An-Najah National University

Faculty of Graduate Studies

Prevalence and Molecular Characterization of *Cysticercus tenuicollis* Cysts in Sheep Slaughtered in Palestine

By

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Dedication

To Family and Friends with Respect and Love

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Thanks for faculty members of Graduate Studies at An-Najah national University for their support during my master program.

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الإقرار

أنا الموقعة ادناه مقدم الرسالة التي تحمل العنوان:

Prevalence and Molecular Characterization of *Cysticercus tenuicollis* Cysts in Sheep Slaughtered in Palestine

الانتشار والتوصيف الجزيئي للكيسات المذنبة دقيقة العنق في الأغنام المذبوحة في فلسطين

أقر بأن ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أية درجة علمية أو بحث علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: اسم الطالب: الاء عزمي يوسف جودكي

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Date: التاريخ: ٢٢/١٢/٢٠١٤

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Prevalence and Molecular Characterization of *Cysticercus tenuicollis* Cysts in Sheep Slaughtered in Palestine

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Abstract

Cysticercus tenuicollis is the metacestode of canine tapeworm *Taenia hydatigena*, which has been reported in domestic and wild ruminants. *C. tenuicollis* infection may constitute a health problem to domestic and wild ruminants and thus a source of economic loss in the meat industry. In Palestine, *C.tenuicollis* infection was not studied and its prevalence is unknown. Therefore, the aim of this study was to estimate the prevalence of *C.tenuicollis* infection among sheep and its molecular characteristics in Nablus, Northern part of the West Bank, Palestine. The study was performed from April to June 2014 and inspection carried out from 1489 sheep slaughtered at the municipal abattoir of Nablus, Northern part of the West Bank, Palestine. The overall prevalence of *C. tenuicollis* was 2.15%. The mean total length of large and small hooks was 38.78 mm and 23.42 mm, respectively. Using Ward's method, the length of the large and small hooks was weakly associated with the variability in mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene. PCR amplification of small subunit ribosomal RNA (*rrnS*) and partial sequencing of mitochondrial (*cox1*) genes were performed for 20 isolates. Ten variable (polymorphic) sites were detected, including 7 singleton

variable sites (SP) at positions 6, 72, 102, 141, 207, 231, 264 and 3 parsimony informative sites (PIP) at positions 51, 213 and 219. The average number of pairwise differences (π) of the *cox1* sequences was 0.0045, suggesting that there was low genetic variation among these isolates. Neutrality tests (Tajima's D and Fu and Li's D) showed that the evolution of *C. tenuicollis* is evolving in a neutral mode. These findings would greatly help to implement control and preventive measures for *C. tenuicollis* in Palestine. Phylogenetic analysis computed by neighbor joining (NJ) from the partial *cox1* gene nucleotide sequences revealed that *C. tenuicollis* isolates were composed of 9 haplotypes and distinguished from the other Taenia species, with the major haplotype comprising 11 out of 20 samples. Furthermore, the Phylogenetic analysis revealed that the Palestinian *C.tenuicollis* isolates were clustered in one clade, along with isolates from Iran, Turkey and Finland. These results confirm circulation of *C. tenuicollis* in different geographical regions.

CHAPTER ONE
INTRODUCTION

1. Introduction

Cysticercus tenuicollis is larval stage (metacestode) of *Taenia hydatigena* (*T. hydatigena*). Adult worms of *T. hydatigena* have been reported to have been found in the small intestines of dogs, cats, mice and wild carnivores, like the wolf and the fox as the definitive hosts [1, 2].

The adult worm of *T. hydatigena* lays eggs which pass out in the feces of the host and are ingested by a wide range of herbivorous animals (intermediate hosts) during grazing. After ingestion, the egg's shell is digested and the oncospheres are liberated and migrate through the intestinal walls, reaching the liver through the hepatic portal system. The oncospheres may remain in the liver or migrate to the omenta, mesenteries or the peritoneal cavity. However, unusual locations like the lungs, the kidneys, brain and reproductive system organs have also been reported [3, 4].

Pathogenicity of adult parasites is not high for definitive hosts. However, the *C. tenuicollis* have serious impacts on their intermediate hosts. The migration of these cysticerci in the liver may cause hepatitis cysticercosa leading to haemorrhagic and fibrotic tracts and serofibrinous peritonitis. In very heavy infections, the migrating larvae

destroy the hepatic cells causing eosinophilic infiltration and severe inflammation that may prove to be fatal [5].

In recent years, it is becoming increasingly clear that greater priority should be given to *C. tenuicollis* because of its economic impact due to condemnation of offal's containing these larvae, particularly in resource-poor countries [6, 7]. Loss in quantity or quality of meat or offal will have financial implications, with reduced payments for carcass contamination or diseased or infected tissues.

In Palestine, the livestock sector is an important one contributing up to 46% of total agricultural income. The small ruminants sector is an important source of income for many Palestinian families, as they produce important products for local consumers and provide employment. In the West Bank and Gaza Strip there are 972.5 thousands heads of sheep and goats, and 39.6 thousands heads of cattle. The livestock sector's total value added was USD 332.6 million in 2011 [8].

Several reports have described the prevalence, the comparative morphology and the genetic divergence of *C. tenuicollis* in different parts of world. In Palestine, abattoir records reported the incidence of carcass condemnations due to *C. tenuicollis*. To the best of our knowledge, no studies concerning *C. tenuicollis* prevalence have previously been conducted in Nablus, Northern part of the West Bank, Palestine. In view of this and considering the financial consequences associated with lamb carcass condemnations due to *C. tenuicollis* infection, the goals of this study were to estimate the

prevalence of *C. tenuicollis* among sheep in the West Bank and identify the molecular characteristics of the *C. tenuicollis* by PCR amplification of small subunit ribosomal RNA (*rrnS*) and partial sequencing of mitochondrial DNA coding for cytochrome c oxidase subunit 1 (*cox*1) gene. This study may add information about strains distribution in this region, which may finally use to find tools in the eradication program of the *C. tenuicollis* infection.

2. General morphology

2.1. Taxonomy and Classification

More than 70 nominal species having been attributed to the genus of *Taenia* [9], and approximately 42 valid species and three subspecies are currently recognized [10]. Taeniidae consists of two genera, *Echinococcus* and *Taenia*. The genus *Taenia* includes a diversity of tapeworm species, including the larvae (metacestodes) and the cestodes (tapeworms), the adult stages of which occur in the intestine of dogs or wild canids. Species of *Taenia* are of significant human and veterinary importance. They parasitize in different hosts, including fish, reptiles and mammals. The adult stage of *T. hydatigena* (Cestoidea; Cyclophyllidea; Taeniidae; *Taenia*) parasitizes and matures in the small intestine of dogs, cats, mice and wild carnivores, like the wolf and the fox [1, 11, 12]. Table 1.1 represents the taxonomy of the *T. hydatigena* according to the “GenBank” database of the NCBI.

Table 1. 1 The taxonomy of the *T. hydatigena* according to the "GenBank" database of the National Center for Biotechnology Information (NCBI).

Category	Taxonomic Classification
Kingdom	*Animalia or Metazoa*
Phylum	*Platyhelminthes*
Class	*Cestoidea*
Sub- class	*Eucestoda*
Order	*Cyclophyllidea*
Family	*Taeniidae*
Species	*T. hydatigena*,
Binomial name	*Taenia hydatigena*
Scientific name	*Taenia hydatigena* Pallas 1766

2. 2. Adult tapeworm

T. hydatigena (Synonym: *Taenia marginata*) or thin – necked bladder worm, the adult stage of *C. tenuicollis*, is very like the *Taenia solium*, but smaller. Its usual length is about 5 meters. The body consists of a head called scolex, followed by a narrow neck and a long strobila. The strobila is composed of linear chain of flat segments called proglottids, and each proglottid is a monoecious (Figure 1.1).

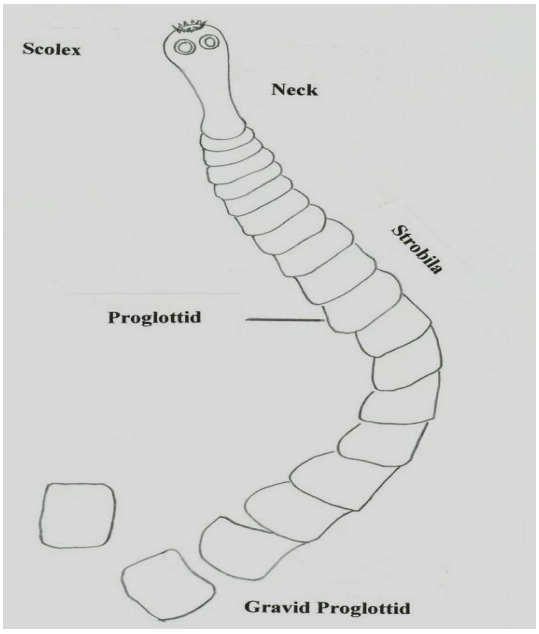

Figure1.1 Diagram of Tania adult tapeworm

The head possesses four suckers and a rostellum with 28 to 33 hooks, situated in two rows hooks (Figure 1.2). These hooks and suckers enable tapeworm to remain attached to the host's intestinal mucosa [13, 14].The lengths of the large hooks of *T. hydatigena* range between 191 and 218 µm, while the small hooks range between 118 and 143 µm [2, 15-18].

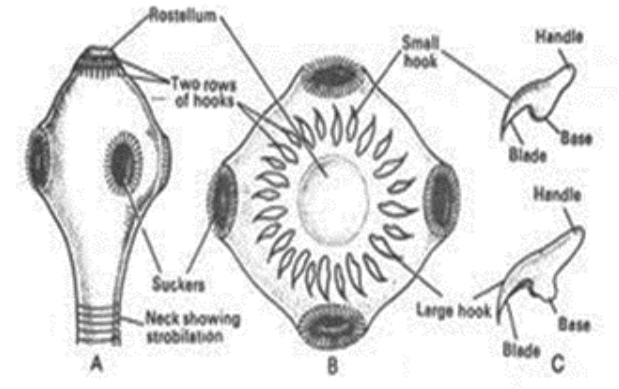

Figure 1.2 Scolex of Taenia: (A) side view; (B) en-face view, (C) hooks

2. 3. Eggs

Taeniid species eggs are spherical or oval 26-34 µm in the diameter and consist of a delicate outer layer (yolk sac), being removed prior to the expulsion of the egg within the proglottid. The second layer, a thick embryophore (polygonal keratin blocks), which gives the egg its radial appearance. On the inside of the embryophore layer is a thin oncospheral membrane and the oncosphere (hexacanth embryo), which contains six hooks and a pair of glands (Figure 1.3) [12, 14, 19]. The eggs of *Taenia* species are morphologically indistinguishable by light microscopy, limiting the diagnosis by fecal examination [20].

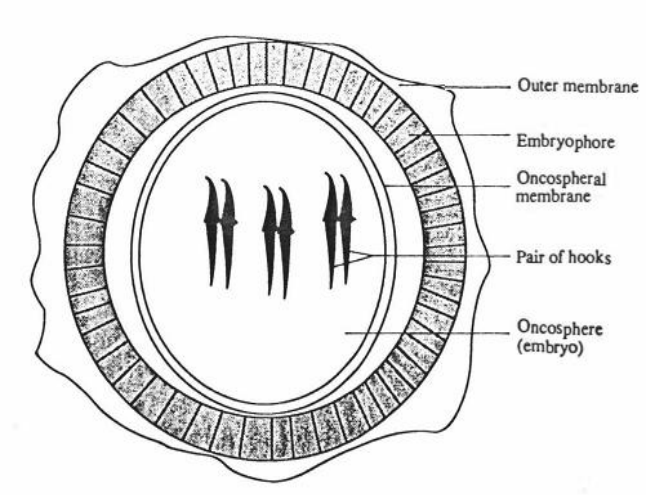

Figure 1.3 Simplified diagram of a taeniid egg.

Adult stage of *T. hydatigena* in dogs produce proglottids which mature, become gravid, detach from the tapeworm, and migrate to the anus or are passed in the stool (approximately 2 per day, each containing about 50,000 eggs). Although each *T. hydatigena* may produce over 100,000 eggs per day, many of them hatch in the small intestine and become inactive [21].

2.4. Metacestode (*Cystcercus tenuicollis*)

C. tenuicollis is the larval stage of *T. hydatigena* tapeworm that considered as the most important parasite of sheep and goats [22]. The adult *T. hydatigena* lives in the small intestines of dogs and other carnivores, segments containing numerous eggs passed in the feces. After the ingestion of eggs, oncosphere within egg, hatches under the influence of gastric juices and bile that break down the embryophore and activate the oncosphere. The oncosphere penetrates the intestinal epithelium, presumably using its hooks and secreted enzymes. The gland secretions act as host-cell lysing agent, thus they assist passage of hooks through the tissues [23, 24]. The penetration in the intestinal mucosa takes around 30-120 minutes of entering the lumen of the small intestine [24]. Once through the epithelium, some oncospheres enter subepithelial capillaries and are carried to the liver via the portal system, where they are transformed to cysticerci [24].

Cysticerci, which arise from the liver, will continue to grow in size and can reach a maximum length of 10 mm in length. The oncospheres which enter the peritoneal cavity become attached to the peritoneum, mature therein the abdominal (peritoneal) cavity of the sheep and can reach 10 mm to 60 mm in diameter.

Cysts contain a clear, jelly-like fluid surrounding a single, immature tapeworm head (scolex) bearing hooks that act as an attachment device for the larvae with the epithelial cells in the host (Figure 1.4).

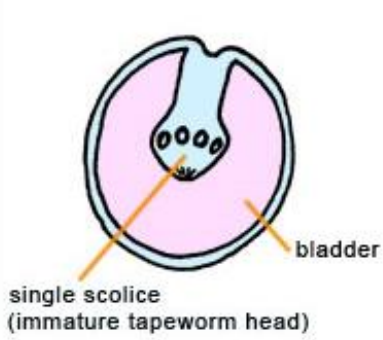

Figure 1.4 A simplified diagram of the larval stage of *T. hydatigena*

Usually, liver damage heals, forming fibrotic tracts, which leads to condemnation at meat inspection. If a sheep swallows a whole proglottid, which may contain 100,000 eggs, death may occur due to massive numbers of developing metacestodes known as cysticercosis hepatica (Figure 1.5) as reported by Carreira et.,al. cited in [25, 26]. *C. tenuicollis* are infective for about two to three months after entering the sheep host [6]. Some cysticerci may survive the lifetime of the host [27]. Cysticerci that die at predilection sites are calcified [28].

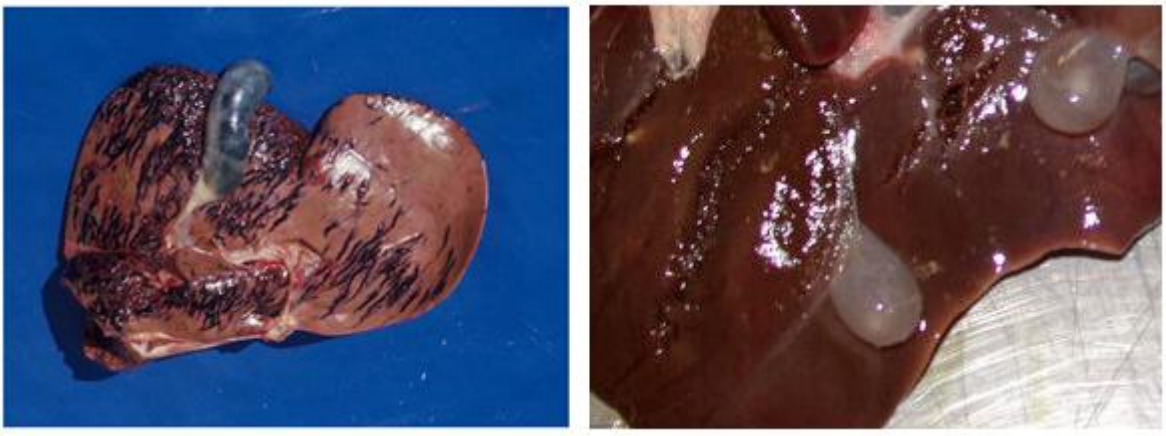

Figure 1.5. A sheep's with severe *hepatitis cysticercosa* (left) and fully developed cysticercus on the surface of the liver (right)

1.3. General life cycles

1.3.1. Basic life-cycle pattern

Life cycle of *T. hydatigena* involves intermediate (a wide range of herbivorous animals) and definitive (dogs or wild Canids) host species; and three distinct stages: eggs in environment, cysticerci in the intermediate host and adult tapeworms in the small intestine of definitive host (Figure 1. 6).

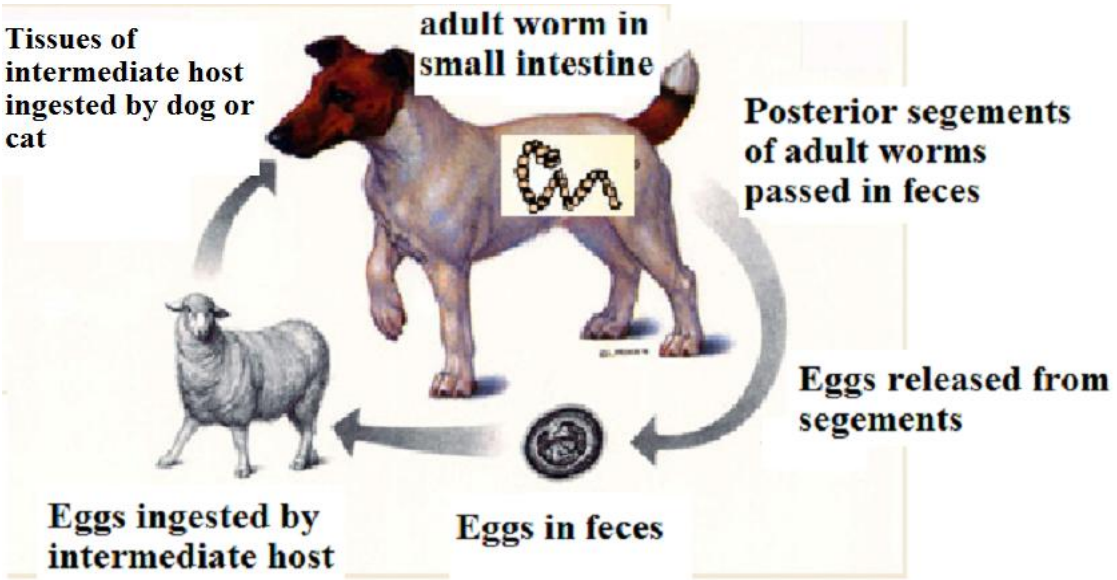

Figure 1.6. Basic life cycle of *T. hydatigena*

1.3.2. Egg survival and dispersion

Eggs of *T. hydatigena* are highly resistant to environmental factors and can remain infective for a long period of time in a suitable environment. Their survival is dependent on temperature and relative humidity. In pasture, Sweatman and Williams [29] found that *T. hydatigena* eggs survived one year after climatic conditions of dry and hot-dry cold Central Otago, New

Zealand (NZ) and one year after rainy weather, but with moderate temperatures on the West Coast of the South Island (NZ). However, the viability of the eggs declined more rapidly under high temperatures, lower humidity, direct exposure to intense sunshine and the presence of tapeworm debris [30]. Heating to 60°C-80°C killed eggs of *T.hydatigena* in less than 5 minutes. On the other hand, *T.hydatigena* eggs can survive freezing conditions [31].

Taeniid eggs disperse at least 80 meters within 10 -19 days from deposition [32]. The potential for dispersion of *T.hydatigena* eggs was recorded by the discovery of *C.tenuicollis* in a population of undomesticated sheep on a remote Scottish Island despite the fact that the nearest definitive hosts were located at a distance of 40 km away [30]. In a pasture, the sheep themselves may be involved in the dispersal of the eggs as they walk through them, but the transfer of eggs over longer distances requires alternative dispersal mechanisms [33]. Flies may be involved in the transport of *T.hydatigena* eggs. In the 1986 Gemmel and Lawson reviewed in [33] found that *T.hydatigena* eggs which had been ingested by flies in field experiments were reported to be viable and caused infection when ingested by lambs.

1.3.3. Hatching and activation

Hatching refers to removal of the thick embryophore of *Taenia* eggs caused by gastric juices and activation refers to discharge of the oncosphere from the oncospheral membrane by the action of bile. In 1967, Laws

[34] reported the use of sodium hypochlorite to effectively hatch *T. hydatigena* , *T. ovis*, *T. pisiformis* and *Echinococcus granulosus* eggs. A series of studies on the composition of the hatching fluids were developed [35], and it has been mentioned that pre-treatment with hypochlorite broke apart of the embryophoral blocks of virtually all the eggs. When this was followed by exposure to a solution containing 10 mg/ml- trypsin, 10% bile and 10% heat-inactivated calf serum, about 50% of the viable oncospheres were activated and escaped from the oncospheral membrane. Bile is one of the most important requirement in the activation of onchospheres and is known to increase the permeability of the onchospheral membrane during the process [36].

1.4. Clinical features and economic impact

1.4.1. Clinical features

The presence of *C. tenuicollis* in ruminants is generally not clinically apparent. However, death may occur due to massive infections of numerous *C. tenuicollis* known as cysticercosis. Deaths can follow due to hepatic hemorrage, mainly in young animals. [22, 26, 37, 38]. Economic loss is mainly due to condemnation of livers and other organs at slaughterhouse. Moderate to heavy infections can result in loss of appetite, diarrhea, jaundice, anemia, and decrease in growth rate leading to increase feed costs. Sheep may also become weak, leaving them susceptible to other infections [6, 39].

On the other hand, liver damages caused by the migration of young *C. tenuicollis*, can create favorable conditions for local growth of some pathogenic microorganisms [40].

The most frequent locations for *C. tenuicollis* are the omenta [25, 37, 41, 42], the mesenteries [43] or the liver [44]. However, unusual locations of *C.tenuicollis* cysts, like the lungs, the kidneys, brain and even the reproductive system had been reported. An aberrant location of *C. tenuicollis* inside the chorion-allantoic membrane of a goat's foetus was reported in Portugal's Northeast [4]. On the other hand, the existence of *C. tenuicollis* cysts attached to the broad ligament and to the uterine tubes also was reported in an abattoir survey on acquired reproductive abnormalities in the ewes. In a certain number of these cases, calcified cysticerci occluded the uterine tubes [45, 46].

1.4.2. Economic impact

The importance of *C. tenuicollis* is the resultant losses encountered during meat inspection when infected carcasses are condemned. The loss due to condemnation of organs by *C. tenuicollis*, particularly liver is of especial significance in countries of low economic output, where sheep and goat production is of particular importance [25, 47]. The estimated annual loss due to the rejection of carcass and organs from of small ruminants slaughtered in Ethiopia to be 65,269 USD or 1,044317 Ethiopian birr [7, 22]. In 2009, more than £500,000 was lost to the English sheep industry due to *C. tenuicollis* [6].

1.5. Detection and diagnosis

1.5.1. Meat inspection — the main diagnostic procedure

Meat inspection at slaughterhouses usually was performed to detect the presence of *C. tenuicollis* cysts in the infected intermediate hosts, although such procedure is insensitive, particularly for lightly infected carcasses. Slaughterhouse meat inspection provides useful information and is an initial indicator for the prevalence of *C. tenuicollis* in an area, but active surveillance is needed to gather more valid epidemiological information and for surveillance of control programs [48]. In addition, meat inspection of *C. tenuicollis* is more difficult if cysts are small or degenerate early, thus allowing more infected carcasses to pass unnoticed. In general, meat inspection procedures detect only about 20–50% of the animals that are actually infected [15].

Meat inspection depends on finding one or several *C. tenuicollis* cysts at necropsy, the presence of only one scolex in the bladder worm and rostellar hook characters, particularly large and small hook lengths.

1.5.2. Serodiagnosis

Immunity in Taeniids is mainly antibody mediated, and thus many serological tests may be employed in the differential diagnosis of larval cestodes [49]. However, all analysis tests using antigen-ELISA (AG-ELISA), antibody- ELISA and enzyme-linked immunoelectrotransfer blot test (EITB) showed variable sensitivity in diagnosis of infected animals

[50]. In contrast with their high sensitivity and specificity when applied to detect human cysticercosis [51]. In spite of ongoing research on the development of serological tests for herd's cysticercosis, using homologous or heterologous antigens, or synthetic peptides to detect circulating parasite antibody, variable degrees of sensitivity and specificity have been reported because of cross reactivity to other parasite [49]. Cross reactivity has been reported to occur with *Fasciola hepatica*, *Fasciola gigantica*, *Taenia ovis* and other parasitic infections [52].

C. tenuicollis fluid reactive antibodies can be detected in the serum of experimentally infected sheep after four weeks of infection [53, 54]. In natural infections, sensitivity of serological tests is lower than experimental infections [55]. It had been reported that 20 lambs, from a group of 29, which had their infected status confirmed, gave false negative reactions by ELISA using *C. tenuicollis* fluid as the antigen. It may be that in natural infections the antibodies are of a transient nature [48].

1.5.3. Molecular techniques

DNA-based methods would not be alternative for meat inspection, however, the combination of meat inspection and the DNA-based methods is required to better understand the nature and significance of intra-specific variation within the *T. hydatigena* species [56]. Different molecular approaches to differentiate *Taenia* to their species have been developed, including restriction fragment length polymorphism (RFLP) analysis, PCR-

linked RFLP analysis (PCR-RFLP), and direct comparison of PCR-amplified DNA sequences [9].

Mitochondrial DNA sequence data have been widely used as genetic markers to examine the population genetic structures of animals, including taeniid cestodes, as it experiences low recombination rates. These sequences have proven useful for not only studying evolutionary relationships among distantly related taxa, but also for species differentiation of parasitic flatworms [47, 57, 58].

Cestodes mitochondrial genomes are similar to eumetazoa but they differ that cestodes lack (*atp8*), the gene that code for ATP synthase subunit 8 [9]. Taeniid cestodes mitochondrial genomes contain 36 genes. These include12 protein-coding genes (3 subunit of cytochrome coxidase, *cox1*, *cox2* and *cox3*; 1 subunit of cytochrome b, *cob*; 7 subunit of NADH dehydrogenase, *nad1-nad6* and *nad4L*; and 1 subunit of ATP synthase, (*atp6*); 22 tRNAs (*trns*) and the small and large subunit ribosomal RNAs, *rrnS* and *rrnL* [9].

All the open reading frames (ORFs) of the12 protein-coding mitochondrial genes are transcribed in the same direction and initiate with GUG or AUG codon [59]. TAG or TAA is used for termination, with five genes (*cox3, nad4L, nad4, nad3 and cox2*) using the TAG stop codon and the remaining seven genes (*cob, atp6, nad1, nad2, nad5, nad6*and*cox1*) using TAA [60]. The *cox1* gene has been found to be useful population genetic marker for *Taenia* and many *cox*1 gene sequence data is available on GenBank [61].

1.6. Distribution and prevalence of *C. tenuicollis*.

Infection of ruminants with *C. tenuicollis* present wherever these ruminants live in association with dogs. *C. tenuicollis* was reported with prevalence of 16.7% in sheep from Germany [62]. In Turkey, *C. tenuicollis* found to be one of the most prevalent *Taenia* species, reported in 27.9% of goats, 26.7% of sheep [63]. A study in an abattoir in Iran, indicated that *C. tenuicollis* was found in 172 sheep (12.87%) and 302 goats (18.04%). The predominant predilection site of *C. tenuicollis* in sheep (84.85%) and goats (82.14%) was the omentum [37]. In Ethiopia, the overall prevalence of *C. tenuicollis* in 845 animals was 24.6%. From a total of 425 sheep and 420 goats examined, 22.8% sheep and 26.4% goats were found positive for C. *tenuicollis* infection. Sheep with poor body (39.8%) were found most infected compared to medium (21.8%) and good (14.5%) body [44]. Another study done in central Ethiopia indicated that *C. tenuicollis* was found in 358 goats (46.6%) and 252 sheep (40.0%), respectively. Adult goats (51.8%) and sheep (47.4%) were more infected than kids (41.4%) and lambs (35.8%), respectively [41].

In Kurdistan Region, Iraq, out of 4716 sheep examined, 31(0.7%) of sheep had *C. tenuicollis* cysts, with absence of these cysts in both goats and cattle. Two years old were more infected (1.7%) than one-year sheep old (0.1%). The heaviest incidence of infection was observed in February 2009 (1.4%) and the lowest was in Jun and July (0.3%) [64]. A study in Mosul municipal slaughterhouse in Iraq, indicated that the prevalence of *C.*

tenuicollis cysts was 2, 10 and 6% in sheep, goats and cattle, respectively. There were no significant differences in the infection rates between males less than one year and older female sheep [65]. In Maharashtra, India, prevalence of *C. tenuicollis* cysts was 15.17 and 18.75% in sheep and goats respectively [66]. A study in the Sokoto abattoir, Nigeria indicated that *C. tenuicollis* was found in 34 (13.03%) of the sheep examined. Prevalence of infection increased with the age of the animals and males had relatively higher prevalence than females [42].

The prevalence of *C. tenuicollis* cysts in sheep slaughtered in England in 2012 varies between 4% and 11%. The heaviest incidence of infection was observed in March and April (11%) and the lowest was in July (4%).

1.7. Immune response to *C. tenuicollis*

1.7.1. General features of immunity

Taeniid cestodes are unique among the helminthes in that protective immune responses can be readily established in their intermediate hosts. This immune response is referred to as concomitant immunity, a term describes that an infected animal is immune to re-infection, while at the same time parasites from the initial infection stay unaffected [67]. The concomitant immunity to *T. hydatigena* was first reported by Sweatman [68] when a high level of protection against *T. hydatigena* was induced in a naive lamb exposed to as few as 50 viable eggs of *T. hydatigena*. Although a considerable degree of protection was achieved after

experimental exposure of hosts to *T. hydatigena* infection, in the field, it is not well clear what number of eggs necessary to develop a protective immune response nor the duration that immunity persists. Craig and Rickard found that antibodies in lamb sera after experimental exposure of hosts to *T. hydatigena* oncospheres peaked approximately 2 weeks after the primary infection and had returned to background levels by 12 weeks post-infection. Moreover, they found that the duration of this immunity depends greatly on host and environmental factors [69].

Both IgG1 and IgG2 have been shown to be the principle immunoglobulins produced by lambs after experimental exposure of hosts to *T. hydatigena* infection [69]. Although IgG2, was of a lower magnitude, its effect level was much more marked than IgG1 in the protection against infection. IgG is the primary immunoglobulin class found in ruminant colostrum and milk. Several subclasses of IgG exist, with IgG1 being the major immunoglobulins in colostrum [70]. Many studies have examined the transfer of immunity from ewes to their offspring via colostrum [71]. The maternal immunity in lambs lasts for 6 - 16 weeks of age against *T. ovis* [72, 73]. A similar phenomenon was also observed with *T. hydatigena*, however, low level of maternal immunity was transferred than T. *ovis*. This could be attributed to many factors like dam genetics and the half-life of IgG1 [74].

The role of antibody in the protection of sheep infection against several taeniid metacestodes was examined using passive transfer of

immunoglobulin. In *T. hydatigena*, a 70-80% reduction in cyst numbers was achieved by transferring 100-120 ml of serum from immunized sheep with *T. hydatigena* oncospheres to recipients [75, 71].

Transferred leukocytes in the presence of serum from infected sheep with *T. hydatigena* killed oncospheres in vitro, while infected sheep serum alone was not lethal to the parasite *in vitro*. Moreover, Fc receptors were not detected on oncospheres. It has been assumed that neutrophils may kill the parasite by producing hydrogen peroxide and the superoxide anions. The function of antibody may be to facilitate attachment of neutrophils to oncospheres by way of their Fc receptors [76].

1.7.2. Cross-reactivity between *C. tenuicollis* and other cestodes

In light of their biological similarities, immunological cross-reactivity occur between *C. tenuicollis* and other cestodes whose life cycles involve sheep as an intermediate host. Despite this fact, *C. tenuicollis* cross-protection against hydatid cysts have been unsuccessful [73].

Studies on cross-reactivity between *C. tenuicollis* and *C. ovis* have produced conflicting results. Varela-Diaz *et. al.*, reported that infections or immunizations with *C. tenuicollis* can induce significant protection against *C. ovis* infections [77]. However, other work found that the presence of *C. tenuicollis* in lambs did not prevent subsequent infection with *T. ovis* [78].

1.7.3. Vaccination against larval cestode infections

In contrast to the situation with the definitive host, taeniid cestodes develop strong concomitant immunity intermediate hosts and in most species immunity is transferred by colostrum. These features have favored the development of practical vaccines against *Taenia* and *Echinococcus*. In the 1977 research done by Rickard *et.al.,* reviewed in [79] published an important advance in the prophylaxis against infection of intermediate hosts with larval taeniid cestodes using the parasite-free supernatant from *in vitro* culture of hatched and activated oncospheres. Recently, a recombinant vaccine against *T. ovis* infection in sheep had been successfully developed using antigens derived from oncospheres [80]. *T. ovis* 45W vaccine (*T. ovis* 45W antigen cloned with β-galactosidase as fusion protein) or GST-45W vaccine (*T. ovis* 45W antigen cloned with *Schistosoma japonicum* glutathione S-transferase (GST) as a fusion protein) with saponin as adjuvant were successful in inducing strong immunity (94% or more) against challenge infection cestode infections [79, 81, 82].

Despite these impressive achievements of recombinant vaccines, many difficulties encountered commercialization and widespread application remain. These difficulties fall into two categories: immunological problems and marketing problems. The immunological problems are (i) antigenic variation in the parasite population and (ii) immunological non-responsiveness by a proportion of sheep to the host-protective vaccine

epitope (s). The marketing difficulties arise because *C. tenuicollis* detected after slaughter. At this time the owner of the carcasses is often not the farmer who raised the stock [79, 82].

1.8. Prevention and control of *C. tenuicollis*

C. tenuicollis do not have serious impact on sheep health. However, they influence on the carcass value at slaughter and cause considerable losses in the sheep industry and thus, restrict market access. *C. tenuicollis* in the internal organs of intermediate hosts are difficult to eliminate with drugs. Therefore, treatment for *C. tenuicollis* in intermediate hosts is not included in any control programme. All control strategies rely on an integrated control programme involving both sheep farmers and dog owners to break the life cycle of *T. hydatigena*.

Sheep farmers should:

1. Prevent the contamination of livestock feed (fresh pasture as well as other stored feed) or water with dog feces that may contain *T. hydatigena* eggs. Additionally, the survival *T. hydatigena* eggs in livestock feed after silage or other processing of hey should be considered.

2. Ensure that carcasses are disposed correctly so farm dogs and any visiting dogs do not have the opportunity for searching and continuing the cycle.

Dog owners should:

1. Not to allow dogs to eat contaminated offal and organs of sheep and goats. If this is not possible, in endemic it is advisable to regularly deworm both farm and guard dogs at least 3 to 4 times a year with a wormer product that control such infections (e.g. praziquantel and epsiprantel).

deworming do not prevent livestock infections with *C. tenuicollis*. There are reports that albendazole and praziquantel are effective, but only at doses higher than the usual therapeutic ones, and results can be unreliable.

2. Prevent dogs from searching or wandering – when not working tie up dogs, keep in a run or kennel.

Remember, after initiating prevention strategies, it is common to see bladder worm on abattoir reports for some time. This is because once infected cysts are present for life. Control could be achieved with time and persistence.

1.9 Objectives

1. To examine the prevalence of *C. tenuicollis* among sheep in Nablus, Nothern part of the West Bank, Palestine.

2. Molecular identification of the *C. tenuicollis* in Nablus, Nothern part of the West Bank, Palestine by PCR amplification of (*rrnS*) and partial sequencing of (*cox 1*) gene.

4. The obtained information will definitely enhance the capacity of the Palestinian Molecular diagnostic laboratories to establish PCR-DNA method combined with DNA sequence analysis for the diagnosis of *C. tenuicollis* in Palestine. In addition, this study introduces preventive measures to reduce unnecessary financial losses caused by carcass condemnations due to *C. tenuicollis* encountered in the ruminant animal industry.

1.10. Justifications

1. The incidence of *C. tenuicollis* in small ruminants according to Palestinian abattoir records by meat inspection.

2. Economic impact attributed to the condemned organs caused by *C. tenuicollis* in small ruminants.

3. The increased interest in the disease as it causes significant economic impacts in different parts of the world.

4. The need of control and prevention program of *C. tenuicollis* infection of small ruminants in Palestine and different parts of the world.

CHAPTER TWO

MATERIALS and METHODS

2.1. Samples collection

During the period of April to June 2014, 1489 sheep slaughtered at the municipal abattoir of Nablus, Northern part of the West Bank, Palestine were visual inspected for the presence of *C. tenuicollis*. The slaughtered sheep were males and females and originated from different parts of the district. To evaluate the effect of age, sheep were classified into two groups: young (less than 1 year) and adult (more than 1 year).

2.2. Parasitological analysis

Visual inspection of the visceral organs was under taken for the presence of *C. tenuicollis*. The number and location of cysts were recorded. Collected cysts were washed with normal saline and transferred into sterile containers for further examinations. *C. tenuicollis* cysts were initially identified according to their feature such as a long-necked single scolex, virtually translucent cyst fluid and rostellar hook morphology [15].

For morphometric analysis, scoleces were mounted in polyvinyl lactophenol with sufficient applied to the coverslip to cause the hooks to lie flat. The scoleces were viewed on light microscope (Light microscopy, Olympus Optical Co., Ltd., Tokyo, Japan) using a (x100) objective lens. Number, length and arrangement of rostellar hooks were done using a 3,0 MPx VisiCam and VisiCam analyzer software. Number of hooks (NH) and

total hook length (TL) of both large and small hooks were done on 10 different scoleces, three large and 3 small hooks per scolex.

2.3. DNA Extraction

The DNA was prepared as described by [83], with slight modifications. Briefly, a part of each individual cyst were cut into small pieces and were lysed in 50 to 60 µl of 0.02 N sodium hydroxide containing 10 to 20 µl of 10 mg/ml proteinase K at 90° C for 30 minutes. After chloroform extraction, DNA samples were used directly for PCR as template DNA.

2.4. PCR and DNA sequencing

Two mitochondrial DNA sequences coding for cytochrome c oxidase subunit 1 (*cox1*) and small subunit of ribosomal RNA (*rrnS*) genes were amplified by polymerase chain reaction (PCR) according to [9].

Primer pairs are (*cox1*) forward 5′-TTT TTT GGG CAT CCT GAG GTT TAT, reverse 5′- TAA AGA AAG AAC ATA ATG AAA ATG; (*rrnS*) forward 5′- AGG GGA TAG GRC ACA GTG CCA GCA TCT GCG G, reverse 5′- AAT TCA TTT AAA GTT ACC TTG TTA CGA CTT ACC TC. Primers (*cox 1*) and (*rrnS*) yield fragments of approximately 446 bp and 558 bp, respectively.

PCR reaction (50 µl) was performed using 2.0 U of Taq DNA polymerase, 1X PCR buffer, 2.5 mM of $MgCl_2$, 0.2 mM of dNTPs, 0.4 µM of each primer and 2 µl of template DNA. DNA amplification was performed using

thermal cycler (Mastercycler Personal, Eppendorf). The PCR conditions were: 5 min at 95ºC (initial denaturation), 35 cycles of 1 min at 95º C, 1min at 50º C and 1 min at 72º C, and finally 5 min at 72º C (final extension). Amplified PCR products using primers *cox 1* were sequenced and the sequences were subsequently have been deposited in the GenBank databases under the accession numbers **KM032284- KM0322303**.

2.5. Sequence homology and phylogenetic analysis

The DNA sequences of (*cox1*) genes of *C. tenuicollis* were compared with previously available sequences from different hosts in NCBI (National Center for Biotechnology Information) using BLAST system. Multiple alignments were done using ClustalW of the Mega 5.05 software. Phylogenetic tree was constructed using the program Neighbor-Joining in the same software. The robustness of the groupings in the Neighbor Joining analysis was assessed with 1000 bootstrap resampling.

2.6. Statistical and bioinformatics analysis

Pearson correlation which was considered significant when $\rho > 0.7$, K-means cluster for morphometric analysis were performed with the program Statistical Package for Social Sciences (SPSS, v. 21). *Cox1* genes were sequenced by dideoxy chain termination method using ABI PRISM sequencer, model 3130 (Hitachi Ltd, Tokyo, Japan). Further comparison with *cox1* gene sequences available in GenBank was made using the BLAST and ClustalW software. Phylogenetic analysis were performed

using the neighbor joining (NJ) method of the Mega 5.05 program. The bootstrap value was set as 1,000 replications with a cutoff value of 50%. Pairwise nucleotide variations of (c*ox 1*) genes and Tajima's Neutrality Test were done using Mega 5.05 program. Tajima's D, and Fu and Li's D test statistic were performed using dnasps program. Tajima's Neutrality Test was considered significant when $\rho > 0.10$.

CHAPTER THREE
RESULTS

3.1. Prevalence of *C.tenuicollis*

Visual inspection of the visceral organs of 1489 sheep carcasses at the municipal abattoir of Nablus, Northern part of the West Bank, Palestine revealed that 32 (2.15%) of the examined sheep were infected with *C. tenuicollis* cysts (Table.3.1). Out of 928 males and 561 females, 24 (2.6%) and 8 (1.4%) were infected, respectively. Among age groups, the prevalence of *C. tenuicollis* was 26 (1.9%) and 6 (4.1%) for young sheep and adult sheep, respectively. Statistical analysis using Z-test revealed no significant effect of age and sex on the prevalence of *C.tenuicollis* (*p* values were 0.06288 and 0.08914, respectively as shown in Table3.1).

Table 3.1: Prevalence of *C. tenuicollis* among sheep examined at the municipal abattoir of Nablus, Palestine

Total number		**Number examined**	**No. (%) infected**
		1489	32 (2.15)
Sex	Male	928	25 (2.7)
	Female	561	7 (1.2)
Age	Young	1343	26 (1.9)
	Adult	146	6 (4.1)

In this study, out of 32 infected sheep, *C. tenuicollis* cysts were found to be more in the liver which was 30 (93.8%) than other organs with statistical significant ($\rho < 0.05$). The number of cysts in each liver of infected sheep

ranges from 1 to 12, with an average diameter of 1.2 cm. Detected cysts were either surrounded by the hepatic tissues (34 cyst) or attached to the liver surface (43 cyst). Most of these cysts were transparent white, with a single scolex appeared as a white spot (Figure 3.1).

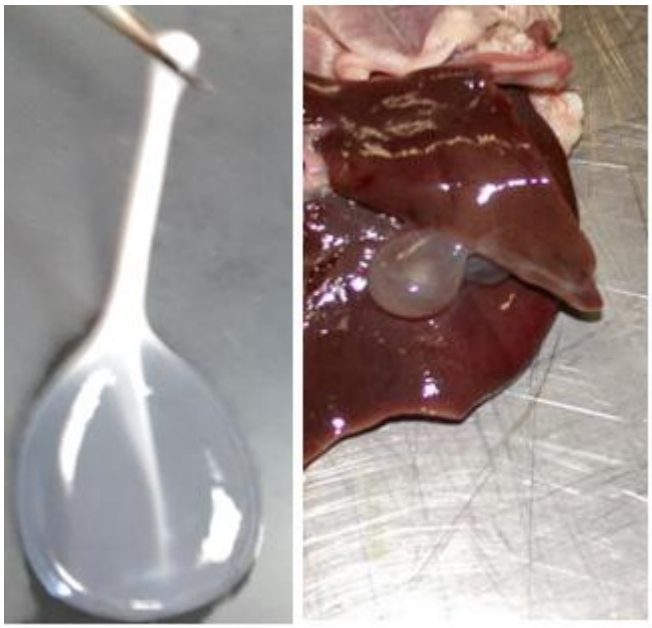

Figure 3.1. *T. hydatigena* cyst from the liver of infected sheep

3.2. Morphometric analysis of larval rostellar hooks

Morphological analysis of the 10 larval rostellar hook indicated that the arrangement of hooks was similar, with two rows of interchanging large and small hooks (Figure 3.2). The means of rostellar hook length are presented in Table 3.2.

Table 3.2. Rostellar hook length measurements of 10 scoleces (mean ± SE., n = 30)

Hooks arrangement	Large and small hooks alternating in 2 rows	
Large hooks	No. of hooks analyzed	30
	Average length (mm)	38.78±10.6
Small hooks	No. of hooks analyzed	30
	Average length (mm)	23.42±6.4

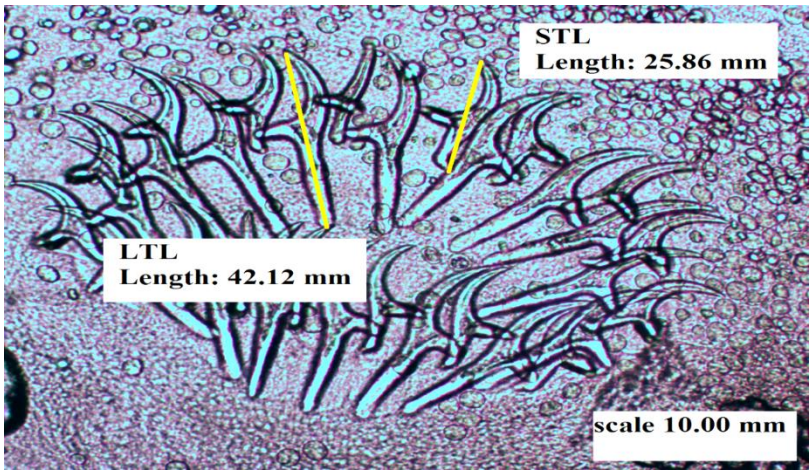

Figure 3.2. Rostellar hooks of *C. tenuicollis* cyst (scale bar = 10 mm).

To quantify the strength of the relationship between the number of hooks and their total length measurements, a correlation analysis was conducted and showed that large and small hook length measurements exhibited a better correlation coefficient score ($\rho > 0.7$) than hook number ($\rho < 0.7$) for strain identification (Figure 3.3).

The discrimination power analysis for rostellar hook length of 10 scoleces computed by Ward's method, a general agglomerative hierarchical clustering procedure revealed just two main clusters (Figure 3.4).

Table 3.3. Correlation for the three variable values, NH: The rostellar hook number, LTL: Long total hook long, STL: Small total hook long. Correlation coefficient, ρ > 0.7 is strong.

Proximity Matrix

	variable Values		
	STL	**LTL**	**HN**
STL	1.000	.943	.538
LTL	.943	1.000	.452
HN	.538	.452	1.000

Figure 3.3. Cluster analysis of Rostellar hook length of 10 scoleces based on the K-means cluster method.

3.3. Sequence analysis of *cox1* gene

Cytochrome c oxidase subunit 1 (*cox 1*) and small subunit of ribosomal RNA (*rrnS*) genes have been described as useful targets for molecular characterization of *C. tenuicollis* species and biovars. The (*cox 1*) and (*rrnS*) genes were amplified by PCR using specific primers. PCR amplification was successfully obtained on all of the isolates (n = 32), for both genes. The amplified DNA fragments size was 446 bp and 558 bp, respectively. A representative gels for the PCR results of both genes are presented in Figure 3.4.

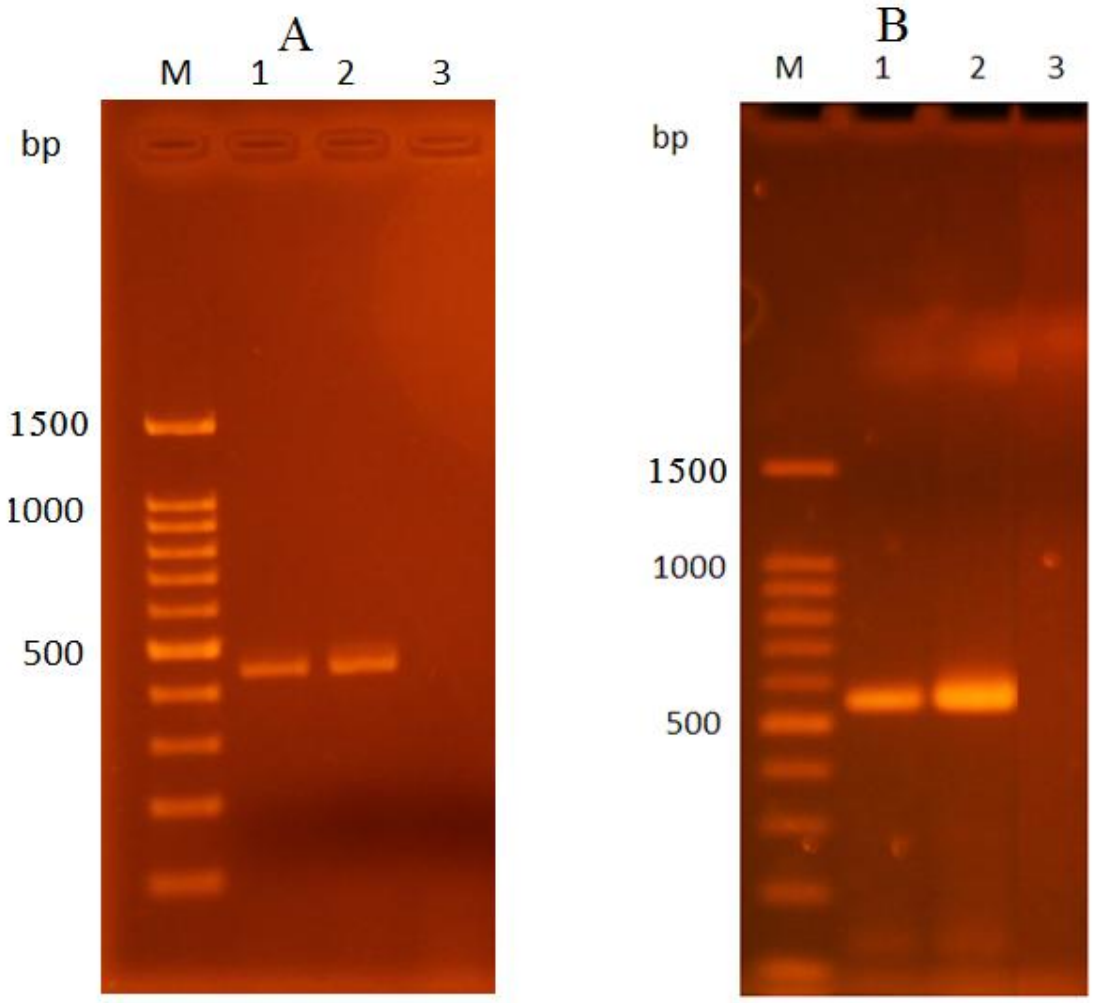

Figure 3.4. PCR of DNA amplification products targeting *C. tenuicollis* DNA. Panel A, PCR products of (C*ox 1*) gene; Panel B, PCR products of (*rrnS*) gene. Lane M, molecular sizes marker (100-bp ladder DNA); Lanes 1 and 2 two *C. tenuicollis* samples collected from the liver.; Lane 3, negative control.

Twenty PCR amplified DNA fragments of the *C. tenuicollis* cysts were used for DNA sequence analysis. For this purpose, partial DNA sequencing of the *cox 1* gene were aligned and compared with other GenBank-accessible gene sequences of *C. tenuicollis* using version 2.0 of BLAST. These sequences were identical to that of the recent sequences of *C. tenuicollis* in the GenBank.

The average nucleotide composition of *Cox 1* sequences in our study was 44.9% (T), 22.6% (A), 23.1% (G), and 9.4% (C), with 67.5% A + T richness. No insertions, deletions, stop codons, sites with alignment gaps or missing data were observed in the tested DNA sequences. Ten variable (polymorphic) sites were detected, including 7 singleton variable sites (SP) at positions 6, 72, 102, 141, 207, 231, 264 and 3 parsimony informative sites (PIP) at positions 51, 213 and 219. The average number of pairwise differences (π) among the *Cox 1* gene DNA sequences determined by Tajima's Neutrality Test was observed to be 0.00445 (Table 3.4).

Table 3.4. Results from Tajima's Neutrality Test

M	*S*	P_s	Θ	π	*D*
20	10	0.029240	0.008242	0.004448	-1.609882

Abbreviations: m = number of sequences, n = total number of sites, S = Number of segregating sites, P_s = S/n, Θ = ps/a1, π = nucleotide diversity, and D is the Tajima test statistic

3.4. Phylogenetic analysis of *cox 1* gene

Phylogenetic relationship of 20 sheep *C. tenuicollis* computed by neighbor joining (NJ) from the partial cox1gene nucleotide sequences shows that there were a total of 9 haplotypes (1-9). As shown in Figure 3.5, Haplotype number one represented 11 *C. tenuicollis* sequences, while the remaining 8 haplotypes contained between one and two *C. tenuicollis* sequences each. The haplotype (gene) diversity, the variance of haplotype ranges from

0.001 to 0.015. Detailed results of nucleotide variation are shown in Figure 3.6.

The neutrality tests were calculated to determine if *Cox 1* gene DNA sequence evolving neutrally or evolving under directional selection. Results showed that Tajima's D=-1.13902 (not significant, $\rho > 0.10$), Fu and Li's D test statistic: -1.91648 (not significant, $\rho > 0.10$).

Phylogenetic tree represented in Figure 3.7 compares the *C. tenuicollis* cysts obtained from the present study with other GenBank-accessible gene sequences of *C. tenuicollis*. A similar topology of *cox 1* phylogenetic tree of our isolates and Iranian *C. tenuicollis*, along with isolates from turkey and Finland was observed, however, the general topology of the *Cox 1* tree is different from other Taeniid isolates.

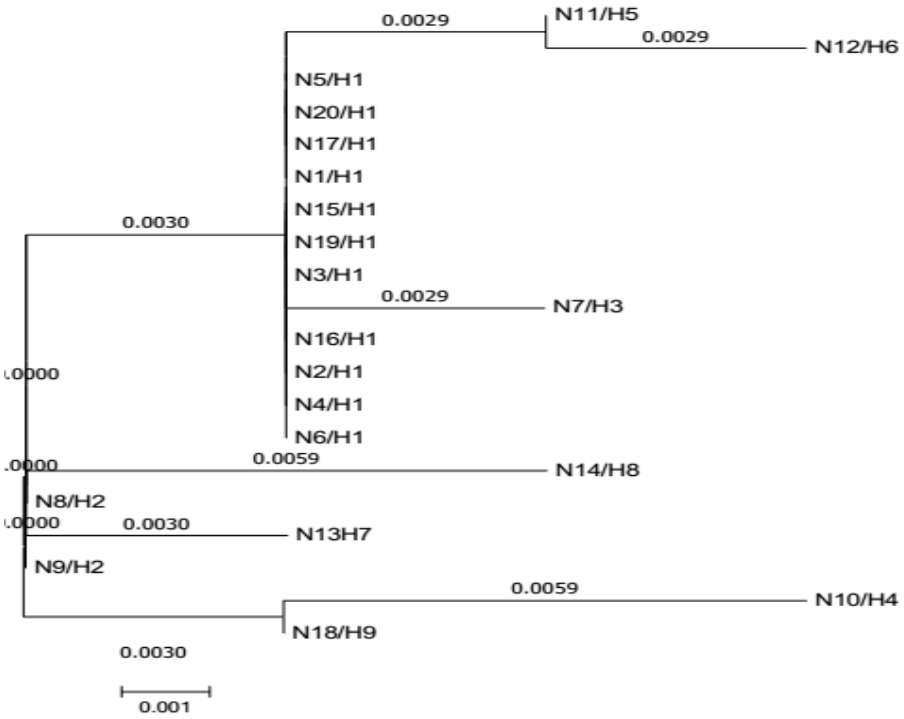

Figure 3.5. Phylogenetic relationship of sheep *C. tenuicollis* computed by neighbor joining (NJ) from the partial (*cox1*) gene nucleotide sequences. Numbers above the branches are genetic distance values. The scale bar represents the estimated number of nucleotide substitutions per nucleotide site.

H9								
H8	**0.009**							
H7	**0.006**	**0.009**						
H6	**0.012**	**0.015**	**0.006**					
H5	**0.009**	**0.012**	**0.003**	**0.003**				
H4	**0.006**	**0.015**	**0.012**	**0.018**	**0.015**			
H3	**0.009**	**0.012**	**0.003**	**0.009**	**0.006**	**0.015**		
H2	**0.003**	**0.006**	**0.003**	**0.009**	**0.006**	**0.009**	**0.006**	
H1	**0.006**	**0.009**	**0.001**	**0.006**	**0.003**	**0.012**	**0.003**	**0.003**

Figure 3.6. Pairwise nucleotide variations between the nine haplotypes of the (*cox 1*) gene DNA sequences. Analysis were conducted using Maximum Composite Likelihood method.

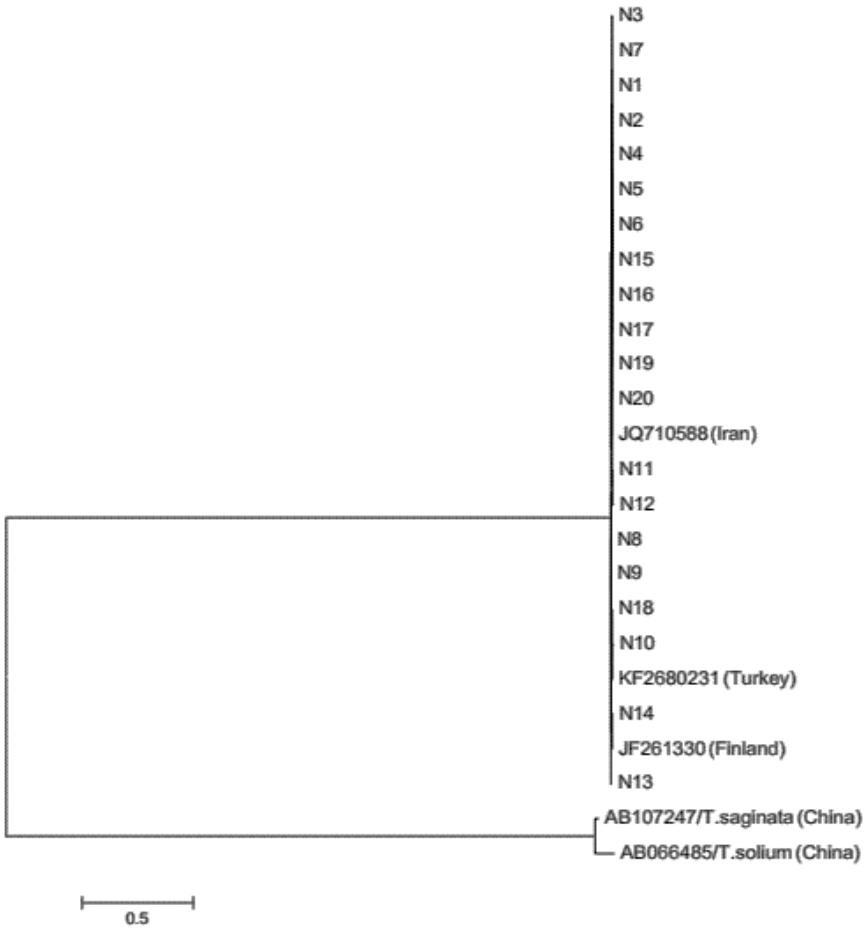

Figure 3.7 . Phylogenetic relationship of sheep *C. tenuicollis* computed by neighbor joining (NJ) from the partial (*cox1*) gene nucleotide sequences, using *T. solium* as an out group strain. The scale bar represents the estimated number of nucleotide substitutions per nucleotide site.

CHAPTER FOUR
DISCUSSION

C.tenuicollis is a serious disease of herbivores with a worldwide dissemination caused by *T. hydatigena* larvae. The disease is manifested by hepatitis cysticercosa in infected sheep. In heavy infections, migration of larvae destroy the hepatic cells, leading to severe inflammation that may be fatal [5]. In recent years, more concern are given to *C. tenuicollis* because of its significant financial implications due to condemnation of offal's containing these larvae, particularly in resource-poor countries [6, 7].

In Palestine, diagnosis of *C. tenuicollis* in animals is based mainly on meat inspection. Up to now, no article has documented the prevalence and molecular characterization of the circulating *C. tenuicollis* in slaughtered sheep in Palestine. This study is the first to address the prevalence and to overview the genotype compositions of *C. tenuicollis* in the northern part of Palestine. The data revealed in this study will give valuable information considering the prevalence of *C. tenuicollis* in Palestine. Moreover, molecular identification of these cysts are vital for implementing control and preventive measures.

A total of 1489 sheep slaughtered at the municipal abattoir of Nablus, Northern part of the West Bank, Palestine were inspected for the presence of *C. tenuicollis*.

The overall prevalence of *C. tenuicollis* in sheep was 2.15%. This is however lower than expected assumed and much lower than those reported from other countries including Nigeria [42] with a prevalence of 13.03% in sheep; Ethiopia [44] with 22.8%; India [66] with 15.17%; Germany [62] with 16.7%.

The prevalence rates for *C. tenuicollis* in nearby countries in the Middle East area were also compared to our results and it was found that rates were higher than or nearly the same as in our study. The rates were: 2% in Iraq [65]; 9.2% in Jordan [84]; 23.27% in Egypt [42]; 1.25% in Saudi Arabia [85] and 26.7% in Turkey [63].

The low prevalence of *C. tenuicollis* in Palestine is probably due, in part, to the grazing behavior and management system in this country [37]. The study area being Nablus area, the sheep pasture-grazing is almost non-existent because the landscape are unsuitable and the lack of rainfall. Thus, animals are owned by farmers under traditional management system, smallholder and backyard management system. In this system, animals are confined at the backyard and fed with cut herbage or prepared straw hey. Secondly, slaughter of animals and disposal of viscera and trimmings is done at the municipal abattoirs. This is very important for the life cycle of *T. hydatigena* not to maintain between the final and intermediate hosts and could also be reason for the low prevalence rate.

Finally, the geographical location of the West Bank, which is between the 31°21` and 32°33` latitude and between 34°52` and 35°32` longitude may

largely contribute to the low prevalence of *C. tenuicollis*. This location makes the area highly influenced by the Mediterranean climate, which is characterized by a long, hot, dry summer and short, cool, rainy winter. These conditions have a significant effect on the viability of the *T. hydatigena* eggs as mentioned elsewhere [30].

It is noteworthy that some limitations of the present study should be considered. Small sample size and sampling period i.e., beginning of summer limit generalization of the prevalence rate observed in our study. Additionally, not including other parts of Palestine are the other associated limitations of our study. Therefore, further studies on larger sample size, including other parts of Palestine in dry and wet seasons of the year are needed in the future.

Also, the present study evaluated the influence of sex, and age of the host on C. *tenuicollis* infection. No statistical significant effects were observed for these two factors on the prevalence of *C. tenuicollis* infection. The dependence of examined sheep on the same grazing system, which leads to equal exposure and opportunity to get the infective eggs of *T. hydatigena* could be considered as a major cause for this issue. The prevalence of *C. tenuicollis* in livers was higher than that in different organs of sheep. Similar observation was reported elsewhere [44]. A possible explanation is that oncospheres are transported to the liver via the portal system, where they mature or, in certain species, may lie dormant [24] suggested that a species- specific stimulus may be present in the organ of predilection of

the host and that may cause the oncospheres to stop there.

The results of scoleces hook morphometry, including arrangement, number and total length (TL) of both large and small hooks were in agreement with rostellar hook characters found in *C. tenuicollis* cysts described previously [2, 86]. It is interesting to note that differences of the total length of hooks showed a better degree of correlation coefficient ($\rho > 0.7$) than hook number ($\rho < 0.7$) for strain identification. However, it worth mentioning that morphometric data based on the length of hooks exhibited low discrimination power, since these data were capable of identifying just two main clusters among the examined scoleces. Moreover, it appears that rostellar hook length could not differentiate between haplotypes 2 and 3 isolates, indicating notable morphological similarities between the two genotypes. Therefore, in the light of these results, the use of rostellar hook length for taxonomic study is inadequate. To this end, molecular methods are required to examine the intraspecific variation of *C. tenuicollis*.

To solve unresolved issues with regards to rostellar hooks length, 20 DNA sequences generated from the *Cox1* gene from 20 individual *C. tenuicollis* were evaluated as a molecular markers for taxonomic phylogenies of *C. tenuicollis*. In this study, the AT-richness of *Cox1* was 67.5%, which was close to percentages of AT bases in the mitochondrial genomes of Taeniid species [9, 17].

The results of our study showed relatively low level of nucleotide variations in (*cox 1*) within the studied isolates (nucleotide diversity Pi (π),

0.00445), suggesting that there was low genetic variation of our isolates. This variation was lower than the values reported in Iran and Poland [13]. Taking into account the conservative nature of (*cox1*), the low genetic diversity within the Palestinian isolates is quite possible because of the lower prevalence rate of *C. tenuicollis*, smaller geographical area and less population size of livestock in Palestine. In conclusion, the low genetic variations observed in this study, provided a good genetic background for future development of prevention and control measures.

Although our pairwise nucleotide variation analysis did not support the existence of defined genetic variants within the isolates, phylogenic analysis have demonstrated the existence of nine *cox1* haplotype with sequence variation of 0.001 - 0.015. Despite this fact, the values of Tajima's D, Fu and Li's D score in this study strongly suggested that *Cox1* gene of *C.tenuicollis* isolates evolving neutrally. Furthermore, the phylogenic tree (Figure 3.7) revealed that the Palestinian *C. tenuicollis* haplotypes were 100% identical to those from Iran and clustered in one clade, along with isolates from Turkey and Finland. These results confirm circulation of *C. tenuicollis* in different geographical regions.

CONCLUSIONS

This study aimed to estimate the prevalence of *C. tenuicollis* infection among sheep and identify the molecular characteristics of *C. tenuicollis* infection in Palestine.

Conclusions drawn from the findings are as follows:

1. The overall prevalence of *C.tenuicollis* in sheep was 2.15%. Infection, with the liver having the highest prevalence (93.8%).

2. Morphometric data of rostellar hooks of *C.tenuicollis* isolates were not strongly associated with the variability in mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene

3. The results of sequence analysis showed that the (cox1) gene sequences were highly conserved among the examined *C.tenuicollis* isolates. However, they were quite different from those of the other Taenia species.

4. Phylogenetic analysis computed by neighbor joining (NJ) from the partial (*cox1)* gene nucleotide sequences revealed that *C.tenuicollis* isolates were composed of 9 haplotypes, clustered in one clade, along with isolates from Iran, Turkey and Finland.

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جامعة النجاح الوطنية

كلية الدراسات العليا

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قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في العلوم الحياتية في كلية الدراسات العليا في جامعة النجاح الوطنية، نابلس- فلسطين.

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الملخص

Cysticercus tenuicollis الكيسات المذنبة دقيقة العنق هي الطور اليرقي من الدودة الشريطية للكلاب (*Taenia hydatigena*) والتي تم تسجيل حالات منها في الحيوانات الأليفة والحيوانات المجترة البرية. الإصابة بالكيسات المذنبة دقيقة العنق قد يسبب مشاكل صحية للحيوانات الأليفة والمجترة والتي بدورها تسبب خسائر اقتصادية في مجال صناعة اللحوم. لا يوجد دراسات مسبقة تبين مدى انتشار الإصابة بالكيسات المذنبة في فلسطين. لذلك، فان الهدف من هذه الدراسة هو تقدير مدى إصابة الأغنام بهذه الكيسات وتصنيفها وراثياً. وقد أجريت هذه الدراسة في الفترة الواقعة بين شهر نيسان وحتى نهاية تموز من سنة 2014. تم فحص 1489 عينه من الأغنام التي تم ذبحها في مسلخ بلدية نابلس، شمالي الضفة الغربية، فلسطين. أظهرت الدراسة الأولية أن نسبة الانتشار العام لهذه الكيسات هو 2.15%. وأظهرت النتائج أن متوسط طول الخطافات الكبيرة والصغيرة المتواجدة على الرؤوس البدائية لهذه الكيسات هو 38.78 ملم و23.42 ملم، بالترتيب. باستخدام التحليل الإحصائي تبين أن طول الخطافات الكبيرة والصغيرة مرتبط بشكل ضعيف مع الجين الموجود في المايتوكندريا والمعروف ب cytochrome c oxidase subunit 1 (*cox1*). تم مضاعفة الجين small subunit ribosomal RNA (*rrnS*)) باستخدام تقنية تفاعل تسلسل البلمرة وايجاد تسلسل الحمض النووي للجين (*cox1* gene) في 20 عينة. تم تحديد 10 مواقع متغيرة وتشمل 7 (singleton variable site (SP)) في المواقع التالية 6 72 102 141 207 231 246 و3 ((PIP) Parsimony) في المواقع 51 213 219. ان معدل الاختلاف في سلاسل الجين (*cox1*) قد بلغ 0.0045. أظهرت التحليلات المعلوماتية الحيوية والمتمثلة في اختبارات الحياد (Tajima's D and Fu

and Li' s D) أن تطور الكيسات المذنبة دقيقة العنق هو تطور نمطي طبيعي ومحايد. ان هذه النتائج سوف تساعد بشكل كبير في مراقبة واتخاذ الإجراءات الوقائية من الاصابة بالكيسات المذنبة دقيقة العنق في فلسطين. إضافة إلى ذلك أظهرت التحليلات المعلوماتية للجين (*cox1*) أن عزلات الكيسات المذنبة تنتمي إلى 9 سلالات متنوعة اختلفت عن أنواع (Taenia) الأخرى.

كذلك تبين أن سلالات هذه العزلات قد تشابهت مع العزلات التي تم دراستها في كل من ايران، تركيا و فنلندا. مما يؤكد ان انتشار هذه السلالات في مناطق جغرافية مختلفة.