

## Preplanned Studies

# Intestinal Protozoan Infections in Patients with Diarrhea — Shanghai Municipality, Zhenjiang City, and Danyang City, China, 2011–2015 and 2019–2021

Yanyan Jiang<sup>1</sup>; Zhongying Yuan<sup>1</sup>; Hua Liu<sup>1</sup>; Jianhai Yin<sup>1</sup>; Yuan Qin<sup>1</sup>;  
Xiaofeng Jiang<sup>1</sup>; Jie Xu<sup>1</sup>; Jianping Cao<sup>1,2</sup>; Yujuan Shen<sup>1,2,#</sup>

## Summary

### What is already known about this topic?

Intestinal protozoa are common pathogens of diarrhea globally. However, the etiology of diarrhea due to intestinal protozoan infections in China is not known.

### What is added by this report?

Based on active syndromic surveillance in Shanghai, Zhenjiang, and Danyang during 2011–2015 and 2019–2021, 89 (1.67%) patients were infected with intestinal protozoa (*Cryptosporidium* spp., *Giardia duodenalis*, *Enterocytozoon bieneusi*, and *Cyclospora cayetanensis*), and positivity rates statistically differed by region and age.

### What are the implications for public health practice?

This was the most comprehensive data collection in investigating parasitic diarrheal diseases in humans. Identification of these protozoa in diarrhea will provide new perspectives for detecting hidden etiological agents of diarrhea as early as possible.

Diarrhea is the most common symptom of disease and the eighth leading cause of death worldwide with more than 1.6 million attributed deaths in 2016. Although syndromic surveillance as a type of active surveillance has been widely applied to safeguard activities of mass gatherings in past years (1), it is now being used to monitor diarrheal disease trends for the early detection of emergent public health events. Furthermore, due to numerous foodborne and waterborne outbreaks of intestinal protozooses, such as cryptosporidiosis (2), giardiasis (3), microsporosis (4), and cyclosporiasis (5) worldwide, interest in these pathogens related to diarrheal disease has increased. However, epidemiological status and characteristics of intestinal protozoan infections in diarrheal populations have not been studied in detail in China. This study aimed to understand the etiology and epidemiological

characteristics of these intestinal protozoan infections in patients with diarrhea from study areas, including Shanghai Municipality, Zhenjiang City, and Danyang City, during the implementation results in 12th Five-Year Plan period and 13th Five-Year Plan period. The results showed that *G. duodenalis*, *E. bieneusi*, and *C. cayetanensis* were detected in these study areas, and *Cryptosporidium* spp. was detected in Shanghai and Danyang, with increases in intestinal protozoan infections during 2011–2015 and 2019–2021. The syndromic surveillance and laboratory surveillance network for testing the capacity of protozoan infections should be jointly strengthened to provide a basis for establishing prevention and control strategies.

Based on an active, prospective study of the National Key Science and Technology Project on Infectious Disease Surveillance Technical Platform, study areas were chosen to represent various administrative levels, economies, population sizes, and geographical areas. A structured questionnaire assessing demographic data and clinical symptoms was administered to each participant. Selection criteria for diarrheal cases were defined as  $\geq 3$  passages of watery, loose, mucoid, or bloody stools within a 24-hour period. Patients referred from other hospitals or patients not initially diagnosed at sentinel sites were excluded from this study. The protocol of this study was evaluated and approved by the ethical committee of the National Institute of Parasitic Diseases, China CDC (2015011, 2019003).

One fecal specimen was collected from each patient, transported to the laboratory within 24 hours in a cooler with ice packs and stored at  $-20^{\circ}\text{C}$ . Following the manufacturer's instructions, genomic DNA was directly extracted from 180–200 mg of fecal specimens using a QIAamp DNA stool mini kit (QIAGEN 51504, QIAGEN, Hilden, Germany), eluted in 200  $\mu\text{L}$  of elution buffer, and stored at  $-20^{\circ}\text{C}$  for pending polymerase chain reaction (PCR) analysis.

*Cryptosporidium* spp., *G. duodenalis*, *E. bienersi*, and *C. cayetanensis* were identified by nested PCR amplifications and sequencing of gene fragments at around 830 bp of the small subunit (SSU) rRNA gene, around 530 bp of the triosephosphate isomerase (*tpi*) gene, around 600 bp of the SSU rRNA gene, and around 500 bp of the SSU rRNA gene, respectively (6). The positivity rates of intestinal protozoan infections in patients with diarrhea were determined, and possible risk factors related to intestinal protozoan infections were assessed by region, age, and sex with the chi-squared ( $\chi^2$ ) test using GraphPad Prism (version 5.0, GraphPad Software, San Diego, CA, USA). Differences were considered statistically significant at  $P < 0.05$ .

From January 1, 2011 to June 30, 2021, an epidemiological investigation on intestinal protozoan infections was conducted among 5,341 patients under surveillance of diarrheal illnesses through the established diarrheal syndromic surveillance network. Among the 5,341 patients, 1,645 patients (1,087 patients during 2011–2015 and 558 patients during 2019–2021) were recruited at the Shanghai Sixth People's Hospital; 1,498 patients (1,032 patients during 2011–2015 and 466 patients during 2019–2021) were recruited at the Affiliated Hospital of Jiangsu University in Zhenjiang; 2,198 patients (1,578 patients during 2011–2015 and 620 patients during 2019–2021) were recruited at the Danyang Hospital.

A total of 89 (1.67%) out of 5,341 diarrheal patients were confirmed to be infected with intestinal protozoa, including *Cryptosporidium* spp. (0.69%), *G. duodenalis* (0.45%), *E. bienersi* (0.28%), and *C. cayetanensis* (0.24%) (Table 1). The highest positivity rate of intestinal protozoan infections was recorded in Shanghai (2.31%), followed by Danyang (1.87%) and Zhenjiang (0.67%). Differences in positivity rate were observed among the study areas ( $P < 0.01$ ), but no statistical difference was observed due to age or gender (Table 1).

The positivity rates of the 4 intestinal protozoa differed during 2011–2015 and 2019–2021; they were 0.70% vs. 0.67% for *Cryptosporidium* spp. (Table 2 and Figure 1A), 0.35% vs. 0.67% for *G. duodenalis*, 0.24% vs. 0.28% for *E. bienersi*, and 0.27% vs. 0.18% for *C. cayetanensis*. Different positivity rates were observed in the study areas during the 2 periods (Figure 1B): 2.21% vs. 2.51% in Shanghai, 0.58% vs. 0.86% in Zhenjiang, and 1.77% vs. 2.10% in Danyang.

## DISCUSSION

The results of this study revealed the changes in positivity rate and epidemiological characteristics of patients with protozoan-related diarrhea in the study areas during 2011–2015 and 2019–2021. Overall, in these 4 intestinal protozoa, the positivity rate of protozoa-related diarrhea was 1.67% by PCR detection. Further analysis of data showed that particular attention was paid to the observation of the increasing positivity rate from 1.57% (2011–2015) to 1.89% (2019–2021). To support this finding, a recent national surveillance study on morbidity analysis of notifiable infectious diseases reported that infectious diarrheal cases have increased from 200,000 (2015–2017) to 1,282,270 (2018) (7). Future research efforts should prioritize diarrheal disease control and prevention, as well as etiology identification. These findings will increase stakeholder's awareness and ability to reduce the risk of infection of parasitic diseases caused by intestinal protozoa.

The findings on intestinal protozoan infections showed wide variations in positivity rate across the study areas — 2.31% in Shanghai, 0.67% in Zhenjiang, and 1.87% in Danyang. These positivity rates of intestinal protozoan-related diarrhea were lower than those of towns in Ethiopia (16.61%) (8) and cities in Pakistan (41.61%) (9). Meanwhile, the positivity rate of each of these four intestinal protozoa was lower than the positivity rate of diarrhea in patients. For *Cryptosporidium* spp., the positivity rate of diarrheal diseases attributable to this protozoan was lower in the present study (0.69%) than in Canada (15.74%) (10). The low positivity rates of intestinal protozoan infections in these geographical areas might be related to local habits of drinking boiled water and eating cooked food/vegetables. Personal hygiene habits, among multiple related factors, affect the positivity rates of intestinal protozoan infections.

The study was subject to some limitations. First, these study areas were in the Yangtze River Delta, which were not representative of the country. Second, data on possible influence factors for intestinal protozoan infections were not collected. These could include drinking boiled water, washing hands before meals and after using toilets, eating unwashed vegetables and fruits, and patterns of animal feeding. Collecting this data could provide evidence for early warning and prediction.

Since numerous animal species have been reported to be infected with *Cryptosporidium* spp., *G.*

TABLE 1. Positivity rates of intestinal protozoa among patients with diarrhea from 3 study areas in China, 2011–2015 and 2019–2021.

Variable	Pathogen									
	Intestinal protozoa		Cryptosporidium spp.		G. duodenalis		E. bienersi		C. cayetanensis	
	No. Positive / No. Examined (%)	$\chi^2/P$ value	No. Positive / No. Examined (%)	$\chi^2/P$ value	No. Positive / No. Examined (%)	$\chi^2/P$ value	No. Positive / No. Examined (%)	$\chi^2/P$ value	No. Positive / No. Examined (%)	$\chi^2/P$ value
Total	89/5,341 (1.67)		37/5,341 (0.69)		24/5,341 (0.45)		15/5,341 (0.28)		13/5,341 (0.24)	
Region	13.81 (<0.01)		20.39 (<0.01)		0.80 (0.70)		0.97 (0.61)		3.00 (0.22)	
Shanghai	38/1,645 (2.31)		22/1,645 (1.34)		6/1,645 (0.36)		4/1,645 (0.24)		6/1,645 (0.36)	
Zhenjiang	10/1,498 (0.67)		0/1,498 (0)		6/1,498 (0.40)		3/1,498 (0.20)		1/1,498 (0.07)	
Danyang	41/2,198 (1.87)		15/2,198 (0.68)		12/2,198 (0.55)		8/2,198 (0.36)		6/2,198 (0.27)	
Age (years)	3.87 (0.14)		0.73 (0.69)		6.14 (0.046)		12.73 (<0.01)		2.85 (0.24)	
Children ( $\leq 14$ )	20/1,437 (1.39)		10/1,437 (0.70)		8/1,437 (0.56)		1/1,437 (0.07)		1/1,437 (0.07)	
Teenagers (15–17)	4/102 (3.92)		0/102 (0)		2/102 (1.96)		2/102 (1.96)		0/102 (1.96)	
Adults ( $\geq 18$ )	65/3,802 (1.71)		27/3,802 (0.71)		14/3,802 (0.37)		12/3,802 (0.32)		12/3,802 (0.32)	
Gender	1.71 (0.19)		3.53 (0.06)		1.34 (0.25)		2.84 (0.09)		0.23 (0.63)	
Male	55/2,935 (1.87)		26/2,935 (0.89)		16/2,935 (0.55)		5/2,935 (0.17)		8/2,935 (0.27)	
Female	34/2,406 (1.41)		11/2,406 (0.46)		8/2,406 (0.33)		10/2,406 (0.42)		5/2,406 (0.21)	

Note: The three cities are: Shanghai, Zhenjiang, and Danyang. Based on the economic development level and population distribution, they represented the large city, the medium city and the small city, respectively. The positive rates of intestinal protozoa in diarrheal patients were determined, and possible risk factors related to intestinal protozoan infection were assessed by region, age, and sex by Chi-square ( $\chi^2$ ) test using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA).

TABLE 2. Change in the positivity rates of intestinal protozoa in patients with diarrhea from study areas in China during 2011–2015 and 2019–2021.

Period	Region	Pathogen, No. of positive/No. of examined (%)				
		Intestinal protozoa	<i>Cryptosporidium</i> spp.	<i>G. duodenalis</i>	<i>E. bieneusi</i>	<i>C. cayetanensis</i>
2011–2015*	Shanghai	24/1,087 (2.21)	11/1,087 (1.01)	4/1,087 (0.37)	3/1,087 (0.28)	6/1,087 (0.55)
	Zhenjiang	6/1,032 (0.58)	0/1,032 (0)	2/1,032 (0.19)	3/1,032 (0.29)	1/1,032 (0.10)
	Danyang	28/1,578 (1.77)	15/1,578 (0.95)	7/1,578 (0.44)	3/1,578 (0.19)	3/1,578 (0.19)
	Total	58/3,697 (1.57)	26/3,697 (0.70)	13/3,697 (0.35)	9/3,697 (0.24)	10/3,697 (0.27)
2019–2021†	Shanghai	14/558 (2.51)	11/558 (1.97)	2/558 (0.36)	1/558 (0.18)	0/558 (0)
	Zhenjiang	4/466 (0.86)	0/466 (0)	4/466 (0.86)	0/466 (0)	0/466 (0)
	Danyang	13/620 (2.10)	0/620 (0)	5/620 (0.81)	5/620 (0.81)	3/620 (0.48)
	Total	31/1,644 (1.89)	11/1,644 (0.67)	11/1,644 (0.67)	6/1,644 (0.28)	3/1,644 (0.18)

Note: The study areas were Shanghai, Zhenjiang, and Danyang.

\* Implementation results of 2011–2015 points out the 12th Five-Year Plan period.

† Implementation results of 2019–2021 points out the 13th Five-Year Plan period.

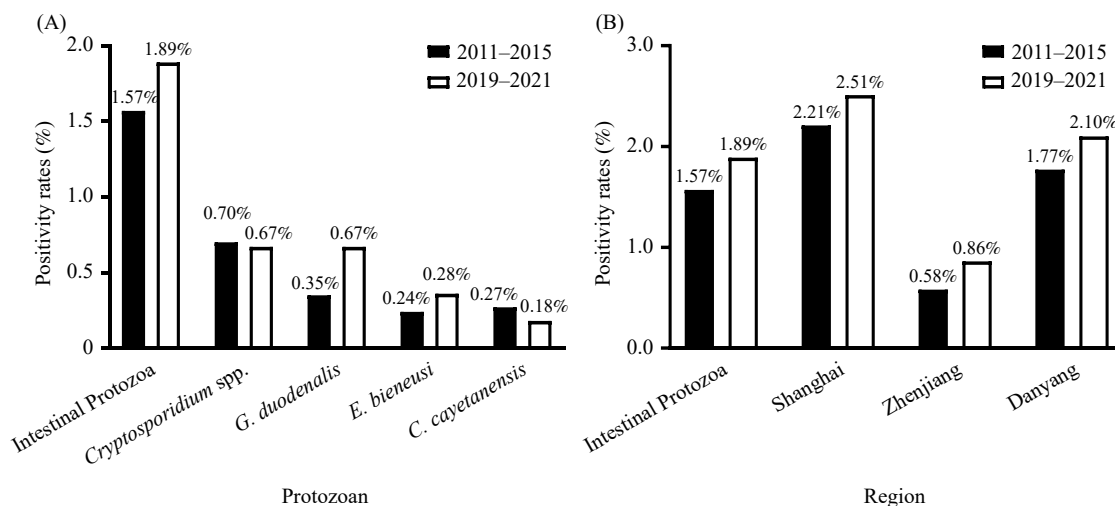


FIGURE 1. Etiology and distribution of intestinal protozoa infection in patients with diarrhea among the study areas, China, 2011–2015 and 2019–2021. (A) Comparison of positivity rates of intestinal protozoan infection during 2011–2015 and 2019–2021 by protozoa; (B) Comparison of positivity rates of intestinal protozoan infection during 2011–2015 and 2019–2021 by region.

*duodenalis*, and *E. bieneusi*, a One Health approach to intestinal protozooses prevention, surveillance, monitoring, control, and mitigation, as well as environmental conservation, should be adopted broadly.

**Conflicts of interest:** The authors declare no competing interests.

**Acknowledgements:** The staff of the Department of Infectious Diseases and Department of Pediatrics, Shanghai Sixth People's Hospital in Shanghai City; Department of Clinical Laboratory, Affiliated Hospital of Jiangsu University; and Department of Infectious Diseases, Danyang Hospital of Jiangsu Province.

**Funding:** Supported by the National Science and Technology Major Program of China (Nos.

2018ZX10713001-004, 2012ZX10004-201, and 2009ZX10004-201), the National Nature Science Foundation of China (Nos. 82072307 and 81772224), the Three-Year Public Health Action Plan (2020–2022) of Shanghai (No. GWV-10.1-XK13), and the National Key R&D Program of China No. 2021YFC2300900).

doi: [10.46234/ccdcw2022.028](https://doi.org/10.46234/ccdcw2022.028)

\* Corresponding author: Yujuan Shen, shenyj@nipd.chinacdc.cn.

<sup>1</sup> National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (Chinese Center for Tropical Diseases Research); Key Laboratory of Parasite and Vector Biology, National Health Commission of the People's Republic of China; World Health Organization Collaborating Centre for Tropical Diseases, Shanghai, China; <sup>2</sup> School of Global Health, Chinese Center for Tropical Diseases Research, Shanghai Jiao Tong University School of Medicine;

Shanghai, China.

Submitted: December 05, 2021; Accepted: February 18, 2022

## REFERENCES

1. Yu JX, Jing HQ, Lai SJ, Xu WB, Li MF, Wu JG, et al. Etiology of diarrhea among children under the age five in China: results from a five-year surveillance. *J Infect* 2015;71(1):19 – 27. <http://dx.doi.org/10.1016/j.jinf.2015.03.001>.
2. Yang X, Guo YQ, Xiao LH, Feng YY. Molecular epidemiology of human cryptosporidiosis in low- and middle-income countries. *Clin Microbiol Rev* 2021;34(2):e00087 – 19. <http://dx.doi.org/10.1128/CMR.00087-19>.
3. Adam RD. *Giardia duodenalis*: biology and pathogenesis. *Clin Microbiol Rev* 2021;34(4):e00024 – 19. <http://dx.doi.org/10.1128/cmr.00024-19>.
4. Zhang Y, Koehler AV, Wang T, Gasser RB. *Enterocytozoon bieneusi* of animals-With an 'Australian twist'. *Adv Parasitol* 2021;111:1 – 73. <http://dx.doi.org/10.1016/bs.apar.2020.10.001>.
5. Li JQ, Cui ZH, Qi M, Zhang LX. Advances in cyclosporiasis diagnosis and therapeutic intervention. *Front Cell Infect Microbiol* 2020;10:43. <http://dx.doi.org/10.3389/fcimb.2020.00043>.
6. Li N, Xiao LH, Wang L, Zhao SM, Zhao XK, Duan LP, et al. Molecular surveillance of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* by genotyping and subtyping parasites in wastewater. *PLoS Negl Trop Dis* 2012;6(9):e1809. <http://dx.doi.org/10.1371/journal.pntd.0001809>.
7. Dong SB, Ren X, Zhang CH, Geng MJ, Zhu YL, Shi LS, et al. Morbidity analysis of the notifiable infectious diseases in China, 2018. *China CDC Wkly* 2019;1(4):47 – 53. <http://dx.doi.org/10.46234/ccdcw2019.016>.
8. Taye S, Abdulkarim A. Prevalence of intestinal parasitic infections among patients with diarrhea at Bereka medical center, southeast Ethiopia: a retrospective study. *Fam Med Med Sci Res* 2014;3(3):1000131. <http://dx.doi.org/10.4172/2327-4972.1000131>.
9. Yakoob J, Abbas Z, Khan R, Tariq K, Awan S, Beg MA. Association of *Helicobacter pylori* and protozoal parasites in patients with chronic diarrhoea. *Br J Biomed Sci* 2018;75(3):105 – 9. <http://dx.doi.org/10.1080/09674845.2017.1420129>.
10. Iqbal A, Goldfarb DM, Slinger R, Dixon BR. Prevalence and molecular characterization of *Cryptosporidium* spp. and *Giardia duodenalis* in diarrhoeic patients in the Qikiqtani Region, Nunavut, Canada. *Int J Circumpolar Health* 2015;74(1):27713. <http://dx.doi.org/10.3402/ijch.v74.27713>.