water

MDPI

Article

Occurrence of *Cryptosporidium* Oocysts in Leisure Pools in the UK, 2017, and Modelling of Oocyst Contamination Events

Rachel M. Chalmers [1,2,*], Lester P. Simmonds [3], Martin Wood [3], Megan Luxford [4], Rob Miller [5] and Rob Johnston [6]

1 Cryptosporidium Reference Unit, Public Health Wales Microbiology and Health Protection, Singleton Hospital, Swansea SA2 8QA, UK
2 Swansea University Medical School, Swansea University, Singleton Park, Swansea SA2 8PP, UK
3 Pool Sentry Ltd., Dale Cottage, Stanton Dale, Ashbourne DE6 2BX, UK; lester@poolsentry.co.uk (L.P.S.); martin@poolsentry.co.uk (M.W.)
4 IDEXX Technologies Ltd., Units 1B & 1C, Newmarket Business Park, Studlands Park Avenue, Newmarket, Suffolk CB8 7ER, UK; Megan-Luxford@idexx.com
5 Latis Scientific Ltd., Unit C1 Acorn Industrial Park, Crayford, Kent DA 1 4AL, UK; Rob.Miller@latisscientific.co.uk
6 Public Health England, Seaton House, London Road, Nottingham NG2 4LA, UK; Rob.Johnston@phe.gov.uk
* Correspondence: rachel.chalmers@wales.nhs.uk

Abstract: *Cryptosporidium* is a major cause of diarrhoea outbreaks linked to swimming pools, but little is known about the frequency of contamination. The primary aim was to investigate the occurrence and concentration, through sampling and modelling, of *Cryptosporidium* oocysts in leisure pools. Secondary aims were to compare detections with operational parameters, provide the evidence-base for guidance, and improve sampling capacity and interpretation for public health investigations. Up to 1000 L pool water was sampled during swim sessions once weekly for 10 weeks from 8 August 2017 at six volunteer pools. Oocysts were detected by microscopy in 12/59 (20%) pool water samples, at least once in each pool; 8/12 (66%) detections were in August when bather loads were highest. At three pools, 1 L filter backwash was sampled weekly and oocysts were detected in 2/29 (7%) samples, following detections in pool water. The probabilities of a bather contaminating the pool ranged from 1 in 1000 to over 1 in 10,000. Monte Carlo analysis showed that when high bather numbers caused contamination on over 70% of days, multiple events per day were more likely than single events. In these generally well-managed leisure pools, *Cryptosporidium* risk related to high bather loads. We conclude that public awareness campaigns for bather hygiene, and reminding pool operators of current guidance for managing faecal accidents, should be ahead of peak swim season.

Keywords: backwash; *cryptosporidium*; occurrence; oocyst counts; swimming pool water; probability modelling

Citation: Chalmers, R.M.; Simmonds, L.P.; Wood, M.; Luxford, M.; Miller, R.; Johnston, R. Occurrence of *Cryptosporidium* Oocysts in Leisure Pools in the UK, 2017, and Modelling of Oocyst Contamination Events. *Water* **2021**, *13*, 1503. https://doi.org/10.3390/w13111503

Academic Editor: Athena Mavridou

Received: 21 April 2021
Accepted: 26 May 2021
Published: 27 May 2021

1. Introduction

The protozoan parasite *Cryptosporidium* is an important cause of gastroenteritis and the most frequently identified pathogen in outbreaks linked to treated recreational waters, especially "leisure" swimming pools, in the United Kingdom (UK) [1,2] and United States (US) [3]. *Cryptosporidium* oocysts (4–6 µm diameter) are shed in high numbers in the faeces of symptomatic patients, and to a lesser extent by recuperating patients and asymptomatic carriers. However, there is a significant risk of infection and illness from ingestion of even small numbers of oocysts [4]. The oocyst wall confers high chlorine resistance on the parasite; whereas bacteria and viruses are inactivated in minutes, *Cryptosporidium* requires up to 10.6 days exposure in water containing 1 mg/L of residual chlorine at pH 7.5, 25 °C, to achieve a 3 $\log_{10}$ reduction in infectivity [5]. *Cryptosporidium* control, therefore, necessitates good pool construction, hydraulics, operation and management, prevention of contamination, effective removal of oocysts by optimal filtration, and inactivation by secondary disinfection with ultraviolet (UV) light or ozone [6,7].

Although oocyst concentration/L of pool water has been identified as the most influential factor on the probability of infection with *Cryptosporidium* in swimming pools [8], it is not tested for routinely and there are no quantitative standards. Furthermore, oocyst concentration can also contribute the greatest degree of uncertainty in risk of infection estimates [9]. Detection is dependent on the efficient recovery of oocysts from samples, and oocyst recovery data from the testing laboratory should be included if oocyst concentrations are to be used to infer health risks, to ensure that risk is not underestimated [10].

Few structured, prospective sample surveys of *Cryptosporidium* in swimming pools have been published and it is difficult to compare results as different sampling frames, methods, detection assays, and outcome measures have been used (Table 1). For example, some report sample prevalence based on the presence/absence of oocysts or *Cryptosporidium* DNA, while others report oocyst counts by microscopy (Table 1). Standard methods for testing drinking water for *Cryptosporidium* have been used for pool water but there are no published validation data for this sample type. The published survey data (Table 1) indicate that to quantify risk from *Cryptosporidium*, pools need to be: (1) sampled repeatedly, or in sufficient numbers, as the parasite may not be present persistently; (2) sampled by passing large volumes of pool water through appropriate filter modules for representative sampling; (3) tested using validated quantification methods; and (4) additionally investigated for appropriate parameters such as bacterial indicator organisms and physiochemical properties to monitor the pool water treatment. Few associations have been reported between pool parameters and *Cryptosporidium* detections (Table 1), although one study in China reported a strong correlation between parasite detection and urea in pool water [11]. Epidemiological evidence suggests seasonal sampling may be more valuable for infection risk assessment than year-round; swimming pool-related cryptosporidiosis outbreaks are reported most frequently in the summer months (June, July and August) in the US [3] and in the summer and mid-autumn (July through to October) in the UK [1,2]. A study in China reported significantly more samples positive in summer (August) than in spring (May) (Table 1; [11]).

The interpretation of survey data is confounded further by how the numbers of oocysts relate to the risk to bathers; the concentrations of oocysts in pool water are likely to vary widely in time and space, with very high relative concentrations in the vicinity shortly after a faecal release. The volume and movement of water in the pool will affect the extent to which oocysts are diluted and distributed, and filter efficiency will determine removal. For example, for a well-mixed pool with filters that have a 90% removal efficiency, it is expected that about 57% of oocysts would be removed in each turnover [12], making the concentration in the pool very dynamic. Hence there is a challenge in assessing the risk to bathers in terms of the peak concentrations that they might have been exposed to on days with detectable contamination.

Currently, there are no published baseline data from structured surveys of *Cryptosporidium* in swimming pools in the UK and it is not known how frequently oocysts are present or at what concentrations. The primary aim of this study was a longitudinal survey, involving weekly sampling for 10 weeks during late summer/early autumn of 2017, to estimate the background occurrence and concentrations of *Cryptosporidium* oocysts in leisure pools. The oocyst concentrations in pool water, averaged during sampling over the course of a day of operation, were used to enable proof of concept probabilistic modelling of the likelihood of there being a measurable input of oocysts into the pool during the day, and whether any such inputs were likely to be single or multiple events. A modelling approach was also used to estimate the magnitude of events in terms of oocyst numbers (and the associated peak concentrations) from daily average data taking account of what is known about the dynamics of oocyst numbers in relation to water circulation and filtration [12].

Table 1. Published sample surveys for *Cryptosporidium* in swimming pools.

Country	Venue, Pool Types and Numbers	Duration of Study	Sampling Frequency	Sample Type	Collection Method	Sample Volume Processed	Oocyst Capture and Detection Method	Pools Positive	Samples Positive	Oocyst Counts Reported	Key findings Relating to *Cryptosporidium*	Reference
France	4 public pools used for babies and children, 1 private club adults pool	November 1998 to January 2000	8× over 1 year	Pool water	Capsule filter	300–600 L	Gradient flotation; IFM	1/5 (20%)	1/40 (3%)	0.6/100 L	Detection was in the private club adults pool	[13]
Greece	3 public indoor pools: adult, children's and hydrotherapy	October 1998 to March 1999	3× over 5 months	Pool water	Wound filter	500–1000 L	Gradient flotation; IFM	1/3 (33%)	1/9 (11%)	0.3/100 L	Detection was in the adult pool	[14]
The Netherlands	7 filters at 5 public pool complexes	May 2000 to June 2001	Biweekly	Back-wash	Grab	1–25 L	Calcium carbonate flocculation; IMS; IFM	7/7 filters	9/150 (6%)	0.11 to 17/L	A high proportion of oocysts were damaged or empty	[15]
	4 toddler pools	June to September 2001	Weekly	Pool water	Capsule filter	200–400 L	IMS; IFM	0/4	0/14	0	Regular reporting of faecal accidents	
	1 learner pool	June to September 2001	Weekly	Pool water	Capsule filter	1000 L	IMS; IFM	1/1 (100%)	7/9 (78%)	0.004 to 2.31/L	Faecal accident; filter cracks	
Italy	10 public pools	May–August 2003	Over two months	Pool water	Capsule filter and/or filter module	180–720 L	IMS; IFM; PCR	1/10 (10%)	1/11 (9%)	2 oocysts (capsule filter) and 4 oocysts (filter module)/480 L	Bather numbers reported. Additional testing by PCR was negative for *Cryptosporidium* but positive for protozoan rDNA	[16]
				Back-wash	Grab	1.1–1.3 L	IMS; IFM	1/3 (33%)	1/3 (33%)	8/1.3 L		
Italy	Not stated	2001–2002	not stated	Pool water	Capsule filter	500 L	IMS; IFM	1/1	1/11 (9%)	4×10^{-2}/100 L	Detection was in an outdoor pool	[17]
Italy	7 public pools	May 2004 to August 2005	in some months	Pool water	Grab	20L	Centrifugation; IFM	2/7 (29%)	6/21 (29%)	0.5–2 /L	5 detections were at one pool complex. Bather numbers reported.	[18]
Greece	5 pools: 3 public indoor and 2 private outdoor pools	1997–2005	2xmonthly then monthly	Pool water	Wound filter	1000 L	Gradient flotation; IFM	0/5	0/462		Bather numbers reported.	[19]
USA	160 public pool filters	Aug–Oct 2006	Once per pool	Back-wash	Grab	1 L	Calcium carbonate flocculation; PCR	3/160 (2%)	3/160 (2%)	n/a	Detections were in small community pools with 1–75 bathers/week used by children and adults	[20]

Table 1. *Cont.*

Country	Venue, Pool Types and Numbers	Duration of Study	Sampling Frequency	Sample Type	Collection Method	Sample Volume Processed	Oocyst Capture and Detection Method	Pools Positive	Samples Positive	Oocyst Counts Reported	Key findings Relating to *Cryptosporidium*	Reference
Egypt	5 swimming pools	6 months	Monthly over 6 months	Pool water	not stated	not stated	Membrane filtration; sedimentation; acid-fast microscopy	1/5 (20%)	1/30 (3%)	not stated	Significant association between poor water quality and bather load; not investigated for *Cryptosporidium*	[21]
Malaysia	1 swimming pool	Oct–Dec 2011		Pool water	Grab	10 L	Membrane filtration; IMS; IFM	0/1	0/1	0		[22]
The Philippines	3 swimming pools	Oct 2012	Once per pool	Pool water	Grab	10 L	Membrane filtration; IMS; IFM	1/3 (33%)	1/3 (33%)	0.6/L		[23]
Belgium	20 public pools: indoors and outdoors	March–Oct 2010	not stated	Pool water	Filter module	60 L	IMS; IFM	0/20	0			[24]
	16 public pool filters: indoors and outdoors	March–Oct 2010	not stated	Back-wash	Grab	2–60 L	IMS; IMF	2/16 (13%)	2	0.03 and 0.23 per L		[24]
China	35 public pools	May and August 2015	Most pools twice	Pool water	Grab	10 L	Flotation; IFM	5/35 (14%)	10/60 (17%)	0–4, mean 0.30 oocysts /10 L	The sample positivity rate was higher in August (24.2%) than in May (7.4%). A strong correlation reported between parasite detection and urea	[11]
USA	127 public pools; indoor and outdoor	July–August 2012	Most pools once, 30 pools > once	Backwash	Grab	800 m	PEG precipitation; PCR	1/127 (1%)	1/160 (1%)	n/a		[25]
China	39 public pools, all outdoors	June to September 2013	not stated	Pool water	Grab	50 L	Hollow fibre ultrafiltration IMS; IFM	3/39 (8%)	3	0.03–0.14 oocysts/L		[26]
Spain	21 indoor heated public pools in 5 towns	Not stated	Once	Pool water	Grab	10 L	Calcium carbonate flocculation; IMS; mZN	5/21 (24%)	5/21 (24%)	3–13 oocysts/L	No statistically significant associations but pools with fewer users (<100) wereall negative, whereas pools with a medium user frequency (100–500) had the highest positivity rate.	[27]

IFM = immunofluorescence microscopy; IMS = immunomagnetic separation; PCR = polymerase chain reaction; mZN = modified Ziehl–Neelsen.

Secondary aims were to provide validation and oocyst recovery data for the methods used for testing swimming pool water and filter backwash, compare detections with chemical, bacterial and operational parameters, provide an evidence-base for guidance, improve sampling capacity and interpretation for public health investigations, and provide data that could contribute to quantitative microbial risk assessment (QMRA).

2. Materials and Methods

2.1. Swimming Pool Recruitment and Description

A convenience sample of six volunteer swimming pools was recruited to the study, three in England, two in Wales and one in Scotland. Table 2 provides the details of each pool. All were free-form leisure pools of varying depth, with features such as slides, wave machines, or lazy rivers. Four were exclusively indoor pools and two had a linked, outdoor area. Two were set at large residential holiday parks and four served local communities. Five were filled solely from the mains water supply and one supplemented with an on-site borehole. Two indoor pools were disinfected with sodium bromide plus sodium hypochlorite, and four pools (two indoor and two incorporating outdoor areas) with sodium or calcium hypochlorite. None used cyanuric acid to limit natural UV light degradation of chlorine. Five pools had medium velocity (<25 m/h) sand filters and one had high-velocity filters filled with glass. All pools used polyaluminium chloride (PAC) as a coagulant. Three pools (including that with high-velocity filters) had secondary disinfection in the form of UV light.

All pools were inspected for construction, operation and management features ahead of sampling, using the checklist available elsewhere online as Appendix 1 of the "Guidance for the investigation of *Cryptosporidium* linked to swimming pools" found at www.publichealthwales.org/cryptopoolguidance (accessed on 23 March 2021).

2.2. Swimming Pool Water and Filter Backwash Sampling

High volume pool water samples were collected on the same day each week for ten weeks from 8 August 2017. At each pool, a gate valve was installed in the return pipework from the swimming pool tank to the filters, after the coarse strainers and circulation pumps. A *Cryptosporidium* sampling rig was constructed for each pool comprising, sequentially, a non-return connector, 13 mm internal diameter flexible PVC hosing, an open-cell reticulated polyurethane filter module in a filter housing (Filta-Max *xpress*™, IDEXX Technologies Ltd., Newmarket, UK), a flow meter (Gardena Water Smart 818, Gardena, Ulm, Germany) and a waste hose. Up to 1000 L water was sampled at a maximum flow rate of 2 L/min. Sampling started when the pools opened in the morning and continued for a minimum of 8 h, and a maximum of 24 h. This enabled sampling through and following peak daily use at each pool.

At three of the pools (D, E, and F) it was possible to also sample 1 L of the initial filter backwash. This was done after the end of the swim session and after completion of pool water sampling, using a single screw cap sample bottle. Filta-Max *xpress*™ filter modules and backwash samples were transported with ice packs for delivery the next day to the laboratory (IDEXX Technologies Ltd., Newmarket, UK).

To monitor the management and control of disinfection, 250 mL water samples were collected for bacteriological testing aseptically in sodium thiosulphate by immersion at 200–400 mm below the surface of each pool [28] at around mid-day on the day of *Cryptosporidium* sampling and transported with an ice pack for delivery next day to the laboratory (Latis Scientific, Crayford, UK). The contents were temperature checked on arrival to ensure <8 °C and tested within 24 h of collection.

Water quality was monitored by sampling at the same time and depth and tested using the pool's own portable devices for pH, residual free chlorine, temperature, and total dissolved solids. Turbidity was measured using devices provided for the project (TB210IR Handheld Turbidity Meter, The Tintometer Ltd., Amesbury, UK).

Table 2. Characteristics of the leisure pools sampled.

Pool Location	Premises, Description, and Date Opened	Details of the Pool Sampled (Other Pools and Treatment May be Present on Site)								
		Pool Water Volume, Shape and Features	**Water Supply**	**Turnover Period (h)**	**Disinfection and Target Free Chlorine Residual**	**Target pH**	**Target Temp °C**	**Secondary Disinfection**	**Filtration and Coagulation**	**Backwash Regime**
A England	Community leisure centre complex of 3 main pools with slides and paddling pools linked to the free form leisure pool. 1990	360 m^3 Freeform, indoor pool with water slides, wild water, geyser, water cannons and wave machine.	Mains	1.5	Sodium bromide plus sodium hypochlorite 2.5 mg/L	8.0	30.5	None	4 medium-rate sand filters, continuously dosed PAC coagulation.	Scheduled twice weekly and as pressure differentials dictate.
B England	Holiday park aquatic centre complex with 8 pools. 1988	1497 m^3 Freeform, indoor and outdoor pool with wave pool, whirlpool, rapids, grotto pool, water cannon, sprays, fountain, geysers, bubble seats.	Mains and borehole	1.58	Sodium bromide plus sodium hypochlorite 1–3 mg/L	7.4–8.2	30	None	12 medium-rate sand filters, continuously dosed PAC coagulation.	Scheduled every 4 days and as pressure differentials dictate.
C Wales	Community leisure centre complex of 3 pools. 2008	85.8 m^3 Free form, indoor pool with slide, splashdown, mushroom spray feature, whirlpool area.	Mains	NA	Chlorine 1.5 mg/L	7.4	30–32	UV light	2 high-rate, glass filters continuously dosed PAC coagulation.	Scheduled once weekly and as pressure differentials dictate.
D Scotland	Community leisure centre complex of 3 pools. 1998	498 m^3 Freeform, indoor and outdoor pool with wild water, fan spray, slides, water cannon, waterfall.	Mains	2.75	Sodium hypochlorite 1.5 mg/L	7.2–7.6	29–31	UV light	5 medium-rate sand filters continuously dosed PAC coagulation. Variable speed circulation pumps used.	Scheduled once weekly and as pressure differentials dictate.

Table 2. *Cont.*

Pool Location	Premises, Description, and Date Opened	Details of the Pool Sampled (Other Pools and Treatment May be Present on Site)							
		Pool Water Volume, Shape and Features	**Water Supply**	**Turnover Period (h)**	**Disinfection and Target Free Chlorine Residual**	**Target pH**	**Target Temp °C**	**Secondary Disinfection**	**Filtration and Coagulation**	**Backwash Regime**
E Wales	Community leisure centre complex of 3 pools. 2008	1325 m^3 Freeform, indoor pool with wave machine, slides, sprays, rapids, water cannon, air geyser, whirlpool area.	Mains	NA	Calcium hypochlorite 1.2 mg/L	7.25	31	UV light	9 sand and pea gravel medium-rate filters continuously dosed PAC coagulation. Variable speed circulation pumps used.	Scheduled 18-day cycle reduced to 9 or 5 days in busy periods, and as pressure differentials dictate.
F England	Holiday park aquatic centre complex of 6 pools. 1980s	299 m^3 Freeform indoor and outdoor pool with slide.	Mains	2.15	Sodium hypochlorite 1–3 mg/L	7.2–7.4	27–30	None	3 medium-rate sand filters, continuously dosed PAC coagulation.	Scheduled once weekly and as pressure differentials dictate.

A standardised form (Supplementary Materials Figure S1) was used to collect *Cryptosporidium* and bacteriological sampling parameters, physical-chemical properties, and daily bather numbers. Information about faecal accidents, arising from the observation of stools in the pool in the seven days preceding sampling, was also collected.

2.3. Cryptosporidium Analysis

Cryptosporidium oocysts in pool water samples were enumerated by standard methods [29] based on elution from the filter, centrifugation, immunomagnetic separation (IMS) (Dynabeads™ anti-*Cryptosporidium*, Applied Biosystems, Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania) and immunofluorescence microscopy (IFM) (EasyStain, BTF, North Ryde, Australia or Crypto-Cel, TCS BioSciences, Botolph Claydon, UK) according to manufacturer's instructions. Additional nucleic acid staining was performed with 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI) (Merck KGaA, Darmstadt, Germany). During IMS, the dissociation procedure to separate the oocysts from the beads was repeated during the first two sampling rounds but was reduced to once as the procedure was lengthy and not fruitful for improved oocyst recovery in test and validation samples (data not shown). The analytical procedure was validated in-house by seeding four 10 L samples of swimming pool water from each of two pools (one chlorinated and one brominated) with 99 pre-stained *Cryptosporidium* oocysts (ColorSeed™, TCS Biosciences, Botolph Claydon, UK) and recording oocyst counts following the first and second dissociation steps.

Cryptosporidium oocysts in backwash samples were enumerated following centrifugation and IFM: the 1 L samples were split between two 500 mL bottles and centrifuged at 1140 ± 20× *g* for 15 ± 2 min, the supernatant removed by aspiration and the pellet re-suspended in 500 mL reverse osmosis (RO) water and the process repeated until the supernatant was clear. The final pellet was re-suspended in 25 mL RO water and transferred to a 50 mL conical centrifuge tube. A further 20 mL RO water was used to rinse the 500 mL bottle and added to the 50 mL tube. The suspension was vortex mixed for 20 s and allowed to stand at room temperature for 20 min, so that fibrous material settled to the bottom of the tube. The supernatant was removed with a pipette and transferred to a clean 50 mL centrifuge tube and centrifuged at 1140 ± 20× *g* for 15 min, and the pellet volume recorded. The supernatant was removed by the aspiration to within 5 mL of the pellet and RO water added to the 7.5 mL mark on the tube. The pellet was re-suspended by vortex mixing for 20 s and transferred using a pipette to measure the volume into a Leighton tube. Sufficient RO water was added to the centrifuge tube and vortex mixed to rinse for 20 s and transferred to the Leighton tube so that the final volume was 10 mL. The sample was stored at 5°C until processing in its entirety by IMS, and the oocysts stained and enumerated as described above. Oocyst recoveries were monitored by spiking each 1 L sample with 99 pre-stained oocysts (ColorSeed™, TCS Biosciences, Biddulph Clayton, UK) according to the manufacturer's instructions.

Cryptosporidium oocyst counts were reported to the project manager within 1 week of sample receipt and positive results discussed with the pool operators, management and local environmental health and health protection teams, in the context of the pool's management, construction and operational performance.

2.4. Detection of Bacterial Indicator Organisms in Pool Water

Colony counts were used to enumerate colony forming units (cfu)/mL after incubation at 37 °C for 24 h for aerobic bacteria, and estimate the most probable number (MPN)/100 mL for total coliforms, *Escherichia coli*, and *Pseudomonas aeruginosa* in pool water samples (Latis Scientific, Crayford, London, UK) using standard methods [28]. Results were reported to pool operators directly, and to the project manager, within 72 h of receipt.

2.5. Statistical Analyses

Data and results were collected weekly and tabulated in an Excel spreadsheet (Supplementary Materials Table S1). Occurrence and average concentration of oocysts per 10 L pool water and per 1 L backwash on the sampling days were compared. Unadjusted and adjusted counts (taking into account oocyst recovery data, and results from the first dissociation only) were used. Bacterial indicator results were classified as satisfactory or unsatisfactory [30]. The associations between oocyst detection and measured parameters were investigated for dichotomous outcome variables using Chi-squared/Fisher's exact test and for continuous outcome variables using t-test or Mann–Whitney U test. Sample volumes and oocyst counts were compared using Spearman's rank correlation coefficient. Median oocyst recovery rates from chlorinated and brominated pools were compared using the Mann-Whitney U test, and from backwash samples from three pools using the Kruskal–Wallis test. Differences with p-values of < 0.05 were considered statistically significant.

2.6. Probabilistic Modelling of Cryptosporidium Contamination Events

These data were used to enable probabilistic modelling of the likelihood of there being a measurable input of oocysts into the pool during the course of a day, and whether any such inputs were likely to be single or multiple events. The likelihood of an 'oocyst event' during the course of a day was based on the notion of a 1 in N chance of a bather contaminating the pool with a measurable quantity of oocysts. If so, the fraction (f) of days when there was likely to be a detectable contamination event was given by Equation (1):

$$f = 1 - \left(\left(1 - \frac{1}{N}\right)^{n}\right) \quad (1)$$

where n was the number of bathers using the pool that day. The greater the daily bather number, the more frequent will be days with detectable oocyst contamination. As the pools varied widely in daily bather number, this was likely to be an important factor contributing to any observed differences between pools in oocyst detection frequency.

To address the question "when oocysts were detected, what is the likelihood that the contamination was from a single bather or multiple bathers?", a Monte Carlo simulation was undertaken. To simulate whether the next bather to enter the pool was a 'contaminator', a random integer was selected between 1 and N. If 'number two' was selected, that designated the bather as being a contaminator. Repeating this selection process n times (where n was the number of bathers entering the pool that day) provided a value for the number of 'number two' events recorded for this trial. For a given combination of N and n, the trial was repeated 1000 times, from which the likely fraction of days with 0, 1, 2, 3 etc. events could be estimated. The fraction of days where the Monte Carlo simulation predicted any contamination should agree with the prediction of Equation (1).

3. Results

3.1. Oocyst Detection, Bacterial Indicator Organisms and Physical-Chemical Properties

The recorded pool operating parameters and the results of testing for bacteriological indicator organisms (Table 3) were mostly compliant with the Pool Water Treatment Advisory Group (PWTAG) standards [30]. The MPN/100 mL was zero in all samples tested for total coliforms, *Escherichia coli* and *Pseudomonas aeruginosa*. Although aerobic colony counts were unsatisfactory (>100 cfu/mL) in 12/57 (21%) samples, none were consecutive samples (Table 3).

A total of 59 pool water samples were tested for *Cryptosporidium* over the ten week period (Table 3). Sample volumes ranged from 60 to 999 L, median 493 L, and were taken over 8 to 24 h periods, as the Filta-Max xpress™ filters sometimes became blocked, even when turbidity was within acceptable limits (Table 3).

Cryptosporidium oocysts were detected in 12/59 (20%) samples. All detections were from the material obtained in the first IMS dissociation. Every pool had at least one

positive pool water sample and up to four (Table 3). Most detections (8/12, 66%) were in August (weeks 1 to 4) when the number of people using the pools was highest (Table 4, Figure 1), and were significantly more likely to be positive than those taken after August (Fisher's exact 2 tailed test $p = 0.045$). There was no statistically significant relationship between *Cryptosporidium* detections and any aerobic colony count (ACC) failures or other measured parameters.

Oocyst counts in pool water ranged from 0 to 1.16 oocysts per 10 L (mean 0.04 oocysts per 10 L; median 0). Although the sample volumes varied, there was no significant difference between the volume of samples in which oocysts were detected (mean 415.9 L, range 60–999.3 L) compared to those in which they were not (mean 530.9 L, range 181.3–992.1 L) (t-value 1.515, $p = 0.135$). There was no correlation between sample volume and oocyst counts (Spearman's rank correlation coefficient 0.071, 95% CI = −0.524–0.620. In spiking experiments, there was no significant difference in the median oocyst recovery percentages from chlorinated pool water (58.1%, range 46.5% to 61.6%) and brominated pool water (54.0%, range 50.5% to 55.6%) ($p = 0.31$). No environmental oocysts (not derived from spiking) were detected in these experimental samples. Environmental oocyst counts from weekly sampling were adjusted using the appropriate median recovery rate, and ranged from 0 to 2.11 oocysts, mean 0.08, per 10 L pool water (Table 4). These data are for pools that differed in size and bather numbers and it is reasonable to assume there was a strong dependency between oocyst counts and (a) the number of bathers and (b) the volume of the pool. Other factors aside, the oocyst count would be proportional to the number of bathers and inversely proportional to the pool volume. In which case, data from pools with different volumes and bather numbers can be compared by multiplying the oocyst concentration by the pool volume and dividing by the bather number. This gives, in effect, the average number of residual oocysts per bather (Table 4), which ranged from 1.7 to 64.1 (mean 15.9, median 8.1).

Filter backwash was sampled from pools D, E and F (Tables 3 and 4). Environmental oocysts were detected in 2/28 (7%) samples from pools E and F. One sample, at pool F, count of 4 oocysts/L, was taken on the day 1.16 oocysts/10 L were detected in the pool water, which was the highest oocyst pool water count recorded for that site. The other detection in backwash was at pool E, where 1 oocyst/L was recorded on the day 0.04/10 L were detected in the pool water.

The median spike recovery for backwash recorded for samples from pool D was 54.1% (range 48.5% to 74.7%), pool E was 55.6% (range 10.1% to 82.8%) and pool F was 56.1% (range 20.2% to 74.7%). Although the variance within the three sets of spike recovery data differed (Bartlett's Chi-square 9.26, df = 2, $p = 0.01$), there was no significant difference in the median values (Kruskal–Wallis H = 0.06, df = 2, $p = 0.97$). The adjusted environmental oocyst counts in the backwash were 2 and 8 oocysts/L.

Fourteen faecal accidents were reported over the 10 week period (Table 3), at least one and up to four per pool (Table 4). Two were liquid, at the same pool. Recorded actions were PWTAG compliant. One report was in the week preceding oocyst detection in the pool water sample (Table 4).

Table 3. Summary of *Cryptosporidium* oocyst occurrence in pool water and backwash samples, aerobic colony counts and physical-chemical properties of volunteer leisure pools from weekly sampling over 10 weeks from 8 August 2017.

Pool	Statistics	Temp °C	pH	Residual Free Chlorine mg/L	TDS ppm	Turbidity NTU	Total Bather Numbers on the Day of Sampling	*Cryptosporidium* Sampling and Detections				Faecal Accidents Reported	Unsatisfactory ACC Tests [a]
								Filter Capsule Flow Rate L/min	Pool Water Volume Examined L	Pool Water Samples Positive/Valid Samples	Backwash Samples Positive/Valid Samples		
A [b]	Mean	30.5	7.8	2.11	1510	0.62	661	1.01	595.7	1/10	ns	2	3
	Range	30.0–30.5	7.40–8.10	1.50–3.00	1100–1900	0.17–1.60	134–1112 [c]	0.69–1.50	486.6–992.1				
B [b]	Mean	30.0	7.95	1.92	1421	0.22	2686	1.01	485.1	1/10	ns	4	2
	Range	30.0–30.0	7.85–8.05	1.54–2.10	1200–1600	0.09–0.41	1223–4056 [c]	0.50–1.43	238.4–687.4				
C	Mean	31.5	7.26	1.83	610	0.50	222	0.91	667.6	2/10	ns	4	2
	Range	31.4–31.7	7.20–7.40	1.46–2.13	460–760	0.29–1.08	199–288[c]	0.06–1.38	60–990				
D	Mean	30.1	7.49	1.50	1560	0.47	600 [d]	0.47	612.47	1/10	0/10	2	2
	Range	29.8–30.2	7.35–7.60	1.10–1.98	1300–2600	0.07–2.40		0.13–0.82	181.3–999.3				
E	Mean	30.2	7.33	2.25	1358	0.27	131 estimated 3000 [e]	0.88	440.32	4/10	1/10	1	1
	Range	29.6–30.7	7.05–7.55	1.53–3.20	1169–1620	0.18–0.42		0.34–1.43	270–684.4				
F	Mean	30.7	7.36	1.80	1674	0.68	994	0.44	212.6	3/9[f]	1/8 [g]	1	2
	Range	30.1–31.3	7.20–7.55	1.23–2.30	1397–2800	0.22–1.37	75–2500 [c]	0.41–0.51	199–246				
All pools	Mean	30.5	Chlorine pools 7.36	1.90	1356	0.46		0.79	502.3	12/59 [f]	2/28 [g]	14	12
	Range	29.6–31.7	7.05–7.60 Bromine pools 7.887.40–8.10	1.10–3.20	460–2800	0.07–2.40		0.06–1.50	60–999.3				

ns = not sampled (inaccessible); TDS = total dissolved solids; NTU = nephelometric turbidity units; ACC = aerobic colony count. [a] valid for 57 samples; three samples were invalidated as one was sent to the wrong laboratory and two arrived at the laboratory >24 h after sampling. [b] Bromine pool; residual is calculated as free chlorine. [c] Number of entries to the pool site on the days sampled. [d] Number of pool users measured in Week 10 only. [e] Estimate based on pool size and type, as the number of pool users in the vicinity at the time of bacterial sampling only was recorded (4–390), rather than the daily total for the pool. [f] One sample was invalidated as it was sent to the wrong laboratory. [g] Two samples were invalidated; one because it was sent to the wrong laboratory, and the other because the spike recovery was zero.

Table 4. *Cryptosporidium* oocyst detections and counts in pool water and backwash samples over 10 weeks from 8 August 2017.

Week	Pool	Bather Numbers on Day of Sampling	Duration of Pool Water Sampling for *Cryptosporidium* min	Pool Water OP 10 L	Pool Water OP 10 L adj	Calculated Number of Residual Oocysts/Bather	Backwash OP L	TDS ppm	Turbidity NTU	Unsatisfactory Aerobic Colony Count	Recorded Faecal Accidents
1	A	977	540	0.07	0.13	4.8	ns	1600	0.39	No	No
1	C	210	810	0.36	0.65	26.6	ns	740	1.08	Yes	No
2	C	288	960	0.17	0.30	9.0	ns	760	0.3	No	No
2	E	3000 [c]	478	0.09	0.16	7.1 [c]	<LOD	1252	0.3	No	No
3	B	3797	480	0.32	0.58	22.9	ns	1600	0.19	Yes	No
3	E	3000 [c]	485	0.04	0.07	3.1 [c]	<LOD	1340	0.39	No	No
4	F	2000	480	1.16	2.11	31.5	4	1640	0.24	Invalid [a]	No
4	E	3000 [c]	490	0.04	0.08	3.5 [c]	1	1340	0.21	No	No
6	F	200	480	0.05	0.09	13.5	<LOD	1515	0.89	Yes	Yes
10	D	600	1440 [b]	0.01 [b]	0.02	1.7	<LOD	1300	0.24	No	No
10	F	140	480	0.17	0.30	64.1	<LOD	1397	0.22	No	No
10	E	3000 [c]	485	0.03	0.06	2.7 [c]	<LOD	1620	0.36	No	No

ns = not sampled; LOD = limit of detection; OP = oocysts per; adj = adjusted for recovery rate; TDS = total dissolved solids; NTU = nephelometric turbidity units. [a] sample arrived at the laboratory >24 h after sampling. [b] prolonged duration of sampling after the pool closed influenced oocyst concentration. [c] estimated as actual total numbers of bathers were not available.

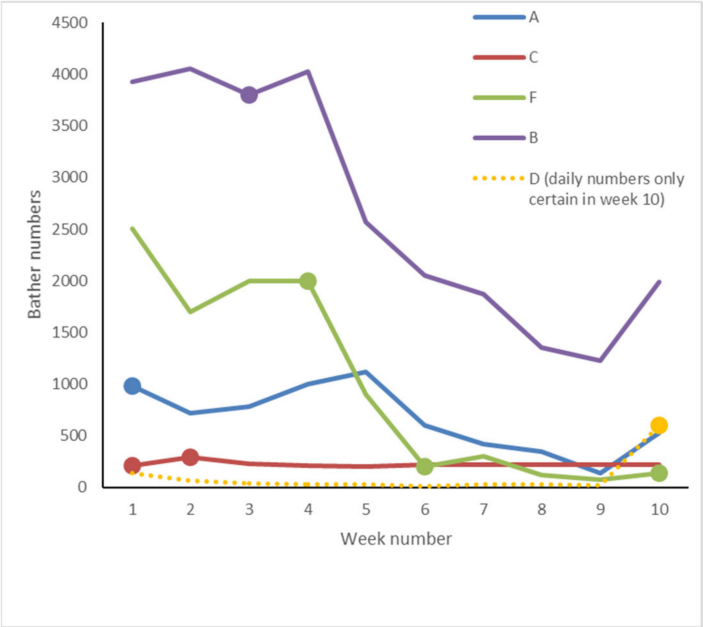

Figure 1. Daily bather numbers and *Cryptosporidium* detections (represented by circles) at five pools (A, B, C, D and F) provided the total bather number for the day of sampling. School holidays likely influenced bather numbers at most pools. Pool D was in Scotland where only sampling weeks 1 and 2 were in the summer holidays and week 10 was in the October holiday. For all other pools sampling weeks 1 to 5 were in the summer holidays.

3.2. Oocyst Modelling

Bather numbers for each of the sampling days were provided by four pools (A, B, C, and F), one pool (D) provided an accurate daily bather number on just one of the sampling days, and one site (E) was unable to provide relevant all-day numbers (Table 4; Figure 1). This provided 41 days when valid *Cryptosporidium* results and daily bather numbers were recorded, with oocysts detected on eight of those sampling days (Figure 1). To investigate further the possible impact of daily bather number on the likelihood of there being recorded oocyst contamination, the data were grouped into two categories, ≤1000 and >1000 daily bathers (Figure 1; Table 5).

Table 5. Observed frequency of *Cryptosporidium* oocyst detection grouped by nominal classes of recorded bather numbers at five pools.

Class	Daily Bather Numbers	Pool	No. Sample Days	No. Positive Sample Days	% Positive Sample Days
1	0–1000 mean = 387	A	9	1	
		C	10	2	
		D	1	1	
		F	6	2	
		Total	26	6	23.1
2	1001–4069 mean = 2411	A	1	0	
		B	10	1	
		F	4	1	
		Total	15	2	13.3
1 and 2	0–4069 mean = 1127	TOTAL	41	8	19.5

Figure 2 shows the overall fraction of days with oocyst detections plotted against the mean (nominal) bather number for the class (from Table 5), and the relationship predicted by Eqn 1 between the fraction of 'positive' days as a function of daily bather number based

on variable probabilities of a bather being a contaminator ranging from 1 in 1000 to 1 in 10,000. The average pool volumes (weighted by the number of days each pool contributed) contributing to the data in classes 1 and 2 in Table 5 were 245 and 1101 m^3 respectively. Smaller pools in class 1 appeared to have a frequency of detectable 'oocyst event days' consistent with there being much greater odds of a bather being a contaminator than larger pools in class 2; much greater numbers of samples would be required to provide a more confident assessment.

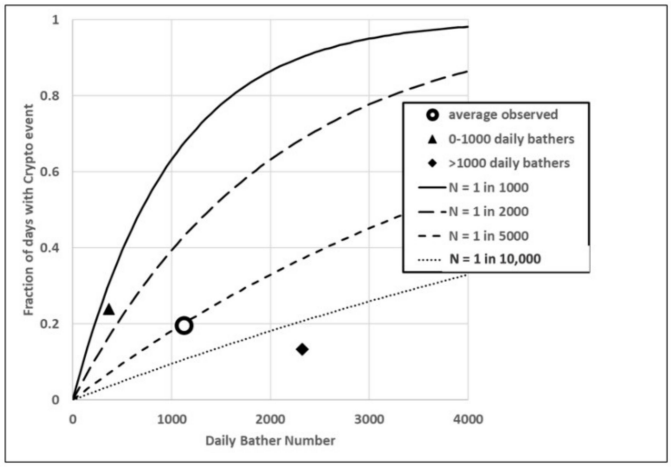

Figure 2. The impact of daily bather number (n) and the probability of a bather being an oocyst shedder (N = 1 in 1000, 1 in 2000, 1 in 5000 or 1 in 10,000) on the fraction of days with detectable Cryptosporidium contamination. Lines show predicted results using Equation (1) and symbols show the observed results.

Pooling the data gave 19.5% of days with *Cryptosporidium* detections, with an average of 1127 bathers per day (Table 5). This corresponded to an average probability of about 1 in 5000 of a bather shedding sufficient oocysts to provide a detectable number in the pool. This probability affected not only the chance of there being oocyst contamination on a given day but also the likely number of contamination events within a day. The results of the Monte Carlo simulation (Figure 3) show the expected frequency of multiple event days for different combinations of oocyst shedder probability and bather number. If there were sufficient bathers (daily bather number) to predict that more than 70% of days had measurable oocyst contamination, then the probability of multiple events exceeded that of there being just a single event. For example, 3000 daily bathers with a 1 in 2000 probability of a bather being an oocyst shedder were expected to give 79% of days with measurable contamination, comprising 46% of days with multiple events and 33% with a single event (Figure 3).

We now turn attention to how the average concentration of oocysts measured over a day in this study could relate to the peak concentration that occurred at the time of a single contamination event. In a well-mixed pool, the removal of particles or turbidity by filtration is expected to reduce the residual concentration in the pool exponentially, where the rate of decline depends on the turnover time and the filter removal efficiency. With effective filtration, the removal efficiency for *Cryptosporidium* oocysts is assumed to be at least 90% [12]. If so, then as a first approximation, it is expected that 57% of any oocysts present at the start of each turnover will be removed during the course of the turnover [12]. The solid line in Figure 4 shows the expected decline in numbers for such a case following a contamination event. The dashed line shows the approximate mean concentrations of oocysts present during each of the 4 turnovers following the contamination event (72%, 30%, 13% and 6% of the initial concentration respectively).

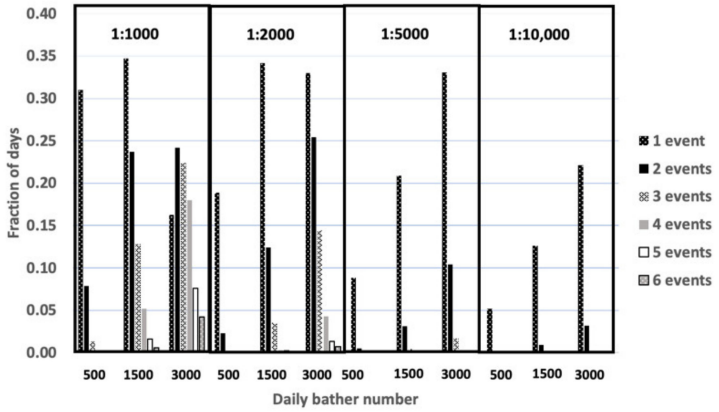

Figure 3. Monte Carlo analysis of the predicted impact of daily bather number (n = 500, 1500 or 3000) and the probability of a bather shedding a detectable number of oocysts (N = 1 in 1000, 1 in 2000, 1 in 5000 or 1 in 10,000) on the number of Cryptosporidium contamination events expected in a single day (expressed as the fraction of days predicted to have one to six events).

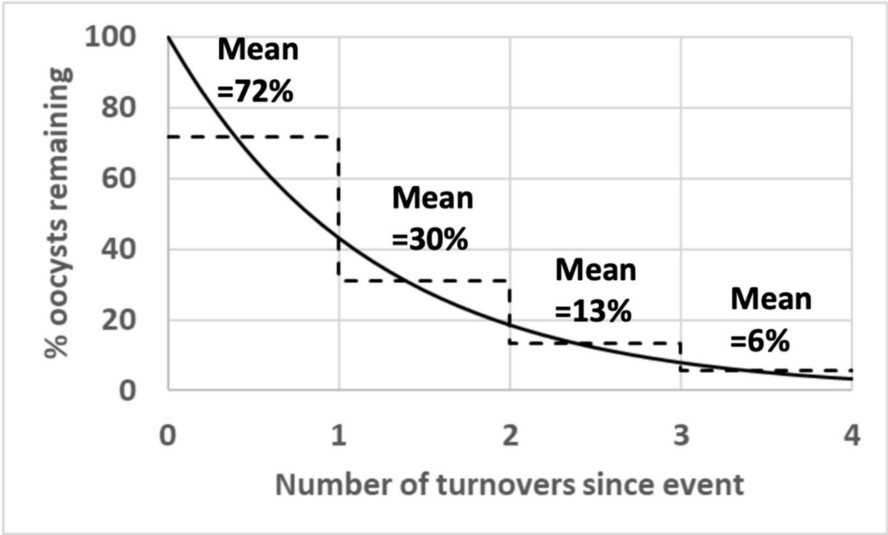

Figure 4. The decline in the concentration of oocysts for a pool where 57% of particles present at the start of each turnover are removed during the course of the turnover period. The histograms show the approximate average percentage of the initial concentration of oocysts expected to be present in the pool throughout each successive turnover period following the contamination event.

In Table 6 these values were used to compute the average expected concentration (as a percentage of the peak) during the course of the day, depending on just when the contamination occurred. The highest concentration of oocysts recorded was 2.11 per 10 L (pool F, week 4) when water was sampled from 08:00 to 16:00 h (Table 4). The turnover time for this pool was within a few minutes of 2 h (Table 2). Table 6 shows, for different presumed times of contamination, the effect of successive 2-h turnovers on the oocyst concentrations in terms of the percentage of the peak value. If the contamination event occurred between 08:00 and 14:00 h, then the measured average concentration over the 8-h sampling period was predicted to be between 31% and 18% of the peak concentration. On this basis we can be reasonably confident that the peak concentration was likely to have been no greater than 5 times the average value measured over the day (i.e., 10.5 oocysts per 10 L in this case of the highest concentration we detected), provided the event was more than one turnover before the end of the sampling period (but could be greater if the event occurred much closer to the end of the sampling period).

Table 6. Average expected concentration of oocysts (expressed as a percentage of the initial peak) during each successive turnover period during the course of a day, depending on just when the contamination occurred, for a pool with a two hour turnover period.

	Turnover Period (h)				
Oocyst Contamination Time (h)	**08:00–10:00**	**10:00–12:00**	**12:00–14:00**	**14:00–16:00**	**08:00–16:00 Mean**
08:00	72	30	13	6	30
10:00	0	72	30	13	29
12:00	0	0	72	30	26
14:00	0	0	0	72	18

4. Discussion

During the summer and early autumn of 2017, pool water samples and operational data were collected from six volunteer leisure pools, and filter backwash samples were collected from three. The results of testing for bacterial indicator organisms and physical-chemical properties indicated that the disinfection at the pools was well managed, but that the *Cryptosporidium* was present in 20% pool water samples and 7% backwash water samples, the latter following detections in pool water. Simultaneous sampling for faecal indicator organisms, pH, residual free chlorine, combined chlorine, and turbidity indicated the pool water treatment performance was good. Even so, a risk of *Cryptosporidium* transmission posed by the contamination of pools was identified, although no outbreaks were associated with the pools during the survey. The oocyst detections in filter backwash coincided with peak detections in pool water, and the number of oocysts detected was higher in filter backwash than in the pool water, demonstrating the role of effective filtration in removing oocysts, and re-enforces PWTAG guidance to mitigate that risk [30]. Previous surveys in other countries have reported *Cryptosporidium* in swimming pool waters and filter backwash in widely varying numbers/frequencies using a variety of sampling regimes and test methods (Table 1). Although many of those pools returned unsatisfactory water quality test results, few statistically significant associations with *Cryptosporidium* detection were identified. Although such indicators are not informative for the presence of this parasite, they are indicative of the management and control of the disinfection system [7]. One study in China reported a strong correlation between parasite (*Cryptosporidium* and *Giardia*) detection and urea in pool water [11]; urea can be an indicator of bather pollution [31] but is not a routine test parameter in the UK. In our study, where bacterial indicator results indicated pool water disinfection was generally well managed, oocyst detections were correlated with peak periods in terms of bather numbers which provides operators with an easily implementable control point. Bather numbers were reported in six of the 16 papers summarised in Table 1 [16,18–21,27] but were not analysed statistically with *Cryptosporidium* detections. In one study, oocysts were not detected in pools with fewer users (<100/day), whereas pools with a medium user frequency (100–500/day) had the highest oocyst prevalence (25%) [27].

This study provided, for the first time, validation data for testing swimming pool water and backwash for *Cryptosporidium*. The 54.8% mean oocyst recovery in validation spiking trials for swimming pool waters was well within the acceptable range for drinking water [32], indicating that standard methods for drinking water are applicable to pool waters. The numbers of oocysts detected were within the ranges reported previously for swimming pools (Table 1) but here we were able to adjust the counts for recovery rates, accommodating in part for the underestimation of oocyst numbers generated by test processes [32].

During our survey, it was notable that no oocysts were detected on 33 (80%) of the 41 days of sampling in the 5 pools where daily bather numbers were recorded, despite over 38,000 bathers using the pools on those days. This implies that (a) there was no long-term (i.e., multi-day) residual presence of oocysts in the pool water following a contamination event and (b) the large majority of bathers were not oocyst shedders. We shall discuss

each in turn and have assumed the sole source of oocysts to be bathers (based on the transmission pathway of [33] not a contaminated water supply. The water providers assured us there were no oocyst detections in the supplies during the sampling period, the pools were operated to high standards and there was no evidence of cross-connections, and any outdoor areas were protected to prevent the entry of animals to the pools. Therefore the *Cryptosporidium*-positive microscopy slides were not subjected to genotyping (which is challenging when small numbers of oocysts are present) to identify the species. Oocyst viability was not tested as there is no standardised or validated test for meaningful results from low numbers, and it is not unreasonable to assume that oocysts captured in pool water in a weekly monitoring regime are viable as they were likely to have been recently shed.

All the samples were taken at seven-day intervals, so there were no sequential days of oocyst monitoring to demonstrate a lack of carry-over from the data we collected. However, significant carry-over was not expected based on information about the removal of oocysts by filtration and the performance of the pool water treatment at the pools. For example, the basis of the UK guidelines for managing diarrhoea incidents is that with effective filtration 99.3% of oocysts are expected to be removed from pool water during six turnovers of circulation [30]. Even if filters are achieving only 50% removal of oocyst-sized particles, the percentage of oocysts removed after six turnovers is still expected to be 90% [12]. The pools studied had turnover times ranging from 1.5 to 2.75 h, so would have achieved at least five turnovers overnight when there were no bathers present. This indicates that even with a relatively poor filtration efficiency of 50%, at least 85% of oocysts would have been removed from the pool even if a contamination event occurred just at the time of closure. Therefore, it is reasonable to assume that if measurable numbers of oocysts were recorded on a given day, then the contamination event(s) responsible were highly likely to have occurred that day.

Although the number of samples was limited, the implications of there being relatively few oocyst detection days was that the probability of a bather being a shedder of a detectable number of oocysts fell within the range of 1 in 1000 to 1 in 10,000 or lower, the average being around 1 in 5000. Therefore it would be expected that most days would not see a detectable oocyst contamination event. A number of demographic, risk factor, environmental and socioeconomic factors affect whether someone is infected with *Cryptosporidium*, and sheds oocysts, and is likely to contaminate a pool including age, sex, location, season and bather type. For example, one study in the UK of young children reported a point prevalence carriage rate of 1.3% (95% confidence interval: 0.3–3.8%) [34]. However, we had insufficient data from this study for multifactorial analysis.

The data for predicted 'oocyst contamination days' shown by the lines in Figure 3 suggest that the range of daily bather numbers reported in this study is within that where the fraction of days where oocysts expected to be recovered is sensitive to daily bather number. This explains why there was a statistically significant greater fraction of 'oocyst contamination' days in August (when bather numbers were greatest) than after August.

The Monte Carlo simulations indicated that multiple contamination events (associated with multiple bathers) were predicted to be most likely when there were more than 70% of days with contamination. When contamination days were less frequent than any oocyst contamination was most likely to be associated with a single bather.

The discussion so far has centred around whether or not there has been contamination on a given day, and the factors affecting the frequency of such days. We now discuss preliminary indications for the possible magnitude of contamination and peak concentration on these days, and the implications for the risk of ingestion of oocysts by other bathers. The only information we have that is indicative of the magnitude of the contamination of the pool water is the average concentration recorded over a period of continuous sampling from before the pool opened to after it closed. The data shown in Table 6 indicate that, as a first approximation, the maximum peak concentration was likely to have been in the order of 10 oocysts per 10 L (i.e., approximately 5 times the detected concentration averaged over 8 h of sampling). If the average ingestion of water by an adult bather is 37 mL [35], then

this would imply a 1 in 27 chance of an adult ingesting a single oocyst at the time of peak concentration. Given this, the importance of secondary disinfection is recommended for such pools is clear [30,36].

It is also notable that it was one of the smaller pools that had the highest oocyst concentration averaged over the sample period. It would be expected that for a given magnitude of event (in terms of numbers of oocysts contaminating the pool) a higher concentration would be found in a smaller pool with less water volume. The increased dilution of oocyst concentrations in a larger pool might have contributed to the observation that it was the smaller pools that seemed to have the higher probability of a bather being a detectable oocyst shedder. For example, at pool C which had a volume of 86 m^3 one of the recorded oocyst concentrations was 0.3 per 10 L. Had the same event occurred at pool B then the extra dilution in this 1500 m^3 pool (assuming perfect mixing) would have reduced the concentration to 0.017 per 10 L which might not have been detected. The normalising of oocyst counts to give the number of residual oocysts per bather (Table 4) provides a means of using these data in future work on QMRA, enabling generalizable models.

A final point to note from this analysis was that if the peak concentration (estimated for pool F, week 4) was 10 oocysts per 10 L, then multiplying by the volume of the pool (299 m^3) and assuming the sampling captured all the oocysts, suggests a total input of around 30,000 oocysts. This number is several orders of magnitude smaller than the 10^8–10^9 oocysts expected from an acute cryptosporidiosis-related diarrhoea event [6] but is within the range expected from asymptomatic individuals [37] assuming a stool mass of 123.6 g [38]. Similar analysis of the four highest concentrations of oocysts recovered in this study suggested total numbers of oocysts in the range 10^4–10^5.

Information collected about reported faecal accidents in this study indicates most were of solid stools and were observed between once and four times, similar to [15] who also found no temporal relationship with positive protozoa findings. Aside from the fact that liquid stools may not be observed and reported, oocyst inputs in this range may well come from asymptomatic infections, recuperating patients and/or simply dirty bottoms. For example, the concentration of *Cryptosporidium hominis* oocysts detected in stools from young children without diarrhoea in a carriage study in the UK was 1.3×10^4 oocysts per gram [34]. This re-enforces the importance of reporting and dealing with faecal accidents, promoting bather hygiene, and excluding recuperating patients from swimming for two weeks after symptoms have ceased.

The timing of the oocyst contamination in relation to the sampling period was also an important factor. The difference between contamination occurring at the start of the day compared to occurring after 3 turnover periods have elapsed is likely to reduce the mean concentration of oocysts detected two-fold. If the study aimed to estimate the total number of oocysts added to the pool, then sampling could be improved by allowing the sampling period to extend for at least two turnover periods following the last possible opportunity for a contamination event i.e., for two turnovers following closing the pool at the end of the day. However, the practicalities of this would need to be considered in the provision of labour to remove sample filters as well as the timing of filter backwashing and backwash sampling. However, if the study aimed to survey the concentration of oocysts bathers are exposed to (e.g., to validate a QMRA), then the sampling period should ideally only cover the period bathers are in the pool. In this study, most sampling periods finished soon after the pool closed whereas at one pool (D) filters were left overnight, in which case there would substantial dilution of the oocyst concentration in the sampled volume because there had been at least 5 turnovers after the pool was closed, which would be expected to remove well over 95% of the oocysts in the water. However, this did not affect the results based on oocyst detections. Though beyond the scope of this paper, future studies of this sort should incorporate a method for estimating the average concentration during the period bathers are in the pool from recovered concentrations over more extended sampling periods.

These preliminary estimates are based on the assumption that pools are perfectly mixed. This may be a reasonable assumption after several turnovers during which oocysts

are transported to the filtration system [39], and especially in this study where there was additional forced mixing due to the presence of pumped water features. However, there will not be a uniform distribution of oocysts in the minutes following localised oocyst contamination. Swimmers in the immediate vicinity at the time would be very likely to ingest oocysts, so the probability of the number of contamination events provides a better indicator of risk. Secondary disinfection such as UV is also unlikely to play a role immediately following a contamination event because this requires time for the circulation of water from the pool to the plant room.

This study has generated quantitative data on the occurrence and concentration of *Cryptosporidium* oocysts in leisure pools in the UK, which will enable contextualisation of test results and provide data to contribute to QMRA of infection risk for the UK population. These data could be used, for example, to validate the approaches developed by Suppes and colleagues [8] and Falk and colleagues [40] to quantify the risk of illness associated with the introduction of *Cryptosporidium* oocysts into pool water by swimmers. Preliminary modelling of oocyst concentration and temporal inputs can be used to guide future studies, for which simultaneous measurements of the filter removal efficiency in the appropriate size range using particle counting would aid the interpretation and modelling of results [12]. An additional, practical benefit of this work is that improved sampling capacity is provided by the six sampling rigs constructed. The findings from this work provide an evidence-base for encouraging seasonal public awareness campaigns for bather hygiene and reminding pool operators of current standards and procedures for managing bather loads and faecal accidents.

5. Conclusions

We conclude that *Cryptosporidium* oocyst enumeration methods for drinking water are applicable for swimming pool water. Oocyst detections are not infrequent in leisure pools, especially during busy periods. Modelling indicated improvement for sampling regimes, and signalled towards oocyst contamination events, although larger studies need to be done. Implications are that public health messages on bather hygiene and guidance for pool operators should be timed ahead of peak season.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w13111503/s1; Figure S1 Standardised Form for data collection at the time of sampling; Table S1 Pool water sampling data.

Author Contributions: Conceptualization, R.M.C., L.P.S. and M.W.; methodology, all authors; validation, R.M.C. and M.L.; formal analysis, M.L. and R.M.; investigation, R.M.C., M.L., R.M. and R.J.; resources, R.M.C., M.L. and R.M.; data curation, R.M.C., L.P.S.; writing—original draft preparation, R.M.C.; writing—review and editing, all authors.; visualization, L.P.S.; supervision, R.M.C. and R.J.; project administration, R.M.C.; funding acquisition, R.M.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by The Pool Water Treatment Advisory Group. The laboratory work was funded by TCS Biosciences, BTF, IDEXX and Latis Scientific, the sampling rigs and their installations were funded by Certikin, Howard Gosling and Total Pool Chemicals Ltd., and Tintometer Limited provided hand-held turbidity meters.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data was available in the supplemental materials.

Acknowledgments: We thank TCS Biosciences, BTF, IDEXX and Latis Scientific for providing laboratory reagents, Certikin, Howard Gosling and Total Pool Chemicals Ltd. for providing the sampling rigs and their installation, Tintometer Limited for providing hand-held turbidity meters, the Local Authorities for undertaking the initial pool check lists, and the management and operators at each pool for participating and taking the samples.

Conflicts of Interest: The authors declare no conflict of interest. R.M.C., M.W., and R.J. are members of the PWTAG Council. PWTAG Council members advised on the practicalities of the pool recruitment and sampling but apart from those listed as authors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Smith, A.; Reacher, M.; Smerdon, W.; Adak, G.K.; Nichols, G.; Chalmers, R.M. Outbreaks of waterborne infectious intestinal disease in England and Wales, 1992–2003. *Epidemiol. Infect.* **2006**, *134*, 1141–1149. [CrossRef]
2. Chalmers, R.M.; Robinson, G.; Elwin, K.; Elson, R. Analysis of the *Cryptosporidium* spp. and gp60 subtypes linked to human outbreaks of cryptosporidiosis in England and Wales, 2009 to 2017. *Parasites Vectors* **2019**, *12*, 95. [CrossRef]
3. Hlavsa, M.C.; Cikesh, B.L.; Roberts, V.A.; Kahler, A.M.; Vigar, M.; Hilborn, E.D.; Wade, T.J.; Roellig, D.M.; Murphy, J.L.; Xiao, L.; et al. Outbreaks associated with treated recreational water—United States, 2000–2014. *MMWR* **2018**, *67*, 547. [CrossRef]
4. Messner, M.J.; Chappell, C.L.; Okhuysen, P.C. Risk assessment for *Cryptosporidium*: A hierarchical Bayesian analysis of human dose response data. *Water Res.* **2001**, *35*, 3934–3940. [CrossRef]
5. Shields, J.M.; Hill, V.R.; Arrowood, M.J.; Beach, M.J. Inactivation of *Cryptosporidium parvum* under chlorinated recreational water conditions. *J. Water Health* **2008**, *6*, 513–520. [CrossRef] [PubMed]
6. Gregory, R. Bench-marking pool water treatment for coping with *Cryptosporidium*. *J. Environ. Health Res.* **2002**, *1*, 11–18.
7. Ryan, U.; Lawler, S.; Reid, S. Limiting swimming pool outbreaks of cryptosporidiosis–the roles of regulations, staff, patrons and research. *J. Water Health* **2017**, *15*, 1–16. [CrossRef] [PubMed]
8. Suppes, L.M.; Canales, R.A.; Gerba, C.P.; Reynolds, K.A. *Cryptosporidium* risk from swimming pool exposures. *Int. J. Hyg. Environ. Health* **2016**, *219*, 915–919. [CrossRef]
9. Pintar, K.D.M.; Fazil, A.; Pollari, F.; Charron, D.F.; Waltner-Toews, D.; McEwen, S.A. A risk assessment model to evaluate the role of fecal contamination in recreational water on the incidence of cryptosporidiosis at the community level in Ontario. *Risk Anal.* **2010**, *30*, 49–64. [CrossRef] [PubMed]
10. Petterson, S.R.; Signor, R.S.; Ashbolt, N.J. Incorporating method recovery uncertainties in stochastic estimates of raw water protozoan concentrations for QMRA. *J. Water Health* **2007**, *5* (Suppl. S1), 51–65. [CrossRef]
11. Xiao, S.; Yin, P.; Zhang, Y.; Hu, S. Occurrence of *Cryptosporidium* and *Giardia* and the relationship between protozoa and water quality indicators in swimming pools. *Korean J. Parasitol.* **2017**, *55*, 129–135. [CrossRef] [PubMed]
12. Wood, M.; Simmonds, L.; MacAdam, J.; Hassard, F.; Jarvis, P.; Chalmers, R.M. Role of filtration in managing the risk from *Cryptosporidium* in commercial swimming pools–a review. *J. Water Health* **2019**, *17*, 357–370. [CrossRef] [PubMed]
13. Fournier, S.; Dubrou, S.; Liguory, O.; Gaussin, F.; Santillana-Hayat, M.; Sarfati, C.; Molina, J.M.; Derouin, F. Detection of microsporidia, cryptosporidia and giardia in swimming pools: A one-year prospective study. *FEMS Immunol. Med. Microbiol.* **2002**, *33*, 209–213. [CrossRef]
14. Karanis, P.; Papadopoulou, C.; Kimura, A.; Economou, E.; Kourenti, C.; Sakkas, H. *Cryptosporidium* and *Giardia* in natural, drinking, and recreational water of Northwestern Greece. *Acta Hydrochim. Hydrobiol.* **2002**, *30*, 49–58. [CrossRef]
15. Schets, F.M.; Engels, G.B.; Evers, E.G. *Cryptosporidium* and *Giardia* in swimming pools in the Netherlands. *J. Water Health* **2004**, *2*, 191–200. [CrossRef]
16. Bonadonna, L.; Briancesco, R.; Magini, V.; Orsini, M.; Romano-Spica, V. A preliminary investigation on the occurrence of protozoa in swimming pools in Italy. *Ann. Ig.* **2004**, *16*, 709–719. [PubMed]
17. Briancesco, R.; Bonadonna, L. An Italian study on *Cryptosporidium* and *Giardia* in wastewater, fresh water and treated water. *Environ. Monit. Assess.* **2005**, *104*, 445–457. [CrossRef]
18. Oliveri, R.; Di Piazza, F.; Marsala, B.; Cerame, G.; Firenze, A.; Di Benedetto, M.A. Occurrence of *Giardia* cysts and *Cryptosporidium* oocysts in swimming pools in the province of Palermo, Italy. *Ann. Ig.* **2006**, *18*, 367–374. [PubMed]
19. Papadopoulou, C.; Economou, V.; Sakkas, H.; Gousia, P.; Giannakopoulos, X.; Dontorou, C.; Filioussis, G.; Gessouli, H.; Karanis, P.; Leveidiotou, S. Microbiological quality of indoor and outdoor swimming pools in Greece: Investigation of the antibiotic resistance of the bacterial isolates. *Int. J. Hyg. Environ. Health* **2008**, *211*, 385–397. [CrossRef]
20. Shields, J.M.; Gleim, E.R.; Beach, M.J. Prevalence of *Cryptosporidium* spp. and *Giardia intestinalis* in swimming pools, Atlanta, Georgia. *Emerg. Infect. Dis.* **2008**, *14*, 948–950. [CrossRef]
21. Abd El-Salam, M.M. Assessment of water quality of some swimming pools: A case study in Alexandria, Egypt. *Environ. Monit. Assess.* **2012**, *184*, 7395–7406. [CrossRef] [PubMed]
22. Kumar, T.; Onichandran, S.; Lim, Y.A.; Sawangjaroen, N.; Ithoi, I.; Andiappan, H.; Salibay, C.C.; Dungca, J.Z.; Chye, T.T.; Sulaiman, W.Y.; et al. Comparative study on waterborne parasites between Malaysia and Thailand: A new insight. *Am. J. Trop. Med. Hyg.* **2014**, *90*, 682–689. [CrossRef]
23. Onichandran, S.; Kumar, T.; Salibay, C.C.; Dungca, J.Z.; Tabo, H.A.; Tabo, N.; Tan, T.C.; Lim, Y.A.; Sawangjaroen, N.; Phiriyasamith, S.; et al. Waterborne parasites: A current status from the Philippines. *Parasites Vectors* **2014**, *7*, 244. [CrossRef]
24. Ehsan, A.; Casaert, S.; Levecke, B.; Van Rooy, L.; Pelicaen, J.; Smis, A.; De Backer, J.; Vervaeke, B.; De Smedt, S.; Schoonbaert, F.; et al. *Cryptosporidium* and *Giardia* in recreational water in Belgium. *J. Water Health* **2015**, *13*, 870–878. [CrossRef]

25. Murphy, J.L.; Hlavsa, M.C.; Carter, B.C.; Miller, C.; Jothikumar, N.; Gerth, T.R.; Beach, M.J.; Hill, V.R. Pool water quality and prevalence of microbes in filter backwash from metro-Atlanta swimming pools. *J. Water Health* **2018**, *16*, 87–92. [CrossRef]
26. Wei, X.; Li, J.; Hou, S.; Xu, C.; Zhang, H.; Atwill, E.R.; Li, X.; Yang, Z.; Chen, S. Assessment of microbiological safety of water in public swimming pools in Guangzhou, China. *Int. J. Environ. Res. Public Health* **2018**, *15*, 1416. [CrossRef]
27. Gracenea, M.; Castaño, S.; Méndez, J.; Lucena, F.; Gómez, M.S. Faecal contamination in public pools in Barcelona province: *Cryptosporidium* spp. and bacterial indicators. *J. Water Health* **2018**, *16*, 762–772. [CrossRef] [PubMed]
28. Standing Committee of Analysts—Methods for the Examination of Waters and Associated Materials. The Microbiology of Recreational and Environmental Waters Series. Available online: http://www.standingcommitteeofanalysts.co.uk/ (accessed on 23 March 2021).
29. Standing Committee of Analysts—The Microbiology of Drinking Water (2010)—Part 14—Methods for the Isolation, Identification and Enumeration of *Cryptosporidium* Oocysts and *Giardia* Cysts. Available online: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/755552/Part_14-oct20-234.pdf (accessed on 23 March 2021).
30. Pool Water Treatment Advisory Group. *Swimming Pool Water Treatment and Quality Standards for Pools and Spas*, 3rd ed.; Pool Water Treatment Advisory Group: Chester, UK, 2017.
31. Khramov, V.A.; Gizzatova, G.L. Urea as an indicator of anthropogenic pollution of water of swimming pools. *Gig. Sanit.* **2006**, *3*, 3–4.
32. US Environmental Protection Agency. Method 1623.1: Cryptosporidium and Giardia in Water by Filtration/IMS/FA. Available online: https://nepis.epa.gov/Exe/ZyNET.exe/P100J7G4.TXT?ZyActionD=ZyDocument&Client=EPA&Index=2011+Thru+2015&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery= (accessed on 23 March 2021).
33. Chalmers, R.; Johnston, R. Understanding the public health risks of *Cryptosporidium* in swimming pools: A transmission pathway approach. *Perspect. Public Health* **2018**, *138*, 238–240. [CrossRef]
34. Davies, A.P.; Campbell, B.; Evans, M.R.; Bone, A.; Roche, A.; Chalmers, R.M. Asymptomatic carriage of protozoan parasites in children in day care centers in the United Kingdom. *Pediatr. Infect. Dis. J.* **2009**, *28*, 838–840. [CrossRef] [PubMed]
35. Dufour, A.P.; Evans, O.; Behymer, T.D.; Cantu, R. Water ingestion during swimming activities in a pool: A pilot study. *J. Water Health* **2006**, *4*, 425–430. [CrossRef] [PubMed]
36. 2018 Model Aquatic Health Code. Available online: https://www.cdc.gov/mahc/pdf/2018-MAHC-Code-Clean-508.pdf (accessed on 23 March 2021).
37. Chappell, C.L.; Okhuysen, P.C.; Sterling, C.R.; DuPont, H.L. *Cryptosporidium parvum*: Intensity of infection and oocyst excretion patterns in healthy volunteers. *J. Infect. Dis.* **1996**, *173*, 232–236. [CrossRef] [PubMed]
38. Rendtorff, R.C.; Kashgarian, M. Stool patterns of healthy adult males. *Dis. Colon Rectum* **1967**, *10*, 222–228. [CrossRef] [PubMed]
39. Alansari, A.; Amburgey, J.; Madding, N. 2018 A quantitative analysis of swimming pool recirculation system efficiency. *J. Water Health* **2018**, *16*, 449–459. [CrossRef]
40. Falk, R.A.; Blatchley, E.R.; Kuechler, T.C.; Meyer, E.M.; Pickens, S.R.; Suppes, L.M. Assessing the Impact of Cyanuric Acid on Bather's Risk of Gastrointestinal Illness at Swimming Pools. *Water* **2019**, *11*, 1314. [CrossRef]