Identification of Genotypes of *Giardia intestinalis* Isolates from a Human and Calf in Japan

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**ABSTRACT.** *Giardia intestinalis* is recognized as a significant pathogen in humans and animals, causing diarrhea. Recent molecular studies indicate that *G. intestinalis* is composed of genetically distinct multiple genotypes. Therefore, it is valuable to distinguish among genotypes in the epidemiology of *Giardia* infection in humans and animals. Although *G. intestinalis* has been found in humans and animals in Japan, the genotype of isolates remains unclear except for several isolates from dogs, because identification has been performed only by conventional microscopy. We report herein the genotypes of *G. intestinalis* isolates distinguished by a phylogenetic analysis. *G. intestinalis* isolates originated from a patient and a calf were found to have Assemblage B and E, respectively.

**KEY WORDS:** genotype, *Giardia*, Japan.

The flagellate *Giardia intestinalis* (syn. *G. lamblia, G. duodenalis*) is a well-known intestinal parasite, which causes an enteric disease in humans, livestock, and companion animals. Transmission of this parasite follows ingestion of viable cysts in contaminated food or water. Recent molecular studies showed that *G. intestinalis* is composed of at least seven genetically distinct but morphologically identical assemblages (Assemblages A to G), and moreover that most of these assemblages appear to have different host preferences, e.g., Assemblages C and D in dogs, Assemblage E in hoofed livestock, Assemblage F in cats, and Assemblage G in rats [2, 10, 11]. On the other hand, Assemblages A and B were recognized as zoonotic genotypes because both were found in several animal species, as well as humans [10, 21, 23].

Although *Giardia* was found in humans, dogs, wild rodents, and some psittaci in Japan [1, 8, 9, 14, 22], there are no reports about *Giardia* infection in cattle in Japan. In addition, molecular analysis has been performed only for the isolates from dogs in Japan [1], and therefore the molecular epidemiology of *Giardia* infection in Japan has been unclear. Since some isolates from animals appear to have zoonotic potential as mentioned above, it is likely that cattle harbor zoonotic genotypes. Therefore, it is important to analyze the isolates from cattle genetically in order to elucidate the epidemiology of *Giardia* infection in animals as well as to control of human giardiasis. In the present study, we obtained isolates from a patient and calf, and compared them phylogenetically with the multiple genotypes of *G. intestinalis* reported previously.

Fecal samples were collected from the patient and calf infected with the Giardia organism. The patient was a 22-year-old Japanese female living in Osaka, who showed diarrhea. She probably contracted *Giardia* organism in Japan, since she had no history of traveling overseas for recent two years. On the other hand, the calf examined was a 6-week-old Holstein bull calf reared on a farm in Hokkaido, Japan, and showed no clinical symptoms such as diarrhea when the fecal sample was collected. The purification of *Giardia* cysts from the fecal samples and the extraction of DNA from cysts were performed following the method reported previously [1]. *Giardia* diagnostic fragments were amplified by the polymerase chain reaction (PCR) with the following primer pairs targeting different gene loci: G7 and G759 for the *Giardia* β-giardin gene (β-giardin), and GDH1 and GDH4 for the *Giardia* glutamate dehydrogenase gene (GDH) [6, 7]. The fragment amplified with each primer pair includes a variable region which can be used to distinguish among the genotypes of *G. intestinalis* [6, 7]. PCR amplification was performed under the conditions reported previously [6, 7], except that Ex Taq DNA polymerase, Ex Taq buffer and dNTP (TAKARA Shuzo Co., Ltd., Otsu, Japan) were used in the present study. Amplification products were subjected to electrophoretic separation using 2% agarose gels, stained with ethidium bromide, and visualized on a UV transilluminator. The PCR products were purified using a QIAquick Gel Extraction kit (QIAGEN GmbH, Hilden, Germany) and sequenced using an ABI PRISM BigDye Terminator Cycle Sequencing FS Ready Reaction kit (PE Applied Biosystems, Foster City, California) on an ABI 310 automated sequencer (PE Applied Biosystems). PCR products were sequenced in both directions using each primer pair mentioned above. Sequences obtained from both isolates were aligned with available nucleotide sequences, reported previously [4, 6, 11–13, 21], from multiple genotypes of *G. intestinalis* using Clustal-X (version 1.63b). Evolutionary distance between different isolates was calcu-
lated with the Kimura 2-parameter method. Trees were constructed using the neighbor-joining algorithm [19]. Branch reliability was assessed using bootstrap analyses (1,000 replicates), and phylogenograms were drawn using the NJplot program [17]. The partial sequences of β-giardin and GDH of both isolates, obtained in the present study, were deposited in the GenBank database under accession numbers AB182124-AB182127.

The partial β-giardin and GDH were successfully amplified in both isolates examined (data not shown). The β-giardin sequences (472 bp) of the patient and calf isolates differed slightly from those of the isolates Nij5 from human and P-15 from pig that were found to have the Assemblages B and E, respectively. Namely, there were 4 and 2 substitutions in the β-giardin sequence of the patient and calf isolates compared with those of the isolates Nij5 and P-15, respectively (data not shown). Furthermore, the GDH sequences (592 bp) of the patient and calf isolates differed slightly from those of the isolates Ad-158 from marmoset and P-15 from pig which were found to have the Assemblages B and E, respectively. There were 4 substitutions in the GDH sequence of the patient and calf isolates compared with that of the isolates Ad-158 and P-15, respectively (data not shown). The close relationship of the isolates from the patient and calf to Assemblages B and E, respectively, was also reflected in the phylogenetic analyses of GDH (Fig. 1B) as well as β-giardin (Fig. 1A): the patient and calf isolates were grouped in Assemblages B and E, respectively.

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Molecular analysis was performed in only 4 isolates of *G. intestinalis* from dogs in Japan, and the dog isolates were found to have a dog-specific genotype, Assemblage D [1]. However, the genotypes of the isolates from humans and other animals were quite unclear, and therefore the present study is the first about the molecular analysis of isolates from human and calf in Japan. The calf isolate examined in the present study was identified as a hoofed livestock specific genotype, Assemblage E. To our knowledge, in other countries 88 isolates (52 isolates in Canada, 22 in Australia, 11 in U.S.A. and 3 in Italy) from cattle were genotyped [3, 5, 15, 18, 20, 23]. The 7 isolates in these countries except for Italy were identified as of zoonotic genotype, Assemblage A [3, 15, 18], and moreover only one isolate in U.S.A. as of the other zoonotic genotype Assemblage B [18]. The other 80 isolates from cattle were analyzed to be the non-zoonotic livestock specific genotype, Assemblage E [3, 5, 15, 18, 20]. Therefore, the genotype Assemblage E is pre-
dominant in cattle, and it is likely that the transmission of zoonotic genotypes from cattle to humans will be of low epidemiological significance. However, in Japan the molecular epidemiology of *Giardia* infection in cattle is quite unclear, because this parasite has not been surveyed in cattle. Therefore, a large-scale survey in cattle including the molecular analysis of isolates is required to reveal whether cattle play a significant role as a reservoir of zoonotic genotypes in Japan.

The genotype Assemblage E was found in only livestock such as cattle, sheep and pigs [2, 10], but recently one isolate from cat was identified as Assemblage E [18]. We suppose that cats, especially kept in farms, play a significant role as a reservoir of the genotype, Assemblage E. Genetic analysis of the isolates from cats, especially kept in farms, is required to confirm this supposition, since the molecular epidemiology of *Giardia* infection in cats is quite unclear in Japan.

*Giardia* infections are clinically important in cattle, especially in calves. Calves are usually infected with *Giardia* between 2 and 10 weeks of age, and clinical giardiasis is observed in calves 3 to 8 weeks of age [16]. The duration of diarrhea by *Giardia* infection is one to 2 weeks, but that of cyst shedding is above 30 weeks [16]. The calf examined in the present study was 6 weeks of age but had no clinical symptoms. We suppose that the animal examined in the present study recovered from illness when fecal samples were collected.

PCR-restriction fragment length polymorphism (RFLP) profiles of β-giardin or GDH was reported to differ among the genotypes, Assemblages A, B and E, but those of the other genotypes are unclear [6, 7]. Therefore, PCR-RFLP of both regions is likely to be a useful tool for distinguishing among Assemblages A, B and E. However, genetic diversity is found even among isolates of the same genotype [10]. Since PCR-RFLP analysis provides only the preliminary information on genetic relationship among the genotypes, we speculated that phylogenetic analysis would be more helpful in elucidating the epidemiology of *Giardia* infection. Therefore, in the present study we analyzed the isolates from a patient and a calf phylogenetically. Consequently, both isolates examined were grouped in Assemblage B or E, and the reference isolates quoted in the present study were also grouped in the Assemblage of each reference isolate identified previously (Figs. 1A, B). Therefore, our study indicates that a phylogenetic analysis based on the sequence data of β-giardin or GDH would be helpful for accurate identification of genotypes of *G. intestinalis* isolates from humans and animals and also for clarification of the genetic diversity in *G. intestinalis* populations.

REFERENCES