Genetic Fingerprint of Unilocular Hydatidosis in Egyptian Camels and Humans Using Nested PCR

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ABSTRACT

The pulmonary and hepatic hydatid cyst fluids were collected from 540 slaughtered camels and 5 human cases in Qalyubia Governorate, Egypt. The prevalence of infection of cystic echinococcosis among camels was 120 (22.2%). The fertility rates of the isolated cysts form camels and humans were 64.5 and 100%, respectively. A nested polymerase chain reaction was used for amplification of mitochondrial NADH 1 gene of Echinococcus granulosus complex in fertile cysts obtained from camels and humans, respectively. Two pairs of primers (EGL1 and EGR2) and (EGL3 and EGR4) were used in 2 amplification steps. First, the outer pair of primer originated from a highly conserve region of NADH1 gene generate a primary 435 bp PCR product. Second, a pair of internal (nested) primer (EGL3 and EGR4), designed to the annealing site of primers (EGL1 and EGR2) yield similar diagnostic amplified DNA bands of molecular size marker at 276 bp in all examined cysts obtained from camels and humans indicating a zoonotic relationship. This study confirms similar fingerprinting patterns of Echinococcus granulosus complex in camels and humans in Qalyubia Governorate, Egypt. Nested PCR for diagnosis of E. granulosis had been used for the first time in Egypt, as far as we know.

INTRODUCTION

Unilocular hydatidosis or cystic echinococcosis (CE) is an important zoonotic disease caused by the larvae of Echinococcus granulosus and represents a major public health problem in many countries around the world including the Mediterranean zone (Pawlowski et al., 2001; Hui et al., 2013). CE produces many health problems in livestock and human, with critical economic consequences due to the cost of medical treatment and morbidity for human cases and losses in animal productivities (Torgerson, 2003). Camels could participate with a vital part in the transmission cycle of the parasite and the epidemiology of the disease, especially in rural communities where dogs (definitive host) infected by ingestion of infected camel carcasses containing the hydatid cysts (Moro and Schantz, 2009). Humans are accidental intermediate host infected by ingestion of food or water contaminated with dog feces containing the infective eggs (Naseri-Moghddam et al., 2011). Human hydatidosis is a symptomatic disease because of the slow growth of hydatid cysts, which typically diagnosed in patients using imaging technique as ultrasonography (Sako et al., 2002). After primary infection, clinical symptoms become evident after 10 years or more (Sako et al., 2011), but early diagnosis and treatment are very crucial for lessening of morbidity and mortality (Sarkari et al., 2007).

Ten genotypes of E. granulosis, EG are recognized worldwide and designated as G1-G10 (McManus et al., 2003). Only 5 strains infect humans, such strains were sheep (G1), Tasmanian sheep (G2), cattle (G5), camel (G6), and pig (G7) strains (Bart et al., 2006). Different molecular strategies and genetic targets have been applied for the identification of E. granulosis, examples of these include cox1 (M’Rad et al., 2005), nad1 and cox1 (Abushewa et al., 2010), and 12sr RNA cox1 and nad1 (Omer et al., 2010; Abdel Aaty et al., 2012; Mogoye et al., 2013). Moreover, NADH dehydrogenase 1 gene (nad1) is the most highly conserved nucleotide sequence among different genotypes of EG-complex (Bowles and McManus, 1993). The nested polymerase chain reaction (nPCR) is the highly sensitive and specific assay when compared to the traditional PCR (Osman et al., 2009).
The aims of the present study were to determine the prevalence of hydatidosis, affected organs, and fertility rate of hydatid cysts in camels and humans. After that we used the nested polymerase chain reaction for revealing of mitochondrial NADH dehydrogenase1 (nad1) gene to investigate the DNA fingerprinting of isolates of cystic echinococcosis obtained from camels and humans.

MATERIALS AND METHODS

Survey and cyst collection
Abattoir survey: The study was carried out on 540 camels (5–15 years old) slaughtered at the official slaughterhouses of Toukh and Benha, Qalyubia Governorat, Egypt, during the period from October 2012 to September 2013, through examination of lung and liver of each slaughtered camel was carried out (Salih et al., 2011).

Isolates from humans: Five hydatid cyst fluids from four livers and one lung (Naseri-Moghaddam et al., 2011) were recovered from humans (3 males and 2 females, 45–55 years old) admitted to Toukh’s hospital and Benha insurance hospital, Qalyubia Governorate, Egypt. The infested samples were sent to the laboratory for further examination.

Parasitological examination: Cyst fluid was aspirated, with a sterile needle, from cysts of infected camels. The contents were examined under a microscope for the presence of protoscolices in the cyst. Cysts with protoscolices isolated from 25 cysts (20 and 5 camel and human cysts, respectively) were used for genetic characterization (Osman et al., 2009), then stored at -20°C till used.

Genetic characterization
DNA extraction: Genomic DNA was extracted from protoscolices as well as from the camel strain G6 (positive control) via a commercially available QIAamp-tissue kit (Qiagen, Hilden, Germany). DNA extraction and purification were done in harmony with the manufacturer’s instructions. Five micro liters of the suspended nucleic acid was run down in the PCR tube having 1 µl of each primer EGL 1 and EGR 2 (100 pmol), and 12.5 µl PCR Master Mix (Jena Bioscience Co. Jena, Germany). Thermal profiles were performed on thermal cycler (T-Biometra) with standard conditions. Every PCR run included positive and negative controls. The positive result is indicative at 435bp.

Polymersase chain reaction: PCR amplification was prepared in 25 µl PCR tube holding 5 µl of target DNA sample, 1 µl of each primer EGL 1 and EGR 2 (100 pmol), and 12.5 µl PCR Master Mix (Jena Bioscience Co. Jena, Germany). The nested pair of primers (EGL 3 and EGR 4) was anticipated to amplify a 276 bp PCR amplicons, internal to the annealing sites of primers EGL 1 and EGR 2. Thermal profiles were performed on a Thermal cycler (T-Biometra). After amplification, 15 µl from each PCR product were loaded over the 1.5% agarose gel stained with ethidium bromide, and then the nPCR products were seen under UV light. Every PCR run included positive and negative controls.

Nested Polyermase Chain Reaction (nPCR): For the nPCR amplification, 2.0 µl of the primary PCR outcome produced by EGL 1 and EGR 2 were relocated to 25 µl PCR tube having 1 µl of each primer EGL 3 and EGR 4 (100 pmol), and 12.5 µl PCR Master Mix (Jena Bioscience Co. Jena, Germany). The nested pair of primers (EGL 3 and EGR 4) was anticipated to amplify a 276 bp PCR amplicons, internal to the annealing sites of primers EGL 1 and EGR 2. Thermal profiles were performed on a Thermal cycler (T-Biometra). After amplification, 15 µl from each PCR product were loaded over the 1.5% agarose gel stained with ethidium bromide, and then the nPCR products were seen under UV light. Every PCR run included positive and negative controls.

RESULTS

Among 540 surveyed camels, 22.2% were infected with CE. Post mortem examination revealed that hydatid cysts infect 51.6% lungs and 27.5% livers. Moreover, mixed infections in lungs and livers, 20.83% were also recorded. Out of the 200 isolated cysts, 64.5% were fertile and the fertility rates of the pulmonary, hepatic, and mixed infections were 75.6, 44.2, and 50.0%, respectively. All human cysts were also fertile (Table 1).

The result of nPCR amplification of mitochondrial nad1 gene of E. granulosus complex in fertile unilocular hydatid cysts gained from infected camels and humans indicated that the outer pair of primers EGL 1 and EGR 2 produced a primary 435 bp PCR product (Fig. 1). The nested primers EGL 3 and EGR 4 induced amplification of a 276 bp amplified DNA bands in all examined (fertile) hydatid cysts infect 51.6% lungs and 27.5% livers. Moreover, mixed infections were 75.6, 44.2, and 50.0% respectively. All human cysts were also fertile (Table 1).

Table 1: Prevalence of hydatosis in slaughtered camels

<table>
<thead>
<tr>
<th>Organ</th>
<th>Infected animals</th>
<th>No. of examined cysts</th>
<th>No. of Fertile cysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>62</td>
<td>123</td>
<td>93</td>
</tr>
<tr>
<td>%</td>
<td>51.7</td>
<td></td>
<td>75.6</td>
</tr>
<tr>
<td>Liver</td>
<td>33</td>
<td>43</td>
<td>19</td>
</tr>
<tr>
<td>%</td>
<td>27.5</td>
<td></td>
<td>44.2</td>
</tr>
<tr>
<td>Mixed*</td>
<td>25</td>
<td>34</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>200</td>
<td>129</td>
</tr>
<tr>
<td>%</td>
<td>22.2</td>
<td></td>
<td>64.5</td>
</tr>
</tbody>
</table>

The study was carried out on 540 camels (5–15 years old)
Political and economic factors play an important role in zoonotic transmission of 

**DISCUSSION**

*E. granulosus* is medically and economically important cyclozoan parasites. Our survey indicated that the prevalence of CE was 22.2%. Hydatid cysts have been reported in camels from almost all countries where camels are raised and our result was nearly similar to that detected in slaughtered camels in Algeria, 24.8% (Bardonnet et al., 2003), Ethiopia, 22.6% (Salih et al., 2011), and Uzbekistan, 25.4% (Torgerson et al., 2006). On the other hand, our prevalence was higher than those previously recorded in several governorates in Egypt, 7.7% among camels in Assiut and Aswan (Dyab et al., 2005), 2.5% in Mansora (Haridy et al., 2006), and 16.25% in Qalyubia (Mahmoud, 2012); 3.6% in Libya (Kassem and Gdoura 2006); 6.5% in Tunisia (Lahmar et al., 2004); 12% in Morocco (Azlaf and Dakkak 2006), and 17.5% in Pakistan (Latif et al., 2010). Our results is lower than those reported in Sudan, 59% and 29.7% (Omer et al., 2010; Ibrahim et al., 2011), Mauritania, 30.1% (Ould Ahmed Salem et al., 2011), Kenya, 61.4% (Njoroge et al., 2002), Saudi Arabia, 32.8% (Ibrahim 2010), Iran, 35.2% (Ahmadi, 2005) and Turkmenistan, 38% (Torgerson et al., 2006).

Considering the slow progression of hydatosis, it would be expected that differences in prevalence rates are more likely owing to changes in the age structure of the livestock populations referred for slaughter, younger animals having typically lower infection rates than older animals (Ibrahim et al., 2011; Salih et al., 2011). The extent of the infection is also influenced by host-related factors, such as sex (Salih et al., 2011), immunity, and presence of concomitant infections (Eckert and Deplazes, 2004). Moreover, other factor influence the prevalence of infection, such as stockbreeding conditions and mode of grassing (Erbero et al., 2010), environmental determinants (Ibrahim, 2010), or presence of large numbers of nomadic or semi-nomadic camel flocks, and their close contact with dogs, providing the transmission of infection to humans.

Our data indicated that the rate of infection in lungs (51.7%) was higher than that of the liver (27.5%). Our observation is in accordance with those noticed in camels in several African countries, such as Mauritania (Bardonnet et al., 2003), Sudan (Ibrahim et al., 2011), Ethiopia (Salih et al., 2011), and Egypt (Haridy et al., 2006; Mahmoud, 2012). In human cases, hydatid cysts developed mainly in the liver followed by lung. Similar finding was reported (Pawloski et al., 2001; Manterola et al., 2008). The fertility of cyst is one of the most important factors in the epidemiology of *E. granulosus*. The fertility rate of hydatid cyst, in the present work, was 64.5% which was higher than that reported in Ethiopia, 50% (Salih et al., 2011) and lower than that recorded in Sudan, 85.4% (Ibrahim et al., 2011). The fertility of cysts varies depending on the hosts and geographical situations (Bardonnet et al., 2003; Ibrahim et al., 2011; Salih et al., 2011). The fertility rates were higher in lungs (75.6%) than those in the liver (44.2%). Our results are in harmony with those reported by other researchers in Egypt (Haridy et al., 2006), Sudan (Ibrahim et al., 2011), and Ethiopia (Salih et al., 2011). Regarding the high fertility rate of cysts in camels, it is indicated that camels in Egypt act as an intermediate host that could maintain transmission of the disease. Camels slaughtered at home without inspection will easily pass on the parasites to dogs and consequently perpetuate the disease in the population. Moreover, all human cysts, used in the present work, were fertile. Similar observation was reported by Mogoye et al. (2013).

Molecular genetic study has been carried out to identify nad1 gene for identification of *E. granulosus (Eg)* complex for hydatid cyst of infected camels and humans. The outer pair of primers EGL1 and EGR2 generated a primary 435 bp amplified products and the nested primers of EGL3 and EGR4 yield diagnostic amplified DNA bands of molecular size marker at 276 bp. The use of nPCR in the present work revealed absence of variation in amplified DNA in all fertile cysts from camels and humans and having high similarity with DNA fingerprinting of *E. granulosus* G6 positive control indicating a zoonotic association (Azab et al., 2004 and Abdel Aaty et al., 2012) in Egypt. Our results are in harmony with that of Osman et al., (2009) as they indicated that the second amplification step using the nested primer EGL 3 and EGR 4 confirmed the specificity of EG complex primary PCR product and increased the
sensitivity of the PCR based assay at least 100 folds. In Egypt, previous molecular representation of human and animal *E. granulosus* was studied through RAPD-PCR (Azab et al., 2004), semi nested PCR, and PCR-RFL (Abdel Aaty et al., 2012; Tawfeek et al., 2009). Consequently, this study is the first work used nested PCR in diagnosis of *E. granulosus* in Egypt, to the best of our knowledge.

**Conclusion:** All examined fertile cysts, in the present study, from camels and humans yield diagnostic amplified DNA fingerprinting of the mitochondrial marker nad1 gene of the *E. granulosus* species. From a public health and veterinary standpoints, our finding about hydatosis leading to great economic concerns in Egypt and an integrated approach for controlling CE should fulfill actions accommodated to improve public health education and controlling home-slaughter habits, meat inspection services, appropriate disposal of infected viscera and dead animals, dog management, such as treatment and elimination of stray dogs. Consequently, application of standardized recording and reporting systems for livestock CE is highly crucial in Egypt.

**Further studies:** Further studies are needed to recognize new molecular investigations at the intra-specific level, as well as viability/infectivity studies in infected intermediate hosts. Such studies could help to elucidate the host range and transmission patterns that sustain CE in a given region. More research is required to determine genotypes of *E. granulosus* in humans and animals in Egypt. Further evaluation of the current situation, especially in dogs, humans, and livestock will improve our understanding on the epidemiology of the disease in Egypt and provide the background for the design of cost-efficient control strategies.

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