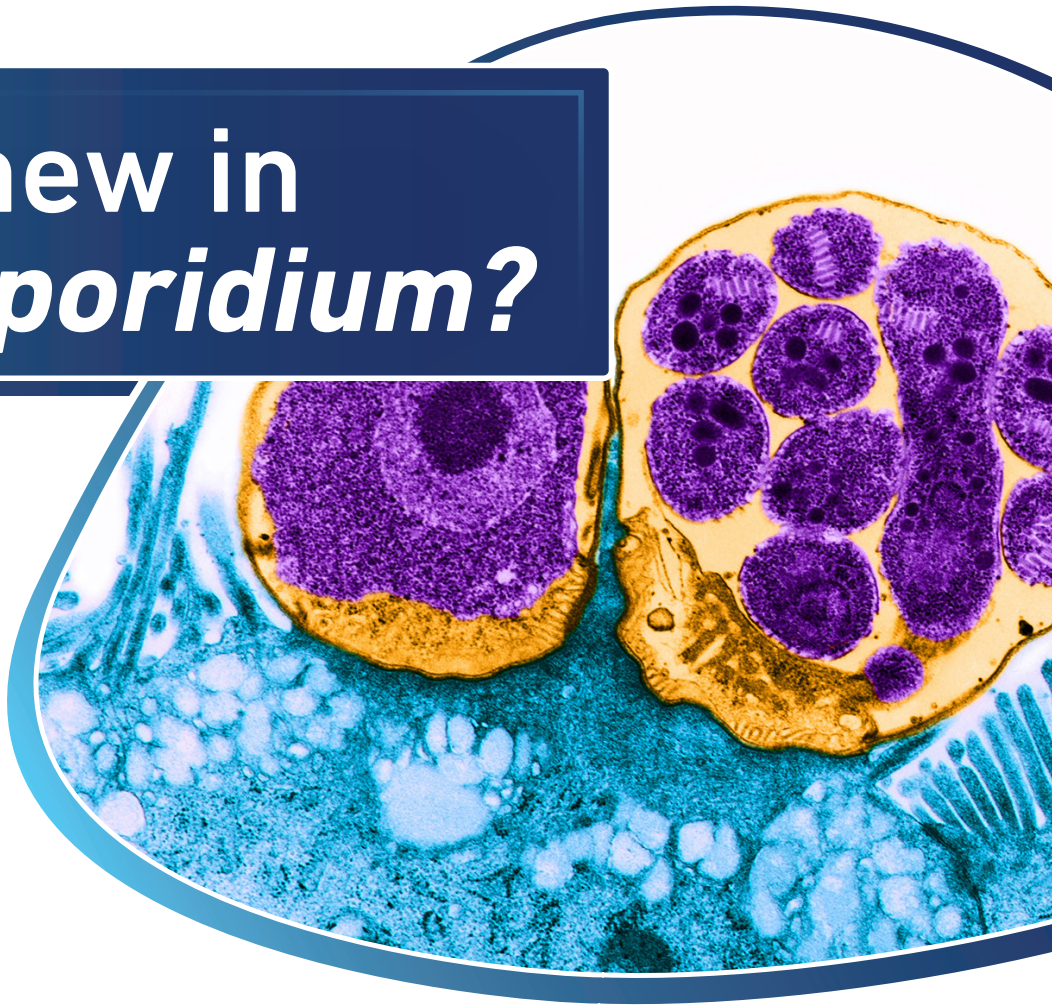
MICROBIOLOGY SOCIETY

What's new in *Cryptosporidium?*



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POSTER ABSTRACT BOOK

The Royal College of Pathologists
Pathology: the science behind the cure

1

An exploration of the awareness of hygienic swimming behaviours and an evaluation of a public health intervention, to reduce the transmission of cryptosporidium.

Hannah Jones

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Abstract

Background: Educating swimming pool users about hygienic swimming behaviours, such as not swimming whilst suffering from diarrhoea and vomiting, is key to reducing the transmission of cryptosporidium.

Methods: To explore awareness of hygienic swimming behaviours and to develop and evaluate a public health intervention to reduce the transmission of cryptosporidium, a study was conducted using an exploratory sequential design. Semi-structured interviews with 28 stakeholders informed the development of a questionnaire completed by 407 swimming pool users. The findings informed the development of a poster to raise awareness of hygienic swimming behaviours, and a small-scale evaluation was conducted with 153 respondents.

Results: Many factors were identified which influenced hygienic swimming behaviours, including current awareness, cultural factors, and the design of swimming facilities. Respondents identified a variety of methods for raising awareness, with a poster in the changing rooms being the most preferred. Positive feedback was provided about the poster, which was perceived as being easy to read and informative. Of note, respondents also reported that the poster had encouraged them to consider their own hygienic swimming behaviours.

Conclusion: A resource was created as part of this study, and it is hoped that it will be used by swimming facilities across Wales, and potentially further, to encourage people to swim, and to do so hygienically. The poster developed had raised awareness of hygienic swimming behaviours and received positive feedback from swimming pool users and endorsement from Public Health Wales.

2

An unusual outbreak of cryptosporidiosis in a British military population in Kenya

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Abstract

Aim

We report preliminary findings and challenges in managing an unusual outbreak of cryptosporidiosis in British military personnel during training exercises in Kenya in 2022.

Methods

Epidemiological and clinical data were recorded on standardised proformas from personnel presenting to medical facilities with diarrhoea, and from symptomatic contacts. Cases were isolated and managed as clinically indicated and outbreak data collated and updated daily. An international multidisciplinary outbreak team met regularly to support the local team and advise on mitigation and control measures. Fresh faecal samples were analysed daily using multiplex PCR (BioFire® FilmArray® gastrointestinal panel).

Results

Between 7-20 Feb 2022, 67 people developed diarrhoea: 12 in the first week and 55 in the second week. Between weeks 3-6, 72 first samples were tested, with a 12-week total of 106. Overall, 63/106 had Cryptosporidium spp. (25 combined with other pathogens); 26/106 other pathogens only; 17/106 no pathogens detected. Epidemiological investigations suggested an initial point source outbreak of cryptosporidiosis related to swimming in contaminated open-air pools, followed by early secondary cases of cryptosporidiosis and later discrete foodborne multiple pathogen diarrhoeal outbreaks. Overall, 187 (14.8%) personnel reported diarrhoea in these 12 weeks and Cryptosporidium spp. were found in 60% of stool samples.

Discussion

Point source outbreaks of cryptosporidiosis are well recognised hazards of swimming pools but have rarely been reported in military personnel. This presentation will focus on the effects of the outbreak on an intensive military training exercise, the diagnostic, investigational and logistic challenges that were met. Detailed molecular epidemiological studies are planned.

3

Cryptosporidium slide genotyping using a new real-time PCR assay reveals high typeability, and diversity within raw water catchments.

Kristin Elwin[1], Guy Robinson[1,2], Heather Ayres[1], Heide Hardiman[3], David Reynolds[3], Stephen Bullock[3], Rachel Chalmers[1,2]

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Abstract

Cryptosporidium is an important cause of gastroenteritis globally and the main agent of waterborne outbreaks caused by protozoan parasites. Water monitoring for Cryptosporidium oocysts is by detection and enumeration using stained slide microscopy. Species identification (known as genotyping) may be undertaken post hoc and remains a specialist test, only undertaken in some laboratories such as in the CRU. The benchmark method is nested PCR-sequencing of part of the SSU rRNA gene, but not all slides are typable and the workflow is cumbersome. We have developed and now use a real-time PCR-sequencing assay based on that gene, using a hydrolysis probe, for the detection and genotyping of all Cryptosporidium spp., which has improved typeability, workflow and turnaround times.

When applied to 115 Cryptosporidium-positive slides, containing 5 to 16 oocysts, mean seven, mode five, collected from Thames Water raw water catchments between 02/2020 and 06/2021 typeability was 82 %. Thirteen different Cryptosporidium species were identified, including the common human pathogens C. hominis and C. parvum, and four named genotypes; multiple species and genotypes were detected from 35 slides. A total of 12 novel Cryptosporidium sequences were also observed. The most common findings were C. ubiquitum on 32 slides and the skunk genotype on 28 slides.

Diverse sources including human, livestock and wildlife faeces contaminate raw waters, indicating the varied nature of interventions that water providers need to take to mitigate against Cryptosporidium.

4

Raising awareness of foodborne cryptosporidiosis through method development, validation and improved risk assessment.

Rachel Chalmers[1,2], Anna Rosa Sannella[3], Barbara Šoba Šparl[4], Gunita Deksne[5], Heather Ayres[1], Ian Woolsey[6], Kristin Elwin[1,2], Lucy Robertson[6], Marco Lalle[3], Simone Caccio[3], Smita Sutrave[7], Tamirat Temesgen[6], Anne Mayer-Scholl[7]

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Abstract

Cryptosporidium has a reputation as a waterborne pathogen and this legacy has meant that foodborne cryptosporidiosis may have been overlooked. For example, in exposure questionnaires, food history may be scant. However, recognised outbreaks have occurred linked to food items such as leafy greens, apple juice, and milk and dairy products.

In 2017, the European Food Safety Authority (EFSA) accepted a mandate for a scientific opinion on public health risks associated with the foodborne parasites *Cryptosporidium, Toxoplasma* and *Echinococcus* which had been identified previously as of importance, but not routinely controlled in food. The published opinion concluded that standardised, validated methods that can be applied across the range of relevant foods are lacking.

Specifically for *Cryptosporidium*, validated and sensitive detection methods are required for development of quantitative risk assessments and efficient control measures; for leafy green vegetables this challenge was taken up by the EFSA-funded IMPACT study, which not only validated a real-time PCR-based detection assay in a ring trial, but also produced guidance for artificial contamination studies that had previously been missing. The latter is now being incorporated into the further development of an ISO standard for the validation of alternative methods against a reference method, addressing viruses and parasites as difficult to culture microorganisms.

This poster will illustrate the *Cryptosporidium* detection assay and summarise the recommended validation procedures.

5

Use of the Polymerase Chain Reaction (PCR) for the Analysis and Enumeration of *Cryptosporidium* Oocysts in Drinking Water

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Abstract

Cryptosporidium presents one of the main waterborne public health threats due to its resistance to chlorine disinfection and ability to cause large-scale outbreaks. The standard method used in the UK water industry for detection and enumeration of *Cryptosporidium* is based on fluorescent microscopy and is laborious and expensive. Molecular methods such as quantitative Polymerase Chain Reaction (qPCR) can be more amenable to streamlining through automation, improving workflows and standardising procedures. We investigated the current use of PCR within the industry, developed and evaluated a qPCR method, and compared it with microscopy to appraise the potential of qPCR for the detection and enumeration of *Cryptosporidium* oocysts in drinking water.

PCR is not widely utilised in the water industry. A qPCR assay incorporating an internal amplification control and calibration curve was developed from a real-time PCR currently used for genotyping, offering practical advantages over the standard microscopic method. Evaluation of this assay demonstrated that detection of *Cryptosporidium* was reliable at low numbers of oocysts; however, enumeration was less reliable and more variable than microscopy. Despite these results, there is potential for the use of PCR-based methods in *Cryptosporidium* analysis if parts of the upstream sample preparation are revised, such as removal of immunomagnetic separation and improved DNA extraction, and alternative technologies for enumeration (such as digital PCR) are explored. However, there are further considerations for both the water industry, drinking water quality regulator and stakeholders in terms of acceptability, from laboratory resources through to interpretation of the detection of DNA vs. oocysts.

6

Implementation of a multi-locus variable number of tandem repeats analysis (MLVA) genotyping scheme for *Cryptosporidium parvum*

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Abstract

A multi-locus genotyping scheme has been validated and rolled out at the national Cryptosporidium Reference Unit for subtyping *Cryptosporidium parvum* from humans and animals involved in human outbreaks. The scheme applies multiple loci variable number tandem repeat analysis (MLVA), following DNA extraction from stools and PCR amplification of seven genetically distant markers (*cgd1-470-1429* (*GRH*), *cgd4-2350-796*, *cgd5-10-310* (*MSF*), *cgd5-4490-2941*, *cgd6-4290-9811* (*MSC6-5*), *cgd8-4440-NC-506*, *cgd8-4840-6355* (*MM19*)) in four-plex and three-plex reactions. Fragment sizing is then performed on the PCR amplicons using the SeqStudio (Thermofisher) genetic analyser. Peaks produced for the DNA fragments at each loci are analysed with Bionumerics (Applied Maths), and "binned" to capture fragment sizes indicative of copy number variation at each locus. MLVA profiles are identified for each sample using the fragment peak binning outcomes, expressed for each locus consecutively in chromosomal order.

In a validation study, the MLVA scheme demonstrated typeability of 0.85 and discriminatory power of 0.99. Variation in copy number at the investigated loci was observed across the samples tested, and a range of *C. parvum* MLVA profiles were identified. The majority (79%) of MLVA profiles in 136 sporadic cases were unique, indicating that genetic clusters should be further investigated for common exposures. A small number of samples contained multiple alleles at one or more loci, indicating the presence of mixed populations of *C. parvum* within samples.

This MLG scheme can be used to infer linkage and is now applied to the investigation of outbreaks. A pilot study for epidemiological surveillance of *C. parvum* is underway.

7

Parapipe: a bioinformatic pipeline for analysing Cryptosporidium NGS data

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Abstract

The necessity to develop methods of processing biological data in a high-throughput manner cannot be understated. The abundance of 'omics data being produced by public health agencies requires robust and clinically validated pipelines to support analysis. Here, we present Parapipe: a bioinformatic pipeline developed to automate, standardise, and streamline the process of dealing with clinical *Cryptosporidium* NGS data and facilitating epidemiological surveys in a clinically validated and reliable fashion. Parapipe is written in the workflow DSL, Nextflow, and is containerised for easy dependency management and implementation on cloud computing and HPC systems. It is designed to replicate the standard workflow a bioinformatician might employ when dealing with short read genomic data, from QC and trimming, to SNP detection and phylogenetic analysis. Extended functionality also supports whole genome assembly, reference guided scaffolding, and higher-level analysis such as VNTR detection and dNdS analysis. This pipeline will be actively developed and maintained, consequently, existing modules are constantly under review for more efficient or effective alternatives, and additional functionality being tested for implementation. This is facilitated by its highly modular nature. As a generalised pipeline, it allows for the analysis of many parasite taxa. Parapipe has undergone extensive validation and testing, both on a modular level, and end-to-end. Testing and validation was carried out using both simulated short read data, and real clinical data from *C. parvum* cluster cases. We hope that Parapipe will become the standard for dealing with Cryptosporidium NGS data by public health agencies.

8

A New Molecular Method for Genotyping *Cryptosporidium* in a Water Utility Laboratory

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Abstract

There have been several waterborne *Cryptosporidium* outbreaks in the UK and of the 60+ known species/ genotypes there are only a few significant human pathogens. New species and genotypes are discovered often therefore accurate speciation is a key consideration in managing contamination incidents.

After water samples are taken, they are washed with PBST, separated using immunomagnetic separation and stained with FITC and DAPI before quantitation using fluorescence and DIC microscopy. Oocysts are isolated from the slide, freeze/thawed (with chelex resin) then DNA is amplified using nested PCR. DNA sequencing is performed and the sequence is compared to different sequences using NCBI-BLAST.

From 41 samples, 58.54% matched the RFLP result. 14 tubes had no direct match between the RFLP species and sequenced. This was due to several reasons including the identification of newer species that were not detailed in our current method and limitations of reading RFLP gels.

These differences included RFLP results changing from *C. parvum* to *C. ditritchi*, "SW species" is now classed as "UK E isolates" and Muskrat/Fox/*C. suis* to Vole (only a few base pairs different). Lastly, *C. cuniculus* was identified as *C. hominus* as the sequences were too close to call via RFLP.

UKAS accreditation has been granted as we successfully compared our RFLP results from our original method and the results were significantly more accurate and current due to the new species being discovered. We are now working on a qPCR method to quantify our samples to improve the accuracy of our results.

9

PARAsite Detection, ISolation and Evaluation - PARADISE

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Abstract

Cryptosporidium and *Giardia* are major contributors to the global burden of gastrointestinal disease in both humans and animals. They are transmitted through direct and indirect routes and can cause outbreaks linked to ingestion of contaminated water and food. Outbreak investigation and source attribution remain difficult. One issue is the genetic variation within species. The PARADISE (PARAsite Detection, ISolation and Evaluation) project is funded through the One Health-European Joint Programme and involves a large European consortium. It works to deliver informative typing schemes and innovative isolation strategies applicable to food and environmental matrices for both parasites. The project has generated large whole-genome sequence datasets for *Cryptosporidium parvum* and *Giardia duodenalis* assemblage B, enabling analysis of genome-wide variability at the European level. The genome data has been used to develop multi-locus sequence typing schemes for these parasites, which have been tested on hundreds of isolates from across Europe and will be validated in ring trials involving PARADISE consortium partners. In addition, metagenomics approaches are being explored as an untargeted method for the detection of foodborne parasites. This involves *in silico* analyses of available metagenomes and experimental work to test the applicability of shotgun- and amplicon-based metagenomics. In parallel, strategies to improve the sensitivity of detection are being developed. This includes selective enrichment of parasites prior to DNA extraction (using aptamers and nanobodies) and from already extracted DNA samples (using hybridization probes). These new methodologies will form the basis for integrated approaches aimed at better controlling foodborne parasites in European food chains.

10

Cryptosporidiosis in young calves in France: scientific and practical aspects

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Abstract

Nowadays, animal health is more than ever a burning issue and overlap with human health is well recognized. In France, 10 to 20% of calves do not reach the age of 6 months. Infectious diarrheal diseases are the main causes of death in young animals. The proportion of cryptosporidiosis linked to symptomatology or the mode of contamination of young animals. In this context, the HealthyCalf project financed by APIS-GENE was developped . Overall, data were collected regarding 836 calves (Holstein + Charolais) over a period of 4 years. Numerous health and epidemiological data were collected such as the age of the animals at the time of the microbiological analyses, their sex, the infectious status of their mothers, their symptoms, the performance of curative or preventive treatments. The performances of two diagnostic tests were evaluated for the detection of Cryptosporidium. The prevalence of Cryptosporidium reached 77% in symptomatic animals, far ahead the ones of Rotavirus, E. coli and Coronavirus. Among animals for whom DNA of Cryptosporidium was detected, only 42% were symptomatic. Speciation and genotyping analyzes demonstrated that calves were not contaminated by same genotypes than their mother. Contamination from the environment seems the most probable origin. Practically, the sensitivity of quick diagnostic test (Speed V-Diar 4®) was better when animals were symptomatic. Vaccination of cows against enteric viruses and bacteria were few effective. To our knowledge, this work represents the most complete field study carried out to date on cryptosporidiosis French farms. Concrete applications can be drawn from the obtained results.

11

Genetic diversity of *Cryptosporidium* spp. in Human and non-human primates (NHPs) in rural and urban areas of Ethiopia

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Abstract

Ethiopian human population and Nonhuman primates (NHPs) have overlapping territories in some regions. NHPs are considered potential sources of zoonotic parasites in humans. This study examined the presence and genetic diversity of *Cryptosporidium* species circulating in both population.

From January to September 2018. Single fecal samples were collected from volunteer patients who visited Wurgessa Health Center (WHC) and Hawassa Health Center (HHC). Fresh stool samples were collected from 94 individuals at WHC and from 93 at HHC. 185 NHPs (177 *Chlorocebus aethiops* and 8 *Colobus guereza*) were also examined. Sequence-based characterization of *Cryptosporidium* has been performed to determine species and genotype.

Genotype data for *Cryptosporidium* spp. were obtained in 48 of 86 human positive PCR samples. Among those genotyped, *C. parvum* was frequently detected. *C. hominis* isolates IaA20, and IdA21 were also identified. Of the 185 NHPs samples, fifty-one were tested positive for *Cryptosporidium* infection. The species detected were *C. parvum* , *C. hominis*, and *C. cuniculus* . Mixed infection with *C. parvum* and *C. hominis* were detected in 2 samples. *C. hominis* IaA20 and C. parvum IIaA17G1R1 were the most prevalent subtypes detected.

These results confirm that *Chlorocebus aethiops* and *Colobus guereza* can be infected with diverse *C. parvum* and *C. hominis* subtypes which are likely associated with human infections. This finding has significant implications for both public health and animal agriculture in Ethiopia.

12

Investigating genetic clusters of *Cryptosporidium parvum* to develop improved epidemiological understanding: Wales and North West England, March to June 2022

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Abstract

Background:

Cryptosporidium outbreaks may require high impact public health measures. However, cases and outbreaks are under-ascertained. Outbreak identification can be improved by multilocus genotyping (MLG). A recently validated multilocus variable number of tandem repeats analysis (MLVA) has shown epidemiologically-unrelated *Cryptosporidium parvum* isolates display high diversity (79% MLGs unique), suggesting genetic clusters might indicate unrecognised outbreaks. We aim to explore if genetic clusters of *C. parvum* can identify otherwise missed epidemiological links.

Methods:

Genetic clusters will be identified using MLVA to investigate *C. parvum* isolates from Wales and North West England, received at the Reference Unit from March-June 2022 (pilot: 28 March-22 April). Epidemiological/exposure data from routine questionnaires will be checked for risk factors and compared to genetic clusters.

Results:

During the pilot, 37 samples were genotyped: 15 in Wales and 22 in NW England, identifying five genetic clusters, each of two cases. One English cluster (55 year-old (yo) female; 22yo male) had no epidemiological links identified; two English clusters and one Welsh cluster are awaiting questionnaires. Further exposure information is awaited for a cross-border cluster (58yo female; 1yo male). Two Welsh cases share an epidemiological link (32yo mother and 1yo son), but MLGs are different.

Conclusions:

From the pilot results of this service development project, there are possible clusters that may have been missed using reported exposure information alone. Further isolates and questionnaires over the study period will provide more details and inform whether a sentinel surveillance structure is useful for *C. parvum* in England and Wales.

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