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Molecular genotyping of *Echinococcus granulosus* in animal and human isolates from Egypt

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ABSTRACT

Despite, Egypt is started to be considered as an emerging endemic area for cystic echinococcosis (CE), no enough data in the literature about the exact status of the genotype in both animals and humans. Therefore, the present study aims to characterize the underlying genotypes that could be responsible for the transmission cycle and for the growing infectivity. Animal isolates were collected from 47 camels and 6 pigs. Human isolates are 31 CE cases including; 21 of hepatic cases, 5 of pulmonary cases and 5 multiple-organ affection cases. Hot-Start specific PCR followed by DNA sequencing for mitochondrial 12S rRNA gene, revealed G1 genotype in one (3.2%) of 31 human isolate only. G6 genotype was detected in all the 53 (100%) animal isolates and in 30 out of 31 (96.8%) human isolate. The Egyptian G6 strain nucleotide sequence revealed 100% homology with an Argentinean reference strain 99% homology with the Kenyan G6 strain. It was concluded that G6 genotype is the predominant genotype in Egypt.

1. Introduction

Cystic echinococcosis (CE) is one of the most important cestodes infections causing significant morbidity and mortality in humans as well as significant economic losses in livestock animals (Eckert et al., 2001). Egypt is one of the countries where CE is of public health importance (Sadjjadi, 2006). The migration of infected people and livestock has great contribution in the flaring up of this problem (Mamuti et al., 2002). To date, six genotypes of the *Echinococcus granulosus* complex have been identified in Africa: the sheep strain (G1 genotype), the Tasmanian sheep strain (G2 genotype), the horse strain (G4 genotype, also referred to as *Echinococcus equinus*), the cattle strain (G5 genotype, *Echinococcus ortleppi*), the camel strain (G6 genotype, *Echinococcus canadensis*) and the lion strain (*Echinococcus felidis*) (Anderson et al., 1997; Macpherson and Wachira, 1997; Sadjjadi, 2006). The extensive intraspecific variation in *E. granulosus* is associated with change in life cycle pattern, host specificity, geographical distribution, transmission dynamics, infectivity to human, antigenicity and sensitivity to chemotherapeutic agents (Romig, 2003; Thompson and McManus, 2002). This may have important implications for the design and development of diagnostic reagents, vaccines and control of echinococcosis (McManus and Thompson, 2003; De la Rue et al., 2006). The G1 genotype is generally considered to be the most widespread *E. granulosus* genotype worldwide, and it is the genotype most commonly involved in CE in humans (Dinkel et al., 2004; Nakao et al., 2008; Thompson and McManus, 2002). However, recent molecular epidemiological studies have revealed that humans can be infected with other genotypes and that the prevalence of infection with these genotypes is higher than previously thought (Kamenetzky et al., 2002). Moreover, within the *E. granulosus* complex there is no scientific evidence that humans are more susceptible to one genotype than another, although some genotypes are more frequently found in humans and some strains like camel strain (G6 genotype) appears to affect human in certain geographical areas but not others (McManus and Thompson, 2003). In the past, the G6 genotype had been identified as the etiological agent only in sporadic cases of CE and it was believed that this strain was less infective for humans or not infective at all (Zeyhle et al., 2010). Recently, several authors reported an increasing prevalence of G6 genotype in different countries (Badaraco et al., 2008; Bart et al., 2006; Dinkel et al., 2004; Guarnera et al., 2004; Kamenetzky et al., 2002; Manterola et al., 2008; Omer et al., 2004; Santivañez et al., 2008). These findings indicate that there is an increasing body of evidence that G6 genotype has a wider distribution than previously thought. Therefore, the present study aimed to clarify the status in Egypt by

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characterizing the *E. granulosus* genotypes from both human and animal isolates.

2. Materials and methods

Human and animal isolates were collected over the period from December 2009 to June 2010 from different Egyptian hospitals.

2.1. Human isolates

Human isolates consist of 31 patients (3–73 years) with confirmed CE by HCF examination or histopathological examination. It included: 21 hepatic CE, 5 pulmonary CE and 5 multiple organ CE. All human subjects were subjected to full clinical history, clinical examination and imaging techniques (chest X-ray, abdominal U/S, and/or CT scan).

2.2. Animal isolates

Animal isolates consist of ninety five cysts (47 camels, 6 pigs and 42 sheep cysts). Isolates were collected from, Al-Marg and Al-Salam abattoirs, Cairo, Egypt. The camel and pig cysts were confirmed to be *E. granulosus* by HCF examination and histopathology. The sheep cysts were confirmed by histopathology to be the cysticercus of *Taenia hydatigena* which is known as *cysticercus tenuicollis*.

2.3. Hydatid cyst materials (HCF, protoscolices and germinal layer)

Hydatid cyst materials (HCF, protoscolices and germinal layer) were processed according to Zhang et al. (1998) with modifications. Hydatid cyst materials (isolates) were obtained from CE patients during PAIR procedure or surgical removal of the cyst.

2.4. DNA extraction

DNA extraction was performed using "QIAamp® DNA Mini Kit" supplied by QIAGEN, Germany (Cat. No.: 51304). The manufacturer protocol for DNA extraction from tissue was used for protoscolices and germinal layer samples, whereas the manufacturer protocol for DNA extraction from fluid was used for HCF samples.

2.5. PCR study

Standard PCR was done according to Dinkel et al. (2004) to detect *E. granulosus* G1 and G5/6/7 genotypes. This PCR study was carried out to amplify the 254 bp fragment corresponding to the mitochondrial 12S rRNA gene. The primers used are according to Dinkel et al. (2004). Briefly, 50 ml reaction mixture was consisted of 200 mM of each dNTP, 2 mM $MgCl_2$, 200 μM of each dNTP, 50 pmol each primer and 2.5 units of QIAGEN HotStarTaq Plus DNA polymerase. A slight modification was done on Dinkel et al. (2004) PCR by adding 1 cycle at 95 °C for 5 min before the 40 cycles of the PCR (Hot Start PCR) and adding 1 cycle at 72 °C for 10 min after the 40 cycles. The PCR program for G1 detection is 40 cycles (denaturation for 30 s at 94 °C, annealing for 1 min at 57 °C and elongation for 40 s at 72 °C). The PCR program for G5/6/7 detection is 40 cycles (denaturation for 30 s at 94 °C, annealing for 1 min at 53 °C and elongation for 40 s at 72 °C). A reference strains supplied by Dr. Mara Cecilia Rosenzvit were used as controls. Amplification products were resolved on a 1.5% ethidium bromide stained agarose gel.

2.6. DNA sequencing

PCR purification kit (AxyPrep PCR Clean-Up Kit Cat. No. AP-PCR-50, from Axygen Biosciences, USA) was used to adsorb DNA to a silica membrane while passing impurities through washing steps that are followed by purified DNA elution. The G5/6/7 PCR was followed by forward and reverse sequencing to detect the specific genotype. Sequencing was done for mitochondrial 12S rRNA gene using ABI 3730xl DNA analyzer. Nucleotide sequence analysis was made using the National Center for Biotechnology Information BLAST programs and databases.

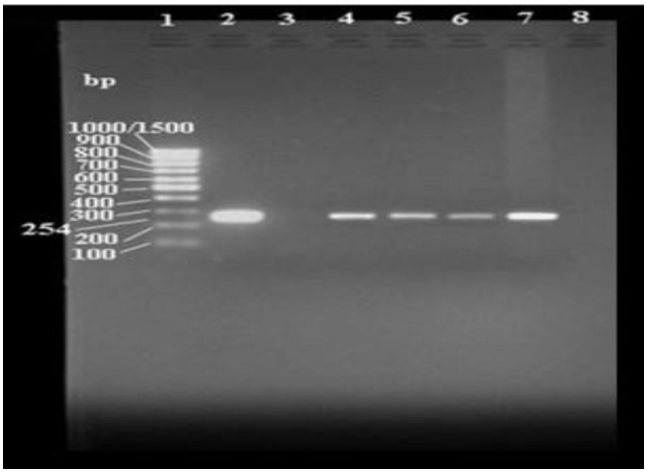

Fig. 1. Results of G5/G6/G7 PCR of human isolates on 1.5% agarose gel electrophoresis stained with ethidium bromide. Lane 1: DNA molecular marker, Lane 2: positive control: reference strain of G6 genotype of cystic hydatid diseases, Lane 3: negative control of PCR (no template DNA), Lane 4: positive human isolate (hepatic cyst, protoscolices), Lane 5: positive human isolate (hepatic cyst, germinal layer), Lane 6: positive human isolate (hepatic cyst, HCF), Lane 7: positive human isolate (from splenic cyst in a multi-organ affection CE case, protoscolices), Lane 8: negative control of samples (*cysticercus tenuicollis* of sheep).

3. Results

Macroscopic and microscopic examination of cysts isolated from sheep revealed that all of them are the larval stage of *Taenia hydatigena*. This was confirmed by histopathological sectioning of the cyst. Out of the 53 animal hydatid cyst isolates, 47 (88.7%) were camels and 6 (11.3%) were pigs. All the hydatid cysts collected from animal isolates in the present work were fertile. Screening of human isolates (obtained from hepatic HCF) for detection of G1 genotype of *E. granulosus* by Hot-Start PCR revealed a specific band at 254 bp in one out (3.2%) of 31 patients while animal isolates revealed no bands at all. Screening of human isolates for the detection of G6/7 genotypes revealed a band at 254 bp in (Fig. 1) 30 (96.8%) out of 31 cases while the same band was detected in all the animal samples (Fig. 2).

Performing the cycle sequencing and nucleotide sequence analysis by BLAST identified G6 genotype in all the animal isolates and in 30 (96.8%) out of 31 human isolates (Genbank ID: GQ476732–GQ476735).

4. Discussion

Interestingly, the present study reported no sheep hydatid cysts were found all over the period of it. In the current work, the collected hydatid cysts of camels and pigs were fertile. This is of great epidemiological importance as the fertile hydatid cysts are responsible for progression of the life cycle and therefore acting as a reservoir for human CE (Dyab et al., 2005). In the present study, G1 genotype was identified in one (3.2%) out of 31 human isolates and its DNA sequencing showed total homology with that in GenBank under accession no. AB297617. G1 genotype was not identified in any of the animal isolates. On the contrary, a study