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PROTOZOOLOGY - SHORT COMMUNICATION

Occurrence of cryptosporidium parvum IIaA17G1R1 in hospitalized hemato-oncological patients in Slovakia

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Abstract

This study aimed to determine the presence of cryptosporidiosis in immunosuppressed patients hospitalized at the Clinic of Haematology and Oncohaematology in a form of routine screening. Samples were collected from November 2019 to February 2020, when the first wave of the Coronavirus pandemic occurred in Slovakia. A total of 36 samples were collected from patients hospitalized at the Clinic of Haematology and Oncohaematology, both from the open ward and the intensive care unit. For the diagnosis of cryptosporidiosis, a nested PCR targeting the gp60 gene and the SSU rRNA locus was used. From the 36 samples, *Cryptosporidium parvum* subtype IIaA17G1R1 was diagnosed in 9 patients (7 from the open ward and 2 from the intensive care unit), all hospitalized at the clinic at the same time, in February 2020. The occurrence of the same species and subtype, *Cryptosporidium parvum* IIaA17G1R1, in 9 patients hospitalized at the same time, both at the open ward and the intensive care unit may suggest a possible transmission occurred at the clinic.

Keywords *Cryptosporidium parvum* · Immunosuppression · Malignancies · Transmission · Slovakia

Introduction

Cryptosporidiosis is a significant infection, causing diarrhea among humans. A recent systematic meta-analysis including findings from 221 publications estimates the pooled global prevalence of cryptosporidiosis as high as 7.6%. The prevalence is highest in people living in low-income countries, people with gastrointestinal symptoms, and in children under 5 years of age (Dong et al. 2020). In developed countries, the prevalence ranges from 3.2% in the EU/EEA to 3.8% in the USA (ECDC 2019; CDC 2019). The infection is transmitted mainly via the fecal–oral route. Contaminated water and food are the most important transmission pathways, but *Cryptosporidium* spp. can also be transmitted by direct contact with an infected human or animal and rarely via air (Hunter and Nichols 2002; Sponseller et al. 2014).

Infections caused by *Cryptosporidium* spp. are one of the most frequent causes of mortality especially in children under 5 years of age (Lozano et al. 2012; Striepen 2013; Shoultz et al. 2016). To this day, 47 valid *Cryptosporidium* species and more than 100 distinguished genotypes have been recognized (Holubová et al. 2019; Bolland et al. 2020; Ježková et al. 2021; Zahedi et al. 2021). Human infections are most commonly caused by *Cryptosporidium parvum*

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and *Cryptosporidium hominis*, although other species and genotypes have been recognized, mainly in humans suffering from immunodeficiency, including *C. meleagridis*; *C. felis*; *C. canis*; *C. cuniculus*; *C. ubiquitum*; *C. viatorum*; *C. muris*; *C. suis*; *C. fayeri*; *C. andersoni*; *C. bovis*; *C. scrofarum*; *C. tyzzeri*; *C. erinacei*; and *Cryptosporidium* horse, skunk, and chipmunk I genotypes (Cacciò et al. 2002; Wolska-Kusnierz et al. 2007; Xiao 2010; Zahedi et al. 2016; Ryan et al. 2016).

In immunocompetent individuals, cryptosporidium gastroenteritis is typically self-limiting, and patients usually recover within 2 weeks. On the other hand, chronic diarrhea is present in immunosuppressed patients (Ryan et al. 2016). Immunosuppression, either impaired or constant, is often associated with oncological diseases or their treatment (Sulżyc-Bielicka et al. 2018). Immunosuppression and diarrhea are known side effects in patients undergoing cancer treatment, although few studies were focused on the occurrence of cryptosporidiosis in patients with malignancies (Hassanein et al. 2012).

In this study, we have focused on the occurrence and subtyping of *Cryptosporidium* spp. in patients with hemato-oncological diseases, hospitalized at the Clinic of Haematology and Oncohaematology. This study is also a follow-up to our previous studies about the occurrence of *Cryptosporidium* spp. in immunosuppressed and immunocompetent patients, animals, and in environmental samples to further expand the knowledge of individual species, genotypes, and subtypes circulating in Slovakia (Hasajová et al. 2014; Danišová et al. 2017; Kalinová et al. 2018; Hatalová et al. 2019).

Materials and methods

From November 2019 to February 2020 a total of 36 samples of feces were collected from diarrheic patients hospitalized at the Clinic of Haematology and Oncohaematology of the Louis Pasteur University Hospital (LPUH) in Kosice. Samples were obtained from patients from the open ward ($n=28$) and the intensive care unit (ICU; $n=8$). Samples were transferred to the Department of Epidemiology, Faculty of Medicine of the Pavol Jozef Safarik University in Kosice and stored at -20 °C until analysis. Characteristics of samples according to the date of sampling are summarized in Table 1. Nucleic acids were extracted using the DNA Sorb-B nucleic acid extraction kit (AmpliSense, Russia) according to the manufacturer's guide. Isolated nucleic acids were stored at -20 °C until the use in PCR. Nested PCR was used for the amplification of the 60 kDa glycoprotein gene (gp60) of *C. parvum* and *C. hominis* since they are the most common species diagnosed in humans (Xiao 2010). After gp60 subtyping, nested PCR using primers targeting the SSU rRNA locus were used for all samples (Leetz et al. 2007) to diagnose other species of *Cryptosporidium* and to ensure that the contamination did not occur during subtyping. For all reactions, a 20 µl mix was prepared using 5 × HOT FIREPol Blend Master Mix Ready to Load (Solis BioDyne, Estonia), 0.1 µM of the respective primers, and 5 µl of DNA template. All reactions were carried out in Biometra T1 Thermocycler (AnalyticJenna). Final products of a length of 450 bp (for primers targeting the gp60 gene) or 250 bp (for primers targeting the SSU locus) were analyzed in 1.5% agarose gel dyed with GoodView™ Nucleic Acid Stain in TBE buffer. Positive samples were sent for sequencing. Final sequences were compared with homologous sequences stored in GenBank using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Subtypes of *Cryptosporidium* were assessed according to the rules of subtyping, using serine-coding trinucleotides TCA, TCT, and TCG at the 5′end of the gp60 gene and other repetitive and non-repetitive sequences following the tandem repeats (Xiao 2010).

The consensus sequences were edited and assembled using BioEdit program. Then sequences were aligned using MUSCLE Multiple Sequence Alignment with reference sequences from GenBank. The phylogenetic tree was constructed by maximum likelihood method using the program MEGA-X (Molecular Evolutionary Genetics Analysis software). Bootstrap values were calculated from 1,000 replicates. *C. tyzzeri* was used as an out-group. The phylogenetic tree is represented in Fig. 1.

Parallel to PCR, all patients from this study were tested for common nosocomial strains of bacteria in the microbiological laboratory of the Louis Pasteur University Hospital.

Table 1 Characteristics of patients' samples according to the date of sampling

Date of sampling	No. of samples	Ward			ICU		
		Male	Female	Total	Male	Female	Total
18 November 2019	8	4	4	8	0	0	0
27 November 2019	10	5	2	7	1	2	3
28 November 2019	3	1	1	2	0	1	1
23 January 2020	6	2	3	5	0	1	1
24 February 2020	9	3	4	7	1	1	2

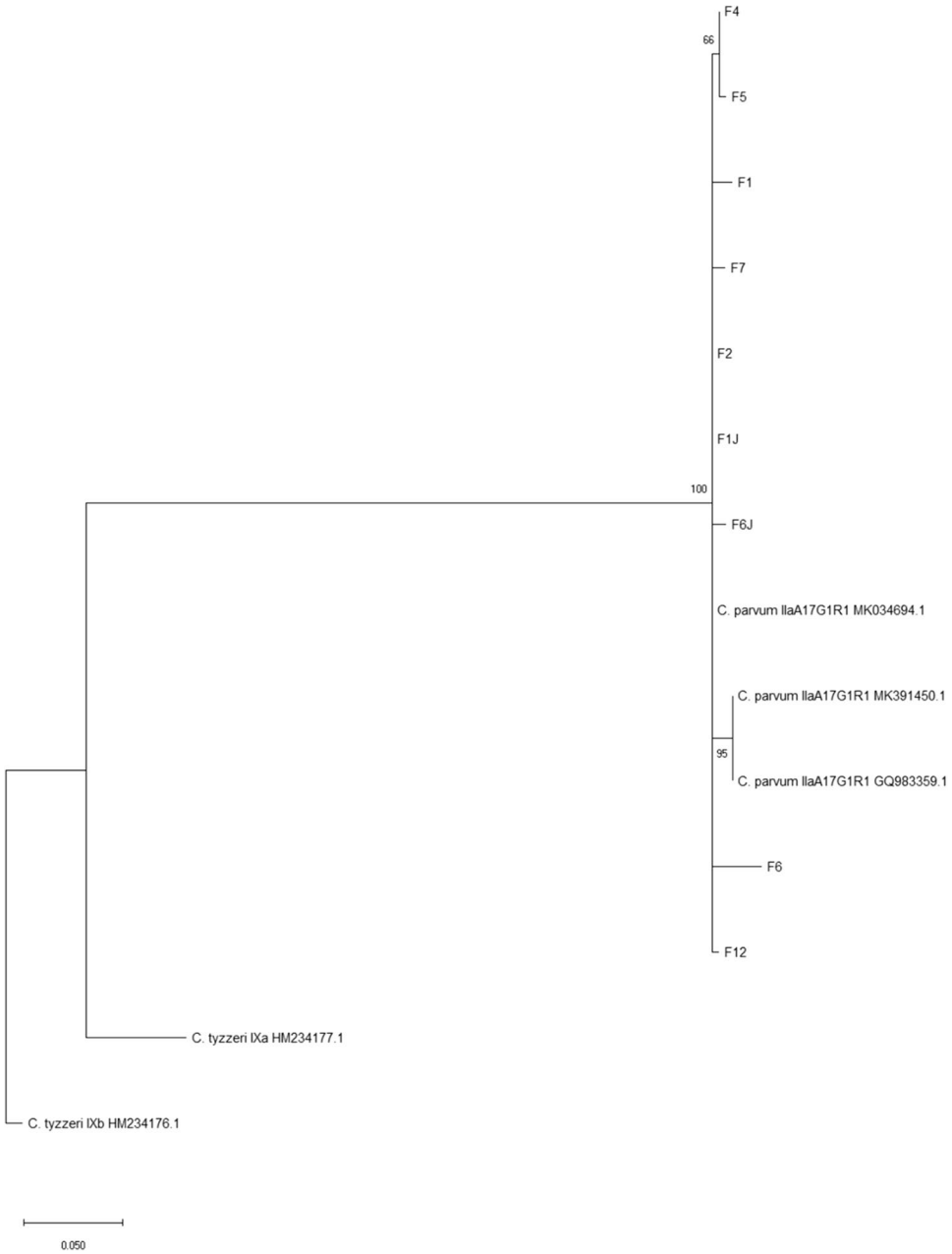

Fig. 1 Phylogenetic tree constructed by maximum likelihood method

Results and discussion

After PCR, from all 36 samples obtained from November to February, only 9 were positive for *Cryptosporidium* spp. using primers targeting the gp60 gene. All 9 samples where *Cryptosporidium* spp. was detected came from February 2020. In these 9 samples from patients hospitalized at the same time, *C. parvum* subtype IIaA17G1R1 was diagnosed in all cases, both in the open ward and the ICU. Primers targeting the SSU region showed positivity for *C. parvum* only in five samples and also obtained in February 2020. The lower rate of amplification by primers targeting the SSU region in comparison to gp60 primers may be explained by their lower specificity and/or low DNA yield in the isolates. All other samples collected from November 2019 were negative. Parallel microbiological examination by the LPUH laboratory showed that one patient positive for *C. parvum* IIaA17G1R1, hospitalized in the ICU during February 2020, was also suffering from *Klebsiella pneumoniae* type CRE (carbapenem-resistant Enterobacteriaceae). Association between PCR results, dates of sampling, and hemato-oncological diseases of the patients are summarized in Table 2.

Cryptosporidium and other opportunistic pathogens are common causes of life-threatening infections with severe or fatal outcomes, especially in immunosuppressed patients

Table 2 Association between PCR results, date of sampling and hemato-oncological diagnoses

Date of sampling	Sample number	Sex	Diagnosis	PCR results
24 February 2020	F1	M	B-NHL DLBCL	*C. parvum* IIaA17G1R1
24 February 2020	F2	M	NS-HL, after allo-SCT	*C. parvum* IIaA17G1R1
24 February 2020	F4	F	PCNSL DLBCL	*C. parvum* IIaA17G1R1
24 February 2020	F5	F	TTP	*C. parvum* IIaA17G1R1
24 February 2020	F6	F†	B-NHL DLBCL	*C. parvum* IIaA17G1R1
24 February 2020	F7	F	AML	*C. parvum* IIaA17G1R1
24 February 2020	F12	M†	AML	*C. parvum* IIaA17G1R1
24 February 2020	F1J	M†	AML, after MUD allo-SCT	*C. parvum* IIaA17G1R1
24 February 2020	F6J	F†	AML	*C. parvum* IIaA17G1R1

Abbreviations: *allo-SCT* allogeneic stem cell transplantation, *AML* acute myeloid leukemia, *B-NHL* B cell non-Hodgkin lymphoma, *DLBCL* diffuse large B cell lymphoma, *MUD* matched unrelated donor, *NS-HL* nodular sclerosis Hodgkin lymphoma, *PCNSL* primary central nervous system lymphoma, *TTP* thrombotic thrombocytopenic purpura

(Iqbal et al. 1999). *Cryptosporidium* spp. are important parasites in cancer patients, in which a severe form of cryptosporidiosis occurs most frequently (Hunter and Nichols 2002). In recent years, several studies investigated the relationship between cancer and parasites (Oliveira 2014; Samuel 2016). Most studies describe infections caused by *Cryptosporidium* as a consequence of immunosuppression caused by cancer and its therapy (Sulżyc-Bielicka et al. 2018). These studies were conducted using samples from patients undergoing chemotherapy, therefore fostering the development of opportunistic infections. In these studies, the prevalence of cryptosporidiosis varied from 1.3 to 80% (Hassan et al. 2002; Al-Qobati et al. 2012). A recent study shows that by 2040, the incidence of cancer is expected to rise to 29.5 million new cases per year (Perera et al. 2021). Due to this fact, the probability of the occurrence of opportunistic parasites in cancer patients will increase.

A study conducted in France between the years 2015 and 2017 declared 210 cases of cryptosporidiosis in immunosuppressed patients with solid organ transplantation (51%), HIV (31%), bone marrow transplantation (8%), and malignancy. From the total 210 cases, 80% of the patients were treated with corticosteroids, 19% were on chemotherapy, and 3% had anti-cytokine biotherapy. Patients on mycophenolate mofetil therapy and solid organ transplantation recipients appeared to be the most vulnerable group, especially during the first 6 months after the transplantation (Costa et al. 2018).

Since the 1980s, numerous outbreaks of cryptosporidiosis have been reported in healthcare facilities around the world, including the transmission of the parasites to healthcare workers from immunosuppressed and immunocompetent patients, and between patients (Baxby et al. 1983; Vandenberg et al. 2012; Feng et al. 2012; Goñi et al. 2015).

In our study, from the total 36 samples obtained from November 2019 to February 2020, none was diagnosed with *Cryptosporidium* spp., except samples obtained in February. In these 9 samples, the presence of the same species and subtype was detected, *C. parvum* IIaA17G1R1, both in patients from the open ward, and also in patients from the ICU. Before hospitalization, none of the patients showed any clinical signs of cryptosporidiosis, suggesting that a possible transmission of the pathogen occurred at the clinic.

C. parvum IIaA17G1R1 was also diagnosed in previous studies from Slovakia, in immunocompetent humans, and also immunosuppressed adults and children, and animals, suggesting it as a very common subtype in our country (Hatalová et al. 2019).

Our study came across limitations. On 11 March 2020, the World Health Organization declared the novel Coronavirus (COVID-19) outbreak a global pandemic (WHO Director-General's opening remarks at the media briefing on COVID19 -March 2020). On the same date, the Government of Slovakia declared a state of emergency and enhanced restrictive measures countrywide, followed by a lockdown of the country for several months (Uznesenie vlády Slovenskej republiky 147/2020 Z.z.). The hospital and the Clinic of Haematology and Oncohaematology closed to visitors during this period, and sanitary regime was increased. For these reasons, new stool samples and samples of water and smears from the environment were not obtained. This fact led us to postpone the assessment of the source of the infection and the transmission mode until the epidemiological situation becomes more favorable. Repeated sampling could not have been conducted from four of the patients, because they are now deceased.

Conclusion

The detection of the same species and subtype of *Cryptosporidium* in 9 patients hospitalized at the Clinic of Haematology and Oncohaematology at the same time, both at the

open ward and on the ICU, indicates that a possible transmission of the pathogen may have occurred. When the situation allows, we will continue sampling to further monitor the situation at the Clinic of Haematology and Oncohaematology. In case of repeated occurrences of cryptosporidiosis, we will conduct an epidemiological investigation to assess the source of the infection and transmission modes of *Cryptosporidium* spp. in patients suffering from hemato-oncological diseases. Along with the staff, these patients should be systematically screened for cryptosporidiosis before and during hospitalization to avoid possible transmission from healthcare personnel to patients and from patients to personnel, eliminating the possibility of an infection.

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Data availability The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Declarations

Ethics approval Approval for the study was obtained from the Ethics Committee of the Faculty of Medicine at P. J. Safarik University in Kosice. The study was performed following the ethical standards as laid down in the Declaration of Helsinki of 1975 and revised in 2008.

Consent to participate Participation in the study was voluntary and anonymous, and informed consent was obtained before the medical examination.

Conflict of interest The authors declare no competing interests.

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