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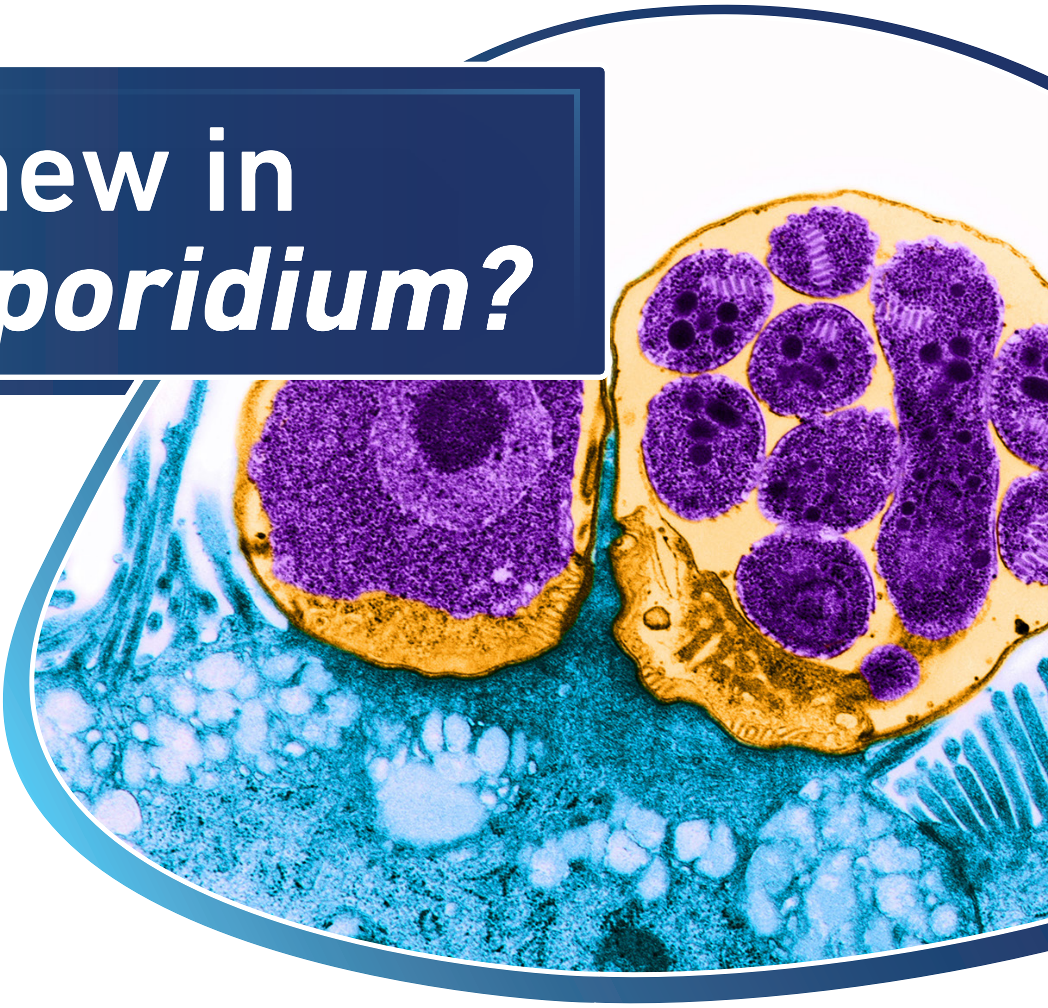
What's new in *Cryptosporidium?*



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Swansea University Bay Campus, Swansea, UK

ORAL ABSTRACT BOOK

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

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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

Do we see changes in the genetic diversity of *Cryptosporidium parvum* genotypes during the calving season within a dairy herd?

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Abstract

Bovine cryptosporidiosis is a common cause of calf enteritis across the United Kingdom, which is caused by an infection with *Cryptosporidium parvum*.

A longitudinal study on *C. parvum* infection was conducted on a dairy farm in Midlothian to examine changes to *C. parvum* genotypes during the calving season in order to identify potential sources of infection. Faecal samples were collected from before and throughout calving with samples being grouped based on the pen of their collection. These pens containing either adult cattle, new-born calves or older calves. We conducted oocyst concentration steps before DNA extraction and 18S PCR/sequencing to identify *C. parvum* positive samples, which were genotyped using the *gp60* gene and a panel of microsatellite markers.

Current data, based on the *gp60* locus, shows that there is one genotype (IIaA15G2R1) present in all calf samples and all but one of the *C. parvum* positive adult samples, regardless of timing or location of their collection. The IIaA15G2R1 *gp60* genotype is the most common *C. parvum* genotype in the United Kingdom. In previous years, an IIaA19G2R1 genotype was predominant in the *C. parvum* positive calf population in this farm, which has not been detected yet during this study. The samples are currently being typed using microsatellite markers and the results will be presented at the meeting. This apparent lack of genotypic diversity that exists from the moment calves are born may indicate that the IIaA15G2R1 genotype has a selective advantage in infecting calves.

4

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.

5

Detection and prevalence of *Cryptosporidum* spp. in wild deer in Scotland

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Abstract

Cryptosporidiosis is a commonly diagnosed enteric disease of livestock and humans which can become infected through accidental exposure to *Cryptosporidium* oocysts in contaminated food/feed and water. Currently, little is known about the prevalence and species of *Cryptosporidium* found in Scottish wild deer and the risks they pose to humans and livestock species. As part of a large-scale study examining enteric pathogens, faecal samples (n=1005) were collected from the four species of wild deer found in Scotland: Red (n=461), Roe (n=408), Sika (n=96), Fallow (n=17) and of unknown species (n=23). Each sample was processed individually to concentrate any oocysts present through a flocculation/sedimentation protocol, prior to DNA extraction using a modified commercial kit method. All samples were tested using a *Cryptosporidium* spp. 18S gene PCR, all positive samples were then sent for sequence analysis and all *C. parvum* positive samples were genotyped at the *gp60* locus.

Results show that 121/1005 (12.04%) samples tested positive for *Cryptosporidium* 18S DNA. Positive samples were detected for all four deer species. Sequence analysis revealed the presence of seven different *Cryptosporidium* species/isolates: deer isolate (n=70), *parvum* (n=24) *ubiquitum* (n=14), *ryanae* (n=5), vole isolate (n=2), *andersoni* (n=1), cervine isolate (n=1) and no species was identified in 4 samples. Overall 24/1005 (2.39%) of samples tested positive for *C. parvum*. Genotyping (*gp60*) was successful for 10/24 samples, all of which demonstrated the IIaA15R1 genotype.

These results suggest that deer could pose a risk to human/animal health, which may not be that significant.

6

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 developpped . 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.

7

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.

8

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.

9

Impact of the COVID-19 pandemic on the epidemiology of Cryptosporidium spp. in England and Wales

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Abstract

Background

In England and Wales, cryptosporidiosis cases peak in late spring and early autumn, attributed to Cryptosporidium parvum infection (spring), usually associated with environmental exposure, or to C. hominis (autumn), usually associated with overseas-travel and activities such as swimming. COVID-19 interventions limited foreign-travel in 2020 with a 75% reduction, but might have increased environmental exposure during ‘staycations’. We investigated the impact on the epidemiology of cryptosporidiosis.

Methods

Confirmed cases of Cryptosporidium species were identified in England and Wales between 01 January 2015 and 31 December 2021. Incidence trends were described by age, sex, location, foreign travel, socio-economic status and ethnicity. An interrupted time-series analysis established if incidence was significantly different to that in the years before the pandemic.

Results

There were 9,046 C. hominis and 12,273 C. parvum cases reported with an annual-average of 1765 and 1895 cases, respectively. There were 174 C. hominis cases and 1,237 C. parvum reported in 2020 (43 cases and 1,560 cases in 2021, respectively), representing a 94% and 26% reduction over the pandemic.

Conclusion

The pandemic had a large impact on Cryptosporidium case numbers, species and peaks: an abrupt reduction of C. hominis cases after a typical start to 2020 (153 cases in the first quarter followed by 21 cases in the following three quarters) and a discernible shift in seasonality for C. parvum, peaking later into summer-autumn. The influence of lockdowns and reduced foreign travel demonstrated the power of minimising personal-contact as an infection prevention and control measure through a natural experiment.

10

Prevalence of *Cryptosporidium* species in dairy cows' farms across Europe

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Abstract

Cryptosporidium genus is comprised of protozoan parasites, which infect a wide range of hosts, causing a disease called cryptosporidiosis. In cattle-farms, the incidence of cryptosporidiosis results in high mortality and, consequently, is a source of considerable economic loss in the livestock industry. Infected animals also might act as major reservoir of *Cryptosporidium* spp., in particularly *C. parvum*, the most common cause of cryptosporidiosis in cattle, and thus pose a significant risk to other farms via breeding centers, to the trading of livestock, and to human health. This study, aimed to assess *C. parvum* prevalence across dairy farms in Belgium, Cyprus, Czech Republic, France, the Netherlands and the UK, and further investigate the zoonotic potential of the circulating *C. parvum* subtypes. To accomplish this, ~2500 cow stool samples, corresponding to 133 dairy-farms from all six countries, were analyzed. Well-established protocols amplifying the 18S-rRNA and gp60 genes fragments, followed by DNA sequencing, were used for the detection and subtyping *C. parvum*; the DNA sequences obtained were further characterised using a combination of bioinformatics and phylogenetics methods. Our results showed 20.8% to 28.3% prevalence of *Cryptosporidium* spp. in all countries. The gp60 subtyping carried out demonstrated a significant number of the *C. parvum*-positives belong to the IIa allelic family, which has been also detected in humans. Consequently, this study highlights how widespread is *C. parvum* in dairy-farms and endorses cattle as a major carrier of zoonotic *C. parvum* subtypes, which subsequently pose a significant threat to human health.

11

Studies on the Health Sequelae of Human Cryptosporidiosis

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Abstract

Evidence is accumulating to suggest that, like some bacterial causes of gastroenteritis, cryptosporidiosis may have longer-term consequences, even in high-income countries. C. parvum in animal models induces pathophysiological features consistent with PI-IBS. This presentation will summarise the findings of studies carried out at the Cryptosporidium Reference Unit, Swansea, including a longitudinal cohort study of patients from an outbreak of C. parvum (1), a prospective study of post-infection sequelae of C. parvum and C. hominis over a 12-month period (2), and a systematic review of the literature (3).

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