Egyptian Veterinary Medical Society
of Parasitology Journal

A new microvariant of G6 genotype in hydatid cyst isolates from camels in Dakahlia province, Egypt

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Abstract

No enough data in the literatures about the exact spread of different *Echinococcus* genotypes in animals and humans allover Egypt. Previous reports illustrated the prevalent relatedness between camels and humans from Cairo and Qaliubya, middle of Egypt, in infection with the G6 gentoype. The present study aimed to underline the genotypes in reared camels from another geographical area of Egypt (Dakahlia province). Hydatid cyst isolates were collected from 10 camels slaughtered at Senbellawine abattoir and their DNA was subjected to the molecular analysis using the cytochrome oxidase subunit I gene (Cox 1). A microvarient of G6 with 5 substitutional positions was reported from all the examined samples. This report illustrates the wide dissemination of G6 genotype throughout Egypt and strength the great role played by camels in maintaining the transmission cycle of this genotype, in addition to its importance as a reservoir for human cystic echinococcosis.

Key words: Camel, *Echinococcus*, hydatid cyst, G6 genotype, Egypt

INTRODUCTION

Hydatid disease is an important zoonotic disease affecting humans and different species of animals all over the world particularly the Mediterranean region, Africa and the Middle East-including Egypt (**Sadjjadi, 2006**). Significant morbidity and mortality in humans as well as marked economic losses in livestock industry are caused as a result of this serious disease (**Haridy et al., 2006**). The primary cause of cystic echinococcosis (CE) is the formation of cystic larval metacestodes stage of *E. granulosus* and *E. multilocularis* in the internal organs of an intermediate host, mainly in the lung and liver. (**Omer et al., 2010; Ibrahim et al., 2011; Salih et al., 2011**).

In Egypt, **Rahman et al. (1992)** reported 31.0% CE prevalence in camels, while **Haridy et al. (2006)** and **Omar et al. (2013)** noted a considerably low rate (5-8%).

Molecular studies have identified 10 genotypes (G1-G10) within 4 *Echinococcus* species (**Cardona and Carmena, 2013**), including two sheep strains (G1 and G2), two bovid strains (G3 and G5), a horse strain (G4), a camel strain (G6), two pig strains (G7 and G9) and two cervid strains (G8 and G10), Nakao et al. (2013). Several reports mentioned an increasing prevalence of camel strain (G6) in different countries (**Kamenetzky et al., 2002; Dinkel et al., 2004; Guarnera et al., 2004; Manterola et al., 2008; Santivanez et al., 2008; Omer et al., 2010**).

By looking to the distribution of different genotypes in Egypt, studies illustrated the dominance of G6 in animals (specially camels) and humans (**Abdel Aaty et al., 2012; Khalifa et al., 2014; Amer et al., 2015; Abdel Aziz and El Meghanawy, 2016**), although G1 and G5 was reported in few cases (**Amer et al., 2015; Abbas et al., 2016**). The collected samples in most of the previous studies were confined to two Egyptian provinces (Cairo and Qaliubya).

Continuing with the previous attempts to understand the phylogenetic aspects of the hydatid disease in Dakahlia province, we selected camels in the current study to investigate the founded genotypes in this animal and to clear out its role in the transmission cycle of the G6 strain.

MATERIALS AND METHODS

Samples, animals and area of study

Ten natively reared camels were slaughtered at Senbellawine abattoir, Dakahlia province (120 km North from the capital Cairo), Egypt. Routine carcasses inspection was done with special attention to lungs and livers for the presence of hydatid cysts. Cysts were dissected out and washed with Phosphate Buffer Saline (PBS). Cysts` protoscolices and germinal epithelia were harvested and stored at –20 °C.

DNA extraction

DNA isolation from 8 cysts was done using the glass beads method according to **Tappeh et al. (2002)**.

Molecular analysis

PCR amplification was performed using the cytochrome oxidase subunit I (Cox1) gene (**Bowles et al., 1992**). Primers pair COI1 (forward) 50-TTTTTTGGCCATCCTGAGGTTTAT-30 and COI2 (reverse) 50-TAACGACATAACATAATGAAAATG-30 were used to amplify Cox1 gene by 30 cycles. Each cycle consisted of denaturation 94°C/30 sec, annealing 55°C/30 sec, elongation 72°C/30 sec, and a final extension72°C/7 min. Reactions were carried out in 35 µL final PCR mixture contained 2 µL of template DNA, 1 µL (25 µM) of each primer, 0.7 µL (10 mM) dNTP mix, 3.5 µL of Taq buffer (10X), 0.35 µL Taq polymerase (5Prime Perfect Taq^{Tm}) and 26.45 µL nuclease free water. Products from PCR reaction were separated on agarose gels (1%) stained with ethidium bromide. Gel bands DNA was purified and commercially sequenced. Bioedit and Mega (6) molecular softwares were used for alignment and phylogenetic purposes.

RESULTS

Using the blast search, analysis of the revealed partial Cox1 nucleotides sections from camel isolates of hydatid cysts confirmed their ligation to the G6 genotype (*E. Canadensis*, camel strain)**.** All the examined samples were identical in their sequences. A new microvariant of the G6 genotype was described from camels in the present study. This new haplotype was variable from the firstly described G6 (M84666 G6) in 5 mutational sites 152 (T-C), 195 (T-A), 256 (G-C), 289 (T-C), 299 (T-A). Additional substitutional position (C315T) was found with G6 sequences reported from camels slaughtered at Basateen abattoir, Cairo, Egypt (AB921058), Figure (1).

Moreover, the Phylogenetic analysis confirmed the alignment results through clustering of the recorded new haplotype within the same clade of G6 genotype reported from different geographical regions of the world, but in a separate branch, and nearer to AB921068 reported from the imported Sudanese camels slaughtered at Basateen abattoir, Cairo, Egypt, Figure (2).

DISCUSSION

Camels constitute important animals for the economy of many countries specially the Arabian ones, in which camel meat is the preferable food. In Egypt, previous studies recorded a high prevalence of camel hydatidosis (**Rahman *et al.*, 1992**). Moreover, a number of molecular reports from Egypt were conducted on both camel and human isolates of hydatid cysts. An important point on which we built our study is the limitation of samples collection in the previous studies to a specific area (Cairo and Qaliubya provinces) in

Figure 1: Alignment of partial Cox1 sequences of Dakahlia G6 isolate in the present study with M84666 (original G6 described by Bowles et al., 1992) and another 2 isolates from camels slaughtered at Basateen abattoir. A new

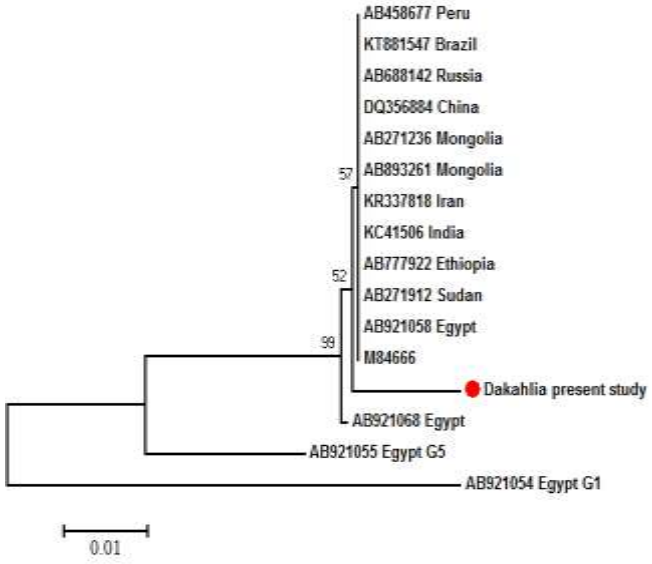

Figure 2: Phylogenetic tree of the Neighbor-Joining type for G6 strain partial Cox1 sequences from different geographical regions. G1 and G5 genotypes were used as an out group. The bootstrap analysis was conducted using 1000 replicates. Scale bar indicates the proportion of sites changing along each branch.

middle of Egypt, in which most of the imported Sudanese and Somalian camels are settled waiting for their slaughter at 2 main abattoirs, Basateen and Toukh. In our study, we collected samples from natively reared camels in another geographical area (Dakahlia province) in North of Delta. The scarcity of camels slaughtered at Dakahlia province was an obstacle we faced.

In the current study, Blast search results of the revealed partial Cox1 nucleotide sequences from isolates of camels slaughtered at Dakahlia province confirmed their genotyping as a camel strain (G6) of *E. canadensis*. Previous reports from Cairo and Qaliubya provinces using different gene markers illustrated the commonness of this strain in both camels and humans CE (**Abdel Aaty *et al.*, 2012; Khalifa *et al.*, 2014; Amer *et al.*, 2015; Abdel Aziz & El-Meghanawy, 2016**). The persistence of G6 strain in the Egyptian environment may be attributed to the close relationship between camels and dogs (**Dinkel *et al.*, 2004**) and the highly prevalent *Echinococcus* infection in dogs (**Mazyad *et al.*, 2007**). Collectively, our results strengthen the hypothesis that camels are influential reservoirs for human CE infection. Globally, G6 strain was predominate in camels at Africa as Libya and Algeria (**Bardonnet *et al.*, 2003**), Sudan (**Ibrahim *et al.*, 2011**) and Mauritania (**Bardonnet *et al.*, 2002**), and at Asia as Iran (**Harandai *et al.*, 2002**).

At the haplotype level using Cox1 partial gene sequencing, a new microvariant of G6 genotype was revealed from our isolates, while **Amer *et al.* (2015)** described another 2 different haplotypes. In addition, G6 strain was revealed previously from buffalo and sheep isolates (**Amer *et al.*, 2015**). The diversity in the recorded haplotypes may be related to the variation of the intermediate hosts that could be infected with this strain.

Focusing on Dakahlia province, 3 genotypes were recorded up till now, G6 from camels (the current study), G5 from cattle (**Abbas *et al.*, 2016**) and G1 from each of cattle (**Abbas *et al.*, 2016**) and buffaloes (**Abbas, 2016**). Another molecular study should be proposed to identify the genotypes in sheep and human, which will promote the understanding about CE epidemiological aspects in this province.

Finally, this report is sorted as a continual study for the previous reports about identification of different strains responsible for echinococcosis in Egypt, which may help in prevention and control of this neglected disease.

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متغير دقيق جديد للنمط الجينى G6 فى معزولات الأكياس العدارية من الجمال بمحافظة الدقهلية، مصر

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الملخص العربى

لا توجد بيانات كافية فى المراجع العلمية عن الانتشار المحدد للانواع الجينية للمشوكات الشريطية التى تصيب الانسان والحيوان فى كل أنحاء مصر. أظهرت التقارير السابقة العلاقة السائدة بين الجمال والانسان فى محافظتى القاهرة و القليوبية بوسط مصر فى العدوى بالنمط الجينى G6 (سلالة الجمل). تهدف هذه الدراسة الى التركيز على الأنماط الجينية التى تصيب الجمال المرباه فى منطقة أخرى من مصر ألا وهى محافظة الدقهلية. فقد تم تجميع معزولات الأكياس العدارية من ذبائح عدد 10 جمال بمجزر السنبلاوين و عزل الحامض الوراثى لهذه المعزولات ثم تم تعرضه للتحليل الجزيئى باستخدام جين Cox1. وأظهرت النتائج وجود متغير دقيق يملك خمس مواضع استبدالية للنمط الجينى G6 فى كل العينات المفحوصة. و يوضح هذا التقرير الانتشار الواسع لهذا النمط الجينى فى كل أنحاء مصر. كما يبرهن على قوة الدور الكبير الذى تقوم به الجمال فى الحفاظ على دورة انتقال هذا النمط الجينى بالاضافة الى أهميتها بوصفها خزان لداء الحويصلات العدارية فى الانسان.