Analysis on the detection rate of *Giardia lamblia* in children of Sichuan province of China

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Research Article

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Abstract

Objective

To investigate the detection rates of *Giardia lamblia* in children of Sichuan province and analyze the susceptible population and the cause of infection.

Methods

The routine fecal examination of a total of 11575 cases were done by normal wet smear microscopy, and the suspected cases were stained with iodine in outpatient children of West China Second University Hospital, Sichuan University from September 1st, 2020 to August 31th, 2021.

Results

In the stools of 11,515 outpatient children, 11 cases of cysts or trophozoites of *G. lamblia* were detected, among which 9 cases were from ethnic minority patients and 2 case were from Han patients. The *G. lamblia* detection rate of Han patients was 0.019%, while that of ethnic minority patients was 0.968%, between which there was a statistically significant difference (P<0.05).

Conclusions

The detection rate of *G. lamblia* in minority patients was higher than that in Han patients. To reduce the spread and epidemic of giardiasis, the epidemic prevention in minority areas should be strengthened and the good hygienic habits of children should be cultivated.

Introduction

*Giardia lamblia*, also known as *Giardia duodenalis*, is one of the most susceptible intestinal parasitic protozoa in children[1]. *G. lamblia* parasitizes the human small intestine, gallbladder, mainly in the duodenum, causing abdominal pain, diarrhea, vomiting and indigestion-based symptoms, called giardiasis[2–3]. *G. lamblia* is a flagellate that does not invade epithelial cells and reproduces asexual only by binary division. They produce resistance stages (*Giardia* cysts) and are released into the environment as a mode of infection for these intestinal parasitic protozoa[3]. *G. lamblia* exhibit a biphasic lifestyle, i.e. dormant cysts or reproductive trophozoites, which propagate by the dichotomy of the trophozoites. Cysts can be found in stools with normal hardness, and trophozoites may be found in cases of diarrhea. At the same time, *G. lamblia* is a zoonotic intestinal parasitic protozoa that may be transmitted from humans to animals[4–5].
G. lamblia is one of the most prevalent parasite infections in the world. It is also a common intestinal protozoa that infects both humans and animals [6]. About 200 million people worldwide are infected with G. lamblia every year, with a high incidence among tourists. So, diarrhea caused by G. lamblia is also called "tourist diarrhea"[7]. The survey of G. lamblia infection status in 11 provinces (or municipalities and autonomous regions) of China shows that the infection rate is 0–16.2%, of which the infection rate of HIV patients and immunodeficiency populations is higher[8–9]. However, the ethnic distribution of giardiasis in Sichuan province of China and the difference in detection rates of different ethnic groups have not been reported. This study was to carry out routine fecal examinations for outpatient children in our hospital and investigate the detection rates of giardiasis in Sichuan province, and to analyze the susceptible people and susceptible causation of this disease.

Materials And Methods

Patients

A total of 11575 cases of outpatient children aged 0- to 12-year-old (mean ±SD 6.85±4.41 years) in West China Second University Hospital, Sichuan University from September 1st, 2020 to August 31th, 2021 were enrolled in this study. Inclusion criteria were as follow: 1) Informed consents were acquired from patients and their guardians, and patients were from 0- to 12-year-old; 2) Patients who had clinical symptoms of abdominal pain, diarrhea, vomiting and indigestion-based symptoms, etc.; 3) Outpatient children with recent diagnosis in our hospital and who were not previously treated with anti-infective drugs for intestinal tract. Exclusion criteria were as follow: 1) Patients who were treated with anti-infective agents for intestinal tract, or with other agents that would cause interference for diagnosis; 2) Patients who were over 12-year-old.

Methods

The routine fecal examination of a total of 11575 cases were done by normal wet smear microscopy, and the suspected cases were stained with iodine. Among which, there were 10,645 Han patients (mean ±SD 6.91±4.68 years) and 930 ethnic minority patients(mean ±SD 5.96±4.05 years) . The found of Giardium cysts or trophozoites were diagnosed as positive giardiasis cases(Figure 1). And then, the nationality, month of birth, living environment, hygiene habits, current medical history, and previous history of all patients were investigated. In our study, the written informed consent was obtained from all participants and the privacy rights of them were also reserved. And all procedures and protocols are in accordance with the Helsinki Declaration as revised in 2013. The study protocol(Medical Research 2020, No.40) was approved by the Institutional Review Board(IRB) of the West China Second University Hospital, Sichuan University on 26th July 2020 before study initiation.

Statistical analysis

The database was established using Excel sheet to calculate the positive rate of giardiasis. The positive proportion of Han and minority outpatient children was calculated using the χ² test correction formula.
\( P < 0.05 \) was considered statistically significant [10].

**Results**

In the stools of 11,515 outpatient children, 11 cases of cysts or trophozoites of *G. lamblia* were detected, among which 9 cases were of ethnic minority patients and 2 case was of Han patients. Among the 9 ethnic minority giardiasis patients, 6 were Tibetans and 3 were Yi nationalities. The total *G. lamblia* detection rate was 0.095%. The detection rate of *G. lamblia* of Han patients was 0.019%, meanwhile that of the ethnic minority patients was 0.968% (Table 1), between which there was a statistically significant difference \( (\chi^2 = 81.12, P < 0.05) \). The detection rates of *G. lamblia* in children of different nationalities were shown in Table 2.

### Table 1

The detection rates of *G. lamblia* in 11,575 children of Sichuan province

| Nationality       | n1(total) | n2(detected) | Detection rates(%) | \( \chi^2 \) | \( P \) |
|-------------------|-----------|--------------|--------------------|--------------|--------|
| Han nationality   | 10645     | 2            | 0.019              | 81.12        | <0.05  |
| Ethnic minority   | 930       | 9            | 0.968              |              |        |
| Total             | 11575     | 11           | 0.095              |              |        |

### Table 2

The detection rates of *G. lamblia* in children of different nationalities of Sichuan province

| Nationality       | N1(total) | N2(detected) | Detection rates(%) |
|-------------------|-----------|--------------|--------------------|
| Han nationality   | 10645     | 2            | 0.019              |
| Tibetan           | 446       | 6            | 1.345              |
| Yi nationality    | 263       | 3            | 1.141              |
| Miao nationality  | 33        | 0            | 0                  |
| Hui nationality   | 32        | 0            | 0                  |
| Qiang nationality | 30        | 0            | 0                  |
| Other nationality | 126       | 0            | 0                  |
| Total             | 11575     | 11           | 0.095              |

Note: Due to the large ethnic minorities of the outpatient children were included, the top six ethnic minorities of them were included in the statistics, and the number of outpatient children with other ethnic minorities was combined.

**Discussion**
*G. lamblia* is a common intestinal parasitic protozoa associated with diarrhoea disease, and also a zoonotic parasite divided into five genotype assemblages of A-E. Studies have shown that the infection rate of *G. lamblia* of patients with HIV and the immunodeficiency population is higher [7–8]. At the same time, the research results showed that *G. lamblia* infection existed in HIV/AIDS patients in Guangxi, and the genotype was mainly assemblage B, and host-specific assemblage C was found[5]. Genotype of *G. lamblia* isolated from different hosts suggests that the host species with the largest infection range of assemblages A and B, appears to be the primary (or possibly only) assemblage of *G. lamblia*. At least in some cases of wild mammalian assemblage A or B infection, there is evidence that the infection was caused by environmental contamination from the *G. lamblia* cysts of human origin[11].

The analytic method of *G. lamblia* directly related to its sensitivity, specificity and detection rate. Currently, *G. lamblia* assays include direct microscopic, staining, immunological methods and molecular diagnosis. The traditional microscopy method is the "gold standard" for *G. lamblia* detection. However, it is time-consuming and low detection rate[12]. Immunological methods, such as immuno-fluorescence, ELISA, have improved sensitivity and specificity compared with traditional microscopy. But the former cannot distinguish insect species, the latter is prone to cross-reactions, all of them cannot accurately quantify the number of *G. lamblia*, and were used poorly in mildly infected fecal samples[13]. With high sensitivity and good specificity, the fluorescent quantitation PCR (FQ-PCR) method, which is one of the preferred methods for the quantitative detection of *G. lamblia*, can detect a large number of samples simultaneously[14]. In recent years, isothermal nucleic acid amplification technology has been widely used in the field of prevention and control of infectious disease, mainly including chain replacement amplification, recombinase polymerase amplification technology and loop-mediated isothermal amplification (LAMP). The LAMP technology has a high degree of specificity and sensitivity, good stability and repetitive in the gene sequence analysis of triose phosphate isomerase (TPI) of *G. lamblia* [15].

In our study, 9 of the 11 giardiasis patients detected in our hospital were ethnic minority children (6 Tibetans and 3 Yi nationalities), and 2 was Han patients. The detection rate of *G. lamblia* in minority patients is higher than that in Han patients. Meanwhile, one of the Han patients had a life history in Yi nationality area and *G. lamblia* have not been detected in other minority children. Considering the intermittent nature of the discharge of the stools and the inexperience of the inspectors, the detection rate of *G. lamblia* in Sichuan Province may be higher [16]. Through analyzing their life history and susceptible reasons, the pathogeny of giardiasis was related to health conditions, economic status, cultural level, unclean eating habits and living habits in ethnic minority areas, as well as related to the animal husbandry that the local residents mainly engaged in[17]. *G. lamblia* has a simple life history and spreads extremely fast. People are infected mainly by ingestion of water and food contaminated with cysts. Improper management of human and animal stools, without washing hands before and after meals, and lack of awareness of epidemic prevention constitute important risk factors for *G. lamblia* infection.

The parents of children work outside, leading to the patients stay in the rural areas under the care of other generations of elders. Due to poor care, the patients may have no personal health position. The children of Kindergarten, nursery, and primary school live together, and play toys with each other can also cause
potential risk factors spreading from person to person [18]. 9 outpatient children with giardiasis were less than 5 years old in our hospital, 2 patients was aged over 5 years (12-year-old), which suggests that children under 5-year-old are more susceptible to Giarasis. Mahdy, et al. have identified children under 12-year-old as populations at high risk for \textit{G. lamblia} infection[19]. Han, et al. suggested that children (0- to 4-years-old) were 17.911 times more susceptible than adults (15- to 64-year-old)[20]. All these suggest that children are more susceptible to giardiasis.

The outpatient children in our hospital are mainly derived from all over Sichuan Province, and many kinds of intestinal protozoa and sporozoa have been detected in their stools. Children with giardiasis often intermittently discharge cysts or trophozoites, which are prone to repeated infection. It is suggested that the primary hospitals should conduct regular census and repeated screening of the population. Insect repellent measures should be taken immediately if the confirmed cases are found. Health and epidemic prevention departments should strengthen the fecal management of human and animal hosts, do a good job in environmental sanitation, and prevent water being polluted. Children should be cultivated to develop good personal hygiene and living habits, and reduce the risk of fecal-hand-mouth-oral transmission and human and animal transmission. At the same time, it is recommended that the local Center for Disease Control and Prevention(CDC) publicize and popularize the relevant knowledge of parasitic diseases to children and their parents in remote areas. For inspectors, we should improve our professional ability and detection experience to reduce the missed detection rate. Immunology and molecular diagnostic techniques based on traditional microscopy should be increased to improve the sensitivity and specificity of \textit{G. lamblia} detection. It is suggested to strengthen the professional training and assessment of the relevant inspectors, and to further improve the detection rate and diagnosis rate of giardiasis.

\textbf{Conclusions}

There was high detection rate of \textit{G. lamblia} in ethnic minority patients, which was mainly related to its personal hygiene, living habits and eating habits. It is suggested to strengthen the epidemic prevention and management in ethnic minority areas. And children's good hygiene habits should be cultivated, so as to reduce the spread and prevalence of giardiasis.

\textbf{Abbreviations}

\textit{G. Lamblia}  
\textit{Giardia lamblia}  
FQ-PCR  
fluorescent quantitation PCR  
LAMP  
loop-mediated isothermal amplification  
TPI  
triose phosphate isomerase
Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent for publication

Informed consent was obtained from all individual participants included in the study.

Competing interests

All authors declares that he/she has no competing of interest.

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Authors' contributions

Zhengqiang Hu drafted the manuscript. Zhengqiang Hu and Yonglin Zhong participated to acquisition of data. Jie Li generated the experimental results. Yunxia Li designed the study and reviewed the manuscript for intellectual content. All authors approved the final version of the manuscript.

Data curation: Zhengqiang Hu, Yonglin Zhong.

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Availability of data and material

All authors declares that the data and material were available.

References

[1] Yu YF. The infection of *Giardia lamblia* and its genotype of HIV infected person and kindergarten children in rural areas of Anhui province. Chinese Journal of Parasitology and Parasiosis, 2016, 34(6): 537-541. (in Chinese)

[2] Zhu MS, Zhu J, Wang SJ, et al. Investigation of infection of *Giardia lamblia* in children of Shiyan City. Health in Chinese schools, 2009, 30(12): 1136-1137. (in Chinese)

[3] Vitaliano AC, Blaine AM. Infections by Intestinal Coccidia and *Giardia* duodenalis. Clin Lab Med, 2015, 35(2): 423-444.

[4] Marc YF, Danielle S, Steven MS. *Giardia lamblia*: Laboratory Maintenance, Lifecycle Induction, and Infection of Murine Models. Curr Protoc Microbiol. 2020, 57(1): e102. doi:10.1002/cpmc.102.

[5] Liu H, Xu N, Shen YJ, et al. Infection and genotype of *Giardia lamblia* among HIV/AIDS patients in Guangxi Region. Chinese Journal of Parasitology and Parasiosis, 2019, 37(3): 321-325. (in Chinese)

[6] Berry, ASF. Johnson, K. Martins, R. Beiting, DP et al. Natural infection with *Giardia* is associated with altered community structure of the human and canine gut microbiome. Sphere, 2020, 5(4):115-119.

[7] Li J, Wang H, Wang R, et al. *Giardia duodenalis* infections in humans and other animals in China. Front Microbiol, 2017, 8: 2004.

[8] Teng XJ, Chen JX, Tian LG. The status of HIV/AIDS patients Combined intestinal protoplasma infection. Chinese Journal of Parasitology and Parasiosis, 2017, 35(6): 607-614. (in Chinese)

[9] Wang L, Xia YT, Tian XH, et al. Analysis of infection and influencing factors of *Giardia lamblia* in people of part of southern China in 2014. The Journal of the Environment and Health, 2018, 35(3): 238-241. (in Chinese)

[10] Li K, He J. Medical Statistics. (7th ed). Beijing: People's Medical Publishing House Co., Ltd., 2018, pp.14–20.

[11] Martin FH. *Giardia* duodenalis genetic assemblages and hosts. Parasite, 2016, 23(13):1-4.

[12] Elsaﬁ SH, Al-Maqati TN, Hussein MI, et al. Comparison of microscopy, rapid immunoassay, and molecular techniques for the detection of *Giardia lamblia* and *Cryptosporidium parvum*. Parasitol Res, 112(4):1641-46.
[13] Zhang XP, Zhu Q, Jiang SF, et al. The comparative study of *Giardia lamblia* and *Cryptosporidium* contamination by two applied detection methods in urban water sources. The International Journal of Medical Parasitology, 2015, 42(6):346-351. (in Chinese)

[14] Li J, Huang DN, Zhang XM, et al. The establishment of a two-step detection technique of double fluorescent quantitation PCR in *Giardia lamblia* and *Cryptosporidium parvum*. Chinese Journal of Zoorbidity, 2020, 36(1):32-39. (in Chinese)

[15] Yu MC, Yang ZW, Wang HR, et al. The establishment of a method for detecting *Giardia lamblia* based on LAMP microfluidic chip. Chinese Journal of Parasitology and Parasitosis, 2021, 39(3): 402-405. (in Chinese)

[16] Chen RX, Zheng ZL. Analysis of parasite egg leakage in fecal examination. The Chinese Journal of Parasitic Disease Prevention and Control, 2004, 17(5): 278-278. (in Chinese)

[17] Hussein EM, Zaki WM, Ahmed SA, et al. Predominance of *Giardia lamblia* assemblage A among iron deficiency anaemic pre-school Egyptian children. Parasitology Research, 2016, 115(4): 1537-1545. DOI:10.1007/s00436-015-4888-y.

[18] Yu XG, Hu W, Li GQ. Preliminary exploration of potential pathogenic mechanism of *Giardia lamblia*. The International Journal of Medical Parasitology, 2015, 42(2): 112-117. (in Chinese)

[19] Mohammed Mahdy AK, Lim YAL, Surin J, et al. Risk factors for endemic giardiasis: highlighting the possible association of contaminated water and food. Transactions of the Royal Society of Tropical Medicine and Hygiene, 2008, 102(5): 465-470. DOI: 10.1016/j.trstmh.2008.02.004.

[20] Han M, Xiao S, An W, Yang M, et al. Co-infection risk assessment of *Giardia* and *Cryptosporidium* with HIV considering synergistic effects and age sensitivity using disability-adjusted life years. Water Research, 2020, 175:115698. (in Chinese)

**Figures**
Figure 1

Cysts and trophozoites of *G. lamblia* 400×

Note: Figure 1-A is the saline wet smear with fluorescent screen background; Figure 1-B is the iodine-stained wet smear. White arrowheads indicate *Giardia* cysts, and red arrows show *Giardia* trophozoites.

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