



## A Study for The Partial Determination of The Prevalence of A Molecular Disease of Cystic Echinococcosis From The Slaughter of One-humped Camel “*Camelus Dromedarius*” In Beheira, Egypt.

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### Abstract

**T**APEWORMS of the genus *Echinococcus* are the cause of the zoonotic disease known as cystic echinococcosis (CE). This work aimed to detect the prevalence, the fertility of cysts, the viability of protoscolices, as well as the molecular and phylogenetic analysis of CE infecting one-humped camels (*Camelus dromedarius*) in Beheria, Egypt. Between August 2022 and July 2023, 156 camels at the Kom Hamada abattoir were checked for the presence of hydatid cysts during routine meat inspection. Using phylogenetic analysis of the Cox1 and Nad1 sequences, the cysts were molecularly identified. The overall prevalence in slaughtered animals was 16.67%. The prevalence in the liver and lungs was 3.85% and 96.15%, respectively. Spring had the highest prevalence (28.11%). The fertility rate was 72.22%. The viability rate was 87.5% from fertile lung cysts. Phylogenetic analysis confirmed the existence of *Echinococcus canadensis* (*E. granulosus* G6). The study area had a high prevalence of CE in one-humped camels in the study area, which requires the implementation of an active control program. Moreover, our data confirms that the genotype involved is *E. canadensis*. Further studies on CE in camels and other ruminants in Egypt should be carried out to illustrate factors associated with this disease.

**Keywords:** Camel, *Echinococcus*, Prevalence, Molecular, Egypt.

### Introduction

The Arabian camel (*Camelus dromedarius*), commonly referred to as the one-humped camel, is a multipurpose domestic animal that is found in specific regions of the world, such as the Middle East. [1, 2, 3]. Camel diseases are not only of veterinary interest but also of public health concern [4, 5]. Tapeworms of the genus *Echinococcus* are the cause of the zoonotic disease known as cystic echinococcosis (CE). Adult tapeworms, which are only 5-7 mm long, are found in the small intestines of carnivorous definitive hosts that are members of the families Canidae, Felidae, and Hyaenidae [6, 7, 8]. The gravid segments are passed to the environment by these carnivores through their feces. Hydatid cysts, or the larvae of the *Echinococcus* species, infect herbivorous

intermediate hosts, including camels, after they eat the eggs. On the intermediate hosts, massive hydatid cyst development, mainly in the liver and lungs, has major pathological consequences [9]. The epidemiology of the disease and the parasite's cycle of transmission appear to be significantly influenced by camels, particularly in rural areas where dogs are infected through the consumption of camel carcasses that contain hydatid cysts [10]. Due to increased mortality, forced slaughter, the condemnation of contaminated offal, decreased breeding value, worse quality and production of meat, milk, or wool, and the high expense of hygienic measures, hydatid cysts result in excessive economic loss [11].

Currently, the *E. granulosus sensu lato* includes four species and ten distinct strains (genotypes G1-11) [12]. Studies using mtDNA analysis have shown

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that *E. granulosus* is actually a complex of species and genotypes with significant genetic variability. Thus, the *E. granulosus sensu lato* has at least ten different genotypes (G1–G10) identified [9]. The strains that are included in this are the G1 and G2 strains of sheep, the G3 and G5 strains of bovinds, the G4 strain of horses, the G6 strain of camels, the G7 and G9 strains of pigs, and the G8 and G10 strains of cervids. Furthermore, new molecular data indicates that a particular strain (G11) of *E. felidis* is probably the source of infections in wild carnivores. This genotype has been documented in lions and hyenas [9, 13]. To form the taxon *E. granulosus sensu stricto* (*E. granulosus s.s.*), G1–G3 cluster firmly together. These variants have a wide geographic distribution and differing degrees of host specificity and are mostly to blame for human infections (particularly G1). All strains of *E. canadensis* belonging to the more distantly related genotype cluster G6–G10 are pathogenic to humans, although to a much lesser degree than strains from *E. granulosus s.s.* [9] and the proposed species *E. intermedius* (G6, G7) are the most recent taxonomic revisions in the genus *Echinococcus* [14, 15]. All other strains, except for the G4 genotype, have been found to infect humans [10, 16].

Cysts from various animals and humans in multiple countries were molecularly characterized using Nicotinamide adenine dinucleotide dehydrogenase subunit 1 (Nad1).

The pig genotype (G7; *Echinococcus intermedius*) was reported in camels in Egypt where the Blast and phylogenetic analysis were based on cytochrome oxidase 1 (Cox1) and Nad1 sequenced genes [17] beside other studies have reported the presence of *E. granulosus sensu lato* genotypes in Egyptian camels (G6, the most common isolate, G1 and G5) according to information derived from the Cox1 gene sequences [18–20]. So, the object of this study is to determine the prevalence and fertility of hydatid cysts, the viability of protoscolices, as well as the molecular and phylogenetic analysis of *E. granulosus* inhabiting camels in the studied area.

## **Material and Methods**

### *Area of study*

Between August 2022 and July 2023, examination of the livers and the lungs of 156 camels for the presence of hydatid cysts carefully occurred as part of a routine meat inspection at the Kom Hamada abattoir, which is located in the Beheira Governorate of Egypt at a latitude of 30°45'45"N and a longitude of 30°41'50"E.

### *Samples*

The detected cysts were individually collected in clean bags, kept in sterile saline, 70% ethanol, labelled (age, seasonal dynamics) and sent to the Parasitology laboratory in the ice box for further examinations within a few hours (2–4 hrs.).

### *Parasitological examination*

The data about the size, distribution, viability, fertility, and seasonal dynamics of cysts was documented. Collected cysts were measured and divided into three sizes: small (diameter <4 cm), medium (diameter 4–8 cm), and large (diameter >8 cm) [21].

Under a microscope, the existence or lack of broad capsules containing protoscolices in the vesicular fluid indicates cyst fertility. [22–24].

The viability of the fertile cysts was tested by putting a drop of vesicular fluid containing protoscolices on a slide with a drop of 0.1% eosin solution. After five minutes, the slide was examined microscopically (power 40 X). The principle behind this test is that viable protoscolices which completely or partially exclude the dye are viable, while ones that take up the dye are not viable [25].

### *DNA extraction*

Three hydatid cysts (fertile and viable protoscolices) were used to extract genomic DNA modified from Maixner *et al.* [26].

Briefly, the selected individual cyst was crushed in a grinding dish by using liquid nitrogen. One gram of ground mixture was picked and mixed with 3ml CTAB extraction buffer (cetyltrimethylammonium bromide 2%, Tris-HCl 1M PH8, EDTA 500 mM PH 8, NaCl 1.4 M and B-mercaptol 0.2%). Then 1.5–2 ml of mixture were transferred to a 2 ml tube and kept in a water bath at 65°C for 15 minutes. The tubes were turned up and down twice, then incubated at 65°C for 15 minutes and centrifuged at 6000 rpm for 5 min.

The pellet contained debris, and nucleic acids were in the supernatant. 1 ml of supernatant using filtered tips was collected and transferred to a clean microtube. Genomic DNA were extracted with 1 ml chloroform-isoamyl alcohol (24 vol. /1 vol.). The tubes were mixed by inverting several times to get an emulsion and then centrifuged at 14000 rpm for 5 min. 0.9 ml of aqueous phase (upper phase) were recovered and transferred to a new microtube, then precipitated with 0.6 volume isopropanol alcohol 70% by inverting the tubes and let at -20°C for 30 min.

The pellets were washed with 1 ml ethanol 70%, vortexed and centrifuged at 14000 rpm for 10 min. Ethanol was removed, and the pellets were dried in speed-vac (on air-dried). The pellets were resuspended with 60–100 micrometre TE 1X buffer (tris 10 Mm EDTA 1Mm PH 8), then kept at room temperature for 30 min. Nucleic acid extract was stored at -20°C until amplification.

### *Molecular characterization*

The genes for cytochrome c oxidase subunit 1 (Cox1) and Nicotinamide adenine dinucleotide

dehydrogenase subunit 1 (Nad1) were both subjected to amplification. Specific primers are used to amplify fragments of mitochondrial genes.

·JB3 primer (5'-TTTTTGGGCATCCTGAGGT-TTAT-3') and JB4.5 (5'-TAAAGAAAGAACATAATG AAAATG-3') for Cox1 as forward and reverse primers respectively [27].

·JB11 (5'-AGATTTCGTAAGGGGCCTAATA-3') and JB12 (5'-ACCACTAATAATTCACCTTTC-3') for Nad1, as forward and reverse primers respectively [28].

PCR products were visualized using electrophoresis with 1.5% Agarose gel in TAE buffer and stained with 0.5 µg/ml ethidium bromide (Sigma). Each gel included a 100-bp ladder, and a sample containing distilled water instead of DNA was used as a negative control. A gel documentation system (SYNGENE) was used to photograph the gels, and the data was analyzed by computer software.

#### *Sequencing and phylogenetic study*

An Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA) used for forward and reverse sequencing of a purified PCR product. Using a ready reaction Bigdye Terminator V3.1 cycle sequencing kit. (Perkin-Elmer/Applied Biosystems, Foster City, CA).

A BLAST analysis (Basic Local Alignment Search Tool) [29] was initially achieved to establish sequence identity to GenBank accessions. For phylogenesis, *Taenia saginata* was used as an out-group species and sequences with greater identity were downloaded from GenBank (Table 1). The Mega 11 software's alignment tool was used to align the sequences [30].

The Tamura Nei model and the Maximum Likelihood method were used to generate a phylogenetic tree [31]. The sequences obtained in this study were deposited into GenBank in order to receive accession numbers.

#### *Statistical analysis*

A statistical application called GraphPad Prism 9 was used to examine statistical significance differences using a Chi-square. P values less than 0.05 were used to determine statistical significance.

### **Results**

#### *Survey findings (Table 2)*

Only 26 of the 156 dromedaries that were inspected had one or more cysts, meaning that the overall prevalence in slaughtered animals was 16.67%.

In terms of cyst organ distribution, only one was in the liver (3.85%), and the others were in the lung (96.15%). The cyst distribution in the lung was significantly more frequent than in the liver ( $\chi^2=44.31$ ,  $P<0.0001$ ).

In terms of the age of the slaughtered animals, camels older than four years had the highest prevalence of infection (21.78%), whereas animals mostly between 1 and 4 years had a lower prevalence (7.27%). The age had a significant ( $\chi^2=5.398$ ,  $P=0.0202$ ) on the prevalence.

In the term of the season, camels had a distinct seasonal pattern of hydatidosis, with the following prevalence: Spring (28.21%), Winter (19.51%), Autumn (10.26%), and Summer (8.11%). However, the statistical analysis ( $\chi^2=6.895$ ,  $P=0.0753$ ) indicated that these changes were not statistically significant.

The animals that were infected had between one and four cysts. Moreover, the diameter of the hydatid cysts that were obtained from the liver and lungs ranged from 1 to 7.5. The cysts that were obtained from the lungs and livers weighed, on average, 35.53g (3–73.3 g).

Thirty-six hydatid cysts that were examined and collected from animals that had been slaughtered had a fertility rate of 72.22%, with 27.78% of the cysts being sterile. Additional microscopic observations revealed that 72.22% (26/36) of the lung's cysts were fertile, as the vesicular fluid contained protoscolices (hydatid sand) (Fig. 1). Whereas 25% (9/36) of the cysts were sterile. Meanwhile, the liver's cyst was fully calcified. The current study found that 87.5% of the protoscolices from fertile lung cysts were viable in terms of the cysts' viability.

#### *Molecular and phylogenetic analysis*

Two bands, measuring 450 bp and 500 bp, were obtained from the PCR amplification of the Cox1 and Nad1 genes, respectively, using specific primers. The current study's sequences corresponding to the Cox1 and Nad1 genes were placed in the GenBank under the accession numbers OR769606 and OR81955, respectively. According to the GenBank database of the Cox1 and Nad1 genes, the BLAST analysis of the sequenced data indicated the presence of an *E. granulosus* G6 genotype isolated from the analyzed hydatid cysts. The Nad1 sequence from the isolate of the current study revealed 100% homology to KT363811 and MZ927665, while the Cox1 sequence from the isolate of the current study revealed 99.72% homology to MK370106 and LC469709 with a minor mutation in base pair number 381 change from G to C. A strong tree linking the G6 isolates from the current study with other *E. canadensis* from different hosts in various parts of the world was revealed by phylogenetic analysis (Figs. 2&3). So, the present study confirmed the existence of *Echinococcus canadensis* (*E. granulosus* G6).

### **Discussion**

Hydatid cysts, which are larval stages of tapeworms that belong to the family Taeniidae and

genus *Echinococcus*, are the source of hydatidosis, a zoonotic parasitic disease. A Hydatid cyst is a bladder-like cyst that develops in a variety of organs and tissues after the oncospheres of an *Echinococcus* tapeworm grow in that particular tissue or organ [32]. The most prevalent presentation of human cystic echinococcosis is likely responsible for more than 95% of the estimated 2-3 million cases globally. [33]. Although there has been some progress in controlling echinococcosis, the disease is still a main public health concern in many other countries, where it is regarded as an emerging and re-emerging disease [8]. Egypt is one of the countries where CE is important for public health [34].

The existing work showed that the overall prevalence of hydatid cysts was 16.67% in slaughtered camels in Beheira. The results of the present study were closely comparable to the earlier study in Kalyobia (16.25%) [35]. While lower than the 39.5% in Beni-seif [36] and higher than the 2.17%, 13.26%, 2.5%, 5.6%, 5%, 10%, 8.32%, 7.7%, 9% and 9.7% in Beheira, [37], Toukh city [38], Mansoura city [39], Giza [36], Aswan [40,17, 41], Aswan and Assuit [42], Assiut [43], Minofyia [44] respectively. The prevalences of 59%, 29.7%, 32.8%, 30.1%, 61.4% and 20% in Sudan [45, 46], Saudia Arabia [47], Mauritania and Kenya [48] and Morocco [49], respectively, were higher than the existing study. According to this study, the prevalence was greater than 3.6% and 6.5% in Libya [50] and Tunisia [51], respectively. Differences in the degree of exposure to infectious eggs in dog faeces, ecological location, environmental conditions, animal rearing method, and hygienic system could all contribute to the variation in prevalence.

The present survey revealed that camels older than four years old had a greater prevalence of hydatid cysts (21.78%) than camels younger than four years old (7.27%). These results were matched with the previous studies [49, 50, 52, 17]. This may be because younger animals are less exposed to *E. granulosus* eggs than older ones, and the course of cyst development is different.

The current study showed that the prevalence in the liver (3.85%) was lower than in the lung (96.15%) and was in line with other studies [53, 17, 14] as the camels were slaughtered at an older age where their liver capillaries will be dilated, and oncospheres passed through the lymphatic circulation to thoracic duct to heart then to lungs so lungs were infected before or instead of the livers [54].

The fertility of cysts plays an important role in the spreading of *E. granulosus*. The present work showed that the fertility rate in the lung was 72.22%, while in the liver was zero. Our findings were in accordance with other studies in which the rate of cysts in the lung was 69.7% and 54.3% in the lung

[55,17], respectively. This could be occurring because lung tissue has a softer consistency, which makes it easier for cysts to form [56].

The prevalence of cysts in Spring and Winter was the highest, followed by Autumn and then Summer. Some studies presented that the prevalence was the highest in winter [25, 57, 17]. These variations could be because of differences in environmental conditions, geographical distribution and pasture among seasons.

The sequence in the current study indicated that the species is *Echinococcus canadensis*, genotype 6 (G6). These findings were in line with the majority of *E. Canadensis* camel species, such as other studies [58, 37, 43, 18, 59, 44]. In Egyptian camels, the G6 genotype was the most common strain [20]. These characteristics supply attractive evidence that camels may be crucial to the preservation of the *Echinococcus* life cycle in livestock intermediate hosts in Asia and Africa. [43]. Global research has shown that camels have the G6 genotype [60, 61]. However, other studies have shown that the G1-G3 complex is the predominant genotype in humans, cattle, camels, sheep, and goats [62, 63] and that G1 appeared in camels [64].

### **Conclusions**

This work demonstrated a high prevalence of CE in one-humped camels in the study area, which requires the implementation of an active control program. Moreover, our data confirms that the genotype involved is *E. canadensis*. Further studies on CE in camels and other ruminants in Egypt should be carried out to illustrate factors associated with this disease.

### *Conflict of interests*

“There are no conflicts to declare”.

### *Funding statement*

“No special grant was obtained”.

### *Author`s contributions*

“Authors contribute equally in this work”.

### *Ethical considerations*

A formal ethical approval (Number: KFS-IACUC/115/2023) was obtained from the ethical committee at Kafrelsheikh University, Egypt. In order to protect animals and decrease their pain, all procedures were completed according to national laws and regulations.

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### *Abbreviations*

CE: Cystic Echinococcosis, Cox1: cytochrome oxidase1, Nad1: Nicotinamide adenine dinucleotide dehydrogenase subunit 1 & G :Genotype

**TABLE 1. GenBank accession number of reference sequences of *Echinococcus* genotypes used in the phylogenetic analysis to compare with genotypes recovered from slaughtered camels in Kom Hamada, Beheira, Egypt.**

Cox1 Accession no.	Locality	Host origin	Genotype	nad1 Accession no.	Locality	Host origin	Genotype
MK644934	South Korea	Human	<i>Taenia saginata</i>	MK644934	South Korea	Human	<i>Taenia saginata</i>
OR769606	Egypt	camel	G6*	OR810955	Egypt	Camel	G6*
LC469709	Southern Monoliga	Human	G6	KT363811	Mauritania	Camel	G6
MK370106	Monoliga	Human	G6	MZ927665	Iran	Human	G6
OP189676	Iran	Cattle	G6	MW183239	Egypt	Camel	G7
OP185208	Iran	Human	G4	KJ663949	China	Human	G10
KX685890	China	Human	G2	MG763764	Iran	Cattle	G5
KX685898	China	Human	G10	KT363809	Tunisia	Donkey	G4
MT796074	Argentina	Pig	G1	KF731955	Iran	Cow	G1
OP185218	Iran	Sheep	G8	LC476661	Iran	Human	G2/3
OP185220	Iran	camel	G9	KF731947	Iran	Camel	G3
OP185209	Iran	Cattle	G5	MK644934	South Korea	Human	<i>Taenia saginata</i>
KX685891	China	Human	G3	OR810955	Egypt	Camel	G6*
OP185211	China	Human	G7				

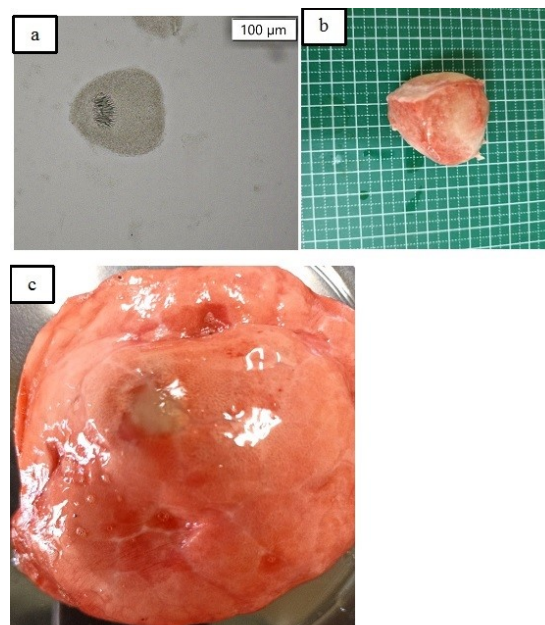
\* Current study

**TABLE 2. The distribution of the recovered hydatid cysts from slaughtered camels in Kom Hamada, Beheira, Egypt, according to age, organ located, fertility and seasonal dynamics.**

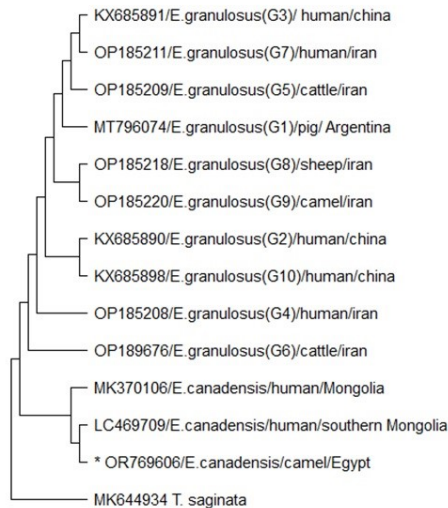
	Age		Fertility			Organ located		Seasonal dynamics			
	1-4 years n (55)	Over 4 y. n(101)	Fertile N (36)	Sterile N (36)	Calcified N (36)	Lung N (26)	Liver N (26)	Winter n (41)	Spring n (39)	Summer n (37)	Autumn n (39)
<b>Number of infected</b>	4	22	26	9	1	25	1	8	11	3	4
<b>Percentage %</b>	7.27	21.78	72.22	25	2.78	96.15	3.85	19.51	28.21	8.11	10.26
<b><math>\chi^2</math></b>	5.398					44.31		6.895			
<b>P-value</b>	0.0202					<0.0001		0.0753			

N number of examined cysts

n number of examined camels

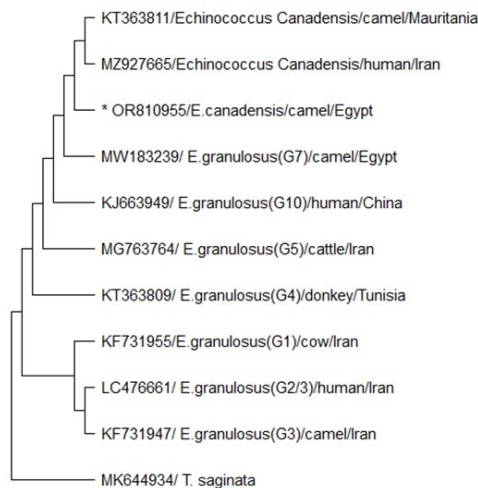
**Fig. 1. Hydatid cysts recovered from slaughtered camels in Kom Hamada, Beheira, Egypt.**

a) Hydatid sand    b) &amp; c) fresh specimens of hydatid cyst in the lung.



**Fig. 2. Phylogenetic analysis of isolated *Echinococcus* genotypes from a one-humped camel in relation to reference sequences of *E. granulosus* and *Taenia saginata* as the outgroups as well the relationships were constructed by Maximum Likelihood, based on Cox1 partial gene of mitochondrial DNA.**

\* Current study



**Fig. 3. Phylogenetic analysis of isolated *Echinococcus* genotypes from a one-humped camel in relation to reference sequences of *E. granulosus* and *Taenia saginata* as the outgroups as well the relationships were constructed by Maximum Likelihood, based on Nad1 partial gene of mitochondrial DNA.**

\* Current study

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## دراسة لبيان انتشارية والتعرف الجيني لداء المشوكات الكيسي في الجمال بمحافظة البحيرة، مصر

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### المستخلص

الديدان الشريطية ( شريطية الكلاب ) من جنس المشوكات هي سبب داء المشوكات الكيسي و هو مرض مشترك حيواني المنشأ قد يصيب الانسان بالإضافة للحيوانات الاخرى مثل المجترات. حيث تكون الديدان الشريطية البالغة في الكلاب اما يرقاتها (المشوكات الكيسية) في احشاء و اعضاء العوائل الاخرى الوسيطة كالمجترات و الانسان. هدفت هذه الدراسة إلى اكتشاف و بيان مدى انتشارية المشوكات الكيسية ، بالإضافة إلى التحليل الجزيئي والتطوري للمشوكات الكيسية التي تصيب الجمال بمحافظة البحيرة، مصر.

في الفترة ما بين أغسطس 2022 و يوليو 2023، تم فحص 156 جملاً في مسلخ كوم حمادة بمنطقة الدراسة بحثاً عن وجود أكياس المشوكات الكيسية أثناء الفحص الروتيني للحوم بالمسلخ. سجلت الإصابات و العوامل المتعلقة بالفحص و عزلت الاكياس و فصحت تحت المجهر الضوئي لبيان خصوباتها (قدرتها علي العدوي) و لتحديد النوع بدقة تم عزل الحمض النووي من تلك الاكياس و استخدام تقنية تفاعل البلمرة المتسلسل لتضخيم و تكثير عدد 2 جين (Cox1 و Nad1) واستخدام التحليل التطوري للتسلسلات الناتجة.

وبلغت نسبة الانتشار الإجمالي في الحيوانات المذبوحة 16.67%. وبلغ معدل الانتشار في الكبد والرئتين 3.85% و96.15% على التوالي. وكان الربيع هو الأعلى انتشاراً (28.11%). وبلغ معدل الخصوبة 72.22%. وكان معدل الحيوية 87.5% من أكياس الرئة الخصبة. كما أكد التحليل الجيني نوع الديدان الشريطية و هو الدودة الشريطية الكلبية مشوكة الراس من النوع 6 و تم رسم شجر النشوء و التطور للنوع المعزول و مقارنته بمثيلاته.

و من النتائج السابقة فيلاحظ ان منطقة الدراسة تشهد انتشاراً عالياً لداء المشوكات الكيسي في الجمال ، الأمر الذي يتطلب تنفيذ برنامج مكافحة فعال. تؤكد بياناتنا جينياً ان النوع المعزول هو الدودة الشريطية الكلبية مشوكة الراس من النوع 6. وينبغي إجراء المزيد من الدراسات حول مرض المشوكات الكيسي في الإبل و المجترات الأخرى في مصر لتوضيح العوامل المرتبطة بهذا المرض ذو الأهمية الطبية و الاقتصادية.

الكلمات الدالة: جمال، المشوكات الكيسية، انتشارية، تحليل جيني، مصر.