Validation and application of *Cryptosporidium* assays for swimming pool waters and filter backwash

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GIG CYMRU NHS WALES | Iechyd Cyhoeddus Cymru Public Health Wales

Today's presentation

1. What is *Cryptosporidium* and how it is transmitted through swimming pools

2. Why look for *Cryptosporidium* in swimming pools?

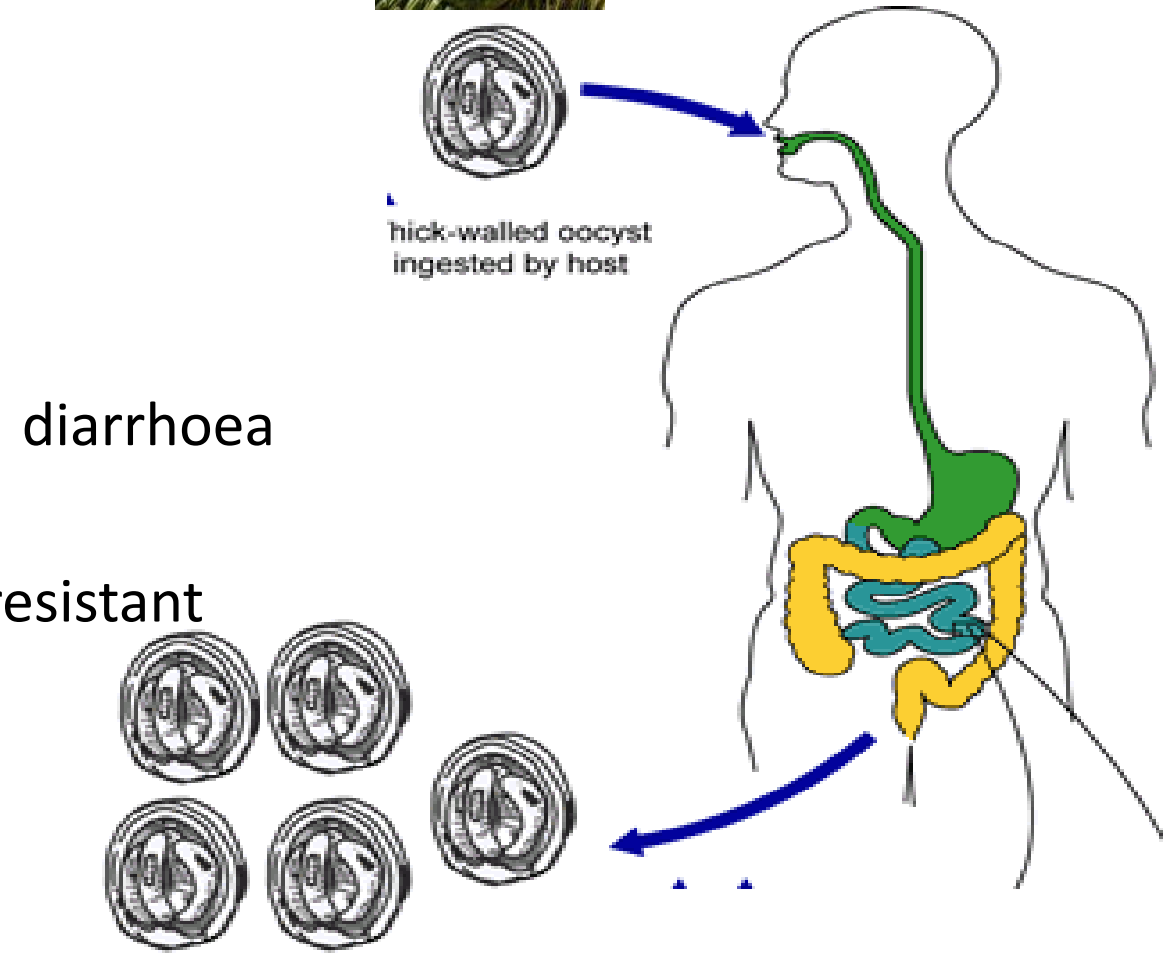
- *transmission, outbreaks*

3. The PWTAG project: A survey of *Cryptosporidium* in UK leisure pools

- *methods validation*
- *Key findings*

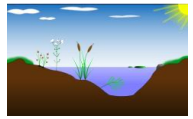
What is *Cryptosporidium*?

- Protozoan parasite: single celled animal, packaged in an oocyst
- Oocysts are ~5 µm in diameter
- Life cycle occurs in the gut:
  no multiplication in environment
  you swallow it and it multiplies in the gut, causing diarrhoea
- Millions of oocysts are shed in faeces: robust and resistant
- Ingesting ONE oocyst could cause illness

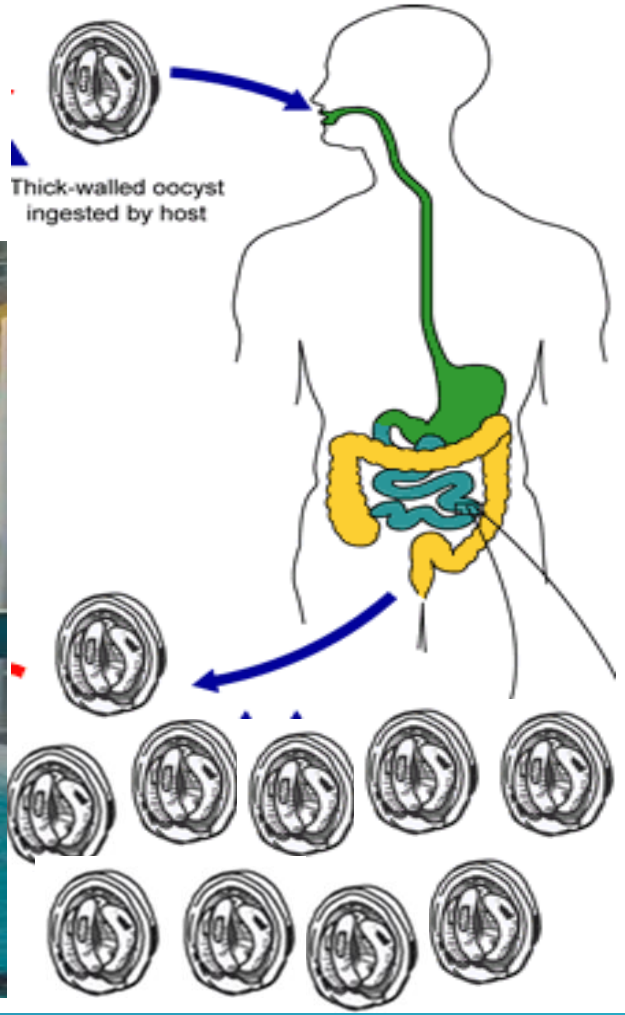


Who is at risk of cryptosporidiosis?

People who:

- Drink contaminated water  
- Eat contaminated food or beverages  
- Travel to less industrialised countries 
- Use recreational water venues 
- Have contact with farmed animals, especially young ruminants 
- Change nappies or toilet young children 
- Have contact with another person with diarrhoea, especially a child 
- Attend child care settings and other institutions
- Have close personal contact

Transmission of *Cryptosporidium* through swimming pools



Symptoms start ~5-7 days later.

Millions of oocysts shed in faeces

Shedding can continue after symptoms stop

Cryptosporidium can evade the cornerstones of swimming pool treatment

Residual disinfection for a 3 log reduction

Pathogen	Chlorine survival* 1mg/L, pH7.5, 25°C
E. coli O157	< 1 min
Cryptosporidium	10.6 days

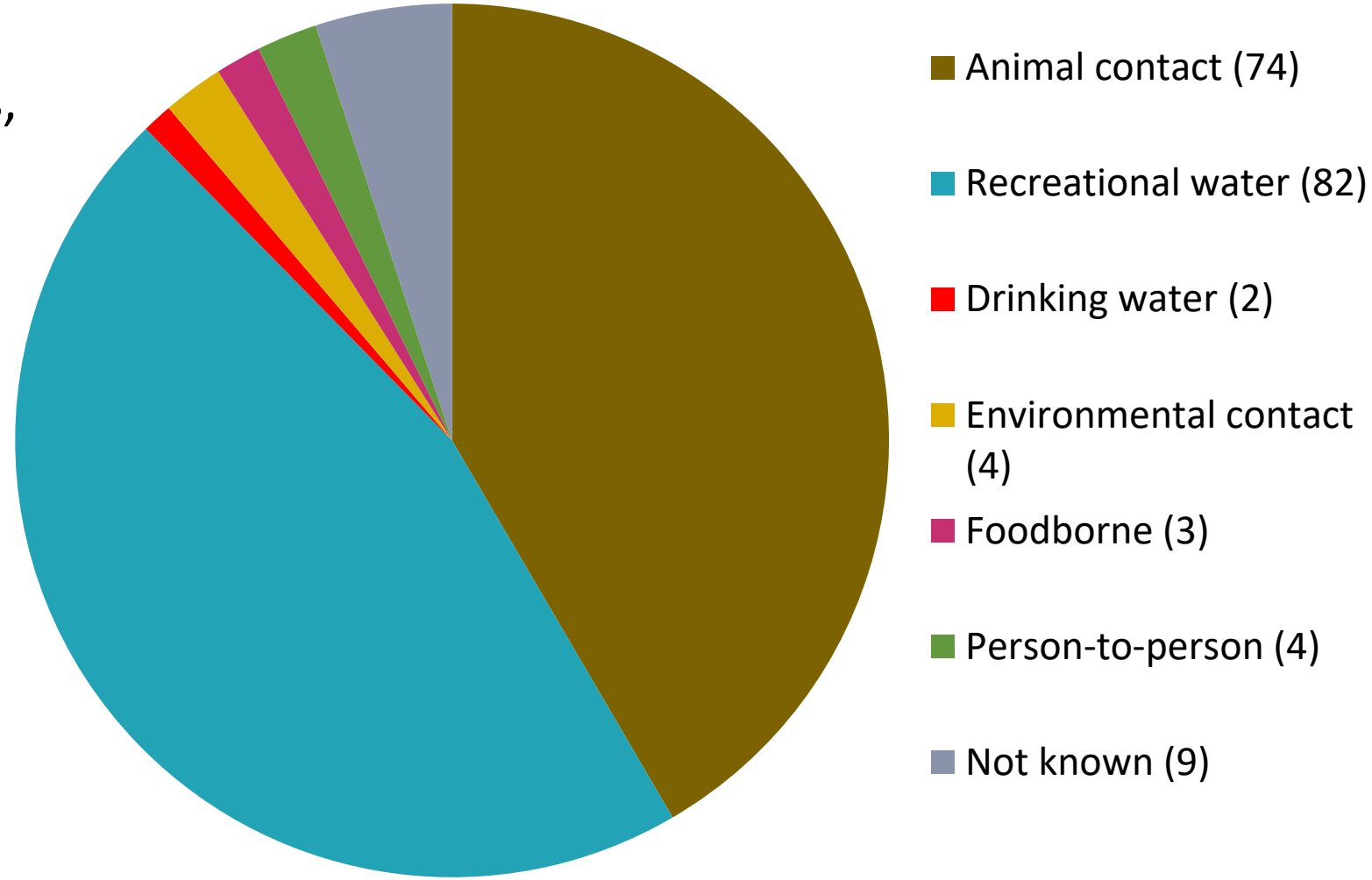
*Source http://www.cdc.gov/healthywater/swimming/pools/chlorine-disinfection-timetable.html

Potentially poor removal by filtration

- designed to provide a physically clean, clear and safe environment, not specifically to remove *Cryptosporidium*
- most effective at low or medium filtration rates with continuous coagulation

178 reported *Cryptosporidium* outbreaks, England &Wales, 2009-2017 (PHE eFOSS and CRU data)

Chalmers *et al*., Parasites and Vectors, March 2019



The PWTAG *Cryptosporidium* pool project

Introduction

- *Cryptosporidium* is not tested for routinely in pools, there are no quantitative standards, and no baseline data are available for the UK.

- Sampling and testing is difficult and expensive, the specialist equipment is lacking, and not all labs can test for it. Validation data for pool samples are lacking.

- Testing as part of an outbreak response requires careful consideration:

➢ investigations may be well after the suspected contamination event

➢ high volume sampling equipment is not readily available

➢ interpretation to inform public health actions needs clarification.

The PWTAG *Cryptosporidium* pool project

Primary Aim:

to investigate the occurrence and numbers of oocysts in UK leisure pools, over 10 weeks during summer and autumn 2017

Secondary aims:

- to inform guidance for pool operators
- to provide data for future quantitative microbial risk assessment
- improve sampling capacity, capability and interpretation for public health investigations.

Why focus on leisure pools?

They are the predominant setting for swimming pool outbreaks

Settings of 82 outbreaks of cryptosporidiosis linked to swimming pools England and Wales, 2009-2017	**Number of reported outbreaks**
Leisure pools	24
Unspecified pools	21
Holiday, caravan and water parks	19
Hydrotherapy pool	5
School and college pools	4
Private clubs and hotels	4
Baby swimming pool	4
Outdoor paddling pool	1

Source: combined dataset of PHE eFOSS national surveillance and CRU data

The PWTAG *Cryptosporidium* pool project

Objectives

1. Evaluate *Cryptosporidium* detection methods for swimming pool and backwash waters.

2. Build and install in-line, high-volume sample rigs with IDEXX Filta-Max xpress® filter modules. Installed in return from pool to treatment plant.

3. Sample up to 1000 L pool water and 1 L backwash water, weekly, for *Cryptosporidium* testing.

4. Sample and test pool waters for bacterial indicators and chemical parameters.

5. Record pool operational data, including bather load and AFRs.

6. Assess the relationships between operating conditions, bather loads, pool parameters, bacteriological counts and *Cryptosporidium* occurrence and oocyst numbers.

The PWTAG *Cryptosporidium* pool project

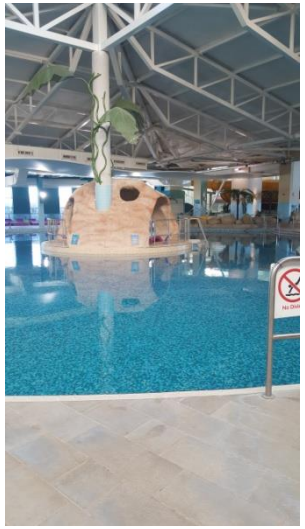
Pool recruitment

6 volunteer free-form leisure pools with features: slides, wave machines, lazy rivers etc.

4 indoor-only pools, 2 linked to outdoor areas.

4 community leisure pools, 2 at large residential holiday parks

All pools inspected using a checklist:
www.publichealthwales.org/cryptopoolguidance/



Standard methods for detection of *Cryptosporidium* in swimming pool waters were validated for drinking water

ISO 15553:2006 US EPA 1623:2012 SCA MoDW "Blue Book" 2010

Continuous sampling large volumes

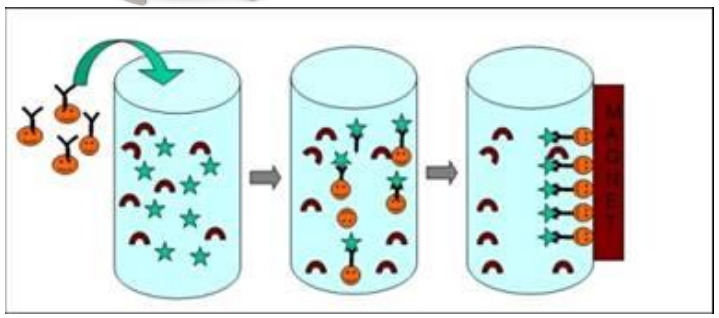
IDEXX Filta-Max xpress® filter modules

elution

centrifugation

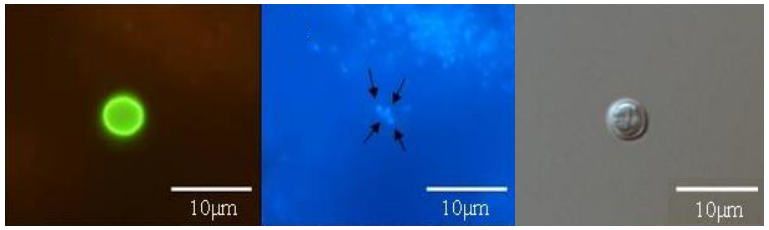
Removal from sample matrix by immuno-magnetic separation

Dynabead anti-Cryptosporidium, Life Technologies



Analysis by immunofluorescence microscopy

Crypto-Cel, TCS Biosciences



oocyst counts.

Need oocyst recovery data from spiking studies: "*Recovery data should be included if oocyst concentrations are to be used to infer health risks*" (Petterson *et al.*, 2007).

How efficient is the method for pool water: chemistry, pH, body fluids, cosmetics, lotions etc?

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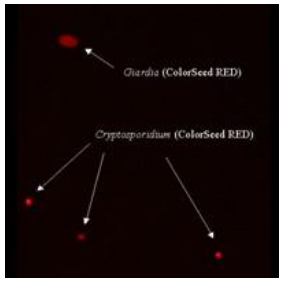
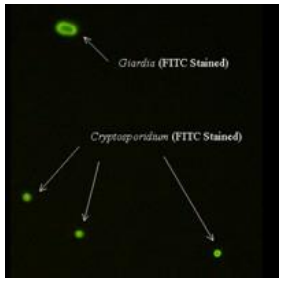
Cryptosporidium spiking trials of pool water samples, using IDEXX Filta-Max xpress® filter modules

- Four, 10 L samples from each of two volunteer pools (one chlorinated and one brominated)
- Spiked with 99 pre-stained *Cryptosporidium* oocysts (ColorSeed™, TCS Biosciences).
- Tested by standard method



Spiked oocysts



Natural oocysts

Image: TCS BioSciences

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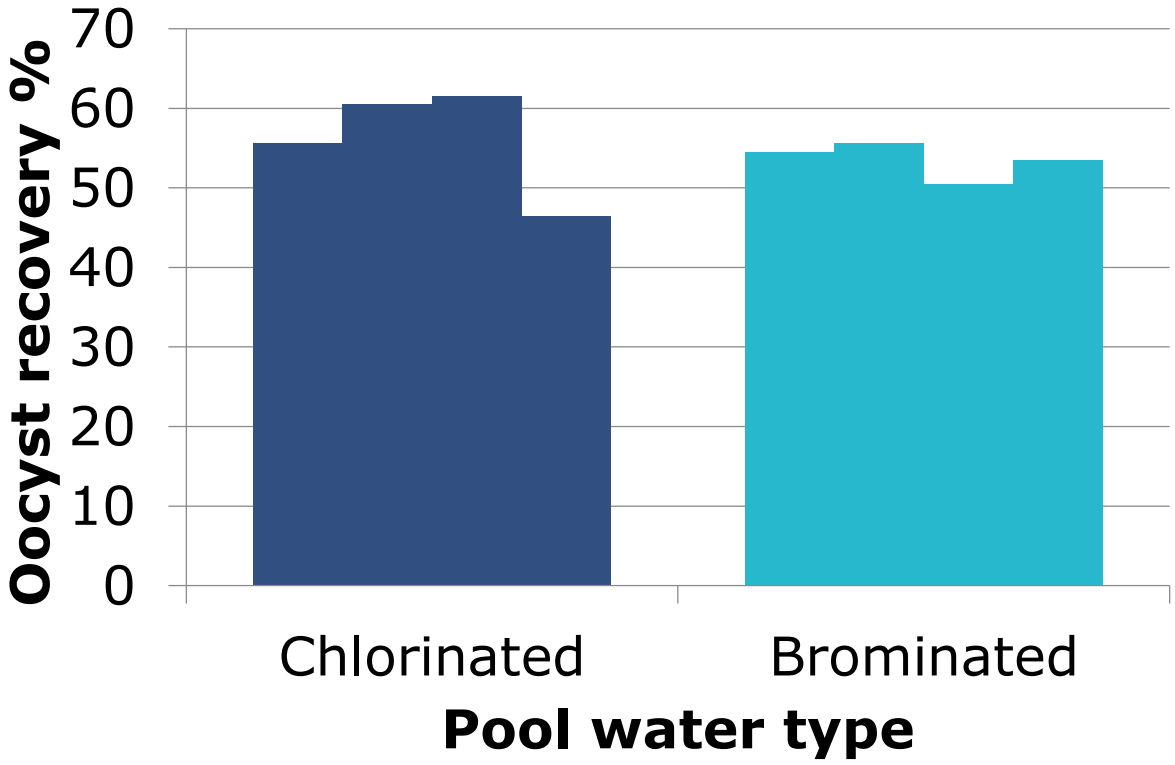
Results – *Cryptosporidium* oocyst recovery data for pool waters spiked with 99 oocysts.



There was no significant difference in oocyst recovery % between pool water types (P=0.31)

Mean recovery 54.8%, range 46.5-61.6% .

This was well within the acceptable range for drinking water (Anon, 2010; McCuin and Clancy, 2003).

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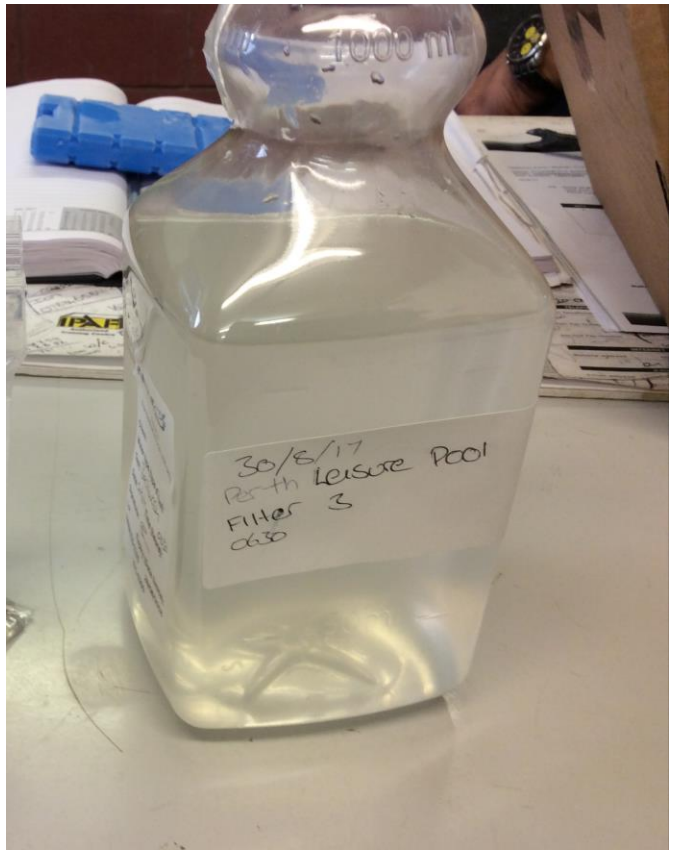
Method – *Cryptosporidium* testing backwash samples

- 1 L backwash samples were available from three pools throughout the study
- Oocyst recoveries were monitored by spiking every sample with 99 pre-stained oocysts (ColorSeedTM, TCS Biosciences).
- *Cryptosporidium* oocysts were enumerated using an in-house method: centrifugation, IMS and IFM



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Cryptosporidium spiking backwash samples, 28 data points

1 sample went to the wrong lab

1 sample failed test

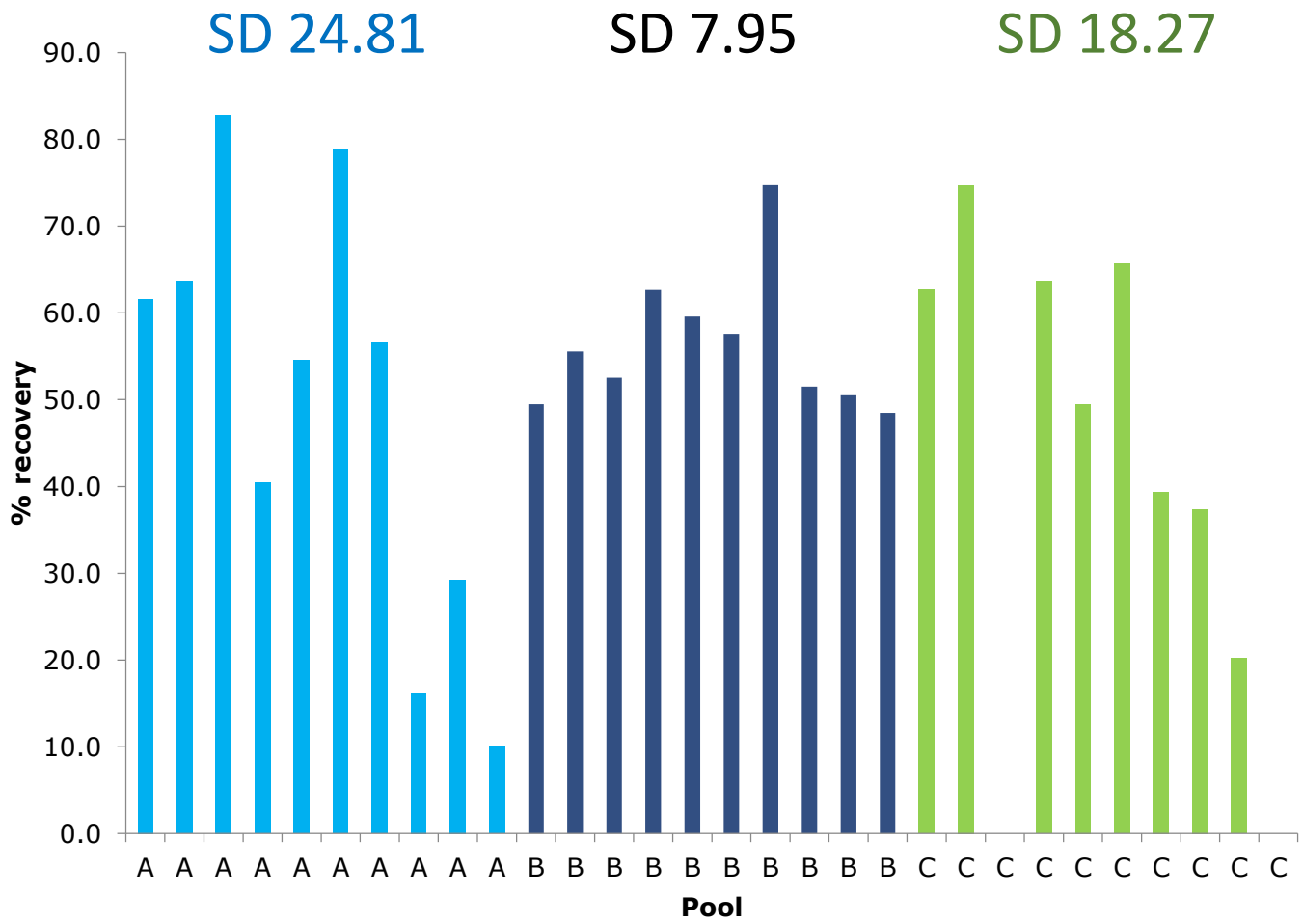
Spike recovery, overall

Range 10.1% to 82.8%

Mean 52.5%

Median 55.5%

Recoveries were variable than pool waters



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Results – *Cryptosporidium* in pool water samples

- Sample volumes were 60 to 999 L, median 493 L, taken over 8 to 24 h

- Oocysts were detected in 12/59 (20 %) samples
  - ➢ 1 sample invalid (sent to wrong lab)

  - ➢ 8/12 (66 %) detections were in August, when bather loads were highest.

- Counts ranged from 0 to 1.16 oocysts per 10 L ; mean 0.04 oocysts per 10 L
- Adjusted counts using the mean recovery rate for pool water ranged from 0 to 2.11 oocysts per 10 L, mean 0.08

The PWTAG *Cryptosporidium* pool project

Results – *Cryptosporidium* in 1 L backwash samples

- 3 pools sampled for 10 weeks

- Oocysts were detected in 2/27 (7%) valid backwash samples, from 2/3 pools
  - 1 and 4 oocysts / L
  - 2adj and 8adj / L
  - The count of 4 (8adj) oocysts was at the same pool and on the same day as the highest oocyst count (23) in pool water
  - The count of 1 (2adj) oocyst was at another pool, also on the same day as oocyst detection in pool water

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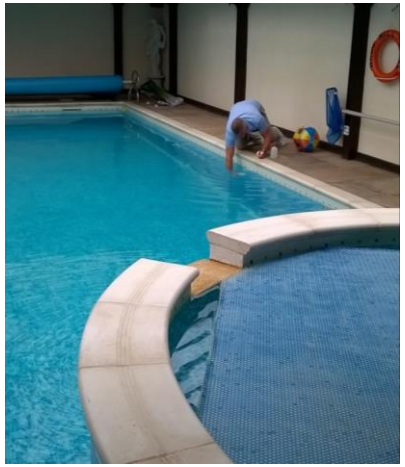

Results - Bacti samples

verified the disinfection system was being managed and controlled

Colony counts of total coliforms*, Escherichia coli,* and *Pseudomonas aeruginosa*

59/60 samples were all zero

➢ 1 sample invalid (sent to wrong lab)

Aerobic colony counts @ 37°C for 24 h

- 12/57 (21 %) samples were unsatisfactory (>100 cfu/ml); none consecutive

➢ 3 samples were invalid (1 sent to wrong lab; 2 arrived >24 h after sampling)

➢ ACC failures and *Cryptosporidium* detections were NOT related; no relationship to NTU

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Visible faecal accident reporting

- 14 AFRs reported in 10 weeks.
- Each pool reported at least one and up to four
- Two were liquid, at the same pool.
- Recorded actions were PWTAG compliant
- 1 report preceeded a *Cryptosporidium* detection at the pool

The PWTAG *Cryptosporidium* pool project

Key findings

- Methods for testing drinking water provided acceptable oocyst recovery rates for swimming pool samples, allowing adjusted counts to be generated to contribute to health risk assessment for *Cryptosporidium* from swimming pools.
- The detection of *Cryptosporidium* was when pools were busiest, suggesting targeted pool operator and health promotion activities are warranted..
- Small numbers of *Cryptosporidium* oocysts detected in swimming pools can be successfully managed by adhering to PWTAG standards
- Improved sampling capacity, capability and interpretation for public health investigations.
- New data on pathogen counts has been generated for UK pools contributing to future quantitative microbial risk assessment (QMRA)

The PWTAG *Cryptosporidium* pool project

Sponsorship and support...without which the project would not be possible

- **PWTAG:** major funder
- **Bourne Leisure:** 6th pool; water for evaluation experiments
- **Certikin, Howard Gosling, Total Pool Chemicals Ltd (Jason Bassett):** sampling valves, rigs, installation
- **IDEXX, especially Megan Luxford:** method evaluation; *Cryptosporidium* courier; sampling training and support; laboratory testing
- **TCS BioSciences Ltd:** ColorSeed
- **Latis Scientific:** bacti shipping and testing
- **Tintometer Limited:** hand-held and in-line turbidity meters
- **Rob Johnston**, Public Health England