Absence of \textit{Cryptosporidium hominis} and dominance of zoonotic \textit{Cryptosporidium} species in patients after Covid-19 restrictions in Auckland, New Zealand

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Abstract

Coronavirus disease-2019 (Covid-19) nonpharmaceutical interventions have proven effective control measures for a range of respiratory illnesses throughout the world. These measures, which include isolation, stringent border controls, physical distancing and improved hygiene also have effects on other human pathogens, including parasitic enteric diseases such as cryptosporidiosis. \textit{Cryptosporidium} infections in humans are almost entirely caused by two species: \textit{C. hominis}, which is primarily transmitted from human to human, and \textit{Cryptosporidium parvum}, which is mainly zoonotic. By monitoring \textit{Cryptosporidium} species and subtype families in human cases of cryptosporidiosis before and after the introduction of Covid-19 control measures in New Zealand, we found \textit{C. hominis} was completely absent after the first months of 2020 and has remained so until the beginning of 2021. Nevertheless, \textit{C. parvum} has followed its typical transmission pattern and continues to be widely reported. We conclude that ~7 weeks of isolation during level 3 and 4 lockdown period interrupted the human to human transmission of \textit{C. hominis} leaving only the primarily zoonotic transmission pathway used by \textit{C. parvum}. Secondary anthroponotic transmission of \textit{C. parvum} remains possible among close contacts of zoonotic cases. Ongoing 14-day quarantine measures for new arrivals to New Zealand have likely suppressed new incursions of \textit{C. hominis} from overseas. Our findings suggest that \textit{C. hominis} may be controlled or even eradicated through nonpharmaceutical interventions.

Introduction

Coronavirus disease-2019 (Covid-19) was declared a global pandemic by the World Health Organization (WHO) on 11 March 2020, leading to a variety of responses by different governments around the world. The nonpharmaceutical intervention strategy of New Zealand was particularly stringent and effectively eliminated a burgeoning Covid-19 outbreak through a nationwide lockdown, physical distancing, improved hand hygiene and ongoing 14-day quarantine for returning travellers (Robert, 2020; Baker et al., 2020a). A four-level alert system (New Zealand Government, 2020) was introduced on 21 March, beginning at level 2 and quickly moving to level 3 on 23 March and level 4 (i.e. the entire nation goes into self-isolation) on 25 March. The level 4 lockdown remained in place until 27 April, when it was reduced to level 3. By 11 May it was lowered to level 2 and finally decreased to level 1 on 8 June (Baker et al., 2020b). The primary intention of these measures in New Zealand was to reduce the transmission of Covid-19, but they also brought about a reduction in influenza and other respiratory viral infections in 2020 (Huang et al., 2021). Similar impacts have also been observed elsewhere (Fricke et al., 2021; Yang et al., 2021), but little attention to date has been given to parasitic enteric diseases.

\textit{Cryptosporidium} species are obligatory intracellular intestinal parasites that infect a wide range of vertebrate hosts, causing a considerable burden of gastrointestinal disease (Xiao et al., 2004; Garcia and Hayman, 2016). In New Zealand, cryptosporidiosis is a notifiable disease with a higher incidence compared to other developed countries (Learmonth et al., 2004). Two species, \textit{C. hominis} and \textit{C. parvum} are responsible for the majority of human infections globally, and in New Zealand (Garcia-R and Hayman, 2017; Garcia-R et al., 2017; Garcia-R et al., 2020b). Of these, \textit{C. hominis} is transmitted primarily through human-to-human contact whereas \textit{C. parvum} is mainly zoonotic with ruminants as the primary reservoir host ([Learmonth et al., 2004; Feltus et al., 2006; Leitch and He, 2011] though see King et al. (2019)]. Clear seasonal peaks in cryptosporidiosis are present in New Zealand. An early autumnal peak for \textit{C. hominis} associated with increased recreational water use and a spring peak for \textit{C. parvum} associated with dairy cattle calving season is observed each year (Learmonth et al., 2004; Lake et al., 2008; Garcia-R et al., 2020b). Transmission of both species is through the faecal–oral route, following contact with infected hosts or water contaminated with the transmissive stages (oocysts) of the organism (Bouzid et al., 2013).
One of the difficulties with monitoring cryptosporidiosis is the lack of morphological characters to identify different Cryptosporidium species. Frequently, diagnosis is based on microscopy by the presence of oocysts in stool samples of infected patients or enzyme immunoassay and species-level identification is rarely attempted. Reliable species and subtype level identification is possible through molecular methods, though diagnostic laboratories moving to molecular diagnostics typically utilize qPCR and report presence/absence, without speciation. The most widely used marker for this purpose is the 60 kDa glycoprotein locus (gp60), upon which the subtyping of Cryptosporidium species is based (Feng and Xiao, 2017). This classification scheme has been used to study the epidemiology of human outbreaks of cryptosporidiosis in many previous studies (O’Brien et al., 2008; Chalmers et al., 2019). Here, we examine the disease dynamics of Cryptosporidium infections using gp60 for species identification and subtype family diversity patterns before, during and after lockdown measures in New Zealand.

Materials and methods
Anonymous Cryptosporidium-positive stool samples were collected between 2015 and 2021 through routine public health investigation activities from symptomatic humans visiting private and public general practitioner (GP) surgeries and hospitals throughout New Zealand. Samples were sent from accredited diagnostic laboratories to the Protozoa Research Unit at Hopkirk Research Institute (Massey University) for molecular analyses under a Ministry of Health contract. Stools were collected in 10-ml screw-cap tubes and stored at 4°C in the laboratory until DNA extraction.

DNA extraction was carried out 1–2 weeks after receiving samples at the Hopkirk Research Institute. Before DNA extraction, the oocysts of Cryptosporidium were physically disrupted using a beadbeater (Tissue Lyser II, Qiagen) at 30 Hz for 5 min. DNA extractions were performed using Zymo Quick-DNA Fecal/Soil Microbe kits following the manufacturer’s instructions. A fragment of the gp60 gene was amplified using a nested PCR (Alves et al., 2003; Waldron et al., 2009) and amplification products were sequenced in both directions using Big Dye Terminator version 3.1 reagents on an ABI 3730XL automated DNA sequencer (Applied Biosystems, Foster City, California, USA). Cryptosporidium gp60 sequences were classified into species and subtype families according to guidelines in Feng and Xiao (2017). While our sampling prior to 2020 included a wide range of regions throughout New Zealand, the 2020 samples were dominated by the Auckland region (118/130). For this reason, we filtered out results from elsewhere and limited our analyses to samples collected from the Auckland region. To check the representativeness of our dataset, we compared it with data from the national notifiable disease surveillance website (https://surv.esr.cri.nz/surveillance/annual_diseasetables.php).

Results
Our dataset included 1502 sequences received from 9 January 2015 to 3 March 2021 (Supplementary Table 1). Of these, C. parvum was the most common (n = 868), followed by C. hominis
Cryptosporidium cuniculus (28) and Cryptosporidium erinacei (8). Seasonal dynamics of C. hominis and C. parvum before 2020 and the impact of the Covid-19 lockdown are illustrated in Fig. 1. Cryptosporidium hominis was commonly found in the first (Q1) and second quarters (Q2) of the year between January and June while C. parvum was more common in the third (Q3) and fourth quarters (Q4) from July to December. The exception to this is in 2020, when no C. hominis was detected after 14 February 2020 (Fig. 1).

Further examination of gp60 subtype families shows the diversity and temporal dynamics of C. hominis and C. parvum (Fig. 2). Within C. hominis, subtype family Ib was detected consistently between 2015 and the beginning of 2020. Other gp60 subtype families in C. hominis appeared as sporadic outbreaks e.g. Id in 2016, Ig in 2017, 2018 and 2019 and/or single cases e.g. Ia in 2015–2019. Within C. parvum, subtype families Ila and IId were consistently detected whilst Ilc and lle appeared as sporadic single cases. Of the other zoonotic Cryptosporidium species, C. cuniculus and C. erinacei were only detected occasionally.

Our data are representative of the total number of cases reported nationally from the notifiable disease database from 2015 to 2019 (between 33% and 46%, Supplementary Table 1). However, in 2020 the number of cases reported to our lab dropped to a lower proportion (18%). This was probably caused by extra stress on diagnostic laboratories that did not send as many samples to our lab for molecular analyses during and following the Covid-19 outbreak. Data from outside Auckland are not included in our analyses due to low numbers in 2020, but in the few samples from outside Auckland which we do have, no post lockdown cases of C. hominis have been reported.

Discussion

In response to Covid-19, New Zealand underwent a nationwide lockdown beginning in late March 2020, with households spending over 7 weeks at home except for essential personal movements like supermarket and hospital visits. Since this time, our surveillance has revealed that C. hominis has not been detected in reported cases of cryptosporidiosis from the Auckland region. This absence has continued until the time of publication during the first quarter of 2021, which in previous years has been the beginning of peak season for C. hominis infections. In contrast, C. parvum and other less common zoonotic Cryptosporidium species have continued typical seasonal patterns. Our data suggest that the national measures for responding to the Covid-19 also interrupted C. hominis transmission. For instance, the closure of all child care centres and schools may have limited C. hominis transmission since these have been linked to previous outbreaks (Goñi et al., 2015). Ongoing managed isolation of visitors or citizens returning to New Zealand since then has also likely reduced new imported cases of cryptosporidiosis.

Prior to 2020, infections by C. hominis were reported seasonally (Garcia-R and Hayman, 2017; Garcia-R et al., 2020b). Furthermore, temporal dynamics of gp60 subtype families generally revealed sporadic infection patterns. Subtype families Ia, Id, Ie, If and Ig appeared at irregular intervals throughout the
study period, which may indicate an importation by visitors or returning travellers from a global pool of *C. hominis* that fail to establish within New Zealand or an opportunistic behaviour of the parasite (Garcia-R and Hayman, 2017; Garcia-R et al., 2020a). The majority of *C. hominis* cases was subtype family Ib, which is also the most common subtype family globally (Feng et al., 2009; Putignani and Menichella, 2010; Segura et al., 2015). These findings imply that despite the hardiness of *Cryptosporidium* oocysts in the environment (Carey et al., 2004), historical *C. hominis* infections in New Zealand arise through importation and that the levels of sanitation and hand hygiene, as well as a low-density population, may be sufficient to prevent the establishment of endemic *C. hominis* infections. Similarly, improved hand hygiene on farms, especially during calving season may help control zoonotic *C. parvum* transmission in New Zealand (Thomas-Lopez et al., 2020).

*C. parvum* sequences were dominated by gp60 subtype families Ila and IId, which both occurred consistently throughout the study period, including during 2020. These *C. parvum* subtype families are primarily associated with ruminants and transmitted through them to humans (Mammeri et al., 2019; Nader et al., 2019). Both have been detected in cattle and sheep in New Zealand (Garcia-R et al., 2017) and evidence from overseas suggests that subtype family IId may be more prevalent in sheep and goat populations (Quilez et al., 2008), but further research is needed to identify if host-specific *C. parvum* subtype families occur in New Zealand’s domestic animals. Overall, *C. parvum* transmission was not interrupted by Covid-19 control measures except perhaps by a small reduction in peak numbers during 2020 Q3 and Q4. Subtype family IIC is rare in New Zealand, with only six cases in our dataset. Previous research shows that unlike other *C. parvum* subtype families, IIC is an anthropoontic (human-adapted) and a potential emerging genotype (King et al., 2019; Nader et al., 2019). While no cases of subtype family IIC were detected after Covid-19 control measures, its rarity before Covid-19 prevents any strong conclusions from our data. Further investigation of gp60 diversity, i.e. trinucleotide repeat regions, may have revealed more details of the transmission patterns of cryptosporidiosis in New Zealand, but this would have been difficult to assess since we do not have descriptive epidemiology or exposure data for our cases. In *C. parvum*, previous research has demonstrated strong associations between animal contact and cryptosporidiosis, though human-to-human transmission is also possible (Chalmers et al., 2019). However, like *C. hominis*, we suggest that human to human *C. parvum* outbreaks in New Zealand are contained through basic sanitation measures.

Our study has limitations in the methodology of data collection stemming from significant changes to human behaviours during 2020 which may have influenced our results. The number of samples we have for 2020/2021 is lower than in previous years and adds uncertainty in the data. As well as potentially being due to a reduction in transmission and cases, since the symptoms of cryptosporidiosis do not closely match Covid-19, people with symptoms of *C. parvum* may have less likely than *C. hominis* numbers at zero. Ongoing monitoring of *Cryptosporidium* species and gp60 subtype families as international travel restrictions ease will provide further information on cryptosporidiosis in New Zealand. Finally, to further investigate the preliminary findings presented here we recommend future studies focus on descriptive epidemiology and exposure data for cases of human cryptosporidiosis in New Zealand.

**Supplementary material.** The supplementary material for this article can be found at https://doi.org/10.1017/S0031182021000974.

**Data.** Sequence data availability is outlined in Garcia-R et al. (2020b).

**Author contribution.** DH conceived and designed the study. PO and AP conducted data gathering. JG and MK performed analyses. MK wrote the original draft article and all coauthors contributed to editing.

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**Ethical standards.** Not applicable.

**References**

Alves M, Xiao L, Sulaiman I, Lal AA, Matos O and Antunes F (2003) Subgenotype analysis of *Cryptosporidium* isolates from humans, cattle, and zoo ruminants in Portugal. *Journal of Clinical Microbiology* 41, 2744–2747.

Baker MG, Kvalsvig A and Verrall AJ (2020a) New Zealand’s COVID-19 elimination strategy. *The Medical Journal of Australia*, 213, 198–200. e191.

Baker MG, Wilson N and Anglemyer A (2020b) Successful elimination of COVID-19 transmission in New Zealand. *New England Journal of Medicine* 383, e56.

Bouzid M, Hunter PR, Chalmers RM and Tyler KM (2013) *Cryptosporidium* pathogenicity and virulence. *Clinical Microbiology Reviews* 26, 115–134.

Bushen OY, Kohli A, Pinkerton RC, Dunpik K, Newman RD, Sears CL, Fayer R, Lima AAM and Guerannt RL (2007) Heavy cryptosporidial infections in children in northeast Brazil: comparison of *Cryptosporidium hominis* and *Cryptosporidium parvum* transmission. *Transactions of The Royal Society of Tropical Medicine and Hygiene* 101, 378–384.

Carey CM, Lee H and Trewors JT (2006) Biology, persistence and detection of *Cryptosporidium parvum* and *Cryptosporidium hominis* oocyst. *Water Research* 38, 818–862.

Chalmers RM, Robinson G, Elwin K and Elson R (2019) Analysis of the *Cryptosporidium* spp. and gp60 subtypes linked to human outbreaks of cryptosporidiosis in England and Wales, 2009 to 2017. *Parasites and Vectors* 12, 95.

Dreessen J, Villarroel-Gonzales D, Cleves S, Reins H and Pulz M (2007) Regionally increased incidence of notified cryptosporidiosis cases due to different laboratory methods. *Gesundheitswesen* 69, 483–487.

Feltus DC, Giddings CW, Schncek BL, Monson T, Warshauer D and McEvoy JM (2006) Evidence supporting zoonotic transmission of *Cryptosporidium* (Weir, 2001). Of the species in our study, *C. hominis* is associated with more severe infections and higher shedding rates (Bushen et al., 2007), potentially making reporting and detection of *C. hominis* more likely than *C. parvum*. Once cryptosporidiosis cases are confirmed, the diagnostic laboratories send stool samples to our lab for genetic analyses, but this may not be nationally representative as evidenced by the lack of samples from outside the Auckland region during 2020. The reasons for this are unclear but may reflect a focus on essential services and over-stressed health systems not having time to send samples since the start of Covid-19.

In conclusion, our study revealed that nonpharmaceutical interventions greatly reduced *C. hominis* in Auckland, New Zealand. Since the March 2020 national lockdown, travel restrictions and mandatory visitor isolation have kept reported *C. hominis* numbers at zero. Ongoing monitoring of *Cryptosporidium* species and gp60 subtype families as international travel restrictions ease will provide further information on cryptosporidiosis in New Zealand. Finally, to further investigate the preliminary findings presented here we recommend future studies focus on descriptive epidemiology and exposure data for cases of human cryptosporidiosis in New Zealand.
Cryptosporidium spp. in Wisconsin. *Journal of Clinical Microbiology* **44**, 4303–4308.

Feng Y and Xiao L (2017) Molecular epidemiologic tools for waterborne pathogens Cryptosporidium spp. and *Giardia duodenalis*. *Food and Waterborne Parasitology* **8–9**, 14–32.

Feng Y, Li N, Duan L and Xiao L (2009) *Cryptosporidium* genotype and subtype distribution in raw wastewater in Shanghai, China: evidence for possible unique *Cryptosporidium hominis* transmission. *Journal of Clinical Microbiology* **47**, 153–157.

Fricke LM, Glückner S, Dreier M and Lange B (2021) Impact of non-pharmaceutical interventions targeted at COVID-19 pandemic on influenza burden – a systematic review. *Journal of Infection* **82**, 1–35.

García-R JC and Hayman DT (2017) Evolutionary processes in populations of *Cryptosporidium* inferred from gp60 sequence data. *Parasitology Research* **116**, 1855–1861.

García-R JC, French N, Pita A, Velalanthirī N, Shrestha R and Hayman D (2017) Local and global genetic diversity of protozoan parasites: spatial distribution of *Cryptosporidium* and *Giardia* genotypes. *PLoS Neglected Tropical Diseases* **11**, e0005736.

García-R JC, Cox MP and Hayman DTS (2020b) Comparative genetic diversity of *Cryptosporidium* species causing human infections. *Parasitology* **147**, 1532–1537.

García-R JC, Pita AB, Velalanthirī N, French NP and Hayman DTS (2020a) Species and genotypes causing human cryptosporidiosis in New Zealand. *Parasitology Research* **119**, 2317–2326.

García RJ and Hayman DT (2016) Origin of a major infectious disease in vertebrates: the timing of *Cryptosporidium* evolution and its hosts. *Parasitology* **143**, 1683–1690.

Goñi P, Almagro-Nieves D, Cieloszyk J, López S, Navarro-Mari JM and Gutiérrez-Fernández J (2015) Cryptosporidiosis outbreak in a child daycare center caused by an unusual *Cryptosporidium hominis* subtype. *Enfermedades Infecciosas y Microbiología Clínica* **33**, 651–655.

Huang QS, Wood T, Iley L, Jennings T, Jefferies S, Daniells K, Nesdale A, Martin M, Baker MG, Taylor S, Trenholme A, Wong C, Lawrence S, McArthur C, Stanley A, Roberts S, Rahnama F, Bennett J, Mansell C, Dijkshoorn L, Solano-Galindo JA, Hagan EA, Davies CR and Grant J (2020) Cryptosporidium species diversity in New Zealand: an overview. *Veterinary Parasitology: Applied and Environmental Microbiology* 1537–1540.

Jokelainen P, Agerholm JS and Stensvold CR (2020) Phylogenetic analysis of *Cryptosporidium parvum* genotypes from humans and domesticated animals in Australia, North America and Europe. *Experimental Parasitology* **118**, 118–121.

Leitch GJ and He Q (2011) Cryptosporidiosis – an overview. *Journal of Biomedical Research* **25**, 1–16.

Mammeri M, Chevillot A, Chenafi I, Thomas M, Julien C, Vallice I, Polack B, Follet J and Adjou KT (2019) Molecular characterization of *Cryptosporidium* isolates from diarrheal dairy calves in France. *Veterinary Parasitology: Regional Studies and Reports* **18**, 100323.

Nader JL, Mathers TC, Ward BJ, Pachebat JA, Swain MT, Robinson G, Chalmers RM, Hunter PR, van Oosterhout C and Tyler KM (2019) Evolutionary genomics of anthroponosis in *Cryptosporidium*. *Nature Microbiology* **4**, 826–836.

New Zealand Government (2020) New Zealand COVID-19 alert levels summary. https://covid19.govt.nz/assets/resources/tables/COVID-19-alert-levels-summary.pdf.

O’Brien E, McNnes L and Ryan U (2008) *Cryptosporidium* GP60 genotypes from humans and domesticated animals in Australia, New Zealand, North America and Europe. *Experimental Parasitology* **118**, 118–121.

Putignani I and Menichella D (2010) Global distribution, public health and clinical impact of the protozoan pathogen *Cryptosporidium*. *Interdisciplinary Perspectives on Infectious Diseases* **2010**, 753512.

Quilez J, Torres E, Chalmers RM, Hadfield SJ, Del Cacho E and Sánchez-Acedo C (2008) *Cryptosporidium* genotypes and subtypes in lambs and goat kids in Spain. *Applied and Environmental Microbiology* **74**, 6026–6031.

Robert A (2020) Lessons from New Zealand’s COVID-19 outbreak response. *The Lancet Public Health* **5**, e569–e570.

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL and Griffin PM (2011) Foodborne illness acquired in the United States – major pathogens. *Emerging Infectious Diseases* **17**, 7.

Segura R, Prim N, Montemayor M, Valls ME and Muñoz C (2015) Predominant virulence *BvAT0G* subtype of *Cryptosporidium hominis* in human isolates in Barcelona: a five-year study. *PLoS ONE* **10**, e0121753–e0121753.

Thomas-Lopez D, Müller L, Vestergaard LS, Christoffersen M, Andersen A-M, Jokelainen P, Agerholm JS and Stensvold CR (2020) Veterinary students have a higher risk of contracting cryptosporidiosis when calves with high fecal *Cryptosporidium* loads are used for fetotomy exercises. *Applied and Environmental Microbiology* **86**, e01220–e01220.

Waldron LS, Ferrari BC and Power ML (2009) Glycoprotein 60 diversity in *C. hominis* and *C. parvum* causing human cryptosporidiosis in NSW, Australia. *Experimental Parasitology* **122**, 124–127.

Weir E (2001) The cryptic nature of cryptosporidiosis. *Canadian Medical Association Journal* **164**, 1743–1743.

Xiao I, Fayer R, Ryan U and Upton SJ (2004) *Cryptosporidium* taxonomy: recent advances and implications for public health. *Clinical Microbiology Reviews* **17**, 72.

Yang Q, Xiao X, Gu X, Liang D, Cao T, Mou J, Huang C, Chen L and Liu J (2021) Surveillance of common respiratory infections during COVID-19 pandemic demonstrates preventive effectiveness of non-pharmaceutical interventions. *International Journal of Infectious Diseases* **105**, 442–447. doi: 10.1016/j.ijid.2021.02.027.