RESEARCH NOTES

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Zoonotic Genotype of *Giardia intestinalis* Detected in a Ferret

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ABSTRACT: *Giardia intestinalis* has been found in a variety of mammals, including humans, and consists of host-specific and zoonotic genotypes. There has been only 1 study of *G. intestinalis* infection in weasels, but the genotype of its isolate remains unclear. In this study, we report the isolation of *Giardia* in a ferret exhibited at a pet shop. The isolate was analyzed genetically to validate the possibility of zoonotic transmission. *Giardia* diagnostic fragments of the small subunit ribosomal RNA, β-giardin, and glutamate dehydrogenase genes were amplified from the ferret isolate and sequenced to reveal the phylogenetic relationships between it and other *Giardia* species or genotypes of *G. intestinalis* reported previously. The results showed that the ferret isolate represented the genetic group A-I in assemblage A, which could be a causative agent of human giardiasis.

The flagellate *Giardia* is a well-known intestinal parasite, which infects a wide range of vertebrate hosts, including humans. At present, 6 species in this genus, i.e., *G. intestinalis* (syn. *G. lamblia*, *G. duodenalis*) in humans, livestock, and other domestic animals, *G. microti* and *G. muris* in rodents, *G. psittaci* and *G. ardeae* in birds, and *G. agilis* in amphibians, which can be distinguished in view of the morphology

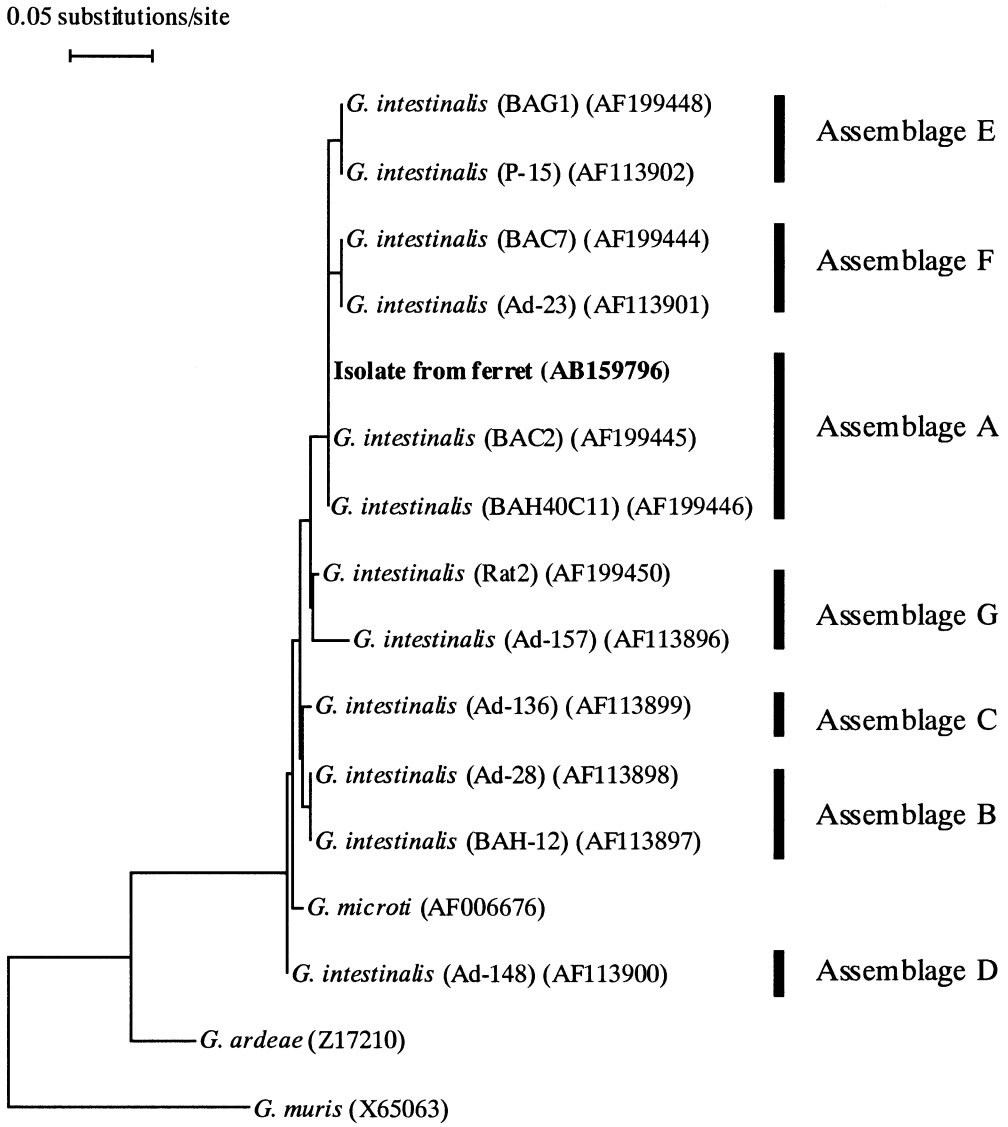

FIGURE 1. Phylogenetic relationships of the ferret isolate to other *Giardia* species and *G. intestinalis* genotypes as inferred by neighbor-joining analysis, based on the nucleotide sequences of the SSUrDNA. Names of the isolates and accession numbers in GenBank are shown in parentheses.

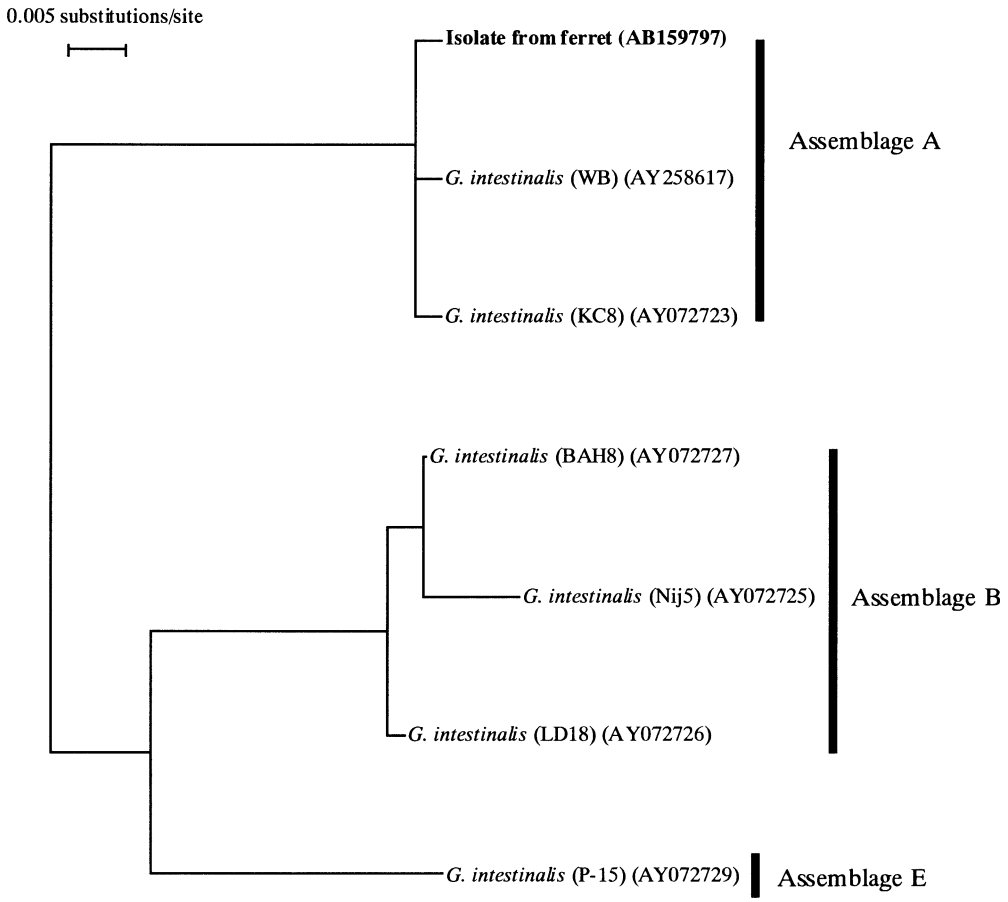

FIGURE 2. Phylogenetic relationships of the ferret isolate to other *Giardia* species and *G. intestinalis* genotypes as inferred by neighbor-joining analysis, based on the nucleotide sequences of the β-giardin. Names of the isolates and accession numbers in GenBank are shown in parentheses.

and ultrastructure of their trophozoites, are recognized as valid (Adam, 2001). However, recent molecular studies have shown that *G. intestinalis* is composed of at least 7 genetically distinct, but morphologically identical, assemblages (assemblages A–G), and, moreover, 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 (Monis et al., 1999; Adam, 2001; Monis and Thompson, 2003). On the other hand, assemblage A consists of isolates that can be classified into 2 genetic groups (A-I and A-II) (Thompson et al., 2000). Genetic group A-I consists of a mixture of animal and human isolates. In contrast, group A-II consists entirely of human isolates. Assemblage B consists of a genetically diverse group of mainly human isolates, but some isolates from animals have been included. Therefore, it is supposed that genetic group A-I in assemblages A and B has the potential for zoonotic transmission (Thompson et al., 2000; Monis and Thompson, 2003).

At present, there has been only 1 study of *Giardia* infection in Mustelidae animals, but the genotype of the isolate remains unclear because identification was performed with only conventional microscopy (Williams et al., 1988). In Japan, the ferret is a popular pet sold in many shops, but a detailed survey of zoonotic pathogens in ferrets has not been performed (Abe and Iseki, 2003). Because *G. intestinalis* is genetically diverse and some isolates from animals appear to have zoonotic potential as mentioned above, it is likely that ferrets harbor ferret-specific or zoonotic genotypes. Therefore, it is important to analyze the isolates from ferrets genetically to elucidate the epizootiology of *Giardia* infection in animals as well as for the control of human giardiasis. In this study, we obtained an isolate from a ferret in a pet shop and compared it genetically with the multiple genotypes of *G. intestinalis* reported previously to validate the phylogenetic relationships.

A fecal sample was collected from a ferret exhibited at a pet shop in Kanazawa City, Japan. This animal showed no clinical symptoms, such as diarrhea, when the fecal sample was collected. The purification of *Giardia* cysts from the fecal sample and the extraction of DNA from cysts were performed following a method reported previously (Abe et al., 2003). *Giardia* diagnostic fragments were amplified by the polymerase chain reaction (PCR) with the following primer pairs targeting the different gene loci: RH11 and RH4 for the *Giardia* small subunit ribosomal RNA gene (SSUrDNA) (Hopkins et al., 1997), G7 and G759 for the *Giardia* β-giardin gene (β-giardin) (Cacciò et al., 2002), and GDH1 and GDH4 for the *Giardia* glutamate dehydrogenase gene (GDH) (Homan et al., 1998). The area amplified with each primer pair includes a variable region, which can be used to distinguish *Giardia* species as well as multiple genotypes of *G. intestinalis*. PCR amplification was performed under conditions reported previously (Hopkins et al., 1997; Homan et al., 1998; Cacciò et al., 2002), except that Ex *Taq* DNA polymerase, Ex *Taq* buffer, and deoxynucleoside triphosphate (TAKARA Shuzo Co. Ltd., Otsu, Japan) were used in this study. Amplification products were subjected to electrophoretic separation using 3% agarose gels, stained with ethidium bromide, and observed on a UV transilluminator. The PCR products were gel purified using a QIA quick 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 the ferret *Giardia* isolate were aligned with available nucleotide sequences reported previously (Baruch et al., 1996; Monis et al., 1996, 1998, 1999; Thompson et al., 2000; Cacciò et al., 2002) from other *Giardia* species and multiple genotypes of *G. intestinalis* using Clustal-X (version 1.63b). Evolutionary distance between different isolates was calculated with the Kimura 2-parameter

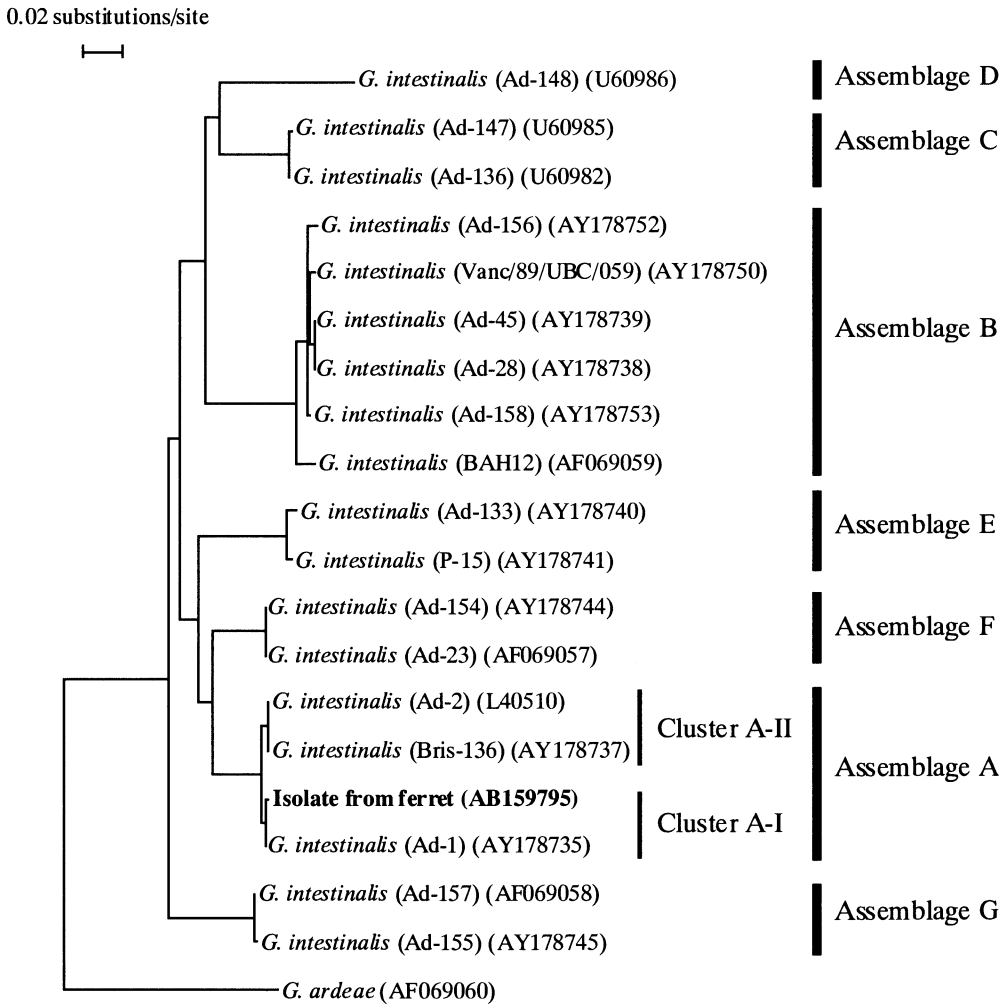

FIGURE 3. Phylogenetic relationships of the ferret isolate to other *Giardia* species and *G. intestinalis* genotypes as inferred by neighbor-joining analysis, based on the nucleotide sequences of the GDH. Names of the isolates and accession numbers in GenBank are shown in parentheses.

method. Trees were constructed using the neighbor-joining algorithm (Saitou and Nei, 1987) and were drawn using the NJplot program (Perrière and Gouy, 1996). The partial sequences of SSUrDNA, β-giardin, and GDH of the ferret *Giardia* isolate, obtained in this study, have been deposited in the GenBank database as AB159796, AB159797, and AB159795, respectively.

The partial SSUrDNA, β-giardin, and GDH were amplified successfully in the ferret isolate (data not shown). The partial SSUrDNA (125 bp) sequence was identical to sequences of the isolates (BAC2 and BAH40C11) found to have the assemblage A. The partial β-giardin sequence (472 bp) of the ferret isolate differed slightly from the sequences of the isolates (WB, KC8) found to have the assemblage A. There were 2 substitutions in the partial β-giardin sequence of the isolate from the ferret as compared with that of the isolate WB or KC8 (data not shown). The partial GDH sequence (592 bp) of the ferret isolate also differed slightly from the sequences of the isolates found to have the assemblage A (Ad-1, Ad-2, Bris-136). There were 1, or 3, substitutions in the partial GDH sequence as compared with that of the isolate Ad-1 or Ad-2 and Bris-136, respectively (data not shown). The close relatedness of the ferret isolate to assemblage A was also reflected in the phylogenetic analysis of β-giardin (Fig. 2) as well as SSUrDNA (Fig. 1): the ferret isolate was clustered with assemblage A. Similarly, the phylogenetic analysis of GDH sequences showed a close relatedness between the ferret isolate and assemblage A, but the ferret isolate was not clustered with the isolates Ad-2 and Bris-136 found to have group A-II but with the isolate Ad-1 found to have group A-I (Fig. 3). At present, the isolates classified into the genetic group A-I have been found in a variety of mammals, e.g., cattle, pig, horse, cat, dog, beaver, and humans, but the isolates in group A-II have been found only in humans (Adam, 2001; Monis and Thompson, 2003). Therefore, on the basis of the results of the phylogenetic analysis performed in this study and of the molecular epidemiological evidence revealed previously, we place the ferret isolate in genetic group A-I, which appears to have zoonotic potential. Although *Giardia* infection in Mustelidae had been confirmed already in a black-footed ferret, *Mustela nigripes*, by light microscopy in 1988 (Williams et al., 1988), since then there have been no reports regarding *Giardia* infection in weasels. Therefore, our study is the first molecular analysis of an isolate from weasels. Epizootiological surveys of zoonotic pathogens in animals reared in pet shops or by breeders have been overlooked, and thus, periodical examinations of pets are needed to prevent infections with zoonotic pathogens.

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Absence of Vacuolar Membrane Involving *Toxoplasma gondii* During Its Intranuclear Localization

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ABSTRACT: Tachyzoites of *Toxoplasma gondii* were located inside the nucleus of both skeletal muscle cells infected in vitro and peritoneal exudate cells collected from infected mouse in vivo. Ultrastructural analysis demonstrated that *T. gondii* invades the nucleus of host cells by the parasite apical region and with constriction of its body. We noted that the rhoptry, a secretory organelle of the parasite that is involved in the host cell invasion mechanism, was empty in the intranuclear *T. gondii*. The parasites were found in the nuclear matrix without evidence of the vacuolar membrane. Frequently, new parasites invaded host cell nucleus, which was already infected. The significance of this nuclear invasion could reflect an alternative route of *T. gondii* for its transitory survival or an escape mechanism from the host immune response during the in vivo infection (or both).

Toxoplasma gondii is an obligate intracellular parasite, which infects a wide variety of warm-blooded vertebrates, including humans and domestic animals. It is an important pathogen in humans, which can cause a serious and potentially fatal disease in newborn infants and in immunocompromised hosts, such as in acquired immunodeficiency syndrome patients (Dubey, 1997; Liesenfeld et al., 1999). The key for *T. gondii* survival is its internalization into the host cell. The process of invasion appears to be complex, involving an oriented active process that requires a motile parasite, its attachment to the host cell surface, followed by the discharge of secretory products from the parasite apical organelles (reviewed by Black and Boothroyd, 2000; Carruthers, 2002). In the host cell, the parasite resides within a modified, membrane-bound compartment, the parasitophorous vacuole (PV), which appears to act as a protective interface, avoiding fusion with endosomes and lysosomes, besides supporting the growth and proliferation of the tachyzoites (reviewed by Sinai and Joiner, 1997; Andrade et al., 2001). The available evidences suggest that the PV is nonfusogenic because the surface determinants from the host cell, which are known to be necessary for membrane fusion events, are excluded from the PV during the parasite invasion (Carvalho and De Souza, 1989, 1990; Mordue et al., 1999; Hakansson et al., 2001).

The intranuclear location of *T. gondii* tachyzoites during the infection of different cell types in vitro has been analyzed by light microscopy (Sourander et al., 1960; Remington et al., 1970; Ogunba, 1972; Azab et al., 1973). The transitory location of microorganisms within the nucleus of host cell, including sporozoites or merozoites from species of *Dobellia*, *Eimeria*, *Isospora*, and *Besnoitia* has also been described. These parasites have been denominated caryotropics because during the endogenous stages they can reside within the host cell nucleus (Roberts et al., 1971; Peka, 1992, 1993). Despite the frequent intranuclear occurrence among coccidians, little is known about the mechanisms or physiological basis for the reported intranuclear parasitism or the significance of this location in coccidian systematics (Atkinson and Ayala, 1987). The majority of these studies have been performed using light microscopy, which did not allow to observe the details from the invasion mechanisms as well as to characterize whether these parasites were surrounded by membranes. In this article we describe, for the first time at the ultrastructural level, the presence of *T. gondii* inside the nucleus of both skeletal muscle cells (SKMC) infected in vitro and peritoneal exudate cells collected from infected mouse in vivo.

Primary cultures of SKMC were obtained from thigh muscles of 18-day-old mouse embryos (for details, see Araújo-Jorge et al., 1986; Barbosa et al., 2000). Three- to 6-day-old cultures of SKMC were infected with tachyzoites (RH strain) of *T. gondii* using a 10:1 parasite–host cell ratio. After 4, 24, 48, and 72 hr of infection, the culture cells were fixed for 30 min at 4 C with 2.5% (v/v) glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.2), washed with the same buffer, postfixed for 30 min at 4 C in 1% of osmium tetroxide, routinely processed for transmission electron microscopy, and examined in a Zeiss EM10C. Another ultrastructural approach was analyzing the peritoneal exudate cells obtained from *T. gondii*–infected Swiss mice after 48–72 hr of infection.

Our results showed that intranuclear tachyzoites in SKMC were directly immersed in the nuclear matrix, without any morphological indication of vacuolar membrane surrounding the parasites (Fig. 1). Cells collected from the peritoneal exudates of *T. gondii*–infected mice also exhibited tachyzoites invading the host cell nucleus (Figs. 2–4), fre-