Molecular characterization of *Giardia* parasite isolated from stool samples collected from different hospitals in Taif City (Saudi Arabia)

Shalaby, I.¹, Gherbawy, Y.¹,²* and Banaja, A.¹
¹Department of Biological Sciences, Faculty of Science, Taif University, Taif, KSA
²Botany Department, Faculty of Science, South Valley University, Qena, Egypt
*Corresponding author email: Youssufgherbawy@yahoo.com
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**Abstract.** *Giardia* parasite is prevalent endemically in Taif city. Infection is more prevalent in children under 5 years old and elderly people. The sickness is more intense in immune-compromised people. The disease is usually diagnosed by stool examination by the microscope, for the identification of the both trophozoite and cyst stages. Usually the disease is overlooked during stool analyses due to the minute size of the parasite and due to the scarcity of infection sometimes. Hence molecular characterization or diagnosis is used as an alternative method for the diagnosis of infection. Molecular characterization is based on polymerase chain reaction (PCR). This technique is regarded as a highly sensitive and accurate method of diagnosis. The prevalence of *Giardia duodenalis* was 15% in stool samples collected from different hospitals in Taif. By means of RAPD technique, most *G. duodenalis* isolates were genetically similar, forming two main groups, with about 60% of similarity one another.

**INTRODUCTION**

*Giardia* is an enteric protozoan parasite that can cause severe gastroenteritis and lengthy diarrhoea in infected humans. *Giardia* has been associated with numerous outbreaks of water-borne diseases (Keister, 1983; Roach et al., 1993; Mahbubani et al., 1998). Currently, giardiasis represents a major public health concern of water utilities in developing and even developed countries (Dykes et al., 1980). In developing countries in Asia, Africa, and Latin America, approximately 200 million people have symptomatic giardiasis (Thompson et al., 2000).

*Giardia* seems to be endemic in Saudi Arabia similar to other tropical and subtropical countries where children are particularly likely to be infected (Farag, 1985; Qardi & Khalil, 1987). The prevalence rate of Al-Sekait et al. (1993) study (13.5%) is similar to other reports in the Kingdom and in other Arab countries (Abu Alsaud, 1983; Farag, 1985; Khan et al., 1989). On the other hand, Eligail et al. (2010) reported that the prevalence rate of *Giardia lamblia* in stool samples from Riyadh region was 0.67%. Consumption of faecal contaminated water may affect the epidemiology of *Giardia* in Saudi Arabia since *Giardia* is more often found in faecal contaminated water (Khan et al., 1989). *Giardia* is a genus of intestinal flagellates that infects a wide range of vertebrate hosts. The genus currently comprises six species, namely *Giardia agilis* in amphibians, *Giardia ardeae* and *Giardia psittaci* in birds, *Giardia microti* and *Giardia muris* in rodents, and *Giardia duodenalis* in mammals. These species are distinguished on the basis of the morphology and ultrastructure of their trophozoites (Adam 2001a). *Giardia duodenalis* (syn. *Giardia intestinalis, G. lamblia*) is the only
species found in humans, although it is also found in other mammals, including pets and livestock (Thompson, 2004). A considerable amount of data has shown that *G. duodenalis* should be considered as a species complex, whose members show little variation in their morphology, yet can be assigned to at least seven distinct assemblages (A–G) based on genetic analyses (Monis et al., 2003).

*Giardia duodenalis* (*G. intestinalis*, Kulda & Nohýnková, 1995) includes organisms recovered from many different mammalian species. The morphological uniformity of these isolates masks a considerable genetic and biotypic diversity and on the basis of available data, the current taxonomy must be considered inadequate (Andrews et al., 1989; Kulda & Nohýnková, 1995; Monis et al., 1999; Thompson et al., 2000; Adam, 2001b, van Keulen et al., 2002).

Techniques that have been used to compare *G. intestinalis* isolates genetically include analysis of allozymes (Andrews et al., 1989; Stranden et al., 1990; Mayrhofer et al., 1995), karyotypes (Sarafis & Isaac-Renton, 1993), restriction fragment length polymorphisms (RFLP) (Nash et al., 1985; Homan et al., 1992), nucleotide sequences (Baruch et al., 1996; Monis et al., 1996, 1999) and random amplified polymorphic DNA (RAPD) (Morgan et al., 1993, Paintlia et al., 1999). RAPD is a DNA fingerprinting technique based on the amplification of undefined segments of the genome by PCR using single arbitrary primers (Williams et al., 1990). The multi-locus character of RAPD allows sensitive detection of polymorphisms in different parts of the genome. If performed with a sufficient number of primers, the method has the potential to provide reliable information about the genetic identity of isolates (Banaja et al., 2008, Shalaby et al., 2011). A drawback of the method is a requirement for DNA samples free of any contaminating DNA. Consequently, applicability of RAPD analysis is limited to axenic *in vitro* isolates which need not represent full spectrum of *G. intestinalis* diversity.

The aim of this work was to assess the prevalence of *Giardia duodenalis* in stool samples from different clinic at Taif region and assess genetic diversity of the collected strains of *G. duodenalis* by means of PCR-RAPD.

**MATERIALS AND METHODS**

**Sample collection**

One hundred stool samples were collected from hospital laboratories in Taif City. Samples were examined macroscopically and microscopically for infection of *Giardia*.

**Parasitological assessment**

Feces samples were examined in the laboratory. Three methods were employed in order to recover enteroparasites: Lutz (Elígio-García et al., 2005) method for eggs, Amato Neto et al. (1963) method to concentrate the protozoa cysts and helminths eggs, and Rugai (Carvalho et al., 2005) method for recovery of larval forms of helminths.

**Purification of *G. duodenalis* cysts from human faeces**

This procedure was performed according to Thompson et al. (1976) with minor adaptations according to Tashima et al. (2009). Faeces samples were diluted in water (1:5), passed through surgical cotton gauze and centrifuged at 700 x g for five min. The supernatant was discharged, the sediment was suspended in water and the process was repeated four more times in order to remove debris. The resulting sediment was suspended in 3 mL water, added 3 mL of 1 M sucrose and centrifuged at 180 x g for 20 min. The cysts in suspension were transferred to another centrifuge tube, suspended in water and centrifuged again at 700 x g for 10 min. The supernatant was discharged, the sediment was suspended in water and the process was repeated four more times in order to remove debris. The resulting sediment was suspended in 3 mL water, added 3 mL of 1 M sucrose and centrifuged at 180 x g for 20 min. The cysts in suspension were transferred to another centrifuge tube, suspended in water and centrifuged again at 700 x g for 10 min. The resulting sediment was suspended in 3 mL of water, added 3 mL of sucrose 0.75 M and centrifuged at 250 x g for 10 min. The cysts in suspension were washed in water to remove the sucrose residues and finally suspended in 1 mL of water.

**DNA extraction**

The purified cysts, ca. 50 per sample (Oda et al., 2005) were centrifuged at 700 x g for five min., the supernatant discarded, and the
pellet received 500 µL of TEN solution (100 µL of 1 M Tris–HCl; 200 µL of 0.5M EDTA, 200 µL of 5M NaCl; pH 8.0). The suspension was then transferred to liquid nitrogen for five min, followed by water-bath at 97ºC for five min. This procedure was carried out three times.

The suspension, after cooling in ice, received 5 µL of lysozyme, mixed and incubated in water-bath at 37ºC for one h. After that, 50 µL of 5% sodium dodecyl sulfate was added, mixed, followed by addition of 2.5 µL of K proteinase (20 mg/mL of H2O) and again incubated in water-bath for 30 min. The suspension was centrifuged at 7500 x g for five min and the supernatant discarded. The pellet was resuspended in isopropyl alcohol and again centrifuged at 15000 x g for five min. The resulting pellet was washed in 70% alcohol and resuspended in 200 µL of TE solution (10 mM Tris; 1 mM EDTA) and the DNA concentration determined spectrophotometrically.

**PCR with arbitrary primers (RAPD)**

The RAPD (Random Amplified Polymorphic DNA) technique (Steindel *et al.*, 1993) was carried out in 25 µL of reaction mixture containing 0.1 U of Taq DNA polymerase (PromegaTM), 0.2 mM of dNTPs, 1.5 mM of MgCl2, 50 mM of KCl, 10 mM of Tris-HCl (pH 8.5), 1 ng of DNA template, and 1 µM of each primer: IMIA5 (5’ AGG GGT CTT G 3’), IMIA8 (5’ GTG ACG TAG G 3’), and IMIA10 (5’ GTG ATC GCA G 3’). The primers were selected randomly among the available primers. Forty-three cycles consisting of denaturation at 94ºC for 60 s, annealing at 50ºC for one min and extension at 72ºC for five min, were performed for each random primer.

**RAPD products analysis**

Computer analysis of RAPD patterns were performed as described by Halmschlager *et al.* (1994) in which the band pattern obtained from agarose gel electrophoresis was digitalized by hand to a two-discrete-character-matrix (0 and 1 for absence and presence of RAPD-bands, respectively) The data of all primers were combined. The analysis data was based on the Nei and Lee coefficient (Nei & Lee, 1979). Dendrograms were constructed by the unweighted paired group method of arithmetic average (UPGMA) based on Jaccard's similarity coefficient by using Phoretex 1D Software (version 5.2).

**RESULTS AND DISCUSSION**

**Prevalence of *G. duodenalis***

Fifteen faecal samples out of 100 were positive for *Giardia*. The positive samples represented 15% of the tested samples (Table 1). Most of the fecal samples were loose, with few exceptions that were diarrhoeic. In Saudi Arabia, Kasim & Elhelu (1983) examined stool samples of 53,306 individuals from the northern, southern, eastern, western and central regions of the Kingdom by wet films and zinc sulphate concentration methods. They reported that five thousand two hundred seventy one (9.9%) stools were positive for *G. lamblia*, and the prevalence of infection was higher in males (64%) than in females (36%). Al-Sekait *et al.* (1993) studied the prevalence of pathogenic intestinal parasites in rural Saudi Arabian schoolchildren aged 6-18 years. Their results showed that the major parasites isolated

<table>
<thead>
<tr>
<th>Giardia duodenalis</th>
<th>Hospital Name</th>
<th>Patient Gender</th>
<th>Patient Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUG 1</td>
<td>Aladwany</td>
<td>Female</td>
<td>10</td>
</tr>
<tr>
<td>TUG 2</td>
<td>Aladwany</td>
<td>Male</td>
<td>8</td>
</tr>
<tr>
<td>TUG 3</td>
<td>Aladwany</td>
<td>Female</td>
<td>35</td>
</tr>
<tr>
<td>TUG 4</td>
<td>Aladwany</td>
<td>Female</td>
<td>55</td>
</tr>
<tr>
<td>TUG 5</td>
<td>Aladwany</td>
<td>Female</td>
<td>10</td>
</tr>
<tr>
<td>TUG 6</td>
<td>Aladwany</td>
<td>Male</td>
<td>8</td>
</tr>
<tr>
<td>TUG 7</td>
<td>King Faisal</td>
<td>Male</td>
<td>15</td>
</tr>
<tr>
<td>TUG 8</td>
<td>Alamen</td>
<td>Female</td>
<td>30</td>
</tr>
<tr>
<td>TUG 9</td>
<td>Alamen</td>
<td>Male</td>
<td>6</td>
</tr>
<tr>
<td>TUG 10</td>
<td>Aladwany</td>
<td>Male</td>
<td>7</td>
</tr>
<tr>
<td>TUG 11</td>
<td>Aladwany</td>
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<td>9</td>
</tr>
<tr>
<td>TUG 12</td>
<td>Aladwany</td>
<td>Male</td>
<td>27</td>
</tr>
</tbody>
</table>

*TUG (Taif University Giardia)*
were *G. lamblia* (13.5%), *Schistosoma mansoni* (3.8%), *Entamoeba histolytica* (2.5%), *Hymenolepis nana* (2.5%), *Ascaris lumbricoides* (2.0%) and *Enterobius vermicularis* (1.0%). El-Sheik & El-Assouli (2001) reported that the prevalence of giardiasis was 3.1% among young children with acute diarrhoea in Jeddah. Their findings varied with the findings of other studies from other parts of Saudi Arabia in which a prevalence of 7.4% and 9.4% (Bolbol & Mahmoud, 1984) was reported. Abahussain (2005) in her study on the prevalence of intestinal parasites among expatriate workers in Al-Khobar (Saudi Arabia) reported that 4 samples out of 17 were positive for *G. lamblia*. Al-Hindi & El-Kichaoi (2008) studied occurrence of gastrointestinal parasites among pre-schoolchildren in Gaza (Palestine). Their results indicated that infection with *G. lamblia* showed the highest prevalence (10.3%) among parasites detected. Also, they reported that giardial infection seemed to be high in all age groups especially in 51-60 month old children (90%). The prevalence of *G. lamblia* was 3.1% among presumably healthy individuals in Lebanon (Saab et al., 2004). From suburbs of the capital city of Asmara (Eritrea), Srikanth & Naik (2004) studied stool samples of 75 farmers who were occupationally exposed, the study revealed that 45% of them harboured *Giardia* cysts. Wadood et al. (2005) showed that the prevalence of *G. lamblia* in Children Hospital Quetta (Pakistan) was very high (32%). Recently, the infection frequency with *G. lamblia* was 1.8% among children in the Kingdom of Saudi Arabia (Aly & Mostafa, 2010).

Our results showed that 5 positive samples (5%) were collected from females and 10 samples (10%) were collected from males. The age of positive samples were ranged from 6 to 55 years. Ten positive samples were collected from persons under 11 years (Table 1). Tashima et al. (2009) studied *G. duodenalis* in children from a daycare center in the region of presidente prudente, São Paulo, Brazil. They reported that, despite the higher proportion of girls among the children (61%), only five presented giardiasis, while 10 boys presented this intestinal parasite. Previous work has also showed higher prevalence of intestinal parasitic infections in males than females, without an apparent reason (Arani et al., 2008).

**Genetic variability of *G. duodenalis* by RAPD**

The fifteen isolates of *G. duodenalis* collected during this study were used to study genetic diversity among *Giardia* population in Taif region. The three primers IMIA5 (5' AGG GGT CTT G 3'), IMIA8 (5' GTG ACG TAG G 3'), and IMIA10 (5' GTG ATC GCA G 3') used in this study, generated a considerable number of amplification products for comparison (Figs. 1-3). Each primer produced from 6 to 9 fragments, depending on the *G. duodenalis* isolate and the primer used in the reaction. A different DNA banding pattern was present in almost every isolate. Tashima et al. (2009) used several arbitrary primers to study genetic variability of *G. duodenalis* isolated from Brazil by RAPD. They reported that each primer produced two to eight fragments, depending on the *G. duodenalis* isolate and the primer used in the reaction. Comparison of each profile for each of the primers was based on the presence (1) versus absence (0) of RAPD amplimers that migrated to the same position in the gel. Bands of the same size obtained by the same primer were scored as identical, but only bands repeatable in at least two experiments with the same primer at different times were evaluated. All three primers revealed high similarities among isolates (Figs. 1 -3). The combined data from all isolates of the *G. duodenalis* were analyzed to produce a dendrogram (Fig. 4). The banding profiles generated by each primer were able to detect genetic variability among the *G. duodenalis* isolates. The grouping analysis resulted in two genetic groups with similarity about 60%. The first group RAPD 1, was formed by nine isolates, all of them obtained from male samples. Also, inside this cluster there were several subclusters according to the source of samples, for example TUG11 and TUG12 isolated from King Faisal hospital samples were clustered together with similarity about
Figure 1. DNA banding patterns from RAPD analysis of *Gardia duodenalis* strains primed by IMIA8 (5’ GTGACGTAGG 3’)

Figure 2. DNA banding patterns from RAPD analysis of *Gardia duodenalis* strains primed by IMIA10 (5’ GTGATCGCA G 3’)

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Figure 3. DNA banding patterns from RAPD analysis of *Gardia duodenalis* strains primed by IMIA5 (5' AGGGGTCTTG 3')

Figure 4. Dendrogram showing genetic relationship of some *Gardia duodenalis* strains produced by RAPD with the three arbitrary primers IMIA5, IMIA8 and IMIA10
85%. The second group RAPD 2, was formed by 6 isolates, all of them were isolated from female samples, with similarity about 65%. Also, strains TUG1, TUG2, TUG3 and TUG4 isolated from samples collected from Aladwany hospital clustered together with similarity about 75% (Fig. 4). Morgan et al. (1993) used RAPD for characterization of fourteen G. duodenalis isolates extracted from different animals and geographical regions, and compared their results with previous data based on isoenzyme analysis of the same isolates. Although the authors found close correlation between the two methods, some inconsistencies were observed. Seven out of fourteen isolates grouped differently when considering each technique, although the 14 isolates had been separated in 10 groups in both cases. In 31 isolates of G. duodenalis, in Brazil, 31 different genetic profiles were found, considering the set of random primers used in the reaction (Tashima et al., 2009). Also, they reported that most of the isolates from children showed genetic profile forming only one genetic group, with about 45% of similarity, except one isolate.

In summary, the prevalence of G. duodenalis was 15% in the tested faecal samples. Also, the level of genetic diversity in Giardia populations is likely to be influenced by a variety of factors. Hence, further study is needed to evaluate the effect of different parameters in the genetic diversity of G. duodenalis population in the all regions of Saudi Arabia.

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REFERENCES


