	26	17.8	26.6	0–105.5

cyanuric acid concentrations carried into blank MilliQ lab water were subtracted from the injection averages of each urine or pool water sample, no statistically significant difference was found using a paired *t*-test between concentrations ($p > 0.6237$). Thus, the reported ingestion volumes were adjusted for cyanuric acid carry-over.

The UHPLC-MS/MS LOD, 0.263 µg/L, is the lowest measured concentration in urine with a $S/N \geq 3$. The method LOD was determined by identifying the lowest calculated ingestion volume after blank subtraction corresponding to a concentration with a $S/N \geq 3$, and is 0.05 mL.

Cyanuric acid recovery from pool water was $103.3 \pm 0.42\%$ based on spiked pool water controls, comparable to efficiencies found by other researchers using similar techniques (Cantu *et al.* 2001a; Dorevitch *et al.* 2011). The recovery of cyanuric acid in urine after SPE was 6%, compared with 32.7% by Dorevitch *et al.* (2011). The majority of cyanuric acid (66.8%) is suspected to have remained in the SAX cartridge following elution steps.

DISCUSSION

Environmental sensors, video analysis, and water ingestion

Reliable tools that estimate water ingestion during swimming are needed to assess ingestion risk factors and groups most at risk of contracting RWI. This study empirically assessed pool water ingestion and associated ingestion volumes with observed activities and behaviors. Novel exposure assessment methods were applied to quantify cumulative time under water, frequency of head submersion, number of splashes to the face, and type of swimming activity. Associations between these parameters and pool water ingestion measured in lap, leisure, adult and child swimmers were compared using videography, environmental sensing, questionnaire, and pool water ingestion quantification techniques. Data from videography and environmental sensor methods were analyzed for significant associations with pool water ingestion, age, and lap and leisure swimming.

Methods used to quantify head submersion frequency by videography were reliable based on inter-observer variability rates <10%, the accepted rate in other videography research

(Ferguson *et al.* 2006). Method variation for submersion frequency was 8.5%. Variability in the submersion duration and face-splash quantification methods were higher, however, indicating a need for improvement (variability in swim duration and face-splash quantification were 12.8% and 27.8%, respectively). Given the low variability between submersion counts quantified by reviewers, the videography method can be reliably applied in future research that assesses head submersion frequency. Without quantitative information on behaviors and activities related to head submersion, inferences about pool water ingestion may be inaccurate.

Comparing videography results with environmental sensors showed the sensors over-estimated swimmer time under water and under-estimated head submersion frequency relative to video analysis estimates because the instruments did not record submersion events that lasted <3 seconds. i-Button thermometers were pre-set to read a temperature every 3 seconds, yet submersions lasting <3 seconds were observed during video analysis. When submersion frequencies are adjusted using a threefold multiplier, sensor estimates are still under-estimated (26 vs. 199.1 submersions/h). The instrument was likely not submerged long enough by participants to capture all submersions, despite controlled trials suggesting 3 second intervals were adequate. Pre-setting the sensors to record temperatures every 1 second rather than every three may improve the instrument's sensitivity in future studies. The environmental sensor was not effective in this study, but can potentially be applied as a head submersion assessment tool in future swimmer exposure assessment if re-calibrated, as the i-Button thermometers were able to identify temperature differentials between air and water. Improvement of method sensitivity may resolve the instrument's capability to record temperature change when it occurs more often than every 3 seconds.

Contrary to RWI outbreak reports and our hypothesis, less frequent submersion tended to be practiced by swimmers ingesting more pool water. Video reviewers noted less frequent head submersion and receipt of more splashes to the face among younger, leisure swimmers who tended to ingest higher volumes of pool water than comparison groups. We recommend additional activities and behaviors be included on outbreak questionnaires or tools, including

engaging in leisure swimming and receiving a splash to the face, as both activities were associated with increased pool water ingestion that was empirically measured.

Leisure swimmers ingested more pool water and submerged their heads less frequently than lap swimmers. During videography, analyzers noted leisure swimmers having short and inconsistent submersions, and lap swimmers consistently and frequently submerging heads during activities like the front crawl. Lap swimmers also appeared to hold their breath under water more frequently than leisure swimmers. Some of the most advanced swimmers had the longest submersion durations because they were able to swim a full half-lap without breathing. Higher skill level may therefore be a factor associated with longer and more frequent head submersion, but less water ingestion. These behavioral differences help explain why water ingestion was elevated among leisure swimmers who, in contrast to the initial hypothesis, had fewer head submersions than lap swimmers. Information from the activity analysis also helps explains why previous researchers have found children ingest more pool water than adults. These results are useful for designing future exposure assessments that should include inquiries or observations of swimmer age, engagement in leisure swimming, and receipt of splashes to the face.

Water ingestion could also be associated with leisure swimming because all children leisurely swam. No difference was found when adult lap and leisure ingestion rates (children excluded) were compared to test this, suggesting ingestion differences between all lap and leisure swimmers are attributable to child leisure swimmers. Children are believed to be engaging in 'micro-activities' during leisure swimming that lead to pool water ingestion other than greater head submersion frequency and duration. A confirmed micro-activity associated with ingestion was receiving a splash to the face. This association could be due to water entering the mouths of swimmers during a splash, or because there is a relationship between being splashed in the face and participating in boisterous activities that lead to water ingestion. Three other micro-activities observed in children and suspected to lead to ingestion were 'bobbing' at the water surface, spouting water for fun, and intentionally allowing pool water to enter the mouth (whether the water was ingested is unknown based on videography, but is

suspected based on ingestion measured by UHPLC-MS/MS). Video reviewers noted less frequent head submersion among younger, leisure swimmers ingesting high volumes of pool water because of 'bobbing' at the water surface with mouths open, as opposed to full head submersion (the activity quantified by videography). Younger swimmers were also observed intentionally allowing water into their mouth and spitting or spouting pool water. Observations by video reviewers suggest children are ingesting more pool water yet engaging in less frequent head submersion because of these activities, which may be related to skill. Bobbing at the water surface while spitting water occurred among younger swimmers who appeared to have difficulty in keeping mouths above water and touching the pool bottom. We hypothesize that 'bobbing' at the water surface is an activity within leisure swimming that leads to pool water ingestion. This behavior can be quantified comparing frequency of <1 s head submersions between age and activity groups, and should be tested as a potential predictor of pool water ingestion in future research. If found to predict ingestion, frequency of <1 s submersions will serve as a more accurate exposure assessment variable than age, as ingestion differs within child age groups.

Pool water ingestion among young, competitive lap swimmers has been empirically assessed in previous research (Allen *et al.* 1982). Results from Allen *et al.* indicate young, competitive swimmers ingest three and one-half times more pool water than non-lap swimmers. This finding is inconsistent with results from the present study, likely because all lap swimming participants were adults. The finding does, however, support our hypothesis that young swimmers, regardless of engagement in leisure or lap swimming, ingest more water than adults because they are less skilled. Future exposure assessments should compare water ingestion rates between child and adult lap and leisure swimmers to confirm skill as an exposure factor related to ingestion.

This study successfully identified activities and behaviors associated with increased pool water ingestion, validated a videography method for assessing swimmer head submersion frequency, and identified behaviors and characteristics suspected to increase pool water ingestion. Quantifying and comparing pool water ingestion with frequency of spitting, spouting, and allowing pool water into the mouth are also recommended for future research. Assessing skill as an

ingestion predictor is also recommended, and can clarify why multiple studies have found children ingest more pool water than adults. Comparing illness probabilities between child, adult, lap, and leisure swimmers by risk assessment is also recommended to identify vulnerable groups and where healthy swimming education efforts should focus.

Cyanuric acid analysis

Cyanuric acid concentrations in urine appear to be underestimated in this study. The recovery analysis following SPE indicates 94% of cyanuric acid was lost during urine clean up. Cyanuric acid recovered in blank controls suggests carry-over from concentrated samples or spikes can occur during injection cycles. Average cyanuric acid concentrations in blank MilliQ water from each injection cycle were subtracted from urine samples to adjust for carry-over. Based on these findings, it is believed more cyanuric acid was ingested by study participants than indicated after data processing. This may explain the inconsistency between ingestion volumes reported in this study and the Dufour *et al.* (2006) study, in which no recovery efficiency or use of blank subtraction was reported (although carry-over into blanks was not reported, developers of the method found cyanuric acid concentrations between sample injections differed by 0.3–1.5% (Cantu *et al.* 2000), suggesting either cyanuric acid carry-over or instrument error occurred). Children ingested pool water at 49 mL/h, and adults ingested 21 mL/h in the Dufour *et al.* (2006) study, compared with 25.7 and 3.5 mL/h in the present study. When cyanuric acid in blanks was not subtracted, the average ingestion of pool water among swimmers in this study was 32.1 mL/h; 59.2 mL/h for children and 9.22 mL/h for adults. The range of ingestion was 0–225 mL/h, compared with 0–205 mL/h reported by Dufour *et al.* Ingestion volumes without blank subtraction are more consistent with previous research, but were not used in the present study due to suspected false-positives/over-estimation of cyanuric acid concentrations from carry-over during UHPLC-MS/MS analysis.

Although a recovery percentage was not reported by Dufour *et al.*, the method developers (Cantu *et al.* 2000, 2001a) recovered cyanuric acid in urine between 89 and 112% compared with 6% in this study, likely due to different SPE and analysis techniques. Methods by Cantu *et al.* (2000)

were also only reproducible for quantifying cyanuric acid ranging 500–125,000 µg/L, which does not fit the lower range of cyanuric acid detected in this study (0.263–900 µg/L). Method sensitivity was higher using UHPLC-MS/MS. The lowest concentration detected and thus the LOD was 0.263 µg/L, compared with a LOD of 100 µg/L found by Dufour *et al.* The inherently high sensitivity of tandem mass spectrometry detection is the reason pool water was diluted 1:20 in this study. Cyanuric acid detected above 900 µg/L was not considered a reliable measurement, as the highest point on the calibration curve was 900 µg/L in this study. A 1 : 20 dilution was considered sufficient because cyanuric acid is typically maintained at between 50 and 100 mg/L in pool water (Johnston 1999).

Cantu *et al.* (2001a) applied three stacked SPE cartridges eluted with hydrochloric acid and dichloromethane. The purpose of using three cartridges was to remove interfering compounds, such as creatinine, in urine prior to analysis. A single SPE cartridge was applied in this study because the extent of interfering compound removal by Dufour *et al.* (applying methods by Cantu *et al.* (2001a)) was not necessary, as tandem mass spectrometry detection is more specific, as well as more sensitive, than UV detection.

Other researchers using similar methods also experienced problems analyzing cyanuric acid in urine. Problems included low recovery efficiencies, interference from matrix substances, and variability between analysis techniques for Dorevitch *et al.* (2011). Dorevitch *et al.* compared cyanuric acid recovery in urine using HPLC-MS/MS and liquid chromatography-diode-array UV detector, and found the HPLC-MS/MS method performed better. Recovery efficiency was 32.7 ± 7.1%. The researchers applied methods by Cantu *et al.* (2001b, c) and Smoker & Krynitsky (2008) using a Hypercarb graphite column and electrospray ionization with tandem mass spectrometry, but applied different urine cleaning methods than our study. Pool water was not diluted because the calibration curve ranged from 0.78 to 78 mg/L, and thus reliably assessed cyanuric acid concentrations typical in pool water. A 7.0 mL urine sample was cleaned with four methyl tert-butyl ether elutions by SPE, reconstituted in HPLC grade water, sonicated, filtered, and analyzed by Dorevitch *et al.* (2011). Only 4.2% (27/665) of urine samples were usable due to insufficient removal of interfering substances in urine

during SPE. Although the percentage cyanuric acid recovery was 26.7% lower in this study, 82.6% of urine samples were usable compared with 4.2% by Dorevitch *et al.*

The inconsistencies in method performance between analytical instruments and SPE techniques confirms that more research is needed to develop a reliable, inexpensive method for quantifying cyanuric acid in urine, and thus pool water ingestion in swimmers. The noted inconsistencies suggest urine processing contributes largely to the success of a method. In this study we were unable to improve recovery efficiency by combining cyanuric acid extraction and analysis techniques from different methods. In future research, a single method developed for extraction and analysis should be applied when analyzing urine for cyanuric acid to avoid problems experienced in this and other studies. The advantages of UHPLC-MS/MS in analyzing cyanuric acid should also be studied. No other cyanuric acid quantification methods have achieved an LOD below 1 μg/L. A method able to detect cyanuric acid in the μg/L range allows broader application of ingestion measurement in swimmers who use pools with low cyanuric acid, and thus may improve exposure assessment.

CONCLUSIONS

This study successfully identified activities and behaviors associated with increased pool water ingestion, validated a videography method for assessing swimmer head submersion frequency, and identified behaviors and characteristics suspected to increase pool water ingestion. Less frequent head submersion appears to be associated with greater pool water ingestion rates because these swimmers were younger and likely less skilled than adults. Video observations suggest children are 'bobbing' with mouths open at water surface to stay above water, and intentionally spitting and spouting water. Outbreak tools should assess leisure activity engagement and number of splashes received to the face among cases and non-cases, as both activities were associated with increased pool water ingestion. Assessing skill as an ingestion predictor is recommended for future swimming exposure assessments to clarify why children ingest more pool water than adults. Quantifying and comparing pool water ingestion with frequency of spitting,

spouting, and allowing pool water into the mouth are also recommended for future research. Comparing illness probabilities between child, adult, lap, and leisure swimmers by risk assessment is also recommended to estimate infection risks from swimming in treated recreational water among different age and activity sub-populations. Risk estimates are needed to identify future research needs related to treated recreational water to improve swimmer health.

ACKNOWLEDGEMENTS

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