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Molecular characterization of cystic echinococcosis: First record of G7 in Egypt and G1 in Yemen

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Abstract

Few molecular studies have identified the current status of cystic echinococcosis in Egypt. The present study aimed to ascertain the genotype(s) of *Echinococcus granulosus* responsible for human hydatidosis in different Egyptian governorates (regions). Animal isolates were collected from 40 camels, 5 pigs and 44 sheep. 27 human isolates were included in the present study. Specific PCR was performed and followed by DNA sequencing for mitochondrial 12S ribosomal RNA gene and BLAST analysis.The sheep cysts were not hydatid cysts. G6 genotype (camel starin) predominates in human, camel and pig isolates. G7 genotype (pig strain) was detected in two human isolates and one pig isolate. G1 genotype (sheep strain) was detected in one human isolate from Yemen and in no animal isolates. This is the first record of G7 in Egypt and G1 in Yemen.

Keywords

Hydatid, Echinococcosis, Genotype, G1, G7, PCR

Introduction

Studying cystic echinococcosis (CE) genotypes is of great epidemiological importance. Molecular studies using mitochondrial DNA sequences have identified 10 distinct genetic types (G1-10) within *Echinococcus granulosus* (Thompson and McManus 2002; McManus and Thompson 2003). To date, 7 genotypes have been identified in Middle East and North Africa: G1, G2, G3, G4, G5, G6 and G7 (Sadjjadi 2006; Omer *et al*. 2010; Ahmed *et al*. 2013; Adwan *et al*. 2013; Cardona and Carmena 2013; Alvarez Rojas *et al*. 2014). Only G1, G4 and G6 have been characterized in Egypt (Abd El Baki *et al*. 2009; Aaty *et al*. 2012; Aboelhadid *et al*. 2013). Few molecular studies have identified the current status of *E. granulosus* genotypes in Egypt. Moreover, Egyptian reports concerning the predominant *E. granulosus* genotype in human and animal isolates are controversial (Azab *et al*. 2004; Abd El baki *et al*. 2009; Tawfeek *et al*. 2009; Aaty *et al*. 2012). Therefore, the present study aimed to ascertain the genotype(s) of *E. granulosus* responsible for human hydatidosis in different Egyptian governorates (regions).

Materials and Methods

The study was conducted from June 2011 to December 2012 and included different Egyptian governorates (regions), see Fig.1.

Ethical consideration

An informed consent was taken from the patients after explaining the aim of the study to them. The study was approved by the Research Ethics Committee, Faculty of Medicine, Ain Shams University.

Human isolates

Human isolates were collected from different Egyptian hospitals of different governorates; 10 from Qalyubiya, 5 from Giza, 4 from Cairo, 3 from Buhayra, 2 from Fayoum, 1 from Sharqiya (Zagazig), 1 from Bani Suwayf and 1 from Menufiya (Fig. 1). The 27 patients had confirmed CE by HCF examination or histopathological examination. The isolates were collected either after surgical removal of hydatid cysts or after PAIR (Percutaneous Aspiration-Injection-Reaspiration) technique. It included: 20 hepatic CE, 5 pulmonary CE and 2 multiple organ CE.

Animal isolates

Animal isolates were collected from different Egyptian abattoirs and consist of 89 isolates (40 camels, 5 pigs and 44 sheep cysts). The camel and pig cysts were fertile and confirmed to be *E. granulosus* by HCF examination. The sheep cysts were

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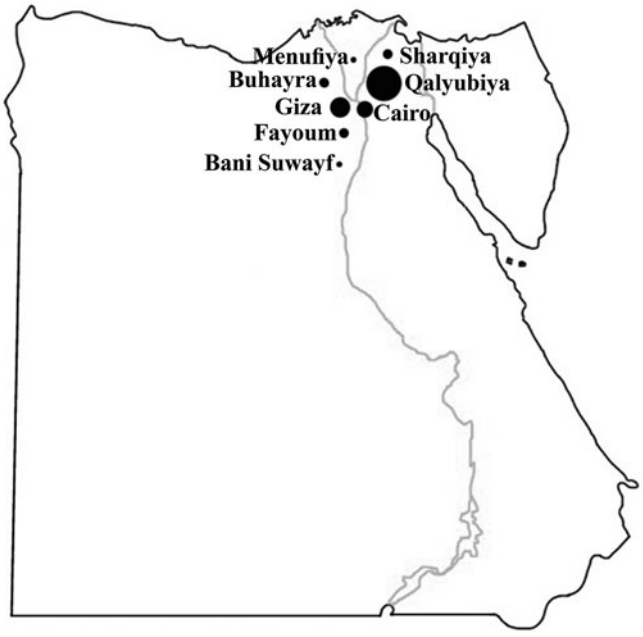

Fig. 1. Map showing distribution of CE patients in Egyptian governorates (regions)

confirmed by histopathology to be the cysticercus of *Taenia hydatigena* which is known as cysticercus tenuicollis.

DNA extraction

Parasitic materials collected from human and animal isolates were processed according to Zhang *et al*. (1998). 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 protoscoleces and germinal layer samples, whereas the manufacturer protocol for DNA extraction from fluid was used for HCF samples.

PCR assay

PCR assay 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 PCR program is 40 cycles (denaturation for 30 s at 94°C, annealing for 1 min at 57°C (for G1) and at 53°C (for G5/6/7), and elongation for 40 s at 72°C). Reference strains supplied by Dr. Mara Cecilia Rosenzvit were used as controls.

DNA sequencing

PCR purification kit (AxyPrep PCR Clean-up Kit" cat. No.: AP-PCR-50, from Axygen Biosciences, USA) was used to purify the PCR products. The G5/6/7 PCR was followed by forward and reverse sequencing to detect the specific genotype. The G1 PCR was also followed by sequencing. Sequencing was done for mitochondrial 12S rRNA gene using ABI 3730xl DNA analyzer. Nucleotide sequence analysis was done by using the National Center for Biotechnology Information BLAST programs and databases.

Results

G1 PCR assay and DNA sequencing for human and animal isolates

G1genotype of *E. granulosus* was detected in one out of 27 CE patients and in no animal isolates. The absence of the G1 genotype in the animal isolates called for further investigation about the positive human case to inspect the source of his infection. The G1 human isolate was from Yemeni patient. He lived in Yemen and came to Egypt 3 weeks before the discovery of his case. He had a history of contact to sheep herd and dog in Yemen. He had a pulmonary cyst measuring 6 cm, indicating its development before his recent residence in Egypt. Therefore, the source of the G1 genotype in the human isolate in the present study was from Yemen.

To confirm the PCR results, the amplified fragments of the G1 PCR assay were sequenced and deposited in the GenBank™ under the accession numbers of KJ801848 (Argentinean reference strain) and KJ801849 (Yemeni human isolate). The obtained sequences were compared with the Brazilian G1 sequence of Dinkel *et al*. (2004) deposited in the GenBank™. The Yemeni and the Argentinian nucleotide sequences are identical to the Brazilian G1 of Dinkel *et al*. (2004).

G5/6/7 PCR assay and DNA sequencing for human and animal isolates

All isolates from camels, pigs and CE patients, included in the current study, were positive for the G5/6/7 PCR. DNA sequencing was performed to characterize the specific genotype of G5/6/7. It revealed that all isolates were of G6 genotype (camel strain) except two Egyptian CE patient isolates and one pig isolate were of the G7 genotype (pig strain). The obtained G7 sequence was deposited in the GenBank™ under the accession number of KM098121. Comparison of the Egyptian G7 genotype with the Slovakian G7 of Dinkel *et al*. (2004) showed 100% identity. The obtained G6 genotype nucleotide sequence is identical to the Egyptian G6 deposited by Aaty *et al*. (2012).

Discussion

In the present study, we expand the research area to include different Egyptian governorates to ascertain the genotypes re-

sponsible for CE in Egypt. All animal isolates were of G6 genotype except one pig isolate which was G7 genotype. In the present study, 4 pigs were found to harbour the G6 genotype. This was previously reported by Dinkel *et al*. (2004) in Kenya and Aaty *et al*. (2012) in Egypt.

None of the sheep isolates were hydatid cysts, although large number of sheep cysts was examined and abattoirs of different regions were surveyed. All sheep cysts were the cysticercus of *Taenia hydatigena* which is known as cysticercus tenuicollis as reported by Azab *et al*. (2004) and Aaty *et al*. (2012). However, Abd El Baki *et al*. (2009); Tawfeek *et al*. (2009); Taha (2012) reported the presence of hydatid cysts in sheep. This controversy may be also due to changes in time and/or place (region) of the studies. Moreover, the cysts of *Taenia hydatigena* may be mistaken as hydatid cysts by some research groups.

In the present work, all tested human isolates were of G6 genotype except two Egyptian patients of G7 genotype and one Yemeni patient of G1 genotype. The absence of G1 genotype and the predominance of G6/7 genotype in all animal isolates (reservoir hosts), coincided with the absence of G1 genotype and the predominance of the G6/7 genotypes in the Egyptian human isolates. Both Azab *et al*. (2004) and Aaty *et al*. (2012) reported that camels are the source of infection and that G6 genotype is predominant in Egypt. The G6 genotype is also predominant in human and animal isolates in Sudan and Mauritania (Bardonnet *et al*. 2002; Dinkel *et al*. 2004; Maillard *et al*. 2007; Omer *et al*. 2010).

For the first time, we report the presence of G7 genotype of *E. granulosus* in Egypt. G7 genotype was reported in human by different authors in Poland, Austria, Turkey and Mongolia (Pawlowski and Stefaniak 2003; Schneider *et al*. 2010; Snabel *et al*. 2009; Jabbar *et al*. 2011).The small numbers of pigs raised and slaughtered may indicate the presence of other reservoir hosts for *E. granulosus* G7 genotype in Egypt. Goats, camels and sheep are reported to harbor G7 genotype in neighboring countries like Sudan and Mauritania (Dinkel *et al*. 2004; Farjallah *et al*. 2007; Omer *et al*. 2010).

In the current work, G1 genotype was identified in one human isolate which was proved to be of Yemeni origin. This study is the first to genetically characterize *E. granulosus* genotypes in Yemeni human isolate and to record the presence of G1 genotype in Yemen. *E. granulosus* G1 genotype was previously reported in countries neighboring Yemen like Jordan, Palestine and Iran (Zhang *et al*. 1998; Yanagida *et al*. 2012; Adwan *et al*. 2013). However, molecular data concerning *E. granulosus* from Arabian Peninsula is lacking.

The contradictory results about the status of G1in the Egyptian reports may be due to change in the region of the study. Moreover, using non-specific techniques like RAPD-PCR (Azab *et al*. 2004; Taha 2012) and RFLP-PCR (Tawfeek *et al*. 2009) may lead to inability to distinguish between the banding patterns (McManus 2002; Dinkel *et al*. 2004). Also, the difference between the recent address and the residence of the patients may lead to misleading data about the presence or absence of G1 genotype in Egypt like in the case of the Yemeni patient in our study. Therefore, tracing the source of CE in patients with history of travelling is mandatory.

Further molecular studies are recommended on different reservoir hosts and regions to explore the *E. granulosus* genotypes in Egypt.

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References

Aaty H.E., Abdel-Hameed D.M., Alam-Eldin Y.H., El-Shennawy S.F., Aminou H.A., Makled S.S., Darweesh S.K. 2012. Molecular genotyping of *Echinococcus granulosus* in animal and human isolates from Egypt. *Acta Tropica*, 121, 125–8. DOI: 10.1016/j.actatropica.2011.10.014

Abd El Baki M.H., El Missiry A.M., Abd El Aaty H.E., Mohamad A.A., Aminou H.A. 2009. Detection of G1 genotype of human cystic echinococcosis in Egypt. *Journal of Egyptian Society of Parasitology*, 39, 711–21

Aboelhadid S.M., El-Dakhly K.M., Yanai T., Fukushi H., Hassanin K.M. 2013. Molecular characterization of *Echinococcus granulosus* in Egyptian donkeys. *Veterinary Parasitology*, 193, 292–296. DOI: 10.1016/j.vetpar.2012.11.019

Adwan G., Adwan, K., Bdir S., Abuseir S. 2013. Molecular characterization of *Echinococcus granulosus* isolated from sheep in Palestine. *Experimental Parasitology*, 134, 195–199. DOI: 10.1016/j.exppara.2013.03.024

Ahmed M.E., Eltom K.H., Musa N.O., Ali, I.A., Elamin F.M., Grobusch M.P., Aradaib I.E. 2013. First report on circulation of *Echinococcus ortleppi* in the one humped camel (Camelus dromedaries), Sudan. *BMC Veterinary Research*, 9, 127. DOI: 10.1186/1746-6148-9-127

Alvarez Rojas C.A., Romig T., Lightowlers M.W. 2014. *Echinococcus granulosus* sensu lato genotypes infecting humans – review of current knowledge. *International Journal of Parasitol*ogy, 44, 9–18. DOI: 10.1016/j.ijpara.2013.08.008

Azab M.E., Bishara S.A., Helmy H., Oteifa N.M., El-Hoseiny L.M., Ramzy R.M., Ahmed M.A. 2004. Molecular characterization of Egyptian human and animal *Echinococcus granulosus* isolates by RAPD-PCR technique. *Journal of Egyptian Society of Parasitology*, 34, 83–96

Bardonnet K., Piarroux R., Dia L., Schneegans F., Beurdeley A., Godot, V., Vuitton D.A. 2002. Combined eco-epidemiological and molecular biology approaches to assess *Echinococcus granulosus* transmission to humans in Mauritania: occurrence of the 'camel' strain and human cystic echinococcosis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 96, 383–386. DOI: 10.1016/S0035-9203(02)90369-X

Cardona G.A., Carmena D. 2013. A review of the global prevalence, molecular epidemiology and economics of cystic echinococcosis in production animals. *Veterinary Parasitology*, 192, 10–32. DOI: 10.1016/j.vetpar.2012.09.027

Dinkel A., Njoroge, E.M., Zimmermann A., Wälz M., Zeyhle E., Elmahdi I.E., Mackenstedt U., Romig T. 2004. A PCR system for detection of species and genotypes of the *Echinococcus granulosus*-complex, with reference to the epidemiological situation in eastern Africa. *International Journal of Parasitology*, 34, 645–53. DOI: 10.1016/j.ijpara.2003.12.013

Farjallah S., Busi M., Mahjoub M.O., Slimane B.B., Said K., D'Amelio S. 2007.Molecular characterization of *Echinococcus gran-*

ulosus in Tunisia and Mauritania by mitochondrial rrnS gene sequencing. *Parassitologia*, 49, 239–246

Jabbar A., Narankhajid M., Nolan M.J., Jex A.R., Campbell B.E., Gasser R.B. 2011. A first insight into the genotypes of *Echinococcus granulosus* from humans in Mongolia. *Molecular and Cellular Probes*. 25, 49–54. DOI: 10.1016/j.mcp.2010.11.001

Maillard S., Benchikh-Elfegoun M.C., Knapp J., Bart J.M., Koskei P., Gottstein B., Piarroux R. 2007. Taxonomic position and geographical distribution of the common sheep G1 and camel G6 strains of *Echinococcus granulosus* in three African countries. *Parasitology Research*, 100, 495–503. DOI: 10.1007/s00436-006-0286-9

McManus D.P. 2002. The molecular epidemiology of *Echinococcus granulosus* and cystic hydatid disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 96, 151–157. DOI: 10.1016/S0035-9203(02)90068-4

McManus D.P., Thompson R.C. 2003. Molecular epidemiology of cystic echinococcosis. *Parasitology*, 12, 37–51. DOI: 10.1017/S0031182003003524

Omer R.A., Dinkel A., Romig T., Mackenstedt U., Elnahas A.A., Aradaib I.E., Ahmed M.E., Elmalik K.H., Adam A. 2010. A molecular survey of cystic echinococcosis in Sudan. *Veterinary Parasitology*, 169, 340–346. DOI: 10.1016/j.vetpar.2010.01.004

Pawlowski Z.S., Stefaniak J. 2003.Cystic echinococcosis (CE) in the Poznan region in the years 1990-2000. *Przeglad Epidemiologiczny*, 57, 579–586

Sadjjadi M.S. 2006. Present situation of echinococcosis in the Middle East and Arabic North Africa. *Parasitology International*, 55, 197–202. DOI: 10.1016/j.parint.2005.11.030

Schneider R., Gollackner B., Schindl M., Tucek G., Auer H. 2010. *Echinococcus canadensis* G7 (pig strain): an underestimated cause of cystic echinococcosis in Austria. *The American Journal of Tropical Medicine and Hygiene*,82, 871–874. DOI: 10.4269/ajtmh.2010.09-0639

Snabel V., Altintas N., D'Amelio S., Nakao M., Romig T., Yolasigmaz A., Gunes K., Turk M., Busi M., Huttner M., Sevcova D., Ito A., Altintas N., Dubinsky P. 2009. Cystic echinococcosis in Turkey: genetic variability and first record of the pig strain (G7) in the country. *Parasitology Research*, 105, 145–154.DOI: 10.1007/s00436-009-1376-2

Taha H.A. 2012. Genetic variations among *Echinococcus granulosus* isolates in Egypt using RAPD-PCR. *Parasitology Research*, 111, 1993–2000. DOI: 10.1007/s00436-012-3046-z

Tawfeek G.M., Elwakil H.S., Awad N.S., El-Hoseiny L., Thabet H.S., Sarhan R.M., Darweesh S.K., Anwar W.A. 2009. Genetic variability of antigen B among *Echinococcus granulosus* Egyptian isolates. *Korean Journal of Parasitology*, 47, 259–264. DOI: 10.3347/kjp.2009.47.3.259

Thompson R.C., McManus D.P. 2002. Towards a taxonomic revision of the genus *Echinococcus*. *Trends in Parasiotology*, 18, 452–457. DOI: 10.1016/S1471-4922(02)02358-9

Yanagida T., Mohammadzadeh T., Kamhawi S., Nakao M., Sadjjadi S.M., Hijjawi N., Abdel-Hafez S.K., Sako Y., Okamoto., Ito A. 2012. Genetic polymorphisms of *Echinococcus granulosus* sensu stricto in the Middle East. *Parasitology International*, 61, 599–603. DOI: 10.1016/j.parint.2012.05.014

Zhang L.H., Eslami A., Hosseini S.H., McManus D.P. 1998. Indication of the presence of two distinct strains of *Echinococcus granulosus* in Iran by mitochondrial DNA markers. *The American Journal of Tropical Medicine and Hygiene*, 59, 171–174

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