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Research Article
Prevalence and Molecular Characterization of Hydatid Cyst Isolates from Cattle in Egypt

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Abstract

Background and Objective: In view of the economic and public health threats of cystic echinococcosis, this study was planned in order to update the prevalence and to determine the genotypes of *Echinococcus* cysts isolates from cattle in Egypt, which has not been stated before. **Materials and Methods:** DNA of the recovered cysts from 500 slaughtered cattle at Mansoura abattoir, Egypt, was subjected to the molecular analysis using the cytochrome oxidase subunit I gene. **Results:** Hydatid cysts were noted in lungs of 2 (0.004%) out of the examined cattle. Two genotypes of *Echinococcus* species were detected, *E. ortleppi* (G5, cattle strain) and *E. granulosus sensu stricto* (G1, common sheep strain). **Conclusion:** This study firstly reported, the genotypes of *Echinococcus* species infecting cattle in Egypt as well as the occurrence of G5 genotype from natively reared animal in Egypt. Moreover, it highlights the role of the sheep-dog cycle in echinococcosis transmission. Such epidemiological data could help in the application of efficient control strategies against this zoonotic disease.

Key words: Hydatid cyst, cattle, prevalence, molecular, *E. ortlepi*, *E. granulosus*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cystic echinococcosis (CE) is a zoonotic infection caused by the larval stage (hydatid cyst) of the smallest canine cestode, *Echinococcus granulosus*[1]. This disease is of major public health and economic importance and it has an endemic status in the Middle East and Arab North Africa including Egypt[2]. In Iran, nearly 1% of admissions to the surgical wards in human are due to cystic echinococcosis[3]. Moreover, several billion dollars are lost in the livestock sector due to mortalities of the infected animals and condemnation of infected organs of slaughtered animals[4].

Infection of the intermediate hosts including cattle with *E. granulosus* results in the formation of hydatid cyst, a fluid-filled cavity surrounded by a thick wall consists of a germinal and laminar layer. Cysts may be fertile (containing protoscolices), or sterile. Only fertile cysts can complete the parasite cycle[5].

Cattle constitute the principle source of meat for human food all over the world. In Egypt, the majority of reports found no infection of the slaughtered cattle with hydatid cyst[6,7], but Haridy *et al.*[8] noted that the prevalence rate was 6.4%.

Sequencing of complete mitochondrial genome was greatly valuable in the phylogenetic studies on genus *Echinococcus*[9]. Molecular studies have identified 10 genotypes (from G1-G10) within 4 haplotypes of the *E. granulosus* complex, *E. granulosus sensu stricto* (G1 common sheep strain, G2 tasmanian sheep strain and G3 buffalo strain), *E. equinus* (G4 horse strain), *E. ortleppi* (G5 cattle strain) and *E. canadensis* (G6 camel strain, G7 pig strain, G8 cervid strain and G10 fennoscandian cervid strain). The status of G9 strain is uncertain[10]. A variety of *Echinococcus* species were found to infect cattle allover the world[11-17] (Table 1).

Few reports are concerned with genotyping of hydatid cyst isolates from human and animals in Egypt. The G6 genotype was prevalent among camels, buffaloes, pigs and humans[18,19] and G1 was revealed from sheep[19], while G4 genotype was isolated from Egyptian donkeys[20]. As far as known, no knowledge is available about the genotypes of *E. granulosus* infecting cattle from Egypt.

The aim of the present study was to update the prevalence of hydatid cyst and to determine which genotypes of the *E. granulosus* complex are found in fertile and non-fertile hydatid cyst isolates from cattle slaughtered in Egypt.

MATERIALS AND METHODS

Specimens collection: Five hundred heads of natively reared slaughtered cattle at Mansoura abattoir, Egypt, were inspected and palpated, especially their lungs and livers, for the presence of hydatid cysts. Cysts were dissected out and washed with Phosphate Buffer Saline (PBS). To investigate cysts` fertility, hydatid fluid and wall fragments were examined under the light microscope for the presence of protoscolices. Protoscolices from fertile cysts and cyst wall (germinal layer after removal of the laminar layer, which could inhibit PCR ampilification[11]), from sterile cysts were harvested. Both were washed three times in PBS (PH 7.2) and stored at -20°C until used.

DNA extraction: DNA was extracted by glass beads method according to Tappeh *et al.*[21]. Protoscolices and/or germinal layer were concentrated in 50 µL in a tube. Then 300 µL of lysis buffer (NaCl 0.1 M, EDTA 0.01 M, tris-HCl 0.1 M, SDS 1%) was added to the sediment of each tube. About 300 µL of 0.5 mm diameter glass beads was added to each tube and shaken vigorously using a dismembrator for 1 min in 900 shakes per minute. About 30 µg of proteinase K (Sigma, USA) was added to each tube containing samples plus 300 µL lysis buffers and incubated at 56°C for 1 h. Then, 300 µL phenol chloroform was added and centrifuged at 5000 rpm for 5 min. After removing the supernatant to a new tube, chloroform was added prior to shaking and spinning at 5000 rpm for 5 min. Subsequently, an equal volume of iso-propanol (Merck, Germany) and 0.1 volume sodium acetate (Merck, Germany) (3 M, pH = 5.2) were added to the supernatant and kept at -20°C for 1 h. Next, it was spun for 15 min at 14000 rpm and the sediment was rinsed by 300 µL 70% ethanol. After spinning for 5 min at 5000 rpm and removing ethanol, the pellet was dissolved in 50 µL distilled water and stored at -20°C.

PCR amplification and gene sequencing: PCR ampli cation was performed using the cytochrome oxidase subunit I (Cox1) gene[22]. The conserved primers COI 1 (forward) 50-TTTTTTGGCCATCCTGAGGTTTAT-30 and COI 2 (reverse) 50-TAACGACATAACATAATGAAAATG-30 were used to amplify the Cox1 gene by 30 cycles. Each cycle consisted of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, elongation at 72°C for 30 sec and a final extension at 72°C for 7 min.

Table 1: Molecular epidemiology of cystic echinococcosis in cattle from different countries

Country	No.	Genetic marker	Genotype	References
Egypt	2	Cox1	G1 (50%), G2 (50%)	Present study
Sudan	62	Cox1 and nad1	G6	Ibrahim *et al.*[40]
	107	12S rRNA, Cox1 and nad1	G6-G7 (99.1%), G5 (0.9%)	Omer *et al.*[39]
	8	12S rRNA, Cox1 and nad1	G6-G7 (75.0%), G5 (25.0%)	Dinkel *et al.*[38]
Ethiopia	14	Cox1	G1-G3 (85.7%), G6-G10 (14.3%)	Hailemariam *et al.*[45]
	1	Cox1, nad1, act2 and hbx2	G1	Maillard *et al.*[46]
Kenya	201	nad1	G1 (99.5%), G5 (0.5%)	Addy *et al.*[42]
South Africa	1	nad1, 12S rRNA	G5	Mogoye *et al.*[41]
Libya	38	Cox1, nad1	G1-G3 (13%), G6 (87%)	Abushhewa *et al.*[15]
	12	Cox1	G1	Tashani *et al.*[47]
Algeria	12	Cox1, nad1, act2 and hbx2	G1	Maillard *et al.*[46]
	20	bg 1/3, Cox1 and nad1	G1	Bardonnet *et al.*[37]
Tunisia	19	Cox1, ef1α	G1	Boufana *et al.*[36]
	4	12S rRNA	G1	Farjallah *et al.*[48]
	166	ITS1 DNA and Cox1	G1	M'Rad *et al.*[12]
Mauritania	20	bg 1/3, Cox1 and nad1	G6	Bardonnet *et al.*[37]
Iran	28	Cox1	G1 (92.8%), G3 (7.8%)	Farhadi *et al.*[32]
	7	Cox1	G1 (72%), G3 (28%)	Pestechian *et al.*[49]
	51	Cox1	G1	Nejad *et al.*[50]
India	14	Cox1	G1 (7.1%), G2 (14.3%), G3 (57.1%), G5 (21.5%)	Pednekar *et al.*[51]
	1	Cox1, nad1 and ITS1 DNA	G1-G3	Bhattacharya *et al.*[29]
China	12	Atp6	G1	Yang *et al.*[52]
Japan	66	Cox1, nad1 and 12S rRNA	G1 (90.9%), G2 (3.0%), G3 (6.1%)	Guoa *et al.*[17]
Pakistan	4	Cox1	G1	Latif *et al.*[53]
Turkey	13	Cox1, nad1	G1	Eryildiz and Sakru[54]
	23	Cox1 and 12S rRNA	G1-G3	Simsek *et al.*[16]
	6	12S rRNA	G1	Kul and Yildiz[55]
Italy	78	Cox1	G1-G3	Casulli *et al.*[56]
	75	Cox1 and nad1	G1 (81.3%), G2 (6.7%), G3 (10.7%), G5 (1.3%)	Casulli *et al.*[13]
	4	Cox1 and nad1	G1	Varcasia *et al.*[57]
France	4	Cox1 and nad1	G1 (50%), G3 (50%)	Umhang *et al.*[30]
Portugal	1	Cox1, atp6, 16S and 12S rRNA	G1-G3	Beato *et al.*[58]
Romania	67	Cox1 and 12S rRNA	G1(47.8%), G2 (34.3%), G3 (17.9%)	Mitrea *et al.*[59]
Moldova	15	Cox1, nad3	G1(60%), G2 (33.3%), G1-G3 (6.7%)	Umhang *et al.*[60]
Bulgaria	99	Cox1	G1-G3	Casulli *et al.*[56]
Hungary	21	Cox1	G1-G3	Casulli *et al.*[56]
Brazil	638	Cox1	G1 (56.6%), G5 (43.4%)	Balbinotti *et al.*[35]
	28	Cox1	G1 (92.9%), G5 (7.1%)	De la Rue *et al.*[61]
Argentina	42	Cox1 and nad1	G1(97.6%), G5(2.4%)	Andresiuk *et al.*[34]
	20	Cox1 and nad1	G1 (70.0%), G2 (5.0%), G5 (25.0%)	Kamenetzky *et al.*[11]
Chille	22	Cox1 and nad1	G1(95.4%), G3 (4.6%)	Espinoza *et al.*[31]
Peru	44	Cox1	G1	Sanchez *et al.*[62]
	16	Cox1 and ef1α	G1-G3	Moro *et al.*[63]

Act2: Nuclear actin 2, bg 1/3: *Echinococcus* genus-specific genomic DNA, Cox1: Mitochondrial cytochrome c oxidase subunit 1, hbx2: Nuclear homeobox 2, ITS1: Ribosomal internal transcribed spacer 1, nad1: Mitochondrial NADH dehydrogenase subunit 1, 12S rRNA: Mitochondrial 12S small subunit ribosomal RNA, ef1α: Nuclear elongation factor 1 alpha

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.

PCR products were separated on agarose gels (1%) stained with ethidium bromide. Sequencing for the purified DNA from the gel was carried out on a 16-capillary DNA sequencer (ABI PRISM 3100 Genetic Analyzer, Applied Biosystem, Foster City, CA). Achieving the reference sequences (Table 2), genotypes identification and sequence alignment were done using GenBank with the BLAST system and the phylogenetic analysis was initiated using the software Bioedit and Mega (version 6).

RESULTS

Prevalence of cystic echinococcosis in cattle: Examination of internal organs (especially livers and lungs) from 500 cattle of different ages at Mansoura abattoir, Egypt, revealed

Table 2: Sequences from GenBank used for phylogenetic tree construction

Genotype	Animal	Country	Accession No.	References
G1	Sheep	Palestine	KC109649	Adwan *et al.*[64]
	Sheep	Jordan	AB688598	Yanagida *et al.*[65]
	Cattle	Tunisia	KM014620	Boufana *et al.*[66]
	Cattle	Ethiopia	AB650530	Hailemariam *et al.*[45]
	Cattle	Turkey	KM100573	
	Cattle	Portugal	HF947574	Beato *et al.*[58]
	Cattle	UK	KP101622	Boufana *et al.*[67]
	Sheep	Iran	JQ250816	Yanagida *et al.*[65]
	Human	India	JX854029	Sharma *et al.*[44]
	Sheep	China	JQ317993	
	Human	Mongolia	AB893249	Ito *et al.*[68]
	Human	Russia	AB688141	Konyaev *et al.*[69]
	Pig	Brazil	KC660075	Monteiro *et al.*[70]
	Sheep	Peru	AB688621	Yanagida *et al.*[65]
G5	Camel	Egypt	AB921055	Amer *et al.*[19]
	Camel	Sudan	JX912709	Ahmed *et al.*[71]
	Cattle	Italy	FJ744757	Casulli *et al.*[13]
	Human	France	KJ624625	Grenouillet *et al.*[33]
	Deer	UK	JX068638	Boufana *et al.*[36]
	Human	India	JX854035	Sharma *et al.*[44]
	Cattle	Brazil	KT382537	
G6	Camel	Egypt	AB921086	Amer *et al.*[19]
E. multilocularis	Rodent	Germany	M84669	Bowles *et al.*[22]
E. felidis	Lion	Uganda	EF558356	Huttner *et al.*[72]
T. saginata	Human	Korea	NC_009938	Jeon *et al.*[73]

presence of hydatid cysts in 2 (0.004%) animals only. Both animals were harbored hydatid cysts in their lungs. By investigation of their fertility, one cyst was fertile, while the other was infertile.

Genotype identification: Results of the blast search of the obtained partial Cox1 sequences revealed the presence of 2 genotypes: G5 (cattle strain, *E. ortleppi*) from the examined fertile cyst and G1 (common sheep strain, *E. granulosus sensu stricto*) from the infertile one.

Sequence polymorphism: There were intra-sequence variations in the 2 revealed genotypes. About 99% identity was noted between DNA sequence of the G1 genotype in the present study and those of G1 reference sequences from different hosts and countries (KC109649 from Palestine, KM014620 from Tunisia, AB650530 from Ethiopia, KM100573 from Turkey, HF947574 from Portugal and JX854029 from India) with single nucleotide substitution (G141A) as shown in Fig. 1. On the other hand, the alignment of our G5 isolate and those on the GenBank (JX068638 from UK and KT382537 from Brazil) revealed 12 nucleotide substitution at position 14 (T-A), 15 (G-A), 18 (T-C), 21 (T-A), 36 (G-A), 41 (G-C), 45 (T-A), 46 (T-A), 47 (T-G), 56 (G-A), 86 (G-C), 165 (T-A) with 97% identity. Additional nucleotide substitution at position 315 (G-A) was found with G5 sequences reported from Egypt (AB921055) and Sudan (JX912709) and at position 344 (C-T) with that reported from Italy (FJ744757) (Fig. 2).

Phylogeny of the revealed haplotypes: Phylogenetic analysis revealed a robust tree associating our isolate of G1 genotype in the same sister group with a variety of G1 genotype (common sheep strain) sequences from different geographical regions of the world, although it was more genetically related to the Palestinian isolate. Concerning the other isolate of the present study, it was clustered with sequences of G5 genotype (cattle strain) and participating in the same clade with those reported from Italy, France, UK, India and Brazil (Fig. 3).

DISCUSSION

Cystic echinococcosis considered as a major constrain for both public health and economic concerns. Recently, this disease has been included in the strategic plan of the World Health Organization (WHO) to control the neglected tropical diseases[23].

In Egypt, conditions are suited for the establishment of dog-livestock cycle for *Echinococcus* species transmission,

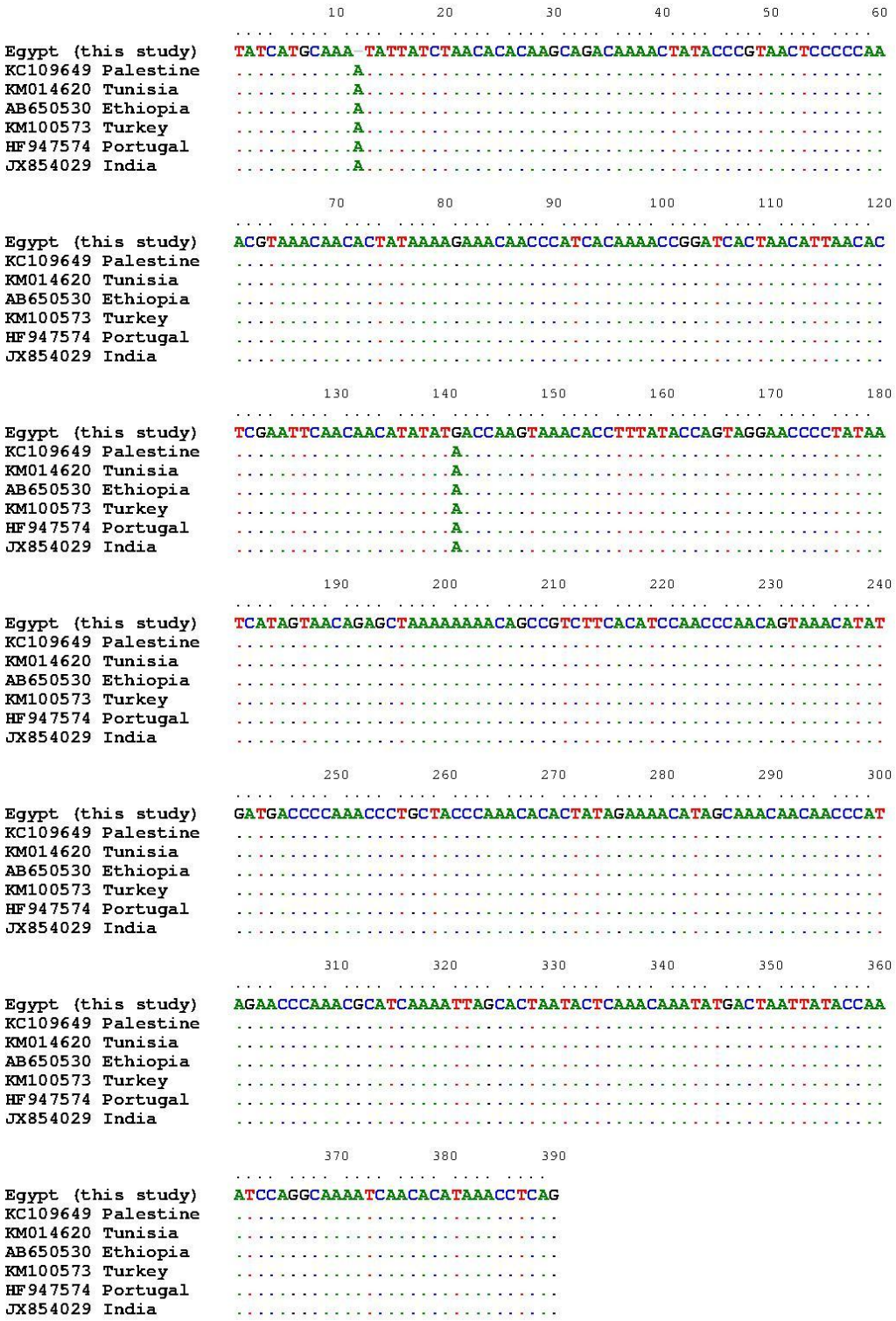

Fig. 1: Alignment of a segment of Cox1 sequences of our G1 isolate from cattle with a number of G1 sequences on the GenBank reported from different countries like Palestine (KC109649), Tunisia (KM014620), Ethiopia (AB650530), Turkey (KM100573), Portugal (HF947574) and India (JX854029). There is one nucleotide substitution at position 141 (G-A)

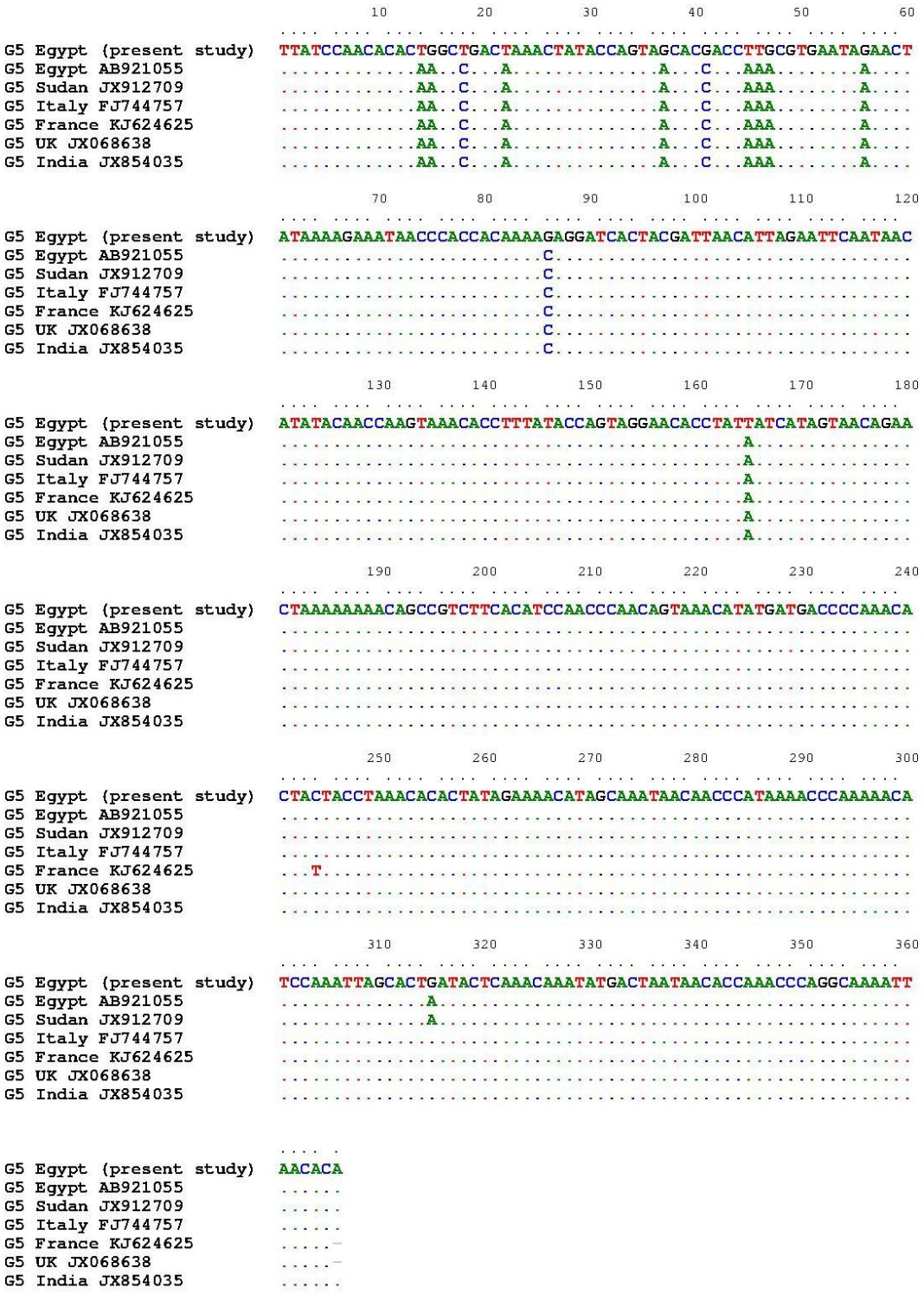

Fig. 2: Alignment of a segment of Cox1 sequences of G5 isolate of the present study with a number of G5 sequences on the GenBank reported from different countries like France (KJ624625), UK (JX068638) and India (JX854035). There is 12 nucleotide substitution at position 14 (T-A), 15 (G-A), 18 (T-C), 21 (T-A), 36 (G-A), 41 (G-C), 45 (T-A), 46 (T-A), 47 (T-G), 56 (G-A), 86 (G-C) and 165 (T-A). Additional nucleotide substitution at position 315 (G-A) was found with G5 sequences reported from Egypt (AB921055) and Sudan (JX912709) and at position 344 (C-T) with that reported from Italy (FJ744757)

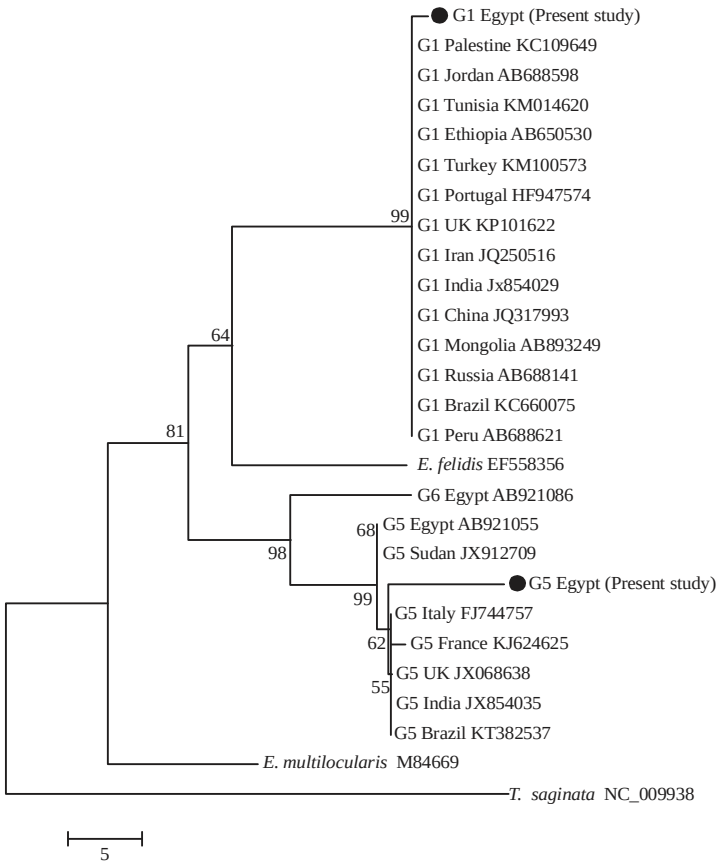

Fig. 3: Phylogenetic tree sequences of G1 and G5 genotypes of *Echinococcus* species and their relationships with the reference sequences of the both genotypes on GenBank. The tree was obtained from partial sequencing of Cox1 gene. Sequences were aligned by ClustalW and the tree was built using the software MEGA (version 6). Scale bar indicates the proportion of sites changing along each branch

like the high prevalence of *Echinococcus* infection among the stray dogs in Egypt[24], contact between animals and dogs in which the later are kept as a tool for protection in animal farms, beside the accessibility of dogs to slaughterhouses and feeding on offals of animal carcasses.

Despite considering as an endemic area of cystic echinococcosis[2], few data are available about this disease in human and animals from Egypt. This study aimed to determine the prevalence and genotypes of hydatid cyst isolates from cattle which constitute the principle source of meat in Egypt.

In the present study, a very low prevalence of hydatidosis was detected. Only 2 (0.004%) out of 500 examined cattle from Egypt were harbored hydatid cysts in their lungs. No infection was noted in the other organs. Previous studies from Egypt found no infection in cattle and 31% prevalence in camels[6,7]. This prevalence is very low than that described in different parts of the world: 15% in Libya[25], 20.5% in Ethiopia[26] and 59.3% in Moldova[27]. The reason for the low prevalence of hydatidosis in cattle from Egypt is unclear, but it may be attributed to the young age of the most slaughtered cattle (short fattening period) with a less possibility of infection. In addition to, the good sanitary condition in the cattle derived farms.

Molecular data about *Echinococcus* species haplotypes are essential to control the spread of cystic echinococcosis because there are differences between the haplotypes in the resistance to anthelmintic drugs[28].

Based on DNA sequences of Cox1 gene, 2 genotypes were detected from cattle in the present study: The G5 genotype (cattle strain) and the G1 genotype (common sheep strain). Among the 10 genotypes of *E. granulosus*, 6 strains were found to infect cattle globally (G1, G2, G3, G5, G6 and G7) as shown in Table 1. The G_{1-3} complex is the predominant genotype for cattle infection all over the world[16,29-32]. Few studies reported G5 from cattle like in France[33], Argentina[34], Brazil[35] and Italy[13]. Considering the Arabic North Africa, G1 was the only noted genotype in cattle from Tunisia[36] and Algeria[37], while, G6 is the only genotype in Mauritania[37]. Moreover, both

G1 and G6 genotypes were reported from the Egyptian nearby countries (Libya and Sudan), although G5 was recorded in Sudan[15,38-40]. In the South African countries (Kenya and South Africa), G5 was noted[41,42]. This is the first report about the genotypes of hydatid cyst isolates from cattle in Egypt.

Four genotypes (G1, G4, G5 and G6) were detected in Egypt. Studies illustrated the predominance of G6 genotype (*E. canadensis*) in humans, camels, sheep and buffaloes[18,19,43], although few cases of G1 genotype were reported from camels and sheep[19]. Moreover, G4 genotype was recorded from equines[20]. Previously, *E. ortleppi* (G5) was reported in one cyst from the imported Sudanese camels slaughtered in Egypt[19]. This stduy considered the first report of this genotype in natively reared animals from Egypt.

At the haplotype level, data inferred from the phylogenetic tree suggested the limited genetic divergence within the G1 and G5 clades and evoked the genetic relatedness between our G1 isolate and that from the Palestinian sheep (KC109649). Palestine is one of the Egyptian neighboring countries and has historical relations with Egypt including human, animal and trade movements. It is assumed that G1 genotype reported in this study could be crossly transmitted between Egypt and Palestine.

Production of fertile or non fertile cysts depends upon the host adaptation for different *Echinococcus* species. It is well known that *E. ortleppi* is best adapted to cattle lungs, while *E. granulosus sensu stricto* is best adapted to sheep[35]. This fact is coincided with our results which showed that the fertile cyst was genotyped under G5 (*E. ortleppi*) and the non-fertile cyst was genotyped under G1 (*E. granulosus sensu stricto*).

In view of the public health importance of the revealed genotypes, the detection of G1 genotype in the present study is interesting in shedding the light on this globally circulating genotype which responsible for the most of human and animals echinococcal infections. Although, sheep are widely reared in Egypt and inturn the dog-sheep cycle might play an important role for transmission of hydatidosis, only 3.2% of human hydatid cysts were genotyped under G1[18]. On the other hand, G5 genotype was reported in human cystic echinococcosis with a limited dispersal[33,41,44].

CONCLUSION

In conclusion, this report is the first about the genotypes of hydatid cyst isolates from cattle in Egypt and sorted as a complementary for the previous reports from other Egyptian animals. A larger extensive study with efficient number of isolates from different geographic regions and different host species, including human, domestic animals and dogs should be carried out for illustrating the epidemiology and transmission dynamics among different *Echinococcus* spp., genotypes from Egypt.

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