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Molecular identification and phylogenetic analysis of *Cysticercus tenuicollis* isolated from sheep in Mosul city, Iraq

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Abstract

The study aimed to determine the infection rate in sheep, analyze the correlation between the rate and certain risk factors, identify larval stage distribution in visceral organs, and provide a molecular characterization of *Cysticercus tenuicollis*. Ninety-six slaughtered sheep and their offal were visually inspected between February and July 2024. Sheep were categorized based on their sex and age. Cysts were observed in the diaphragm, liver, lung, mesentery, and omentum after the animals were slaughtered. The 12SrRNA gene was amplified and sequenced. For statistical analysis, the Chi-square test was applied. The overall infection rate was 16.66% (16/96). Males showed a higher rate 17.8% than females 13.04%, with a statistically significant. The rate was higher in adult males 21.56% and females 14.28% than in young males 9.09% and females 11.11%. March had the lowest rate 6.66%, and June had the highest 31.57%. The most common site of *C. tenuicollis* cyst was in the omentum 68.75%, followed by mesentery 31.25%. Successful 12SrRNA gene amplification was achieved in all 16 positive cases, and the amplified products were 490 bp. GenBank accession numbers were registered. Genetic relatedness revealed that the local *C. tenuicollis* isolates were more closely related to isolates from Sulaymaniyah and Iran. The findings showed that sheep are commonly infected with cysticercosis, which may result in financial losses due to organ condemnation. Thus, it is important to appropriately dispose of butcher materials as they could have contributed to the disease prevalence.

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Introduction

The larval stage of *Taenia hydatigena* is known as *Cysticercus tenuicollis*. In order to complete its life cycle, *T. hydatigena* needs two different hosts. Dogs and other wild carnivores are definitive hosts where the adult parasites live in their intestines. The intermediate hosts become infected by consuming ova from contaminated pasture. These hosts are typically sheep, goats, and, less frequently, cattle and other wild species (1,2). Eaten ova develop in the intestinal tract and later develop cysticerci migrating to the liver and further visceral organs such as the heart, lung, and kidney (3). Mature cysticerci often exist in the mesentery, peritoneum, omentum, and, less commonly, the pleura and pericardium (4). The clinical manifestations in an intermediate host differ based on the level of infection. Most infections are chronic, asymptomatic, and typically undetectable until after slaughter (5). A significant number of larvae migrate during severe infections, resulting in peritonitis, pneumonia, and severe traumatic hepatitis. Furthermore, signs of colic, appetite loss, emaciation, and unthriftiness may be present (6). Lambs with severe hepatic and pulmonary infections have been reported to have a 19% death rate (7). Consequently, *C. tenuicollis* infection can be deleterious to intermediate hosts and cause financial losses for the meat industry (8,9). It has been demonstrated that the prevalence of this cestode infection varies depending on the geographical location. The percentage of sheep infected in

Egypt, Brazil, Germany, and Australia varied from 11.4% to 19% (9,10). The infection percentages in sheep and goats in Turkey are more extensive, ranging from 56.8 to 65.6%, respectively (11). Furthermore, cysticercosis is a prevalent infection of small ruminants in Iran. According to one study, 12.87% of sheep were assessed to be infected with *C. tenuicollis* (12). In Iraq, the prevalence of *T. hydatigena* was well-documented; nevertheless, the studies all addressed infections in intermediate hosts (13-19). According to research conducted at a slaughterhouse in Mosul, the occurrence of *C. tenuicollis* in sheep, goats, and cattle was 2, 10, and 6%, respectively (20). Various serological tests have been employed because *T. hydatigena* immunity is largely antibody-mediated. The sensitivity and specificity of these tests vary because of their cross-reactivity with other parasites (21). Additionally, inspecting the meat at slaughterhouses provides a preliminary sign of the presence of *C. tenuicollis* in a region. However, this approach is insensitive, particularly for carcasses with minor infections or if the cysts are small or form too soon. Therefore, meat inspection methods only detect 20–50% of diseased animals (22). While DNA-based techniques are not a substitute for meat inspection, combining the two approaches will be most helpful in confirming the presence of C. tenuicollis infection. Molecular techniques are also crucial in cases where morphological features make it difficult to distinguish C. tenuicollis from other metacestodes, such as hydatidosis, which has a completely different pathogenicity and necessitates various control programs (23,24). Studies on the genetic characterization of T. hydatigena have been conducted globally (25,26).

The molecular description and genetic relationship of *T. hydatigena* have not yet been determined in Mosul city; more research is necessary to offer a thorough understanding of this taeniid member. Thus, the study aimed to determine the infection rate of *C. tenuicollis* in slaughtered sheep at butcher shops in Mosul, analyze the correlation between infection rate and certain risk factors, identify the distribution of *C. tenuicollis* in visceral organs of sheep, provide molecular characterization and genetic relatedness of *C. tenuicollis* via 12SrRNA gene amplification, sequencing, and phylogenetic analysis.

Materials and methods

Ethical consent

Under approval issue number UM.VET.2024.062, the sample collection methods were approved in January 2024.

Sample collection

A total of 96 sheep (73 males; 23 females) from various regions in Mosul city that were slaughtered at butcher shops between February and July 2024 were visually inspected. The slaughtered sheep were native breeds and divided into two groups, young (one-year-old or less) and old (greater than one year), to assess age's impact. Months of samples were also taken as a risk factor for *C. tenuicollis*. Following the slaughter, the diaphragm, liver, lung, mesentery, and omentum were checked for the presence of *C. tenuicollis* cysts.

Visual examination of *C. tenuicollis* cyst

The cysts were counted, and their locations were noted. After being cleaned with regular saline, collected cysts were placed in sterile containers for further testing. Initially, characteristics of *C. tenuicollis* cysts, such as a long-necked alone scolex, almost transparent cyst fluid, and rostellar hook shape, were used to identify them (27).

Genomic DNA extraction

According to the manufacturer's guidelines, the DNA tissue extraction kit (Geneaid, South Korea) was used to extract the cysts' whole genomic DNA. Until it was used, DNA was stored at -20°C.

PCR and gel electrophoresis

The 12SrRNA gene of *C. tenuicollis* was amplified using PCR. Six microliters of nuclease-free water, two microliters of DNA, ten microliters of master mix (AddBio Inc., South Korea), and one microliter each of the forward (5'-AGGGGATAGGACACAGTGCCAGC-3') and reverse (5'-CGGTGTGTACATGAG CTAAAC-3') primers were included in a 20 µl (28). Amplification was carried out under these conditions using a thermocycler (Bio-Rad, USA): one cycle of 95°C for 10 minutes, followed by 35 cycles of 45 seconds at 95°C, 45 seconds at 61°C, and 45 seconds at 72°C. After that, a cycle of 72°C for seven minutes was chosen for the final extension. The processes were ultimately cooled to 4°C. The amplified products were verified using a 1.5% agarose gel prepared with 1x Tris-Borate-EDTA buffer and a red-safe DNA colouring solution (GeNetBio, South Korea). A digital camera (Bio-Rad, USA) and UV transilluminator were employed to view the results. A 100 bp DNA marker (AddBio Inc., South Korea) was added for each electrophoresis.

Sequencing and phylogenetic analysis

After PCR amplification, the amplicons were sequenced (Macrogen, Korea). Using BLAST, the obtained gene sequences were compared with *C. tenuicollis* 12SrRNA gene sequences from other countries that had already been recorded in the GenBank. Using MEGA 5.5 software, multiple alignments were carried out using the MUSCLE program. The neighbour joining (NJ) method of the MEGA program was used to carry out the phylogenetic analysis of the 12SrRNA gene. One hundred bootstrap resampling was used to evaluate the robustness of the groups in the neighbour-joining tree (29,30).

Statistical analysis

The Chi-square test was carried out using the SPSS V25 software, taking into account all of the data that had been analyzed and setting the significance threshold at P≤0.05 (31).

Results

Rate of infection according to sex and age

Out of 96 sheep, 16 had *C. tenuicollis* cysts found after postmortem examination, representing a 16.66% overall infection rate (Table 1). There was a statistically significant difference in the infection rates between males 17.8% and females 13.04%. There was a significant difference in infection rates between the two age groups. Compared to young males 9.09% and females 11.11%, adult males 21.56% and females 14.28% had higher infection rates (Table 1).

Table 1: Rate of *C. tenuicollis* infection in slaughtered sheep according to sex and age

Sex	Age	Total examined	Positive n (%)
Male	Young	22	2(9.09)[a]
	Adult	51	11(21.56)[b]
	Total	73	13(17.80)[d]
Female	Young	9	1(11.11)[a]
	Adult	14	2(14.28)[c]
	Total	23	3(13.04)[c]

Vertical letter differences are significant at P≤0.05.

Rate of infection according to month of sampling

June 2024 was found to have the highest recorded infection rate of *C. tenuicollis* cysts (31.57%; 6 out of 19 positive cases) during the study period. The lowest infection rate of 6.66% (1 out of 15) was recorded in March. There were noticeable variations over the months, and the infection rates in February, April, May, and July were 8.33, 14.28, 14.28, and 20%, respectively (Figure 1).

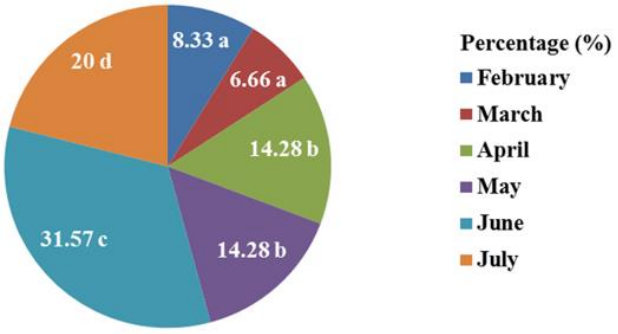

Figure 1: Infection rate with *C. tenuicollis* according to month of sampling. Letter differences are significant at P≤0.05.

Occurrence of *C. tenuicollis* in the abdominal cavity of slaughtered sheep

The results revealed that of 96 slaughtered sheep, the omentum (68.75%; 11 out of 16 positive cases) and the mesentery (31.25%; 5 out of 16) had a higher number of *C. tenuicollis* cysts than the diaphragm liver, and lung 0%, which had no cysts reported with statistically significant. The cyst characteristics were a thin, transparent, milky white wall and a white to yellowish fluid. One to three cysts, each with an average size of 3 to 6 cm, were found in each omentum and mesentery of infected slaughtered sheep (Figure 2).

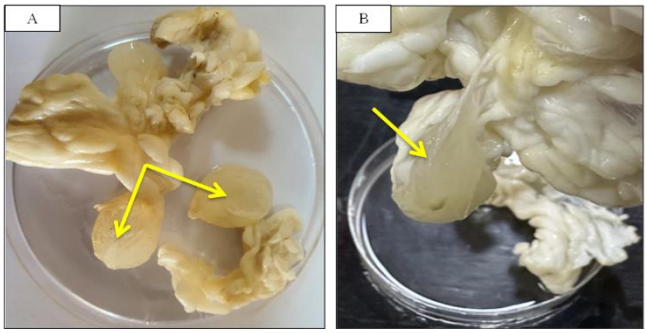

Figure 2: *Cysticercus tenuicollis* cysts differed in size and position in the omentum (A) and the mesentery (B) (arrow).

Molecular characterization of *C. tenuicollis*

All isolates (16 positive cases) had successful 12SrRNA gene PCR amplification. The final amplified products were about 490 bp (Figure 3).

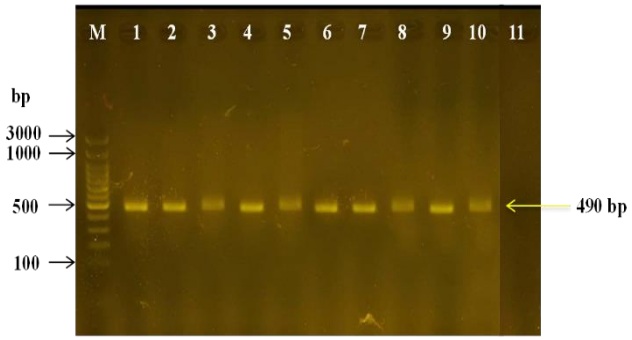

Figure 3: Electrophoresis of PCR products (490 bp) targeting *C. tenuicollis* 12SrRNA gene on the agarose gel (1.5%) stained with 3µL red safe DNA colouring solution. Lane M is the 100-bp DNA marker; positive samples are found in lanes 1 to 10; negative control is found in lane 11.

Sequencing and phylogenetic analysis

The local isolates of *T. hydatigena* with accession numbers LC746810.1 and LC749827.1, identified by sequence analysis of the 12SrRNA gene, exhibited 100% similarity with the previously published isolates from

Sulaymaniyah-Iraq (AM.Suli-16; MK858248.1) and *T. hydatigena* voucher MFTH 0053 (JQ717215.1) from Iran, which also demonstrated 100% similarity (Table 2). However, the local isolate exhibited 99.79% similarity to the Chinese isolate (MT784896.1). A 99.77% similarity was observed between the sequence of the local isolate and the published *T. hydatigena* isolates from Sulaymaniyah- Iraq, Iran, and Egypt, respectively, with accession numbers MK858233.1, KU745527.1, and OL470131.1. Furthermore, it was shown that the local isolate and the Bangladeshi *T. hydatigena* 12SrRNA gene sequence (LC672189.1) had 99.59% identity. Accession number MK858250.1 of *T. hydatigena* was determined to be 99.54% identical to the local isolate. The Iranian 12SrRNA gene sequence (JQ717246.1) showed 99.49% identity with the local isolate. With accession numbers OR063932.1, FJ518620.1, MK858249.1, JQ717211.1, and KX084714.1, the local isolate sequencing indicated 99.38%, 99.31%, 99.23%, and 99.1% recognition with previously reported *T. hydatigena* strains from Iraq, China, Sulaymaniyah-Iraq, and Iran, respectively. The local isolate's identification was 97.64% and 95.46% similar to known isolates of *T. hydatigena* from Egypt and Japan, respectively, with accession numbers OL470128.1 and NC024589.1 (Table 2).

Table 2: Sequence identity between local *Taenia hydatigena* and other isolates

	Accession number	Country	Identity (%)
1	MK858248.1	Iraq	100
2	JQ717215.1	Iran	100
3	MT784896.1	China	99.79
4	MK858233.1	Iraq	99.77
5	KU745527.1	Iran	99.77
6	OL470131.1	Egypt	99.77
7	LC672189.1	Bangladesh	99.59
8	MK858250.1	Iraq	99.54
9	JQ717246.1	Iran	99.49
10	OR063932.1	Iraq	99.38
11	FJ518620.1	China	99.38
12	MK858249.1	Iraq	99.31
13	JQ717211.1	Iran	99.23
14	KX084714.1	Iran	99.1
15	OL470128.1	Egypt	97.64
16	NC024589.1	Japan	95.46

Additionally, eighteen 12SrRNA gene sequences from various *T. hydatigena* isolates were combined into a neighbour-joining phylogenetic tree. The tree's confidence was ensured by using a 100 times bootstrap value. These sequences' phylogenetic analysis mostly identified clades among the 12SrRNA gene sequence members (Figure 4). Based on the generated phylogenetic tree, it has been determined that the 12SrRNA gene is preserved in all *T. hydatigena* isolates, and the local isolates were more closely related to AM.Suli-16 (MK858248.1) and *T. hydatigena* isolate LE-49s-f (KX084714.1) from Sulaymaniyah-Iraq and Iran, respectively (Figure 4).

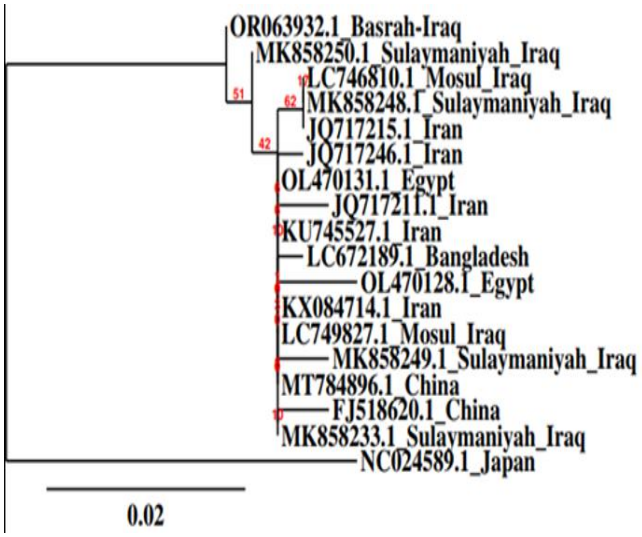

Figure 4: Neighbour-joining phylogenetic tree between local *T. hydatigena* isolate (LC746810.1, LC749827.1) and other isolates have recorded in the GeneBank.

Discussion

In the present investigation, 16 of the 96 sheep examined had an overall infection rate of 16.66% due to *C. tenuicollis*. When compared to other earlier results from our country, this percentage was seen to be relatively high. According to Al-Saqur and Gorani's (32) findings, 1% of sheep in Basrah were infected with *C. tenuicollis*, and Abass and Rahif found that 14.22% of the sheep at the Baghdad slaughterhouse were infected (33). Moreover, 32 sheep (7.4%) out of 427 at the Al-Diwania slaughterhouse had cysticercosis (14). In another research, 13395 sheep were examined at the Sulaimani Abattoir, and the prevalence of *C. tenuicollis* was 2.63% (34). Research conducted in Mosul between 2009 and 2010 at the slaughterhouse and surrounding meat markets found that 2% of sheep in the area had *C. tenuicollis* (20). Conversely, the percentage attained is lower than the previously reported results in the governorates of Baghdad and Karbala, which were 21 and 32.5%, respectively (15,18). According to a comprehensive review of published research, geographical differences exist in tenicollosis infection. In Iran, the infection rate in sheep was 12.87%, but in Jordan it was 9.2%. According to studies conducted in Turkey, the infection rates varied from 12.13 to 56.7% (12,35-37). *Cysticercus tenuicollis* has also been recorded by many researchers from other parts of the world. There have been reports of a 37.03% in sheep in India and a 21.4% in Nigeria among sheep. In Germany, 16.7% of sheep had the infection. Of the 600 sheep tested in Ethiopia, 223 (37.2%) had *C.*

tenuicollis (38). Factors such as temperature variations, environmental conditions, pasture contaminating levels, and the methods used to raise and graze these animals which could facilitate the spread of the disease among ruminants and other dogs, as well as the existence of stray dogs in pastures and near slaughterhouses may all be considered to explain variations in prevalence (39-43). In the current study, there was a statistically significant difference in the infection rates between males 17.8% and females 13.04%. This finding is consistent with studies by Saulawa *et al.* (44), who found that the prevalence was 13.66% in males and 11.54% in females, and Mekuria *et al.* (22), who found that male animals had a higher rate 25.5% than female 23.6%. This finding was at odds with that of Omar *et al.* (10), who found that the prevalence was greater in female sheep, 17%, compared to 7% in males. Additionally, Mirzaei and Rezaei (45) reported that the infection rate was greater in female sheep at 6.54% than in males at 2.27%. The differences in results based on the sex of the animals were caused by variations in physiological and business-related, where most males were chosen for weight gain and females for reproduction. As a result, the difference may be attributed to the fact that a majority of males are slaughtered (46-50). Sheep in the present investigation were divided into two ages (young and adult). Compared to young males 9.09% and females 11.11%, adult males 21.56% and females 14.28% had higher infection rates with a statistical difference. This finding is consistent with the findings of Haddawee *et al.* (15), who reported a prevalence of 27.5% in young sheep and 37.5% in adult sheep, as well as Guadu *et al.* (51), who reported greater infection rates in older sheep 38% compared to young 34.6%. Considering that adult sheep have a longer lifetime and eat more eggs when grazing than young sheep, it is possible that this explains the observed variance in infection rates between the two age groups of sheep (52-54). The findings also revealed that the highest infection rate of *C. tenuicollis* cysts was observed in 2024 June 31.57%, while the lowest rate was observed in March 6.66%, with a statistically significant difference. The present findings are consistent with those of Al-Sudani and Al-Amery (18), who found that the lowest rate 10% was identified in March, while the highest rate 40% in sheep was verified in June. However, the findings differed according to Ghaffar (55), who reported that the highest prevalence was recorded in February 1.4% and the lowest in June and July 0.3%. Month-to-month variations in infection rates may be caused by changes in humidity, temperature, or the degree of grazing field contamination caused by stray dogs' unrestricted movement. Uncontrolled dog movement on lush pastures and near food storage also contributed to a rise in the infection rate in sheep (56-58). During this study, it was found that *C. tenuicollis* was mainly predisposed to the omentum and mesentery. Other investigations showed similar results, indicating that the mesentery has the second largest incidence of *C. tenuicollis* and that the omentum is the prevalent preference location (59-61). However, the result was at odds with Mirzaei and Rezaei's (45) findings, which showed that the liver had a notably higher prevalence of cysts than other organs. The omentum has a larger surface area than other tissues in the peritoneal cavity, which may be the explanation for *C. tenuicollis* attraction to it (45). The results also showed that the 12SrRNA gene had been effectively amplified in all positive cases using PCR. After amplification, the final products were around 490 bp. The most commonly employed gene for molecular analysis, studying the phylogeny, and development of parasites is 12SrRNA (62). The local isolates were compared phylogenetically to the reference isolates in the Genebank using the 12SrRNA gene sequence. The results demonstrated that the local isolates were closer to AM.Suli-16 and *T. hydatigena* isolate LE-49s-f from Sulaymaniyah-Iraq and Iran, respectively. This might be because Sulaymaniyah province and Iran, near Mosul city, have similar temperatures and animal habitats. Therefore, knowing the parasite's genetic identification will be essential for controlling this parasitic disease (63). The local isolates showed different sequence identities from others recorded in China, Bangladesh, Egypt, and Japan. The parasite seems to have been crossing the world for an extended time via animal transportation. Transmission across intermediate hosts may potentially enhance the possibility of genetic variation among various worldwide parasite species (64-66).

Conclusion

There was a notable rate of *C. tenuicollis* infection in slaughtered sheep at butcher shops in Mosul. It was also seen that the cyst was distributed throughout the omentum and mesentery. The three most important risk factors for cysticercosis are sex, age, and sample month. Genetic relatedness revealed that the local *C. tenuicollis* isolates were more closely related to isolates from Sulaymaniyah-Iraq (MK858248.1) and Iran (KX084714.1). Incorrect handling of butcher shop waste and giving dogs off from infected sheep have contributed to the prevalence of the disease in the area.

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Conflict of interest

The authors declare that they have no conflicts of interest related to the publication of this work.

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الكشف الجزيئي والتحليل الوراثي للكيسانية المذنبة رقيقة العنق المعزولة من الضأن في مدينة الموصل، العراق

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الخلاصة

هدفت الدراسة الحالية إلى تحديد نسبة الإصابة في الضأن، تحليل العلاقة بين نسبة الإصابة وبعض عوامل الخطورة، تحديد تواجد الطور اليرقي في الأعضاء الحشوية، والتوصيف الجزيئي للكيسانية المذنبة رقيقة العنق. تم فحص ٩٦ من الضأن المذبوحة وأحشائها عيانياً للفترة الممتدة من شباط الى تموز ٢٠٢٤. تم تصنيف الضأن بناءً على جنسها وعمرها. فحص الحجاب الحاجز، الكبد، الرئة، المساريق، والغشاء المعوي الشحمي بعد ذبح الحيوان للتحري عن تواجد الكيسانية المذنبة رقيقة العنق. تم تضخيم جين 12SrRNA وإيجاد تسلسلهِ. استخدم اختبار مربع كاي لغرض التحليل الإحصائي. كانت نسبة الإصابة الإجمالي ١٦،٦٦٪ (٩٦/١٦). أظهر الذكور معدل أعلى ١٧،٨٪ من الإناث ١٣،٠٤٪، مع وجود فرق معنوي. كانت نسبة الإصابة أعلى في الذكور البالغين ٢١،٥٦٪ والإناث ١٤،٢٨٪ مقارنة بالذكور صغار العمر ٩،٠٩٪ والإناث ١١،١١٪. أقل نسبة للإصابة سُجلت في أذار ٦،٦٦٪ وأعلى نسبة في تموز ٣١،٥٧٪. أعلى نسبة لتواجد الكيسانية المذنبة رقيقة العنق في الغشاء المعوي الشحمي ٦٨،٧٥٪ يليه المساريق ٣١،٢٥٪. ضُخم الجين 12SrRNA بنجاح من جميع العينات الموجبة والتي كان عددها ١٦ وبحجم ٤٩٠ زوج قاعدي. سُجلت تسلسلات الجين في بنك الجينات. أظهرت العلاقة الورائية بأن العزلات المحلية للكيسانية المذنبة رقيقة العنق كانت أكثر ارتباطا بالعزلات المسجلة في السليمانية وإيران. بينت النتائج بأن الضأن مصابة بشكل كبير بداء الكيسات المذنبة وقد يؤدي ذلك إلى خسائر اقتصادية بسبب إتلاف الأعضاء المصابة. لذلك، فمن المهم التخلص بشكل صحيح من مخلفات محلات الجزارة، لأنها تساهم في انتشار المرض.