Occurance of hydratic cyst in camels (*Camelus dromedarius*) and their effect on meat quality

Amer R. Abdel Aziz1, Rasha A. El Meghanawy*2, Fatma F. M. Ibrahim3, and Dina I. El Zahaby4.

1Department of parasitology, Faculty of Veterinary Medicine, Souhag University, Souhag, Egypt. 2Department of parasitology, Animal Health Research Institute (AHRI), Shebin El koom, Menoufiya, Egypt, 3Department of Biochemistry, Animal Health Research Institute (AHRI), Shebin El koom, Menoufiya, Egypt, 4Department of food hygiene, Animal Health Research Institute (AHRI), Shebin El koom, Menoufiya, Egypt,

Abstract

Hydatidosis is caused by *Echinococcus granulosus* which is one of the important parasitic disease in warm blooded vertebrates and occasionally in many other mammal species. Their were no enough informations available regarding haematological profile, serum enzyme activity, chemical changes, and mineral profile in one humped camels (*Camelus dromedaries*) from Egypt with pulmonary hydatid cyst. The objective of the present study was to investigate the changes in complete haematological profile and also changes in some biochemical parameters in serum samples, such as; Total Protein, Albumin, Globulin, A/G ratio, serum liver enzyme activity such as; Alanine Amino Transaminase (ALT), Aspartate Amino Transferase (AST), bilirubin, glucose, cholesterol, also, kidney function activity as; Urea, Uric acid, and Creatinine, also, the level of complete Minirals profile, such as; Calcium, Inorganic phosphorus, Magnesium, Sodium, and Potassium were also done. Meat chemical composition of healthy and infected camels with *Echinicoccus granulosus*, was also made as nutrition value, proteins, fats, ashes, mineral elements, as, calcium level in meat samples was measured and compared to samples from healthy camels at abattoir, a total of Fifty samples of meat, serum and hydatid cysts from lungs were collected from camels admitted to slaughter houses at Qalyubia Governorate, Egypt. Results concluding that; meat from the camel infected with echinococcosis is of poorer quality as compared with muscle tissue of the healthy animal as well as being biologically inferior, there were a significant increase in leucocytic count, neutrophils and eosinophils while the other haemograms are non significant. More over significant increase in total protein, and globulin in sera of infected camel with hydatidosis when compared with the non infected ones. Decreased in A/G ratio reflected the changes observed in albumin and globulin fractions. A significant elevation of the activity of AST, ALT and bilirubin level ($p<0.05$), but a significant decrease in glucose value, and there is no alteration in serum cholesterol, urea, uric acid and creatinine level was observed. Concerning calcium and inorganic phosphorus levels, they were significant decrease, and this study showed non-

significantly changed in sodium, magnesium and potassium levels. On the other hand; in the present study, DNAs were extracted from protoscolices and/or associated germinal layers of hydatid cysts using a commercial kit. The (18 SSU r RNA gene) was used as targets for polymerase chain reaction (PCR) amplification. PCR products were purified and partial sequences were generated. Sequences were further examined by sequence analysis and subsequent phylogeny to compare these sequences to those from known strains of EG circulating globally. The phylogenetic analysis showed that 98% (n = 49) of the isolates clustered with *Echinococcus canadensis* genotype 6 (G6). Conclusions: It is clear that activity of the specific enzymes of serum and the haematological, chemical and biochemical profile can be helpful in diagnosis of pulmonary hydatid cyst infection of one humped camels.

Keywords: *Echinococcus granulosus*, (18SSUr-RNAgene), hematological , biochemical, Phylogenetic analysis, Egypt.

1-Introduction

Cystic echinococcosis is a zoonotic parasite belonging to the genus *Echinococcus* (Family Taeniidae). The final host is carnivores, and intermediate hosts include camels, sheep, goats, cattle, small rodent, wild herbivores and humans. This parasite might inhabit especially in liver and lung than other organs (e.g., kidneys, spleen, brain, bones, and heart) (**Bakır *et al.*, 2012**). Hydatidosis is an economically important disease as it causes severe problems in different species animals especially in the industrial and semi-industrial cattle farms by close contact with the final host via domestic and wildlife reservoirs (**Umesh *et al.*, 2010**). Echinococcosis can be accurately evaluated in Definitive Hosts and Intermediate Hosts (humans), respectively based on the patient's history, clinical findings, hematological serum, biochemical profiles and serological testing, (**Elshazly *et al.*, 2009**). The liver is one of the essential organs and largest gland of the body (**Schmucker, 2005**), sensitive to different parasites and disease conditions which affect the total health state of the animal (**Ahmedullah *et al.*, 2007**). The liver, which plays a serious role in lipid, carbohydrate and protein metabolism, performs tasks such as bile construction, vitamin storage and the biotransformation of drugs and toxins. In addition, the liver plays a role in immune functions (**Schmucker, 2005**). The hepatic enzymes such as Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), serum glutamate-oxal-acetate transaminase (SGOT), and serum glutamate pyruvate transaminase (SGPT) are principal enzymes that primarily represent hepatocellular necrosis and cholestasis, respectively. So, there are of special usefulness in the diagnosis of serious hepatic diseases (**Kim *et al.*, 2008**). It is clear that activity of the specific enzymes of serum in the infected serum of camels help to diagnosis of hydatid cyst. In farm animals, infested by hydatid cysts may not

show any signs and symptoms, hydatid infection may be detected at slaughter or at post mortem examination and recognized as round swelling in the liver and lungs in of infected food animals and may be more common in older animals. The purpose of the present study has been performed to evaluate activity of the specific enzymes of serum in the infected camels, and some haematological, biochemical, chemical, and some minerals that may help in diagnosis of hydatid cyst, also PCR and phylogeny for isolated strain will be done.

2. Material and Methods

2.1. Study area and Samples collection:

In this descriptive-cross sectional study, fifty humped camels Tokh abattoir in Qaliobia Governorate, Egypt, from May 2015 to August 2015. This Province is located at the Northern to Cairo about 35 km from the capital of Egypt. They included 30 females and 20 male with age range between, 4 to 8 years. Before slaughter, an anti mortem examination was done on all camels during which their age and gender were evaluated. The age was determined by examination of the teeth as described by Kelly (**Kelly, 1975**). Blood samples were collected before slaughter, from the jugular vein into vacationer tubes, with and without anticoagulant for each collected sample. After clotting for 3 hours at 4°C and centrifugation (1500G, 10 min, 4°C), sera were carefully harvested and stored at -20°C until analysis (**Djokovic *et al*., 2013**) from infected and healthy animals. After slaughtering, post mortem examination procedure employed visual inspection, palpation and systematic incision of each carcass, visceral organs particularly the lung targeted lesions consistent with hydatidosis (**Swai and Schoonman, 2012**). A ten samples from healthy animals as a control group and a total of fifty meat samples and hydatid cyst from infected lungs were collected, and measurements were taken in mm, and incision were made into cyst to collect both hydatid sand and the germinal layer of fertile cyst which containing protoscolices, The aspirates were transferred to clean sterile tubes to which 70% alcohol was added as preservative and stored at -20 °C until used according to **Osman, *et al*., (2009**). For molecular studies (PCR, and phylogeny).

2.2. Hematological studies, Biochemical studies, and mineral profile:

Haematological examination for the tested blood samples included total erythrocytes count (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV), and leucocytes count (WBCs), the total erythrocytes (RBCs) and leucocytes (WBCs) were counted according to **Thompson (1980).** Hemoglobin concentration (Hb) was

measured according to **Crosby *et al.*, (1954),** packed cell volume (PCV) was determined by the micro-haematocrite method of **Schalm (1986).**

Biochemical examination for serum samples included determination of serum total protein level , albumin, globulin level, urea, alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), serum total cholesterol, bilirubin, glucose, urea, serum uric acid, creatinine, serum calcium, inorganic phosphorous, magnesium, sodium and potassium.

Estimation of serum total protein was determined by Biuret method as described by **Weichselbaum (1946)**, Serum albumin level was determined according to **Dumas *et al.*, (1971).** Serum alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) were determined according to **Reitman and Frankel (1957).** The serum bilirubin was determined according to **Sherloch (1951).** The serum glucose level was determined according to **Siest *et al.*, (1981)**.The serum cholesterol was determined according to **Richmond (1973).** The serum urea determined according to **Haisman and Muller (1977).** The serum creatinine was determined according to **Siest *et al.*, (1985).** Serum calcium was determined as described by **Gindler and King (1972).** Serum inorganic phosphorous was determined according to **Kliching and Freiburg (1951).** Serum magnesium level was determined as described by **Gindler (1971).** Serum sodium and potassium were estimated using the flam photometer (Corning photometer 410) according to **Dawborn et al. (1965).**

2.3. Meat chemical composition:

Meat chemical composition of healthy and infected camels with *Echinicoccus granulosus*, was also made, as, nutrition value, proteins, fats, ashes, mineral elements, as, calcium level in meat samples was measured and compared with commercially available kits using an automated analyzer. Meat samples were placed in small sterile polyethylene plastic bags in icebox and transferred immediately to the laboratory. The samples were kept frozen at -18°C until the chemical analysis.

a-Determination of protein contents according to **A.O.A.C (2000):** the samples were estimated by the Kjeldahl method.

b-Determination of fat contents according to **APHA (1985):** the fat% of samples were estimated by the soxhlet extraction.

c-Determination of Vitamins (A, E, B1and B2) by HPLC system .

d-Determination of Calcium %(Digestion procedure according **Stanek *et al.*, 2013) .**

e-Determination of Ash contents according to **A.O.A.C (2000).**

2.4. DNA Extraction from intact cysts:

The suspensions containing protoscolices and/or associated germinal layers were washed in nucleic acid free water to remove excess alcohol. Extraction of DNA from hydatid cysts was made possible using a commercially available QIAamp tissue kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Briefly. The DNA yield was used in the PCR amplification (**McManus and Thompson , 2003**).

2.5. Polymerase chain reaction

The primers were selected from ribosomal RNA subunit (SSU r RNA) gene, and used in this study. For the first amplification step, a pair of outer primers JBl1: 5' AGATTCGTAAGGGGCCTAATA 3' and JB12: 5' ACCACTAACTAATTCACTTTC 3' were used to amplify a 530 bp PCR product from EG isolates. A stock buffered solution containing 150 µl 10x PCR buffer, 100 µl of 25 mM $MgCl_2$, 12.5 µl of each dATP, dTTP, dGTP and dCTP at a concentration of 10 mM was prepared in 1.5 ml eppendorf tube. Each 0.5 ml PCR reaction tube contained 2 µl of the primers at conc. Of 20 P/mol, 1 µl (5.0 U) of Taq DNA polymerase (QIAGEN), 5.0 µl of the target DNA and 42 µl of the stock buffered solution. For nested PCR, 2 µl of the primary PCR product was used as DNA template. The thermal cycling profiles were as follows: a 2 min initial incubation at 95°C, followed by 40 cycles of 95°C for 1 min, 55°C for 30 sec and 72°C for 45 sec, and a final incubation at 72°C for 10 min. On a Techne TC-412 thermo-cycler (Techne, Staffordshire, UK). Following amplification, 15 µl from each PCR stained with ethidium bromide containing amplified products were loaded onto 1.0% agarose gel and electrophoresed for 1 h. The PCR products were easily identified following visualization under UV light **(Mohamed *et al.*, 2013)**.

2.6. Sequence analysis and construction of phylogenetic tree

The primary PCR products were purified using QIAquick PCR purification kit (QIAGEN) and sent for sequencing to commercial companies (Seqlab, Göttingen, Germany, and Macrogen, Seoul, Korea). Resulted sequences were edited and aligned using BioEdit software (Ibis Biosciences, Carlsbad, CA, USA). The Basic Local Alignment Search Tool (BLAST) of NCBI (National Center for Biotechnology Information, Bethesda, MD, USA) was used to confirm the identity of the generated sequences in relation to the GenBank nucleotide database. The phylogenetic trees were constructed using Unweighted Pair Group Method with Arithmetic mean (UPGMA) implemented in MEGA software version 5.0 (Tamura K, et al., 2011).The

country of origin, the GenBank accession numbers and the genotype were given for each EG isolate when available. Bootstrap analysis was applied and values were given at relevant nodes of the constructed tree. Corresponding nucleotide sequences of *Taenia saginata* with GenBank accession numbers (NC_009938); were used as out groups in the constructed phylogenetic trees **(Mohamed *et al.*, 2013)**.

3- Results and Discussion:

All collected cysts were fertile, and the size was ranged from 8-20 mm, haematological and biochemical analysis of serum were commonly used to monitor health status and disease diagnosis in different animals, in these studies (**Table, 1, 2**) realistic values of different biochemical changes were analyzed in camels suffering from hydatidosis. Concerning to Haematological results, the present results showed significant increase in leucocytic count, neutrophils and eosinophils while the other haemograms are non significant. These finding were supported by those obtained by **Sanaa (2009)** who stated that, parasites induced an increase in eosinophil count especially in cases that infected tissues **(Coles, 1986).** As neutrophils were considered one of the defense mechanism against any strange body , as they showed also showed remarkable increase.

The biochemical analysis of sera of infected camels showed that, significant increase in total protein, and globulin in sera of infected camel with hydatidosis when compared with the non infected ones. This result agreed with **Avez *et al.*, (2007)** and **Hammad (2008)** which could be referred to the body response to infection with hydatid cysts, as the total protein increased due to increase in globulin value which occurred due to functional activity of the reticulo-endothelial system. The significant decrease in albumin may be attributed to the reduction of its synthesis in the infected liver with hydatid cysts. Similar results were obtained by **Avez *et al.*, (2007) and Hammad (2008).**

The decrease in A/G ratio reflected the changes observed in albumin and globulin fractions. The significant elevation of the activity of AST, ALT and bilirubin level were also observed by **Hammad (2008).** The significant decrease in glucose value in affected animals may result from the decrease in hepatic glycogensis of the infected liver **(Coles, 1986)**. This result contradicts with that of **Hammed (2008)** who mentioned significant increase in glucose level in infected camel. On the other hand, no alteration in serum cholesterol level was observed, which reflected a variable degree of liver infection. These results were contradicted with **Hammad(2008)** who observed significant decrease in cholesterol level. As well, in this study, no change were observed in urea, uric acd and creatinine. These results agreed with the results of **Sanaa (2009).**

Concerning to calcium and inorganic phosphorus level (**Table, 3**), they were significant decrease in the infected camels with hydatid cyst. This finding coincided with the obtained result by **Avez *et al.,(*2007)** and may be due to the decrease in amount of protein-bound calcium in blood as a result of infection of liver with hydatid cysts, as the infected liver decrease synthesis of protein. The reduction of phosphorus level may be attributed to the decrease of serum calcium to keep the Ca/P ratio in blood constant. Magnesium level was non – significantly changed. This result was contradicted with the result obtained by **Avez Avez *et al.,*(2007)** who recorded a significant decrease in magnesium level of infected camel with hydatid cysts. This study showed non- significantly changed in sodium and potassium levels and this result agreed with that recorded by **Sanaa (2009).**

Concerning the Meat chemical composition, The results in Table (4), revealed that there is decrease in a protein level of the camel infected with echinococcosis in compare with the muscle tissue of healthy camel depends on the intensity of an invasion. According to the present research , the muscle tissue of healthy camel contains a protein average of 21.83 g and in infected animals this indicator was lower by 4.2% ($p<0.05$). The content of fat in muscles of the infected animals also decreased ($p<0.05$) considerably and was 37% less than in healthy animals. Results of our research indicate that the echinococcosis has an impact on a mineral exchange too. In muscle tissue of infected camels we observed an increase in the content of ashes and decrease in the concentration of calcium. For example, in muscles of healthy camels the share of ashes was 0.89 g and level of calcium reached 0.62 mg whereas in samples from the infected camels these indicators were higher by 11.78% ($p<0.05$) and lower for 5.0% ($p<0.05$), respectively.

According to the obtained data, echinococcosis also considerably influences to the content of vitamins of muscle tissue as showen in table (5). So, practically all studied vitamins in samples from infected camels were present in lower concentration than in healthy camels. However, the strongest influence of the infection was noted on vitamins B1, B2 and E which contents in muscles of the infected animals was(0.109, 0.165 and 0.495 mg/100g) less ($p<0.05$) for 8.4% , 26.3% and 29.3% than in healthy animals, respectively while vitamin A was 0 in both healthy and infected camels .

It has been experimentally demonstrated that helminthoses cause the reduction of the content of general protein in liver, muscles, blood and other tissues, the decrease in the contents in organs of B vitamins (**Yampolskiy, 1981).** Studies of the chemical composition of meat and meat products of camle infected with echinococcosis established that the maintenance of protein and fat decreases. The liver and other organs of camle with echinococcosis display serious pathology

compared to organs of healthy animals (**Blochina, 2009).** The biochemical analysis also showed that in the meat of camels infected by hydatidosis, in comparison with muscle tissue of healthy camels, the amount of protein, fat and calcium significantly decrease. Besides, in the meat of animals infected with echinococcosis there is a substantial increase of amount of ashes.

These results testify that in the camel, echinococcosis causes a complex of biochemical changes. Along with disruption of the synthesis of protein and the vitamin balance, in particular, the sharp insufficiency of vitamins A, E, B1, and B2, also indicated that economic losses not only from the condemnation of infected viscera, but also from decreases in yield and quality of meat and delayed performance and growth; so we can concluding that the specified biochemical changes in the muscle tissue of infected camels are the cause of the decrease in the biological value of meat. Results of the present research allow concluding that the meat from the camel infected with echinococcosis is of poorer quality to the muscle tissue of the healthy animal as well as being biologically inferior.

The PCR-based assay with primers specific for (18 SSU r-RNA genes) yielded amplification products from of all of the fifty hydatid cysts obtained from naturally infected camels produced a primary 530 bp PCR product. The sequences obtained from the PCR products were found to align in the GenBank confirming the cysts to contain the EG complex, and showed 100% homology among 49 out of 50 EG isolates recovered in this study. To investigate for the relationship between these EG isolates and the other EG genotypes identified globally, phylogenetic trees were constructed (Figures 1). Forty nine (98%) of EG isolates (represented by one sequence in the tree) clustered with *E. canadensis* genotype 6 (G6), the camel genotype, of EG complex obtained from other parts of the world with a strong bootstrap. The circulation of a major variant (G6) Egyptian camels suggests that specific mechanisms are responsible for its persistence in this area. This is probably due to close relationships between dogs and camels in the study area **Dinkel *et al.*, (2004)**.

In rural communities with resource-poor settings, such as this study area, the practice of animal slaughtering is usually performed in the open space. Under these conditions, dogs would have free access to feed on livestock viscera, which may harbor hydatid cysts, the infective stage. Therefore, it is believed that this practice of livestock slaughtering could effectively contribute towards the persistence of the camel genotype (G6) in the study area. The addition of EG strains sequences from Egypt enhances our understanding of the expansion and, to some degree, maintenance of the parasites in the intermediate hosts. Ongoing surveillance and EG

strains characterization should also aid in determining the distribution of this cestode parasite in the country. As more sequencing data and prediction tools become more accurate and available, these data will provide the public health authorities an opportunity to anticipate and prepare for treatment and subsequent control programs for the disease. Our findings confirms a previous study done using RAPD-PCR for characterization of human and animal hydatid cysts, it has been shown that human and camel isolates were the most related pair and camels are important hosts for the transmission of human hydatidosis **Nashwa *et al.*, (2014)**.

Similarly, performing the cycle sequencing and nucleotide sequence analysis identified the G6 genotype in 30 (96.8%) out of 31 human isolates in Cairo, Egypt). The extensive intraspecific variation in *E. granulosus* is associated with change in the life cycle pattern, host specificity, geographical distribution, transmission dynamics, infectivity to human, antigenicity, and sensitivity to chemotherapy **Abdel Aaty *et al.*,(2012).**

4. Conclusion

In conclusion, echinococcosis causes a complex of haematological and biochemical changes. Along with disruption of the synthesis of proteins and the vitamin balance, in particular, the sharp insufficiency of vitamins A, E, B1, and B2, there were observed shifts in a lipidic exchange that was expressed as a noticeable reduction of the monounsaturated and polyunsaturated fatty acids level, also the result of this study indicates the circulation of *E. canadensis*, (G6) in the one humped camel in Egypt for the first time in Qalyubia governorate, Egypt, and it is the second study that revealed *Echinococcus* genotype of *E. canadensis* (G6) in camels. In addition, this investigation expands on the existing data on sequences generated from EG isolates recovered from the one humped camel in such areas Such epidemiological data could guide the application of efficient control strategies of CE in Egypt.

Competing interests: The authors declare that they have no competing interests.

Authors' contributions

Rasha A. El Meghanawy collected preserve, and storing blood, serum, and hydatid cyst samples, extracted the DNA and, **Fatma F. M. Ibrahim, conduct the haematological, biochemical, mineral profile work, Dina El Zahaby make the meat composition analysis, Amer R. Abdel Aziz**: optimized the polymerase chain reaction-based detection assay; analyzed the sequences and designed the study;

designed the experiment; edited the sequences and helped with experimental design. **Fatma F. M. Ibrahim**, and **Amer R. Abdel Aziz** designed the experiment and prepared the final manuscript. All authors read and approved the final version of the manuscript.

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Table (1): Haematological profile of camels infected with hydatid cyst and non- infected control group

Parameter	Control camels	Infected camels
RBCsx106	10.5± 0.31	9±0.41
Hb gm/dl	12.8± 0.11	11.23±0.24
Pcv%	32.23± 0.34	34.62±0.88
WBCsx103 /ul	11.80± 0.45	17.2±0.33*
Neutrophils	43.7± 4.5	52.61±1.86*
Lymphocytes	47.51±0.61	44.42±2.43
Monocytes	2.11± 0.43	2.88±0.61
Eosinophils	2.34± 0.23	5.1±0.33*
Basophils	0	0

Table (2): Some biochemical parameters of camels infected with hydatid cysts and non-infected control group.

Parameters	Control camel	Infected camels
Total Protein g/dl	7.45±0.21	8,45±0.43*
Albumin g/dl	3.52±0.86	3.11±0.77*
Globulin g/dl	4.22±0.11	5.53±0.65*
A/G	0.83± 0.11	0.56±0.43*
ALT U/L	12.30± 0.54	35,3±1.12*
AST U/L	45.63± 2.69	185.45±3.67*

Bilirubin mg/dl	0.33± 0.63	0.79±0.15*
Glucose mg/dl	88,06± 2.03	78,00±3.21*
Cholesterol mg/dl	135± 4.56	133.3±3.1
Urea mg/dl	37.87± 1.42	42,2±1.88
Uric acid mg/dl	9.65±0.77	10.45±0.76
Creatinine mg/dl	0.76±0.06	0.97±0.05

Table (3): Minirals profile in hydatid cyst infected camels and non-infected control camels

Parameter	Control camels	Infected camels
Calcium mg/dl	10.22±0.27	8.65±0.21*
Inorganic phosphorus mg/dl	6.87±0.17	5.06±0.23*
Magnesium mg/dl	2.43±0.12	2.2±0.1
Sodium m Eq/l	133.2±1.12	127±1.32
Potassium m Eq/l	3.45±0.06	2.76±0.07

Table 4 Statical analysis of meat chemical composition of healthy and infected camels with *Echinicoccus granulosus*.

Indicators	Meat of camels	
	Healthy camels	Infected camels by echinococcosis
Nutrition value (g/100 g)		
Proteins	18.91±0.19*	16.03 ±0.04
Fats	3.2 ±0.08*	2.724±0.44
Ashes	1.21 ±0.02*	1.51±0.02
Mineral elements (mg/100 g)		
Calcium (Ca)	0.53±0.07	0.62±0.05*

*p<0.05 value differ significantly.

Table5 Vitamins in meat of healthy and infected camels with *Echinicoccus granulosus*.

Vitamins mg/100g	Healthy camels	Infected camels by echinococcosis
Vitamin B 1	0.12±0.02	0.109±0.003
Vitamin B 2	0.18±0.01	0.165±0.021
Vitamin E	0.70±0.09	0.495±0.02

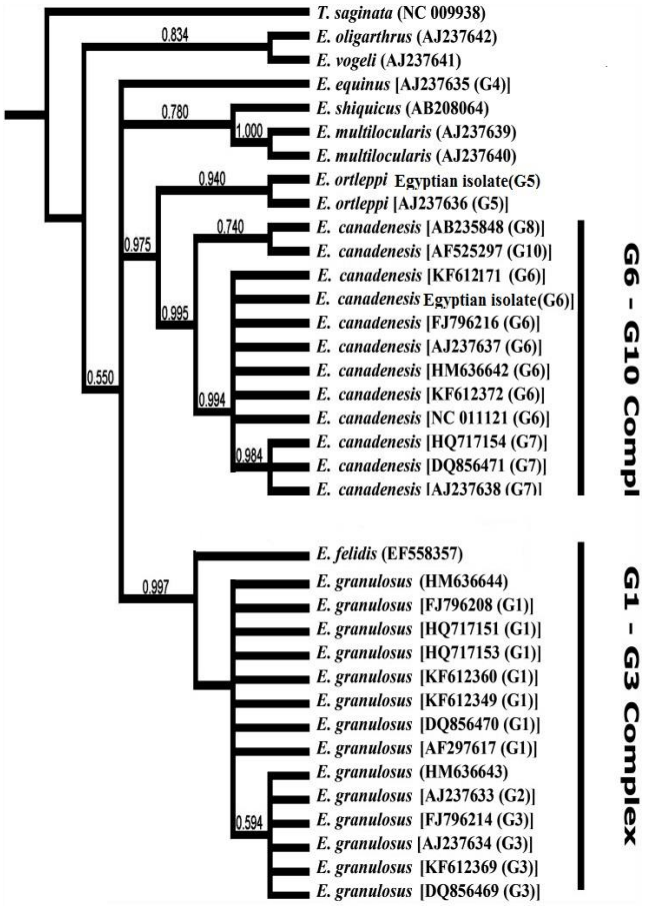

Figure 1: Phylogenetic relationship of Echinococcus granulosus-complex genotypes recovered from Egyptian camels and other genotypes identified globally.

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