Infection of Egyptian domestic rabbits, Oryctolagus cuniculus, with Cysticercus pisiformis (Cestoda: Taeniidae): morphological, molecular, and histopathological diagnostic tools

Nermean Moamen Hussein (nermeanmohu@yahoo.com)
South Valley University https://orcid.org/0000-0002-6003-5710
Soheir A. H. Rabie
South Valley University Faculty of Science
Wafaa A. Abuelwafa
South Valley University Faculty of Science
Mouchira M. Mohi Eldin
South Valley University Faculty of Veterinary Medicine

Research Article

Keywords: Cysticercosis, Cysticercus pisiformis, Morphology, Molecular analysis, Histopathology, Oryctolagus cuniculus, Egypt

Posted Date: November 16th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3597278/v1

Abstract

Cysticercosis raises the alarm for domestic rabbits since it has a significant impact on their health, productivity, and nutritional value. In this study, several white to cream-colored cysts were detected in the mesentery of the stomach and intestine, as well as embedded in the liver of domestic rabbits collected from Qena Governorate, Egypt. Our morphological and molecular analyses of these cysts reveal that they are *Cysticercus pisiformis*, the larval stage of *Taenia pisiformis*. Gross and histopathological investigations of infected organs were documented. To our knowledge, this is the first geographical report on *cysticercus pisiformis* in domestic rabbits in Qena Governorate, using current morphological, molecular, and histopathological methodologies that provide critical data for developing diagnostic targets for *C. pisiformis*.

Introduction

Rabbits are one of the most commonly used laboratory animals, being used in investigations all over the world to examine numerous biological aspects (Mapara et al. 2012). Rabbits, like other animals, are susceptible to a variety of infectious and non-infectious agents, resulting in a wide range of diseases and economic losses (Varga 2014). Domestic rabbits serve as intermediate hosts for *Cysticercus pisiformis*, which is a larval stage of *Taenia pisiformis* (Taylor et al. 2016). The economic importance of *T. pisiformis*-induced cysticercosis in rabbits has grown due to its influence on animal welfare and output (Domnguez-Roldan et al. 2018).

The majority research on Egyptian domestic rabbits has focused on the morphology and histopathology of their coccidian and nematode parasites, while little is known about *Taenia pisiformis* and its metacestode, *Cysticercus pisiformis* (see Rashed et al. 1991; Radwan et al. 2014). Given the scarcity of *T. pisiformis* material, the current study used light, scanning, and transmission electron microscopy, as well as molecular and histopathological investigations, to look for *C. pisiformis* in domestic rabbits in Qena Governorate, Egypt, in the context of our continuing research on parasites infecting Egyptian domestic rabbits (see Hussein et al. 2022; Rabie et al. 2022).

Materials and Methods

Sample collection:

Domestic rabbits were collected from several locations in Qena Governorate, Egypt, between October 2018 and October 2020. They ranged in age from young to adults. The collected rabbits weighed between 350g-2700g. When they were dissected, white to cream-colored cysts were found in a variety of organs and viscera, including the liver, mesenteries, and stomach. Freshly collected cysts were photographed, placed in a sterile Petri dish, and sent to the Parasitology Laboratory, Department of Zoology, Faculty of Science, South Valley University, Qena, Egypt.

Morphological analyses:

Light microscopy

The recovered cysts were flattened between two slides, stained with acetic acid alum carmine, dehydrated in ethanol alcohol concentrations of 70%, 80%, 90%, and 100%, clarified in xylene, and mounted in DPX. The first cyst diagnosis was based on morphological traits such as scolex form and virtually translucent cyst fluid.

Ten samples were measured in micrometers using the calibration software LAS EZ (version 1.8.0). Drawings were created using copies of the collected worms' figures, which were subsequently modified in Adobe Photoshop CS6 (version 13.0 x32).

Scanning electron microscopy

Specimens were fixed in 3% glutaraldehyde in 0.1 mol/L Phosphate Buffer Solution for 1–2 hours, then dehydrated in a series of ascending concentrations of ethanol (70%, 80%, 90%, 100%; 5 minutes per change). They were air-dried for a few minutes before being mounted on an aluminum stub with a double-phase sticker and coated in a sputter coating unit with gold-palladium

(Radwan et al. 2014). Finally, worms were investigated using a JEOL-JSM-5500 LV Scanning electron microscope (Jeol, Japan) with a 20 kV accelerating voltage in South Valley University's Central Laboratory.

Transmission electron microscopy

For 24 hours, samples were immersed in a 0.1 M cacodylate buffer (pH 7.3) containing 3% glutaraldehyde. Dehydration occurred in an ascending ethanol series after 4 hours of post-fixation in 2% OsO4. Samples were processed and fixed in an Araldite-embedding medium in accordance with standard technique. Semithin sections were cut and stained with toluidine blue for light microscopy. In the Central Laboratory of South Valley University, ultrathin sections were stained with uranyl acetate and lead citrate (Fan et al. 2012; Radwan et al. 2014) and examined with a JEOL-JEM-1010 electron microscope at 80 kV.

Molecular analysis:

DNA extraction and sequencing

Specimens were extracted from ethanol and then washed with distilled water to remove all residues of alcohol. Individual samples were deposited in 1.5 mL microcentrifuge tubes, and genomic DNA was extracted with a small kit (Quick-DNATM Fungal/Bacterial Miniprep Kit). The oligonucleotide primers JB10 (forward: 5'-GATTACCCGCTGAACTTAAGCATAT-3') and JB9 (reverse: 5'-GCTGCATTCACAAACACCCCGACTC-3') were used to amplify partial domain D1 of the 28S rDNA gene (Bowles and McManus 1994). Procedures for PCR amplification, gel electrophoresis, purification, and sequencing were followed in accordance with Hussein et al. (2022).

Sequences of cestodes (partial 28S rDNA) from GenBank

This study used GenBank to obtain partial 28S rDNA sequences for closely related taeniid cestodes. GenBank produced 12 partial 28S rDNA sequences from cestodes of the Family Taeniidae (together with an outgroup from the Family Onchobothriidae). These sequences were compared to the current partial 28S rDNA sequence (Table 2).

Phylogenetic analysis

The sequences were modified in GeneStudio TM Profissional Edition (version 2.2.0.0) and aligned in MEGA X software (version 10.0.5). The obtained DNA sequence was compared to those found in the GenBank database. The maximum likelihood (ML) method, as implemented in MEGA X software (version 10.0.5), was used to construct a phylogenetic tree from partial 28S rDNA sequencing data. The outgroup was *Uncibilocularis okei* of the Onchobothriidae family. The Tamura 3-parameter (T92) model was chosen, with 5 rate categories among sites (a discrete Gamma distribution (+ G)). 1000 replications were used in the bootstrap procedures.

Histopathological analysis

Hussein et al. (2022) detailed how tissue samples were obtained from the gastrointestinal tract and preserved in 10% formalin for histological investigation. Following adequate fixation, the tissue blocks were embedded in paraffin. Hematoxylin and eosin (H&E) were commonly employed to stain 5- µm -thick tissue sections.

Results

Cysticercus pisiformis infected 60 (30%) of 200 domestic rabbits. Infected rabbits had many oval cysts related to the liver (Fig. 1A–B) and numerous viscera (Fig. 2A–C). Table 1 and Figs. 3–9 depict the morphological and morphometric properties of *C. pisiformis*.

Taxonomic summary

Species: *Cysticercus pisiformis* (F: Taeniidae)

Host: Domestic rabbits, *Oryctolagus cuniculus* (F: Leporidae).

Locality

Qena Governorate, Egypt.

Site of infection

Stomach, mesenteries, and liver

Prevalence

30% (60 out of 200).

Intensity

1-300 cysts per infected rabbit.

Morphological analyses

Light microscopy (Table 1; Figs. 3–6):

Cysticerci emerged as very small undifferentiated immature ovoid cysts attached to the surface of the liver. Although hooks and suckers were not discernible, the scolex generated an invaginated thickening at the anterior end of the developing metacestodes (Figs. 3 and 6A). An invaginated notch at the body's tip revealed the presence of the invagination canal (Figs. 3 and 6A).

Metacestodes proceeded through a pre-final stage of differentiation as cysticerci developed. The presence of suckers and invaginated rostellum in the scolex distinguished this stage. The bladder swelled. At the base of the rostellar cone, 20 taenoid hooks were placed in two rows (Fig. 4). Suckers appeared on the sides of the scolex (Fig. 4A–B and 4D–E).

The cysticercus was well developed, and the scolex was clearly defined and evaginated outwardly (Figs. 5A–D and 6B–C). The length was 3255.18μm (2915.25-3595.11μm) and the width was 697.19μm (641.56-752.83μm). The scolex was 739.67μm (243.09-1180.31μm) in diameter, with four cup-shaped well-developed suckers and a rostellum armed with two rings of 20 taenioid hooks each (Figs. 5A–D and 6B–C). Large hooks had a length of 209.81μm (100.93-366.49μm), while small hooks had a length of 132.5μm (76.4-253.32μm) (Figs. 5E–G and 6D). The blade of a mature hook appeared to be well-formed, while the handle and guard were short and grew in length. The suckers had a thick muscular wall and a diameter of 233.15μm (115.96-370.71μm).

Scanning electron microscopy (Figs. 7&8):

An invaginated notch at the tip of the body revealed the presence of an invagination canal in the cysticerci of the liver (Fig. 7A–B). Furthermore, the body was covered with corrugated tegument, with no obvious microtriches (Fig. 7A–B). The invagination canal was obvious in the pre-fully developed cysticerci (Fig. 7C). The microtriches were pointy and covered the wrinkled tegumental surface of the body (Fig. 7D).

Suckers and rostellum were found in fully mature cysticercus (Fig. 8). A thin tegumental membrane coated the rostellum and hooks. The rostellum looked like an umbrella covering the top of the scolex (Fig. 8A–B and 8D–F). There was an antler-like hook bifurcation, and the long hooks looked like chicken claws (Fig. 8A–B and 8D–F). Suckers resembled a smooth-walled circular cavern (Fig. 8).

Transmission electron microscopy (Fig. 9):

Microtriches appeared to create a base on the tegument, which frequently encompassed a dense core, a dense, pointed shaft, and a base plate (Fig. 9A). The distal cytoplasm was visible as unevenly scattered bundles of circular and longitudinal muscles beneath the basement lamella of cysticerci (Fig. 9B–C). The endoplasmic reticulum, ribosomes, glycogen granules, and dense bodies are abundant in the perinuclear cytoplasm of tegumental cells (Fig. 9B and 9D). Fibrillar and membranous structures, lucent vesicles, and opaque and dense masses were found in the parenchymal matrix (Fig. 9B–C).

Molecular analysis (Tables 2 and 3; Fig. 10):

The 28S rDNA gene domain D1 of the Taeniidae-related *C. pisiformis* was amplified and sequenced. The PCR amplification ranged from 200 to 300 bp. The sequencing data of *Cysticercus pisiformis* (220 nucleotides) was deposited in GenBank under the accession number MZ576207.

The obtained sequence was aligned with 11 *Taenia* reference sequences (Table 2): four *Taenia taeniaeformis* sequences, three *Taenia saginata* sequences, a single sequence each of *Taenia solium*, *T. multiceps*, *T. hydatigena*, *T. pisiformis*, and *Unicibilocularis okei galli* (Onchobothriidae) as an outgroup. All 13 sequences (including the outgroup) were aligned over 228 positions.

The resulting phylogenetic tree is depicted in Fig. 10 using the Maximum Likelihood (ML) method. A phylogenetic dataset based on the partial 28S sequence revealed that the Taeniidae family is monophyletic. *Taenia saginata* sequences clustered together (ML = 99). *Taenia solium* and *T. multiceps* combined to produce a weak support cluster (ML = 42). *Taenia hydatigena* (ML = 65) formed a basal clade with *Taenia solium*, *T. multiceps*, and *T. saginata*. The *Taenia pisiformis* clade is a member of the monophyletic group that includes *Taenia solium*, *T. multiceps*, and *T. pisiformis*. *Taenia taeniaeformis* sequences clustered together with a high support value (ML = 99). The current *C. pisiformis* grouped with *T. pisiformis* and had a high bootstrap value (ML = 97).

The genetic difference between *C. pisiformis* and *T. pisiformis* was just 3.6%. The variations in *Taenia saginata* sequences ranged from 0.5–1.5%. *Taenia solium* and *T. multiceps* differed by 3.5% (Table 3). As a result, genetic distances back up the results reached from the phylogenetic tree (Fig. 10).

Histological results (Fig. 11):

The scolex of *C. pisiformis* was histologically composed of four suckers, each having an outer membrane and interior muscle fibers (Fig. 11E–F). On the rostellum, there were two rows of hooks encircled by anterior muscle fibers (Fig. 11H). The invagination canal was encased in an outer membrane (Fig. 11I). The exterior layer of the cyst wall was made up of microtrichs, distal cytoplasm, and vesicles (Fig. 11C). *Cysticercus pisiformis* internal structures were embedded in the parenchyma and supported by connective tissue (Fig. 11A–B, 11D, and 11G).

Pathological Findings (Figs. 13–18):

The pathological results in the stomach revealed gravid segments with eggs surrounding *C. pisiformis* oncospheres entering and moving among the gastric wall (Fig. 13). Mucinous degeneration caused damage to the stomach epithelium and gastric glands, resulting in increased mucin synthesis from the degenerated gastric mucosa and gland (Fig. 14A–B).

Furthermore, the rabbits' livers were severely infested with *C. pisiformis*, which has a shallow depression in the anterior position of the scolex and a segmented body that penetrates the hepatic parenchyma, surrounded by a dense fibrous capsule, and causes hepatic cell compression (Fig. 15A–C). The migration of cysts among hepatocytes caused coagulative necrosis in hepatocytes with extravasated RBCs. The majority of the liver parenchyma suffers from fibrinopurulent hepatitis, which is characterized by neutrophil cell aggregation scattered among the hepatic cells and an abnormal accumulation of fibrous tissue proliferation that replaces significant areas of liver damage. Moreover, diffuse granulomatous areas were observed in the liver, which was characterized centrally by a caseated dead cyst, surrounded by aggregation of polymorphonuclear cells, primarily neutrophils and a few eosinophils, followed by round cells, particularly macrophages, and lymphocytes, and surrounded by a thick wall of fibrous tissue proliferation (Fig. 16A–C and Fig. 17). The portal areas showed periportal fibrosis, which manifested as hyperplasia in the epithelial lining of the bile duct, obstructing its lumen, and significant fibrous tissue growth replacing the necrotic cells around the portal triad (Fig. 18).

Discussion

Cysticercus pisiformis is a well-known parasite of lagomorphs (intermediate hosts), particularly rabbits (Stancampiano et al. 2019). Previous research on *C. pisiformis* relied solely on morphological, histological, or molecular characteristics, as well as

host specificity and/or overall infection prevalence, but this study covered all morphological, morphometric, molecular, and histopathological characteristics related to *C. pisiformis* as integrated diagnostic tools for monitoring this parasite in Egyptian domestic rabbits.

The overall prevalence of infection in the current study (30%) differed from that found by Kang 1987 (21.6%), Al-Moula 2005 (4.3%), Daoud et al. 2005 (68.18%), Szkucik et al. 2014 (4.74%), and Mogalli 2020 (76.7%). Differences in infection prevalence could be attributable to rabbit food, therapeutic techniques, and environmental factors (see Rabie et al. 2022).

Three stages were seen for *C. pisiformis* and morphologically described in this study, which is consistent with Radwan et al. (2014) when they defined the developmental stages of *T. pisiformis* from eggs to cysticerci. The morphology of cysticerci in the liver mirrored that of Graham-Brown et al. (2018), who described them as tiny, transparent, fluid-filled cysts with a broad anterior and a narrow, tail-like posterior. Notably, the morphology of fully developed cysticerci matched that described by Kang (1987), who noted the presence of four suckers and a rostellum on the top of the identified scolex in the cyst, as well as hooks grouped in two rows, the large-type inner and small-type outer hooks. The current study's samples had fewer hooks (40 taenoid hooks) than Kang (1987) (44 taenoid hooks) and overlapped with Radwan et al. (2014) (40–42 taenoid hooks), implying that hook number is variable and may depend on the age, development of specimens, or practical manipulation. Previous research did not include drawings of the *C. pisiformis* stages; hence, this study may be regarded as the first to provide illustrations of the *C. pisiformis* stages.

The SEM results revealed the presence of a notch at the top of the cysticerci in the liver, reflecting the invagination canal. This finding was similar to Radwan et al. (2014), except that the invagination canal was more apparent in our pre-fully grown cysticerci than Radwan et al. (2014). Furthermore, SEM analysis revealed a bifurcation of hooks like antlers on the rostellum of fully mature cysticerci, with the long hooks resembling chicken feet. These findings are consistent with those of Pan et al. (2014) and Pan et al. (2016). Suckers in our study resembled a spherical cave with a smooth wall, similar to those described by Pan et al. (2014). According to the TEM data, microtrichs coated the tegument of the cyst wall. In the parenchymal matrix and distal cytoplasm, muscle bundles and vacuoles developed, respectively. These results agree with those of Fan et al. (2012) and Radwan et al. (2014).

Our phylogenetic tree has a topology that is similar to that of Moore and Brooks (1987) and Zhang et al. (2007), where *T. pisiformis* formed a basal clade with *T. hydatigena*, *T. multiceps*, *T. solium*, and *T. saginata*. Furthermore, *T. taeniaeformis* from rodents and felids was basal to all other *Taenia* spp. from lagomorphs, canids, ruminants, and humans, as reported by Zhang et al. (2007). For the partial 28S rDNA gene, there is only one sequence for adult *T. pisiformis* in the GenBank database, which was reported by Zhang et al. (2007). To our knowledge, this is the first work in Egypt that provides molecular proof of *T. pisiformis* cysticercosis in an intermediate host, such as the domestic rabbit.

The current study focused on the histology of *C. pisiformis*, particularly the scolex and cyst wall features. The current findings agree with those of Sun et al. (2008). Radwan et al. (2014) showed a histological image of a fully developed cysticercus showing the scolex and invagination canal, which was compatible with the current findings.

The current pathological findings indicated that the migration of *T. pisiforms* gravid segments along the stomach wall resulted in a significant infestation of *C. pisiforms* and, later, necrosis, granuloma formations, and, finally, liver fibrosis. The severe liver lesions were attributed to the worms migrating across the body, inflicting damage to the host's tissues directly through their activity or metabolism or indirectly through host defense mechanisms. Necrosis was produced by parasite proliferation in enormous fluid-filled cysts in the liver and their spread, which caused pressure atrophy in hepatic cells (Wakelin 1996). Meanwhile, when the contents of the cyst are attacked by the host, degeneration in their membrane and scolex occurs as a result of the cellular response, and it shrinks and is replaced by fibrotic tissue (Garcia et al. 2014).

In conclusion, the current findings indicate that the taeniid species infecting Egyptian domestic rabbits is *C. pisiformis* Bloch 1780. We are aware of no data on *C. pisiformis* from Qena Governorate, Egypt. As a result, the current study could be the first to report morphological, morphometric, molecular, and histopathological characteristics of *C. pisiformis* in Egyptian domestic

rabbits. The full scenario of this infection on Egyptian rabbits is currently unknown, and more research is needed. Our findings can be used to monitor parasite population fluctuations and develop future control tactics.

Declarations

Acknowledgments We thank the Department of Zoology, Faculty of Science, South Valley University, Qena, Egypt, for providing the required facilities and assistance in collecting domestic rabbits. We would also like to thank South Valley University's Central Laboratory for their direct cooperation and technical assistance with scanning and transmission electron microscopic examinations. We would like to express our full gratitude to Dr. Ali Mansour, Department of Zoology, Faculty of Science, South Valley University, Qena, Egypt, for reviewing and analyzing the final draft of the work, as well as providing all useful comments.

Author contributions Soheir A. H. Rabie: supervision, writing—review, and editing. Wafaa A. Abuelwafa: sample collection, methodology, and writing—original draft. Mouchira M. Mohi ElDin: writing—review and editing the histopathological part. Nermean M. Hussein: supervision, writing—review and editing. All authors reviewed and approved the final manuscript.

Funding The authors have not disclosed any funding.

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval All relevant institutional, national, and international criteria for the care and use of animals were followed. The Scientific Research Ethics Committee, Faculty of Veterinary Medicine, South Valley University, Egypt approved the study (approval number: 25/13/11.2021).

References

1. Al-Moula IDH (2005) Study of some endo and ecto parasites in domestic rabbits in Mosul/Iraq. Iraqi J Vet Sci 19(2):143–153
2. Bowles J, McManus DP (1994) Genetic characterization of the Asian *Taenia*, a newly described taeniid cestode of humans. Am J Trop Med Hyg 50(1):33–44
3. Caira JN., Jensen K, Waeschenbach A, Olson PD, Littlewood DTJ (2014) Orders out of chaos–molecular phylogenetics reveals the complexity of shark and stingray tapeworm relationships. Int. J Parasitol 44(1):55–73. https://doi.org/10.1016/j.ijpara.2013.10.004
4. Daoud MS, Rhymah MSh, Sulaiman EG (2005) Natural infection of *Cysticercus pisiformis* in domestic rabbits and a study of experimental infection in the final host (Dogs). Iraqi J Vet Sci 19(2):169–174
5. Fan XP, Shang QY, Zhang XY, Han JH, Sun XL (2012) Observation on ultrastructure of Rabbit *Cysticercus pisiformis*. J Anim Vet Adv 11(22):4205–4209
6. Garcia HH, Rodriguez S, Friedland JS (2014) Immunology of *Taenia solium* taeniasis and human cysticercosis. Parasite Immunol 36(8):388–396. https://doi.org/10.1111/pim.12126
7. Graham-Brown J, Gilmore P, Harcourt-Brown F, Eastham H, Williams D (2018) Lethal cysticercosis in a pet rabbit. Vet Record Case Reports 6(3), e000634.
8. Hussein NM., Rabie SA, Abuelwafa WA, ElDin MMM (2022) Morphometry, molecular identification and histopathology of *Passalurus ambiguus* Rudolphi, 1819 in domestic rabbits (*Oryctolagus cuniculus*) in Qena, Upper Egypt. J Parasit Dis 46(2):511–525. https://doi.org/10.1007/s12639-022-01477-3
9. Kang YB (1987) Incidence and morphology of *Cysticercus pisiformis* (*Taenia pisiformis* Bloch 1780: Taeniidae) collected from rabbits in Korea. Korean J Vet Res 27(1):101–108
10. Lee SU., Chun HC, Huh S (2007) Molecular phylogeny of parasitic Platyhelminthes based on sequences of partial 28S rDNA D1 and mitochondrial cytochrome c oxidase subunit I. Korean J Parasitol 45(3):181–189. https://doi.org/10.3347/kjp.2007.45.3.181
11. Mapara M, Thomas BS, Bhat KM (2012) Rabbit as an animal model for experimental research. Dent Res J 9(1):111–118

12. Mogalli NM (2020) First report of *Taenia pisiformis* Cysticercus infestation in Domestic rabbits in Hajjah city Yemen. Int J Vet Sci Res 6(2):159–163. https://doi.org/10.17352/ijvsr.000068
13. Moore J, Brooks DR (1987) Asexual reproduction in cestodes (Cyclophyllidea: Taeniidae): ecological and phylogenetic influences. Evolution 41:882–891. https://doi.org/10.1111/j.1558-5646.1987.tb05861.x
14. Pan Y, Li R, Yin M, Tang H, Feng C, Zhang H (2016) Ultra-morphological comparative observation on scolex of hook and no-hook *Cysticercus pisiformis*. Acta Veterinaria et Zootechnica Sinica 47(12):2493–2500
15. Pan Y, Zhang L, Wang S, Li R, Liu Z, Sun Y, Gadahi JA, Liu X (2014) Ultrastructural changes of scolex on *Cysticercus pisiformis* during relative rest and motion states. Agricultural Science & Technology 15(1):111–115.
16. Pinto RM, Gomez DC, Menezes RC, Gomez CT, Noronha D (2004) Helminths of rabbits (Lagomorpha, Leporidae) deposited in the Helminthological Collection of the Oswaldo Cruz Institute Rev Bras Entomol 21(3):599–604. https://doi.org/10.1590/S0101-81752004000300023
17. Radwan NA, El Sefy MN, El Din SAN, Abou Shafeey HE, Sharaf SE, Khalil AI (2014) *Cysticercus pisiformis*: ultrastructural transformation of the tegument during development from oncosphere to cysticercus. Parasitologists United Journal 7(1):13–26. https://doi.org/10.4103/1687-7942.139686
18. Rabie SA, Abuelwafa WA, Hussein NM (2022) Occurrence of *Eimeria* species (Apicomplexa: Eimeriidae) in domestic rabbits (*Oryctolagus cuniculus*) in Qena Governorate, Upper Egypt. J Parasit Dis 46(3):811–832. https://doi.org/10.1007/s12639-022-01494-2
19. Rashed RM, Whitfield PJ, Lewis JW (1991) The epidemiology of *Taenia pisiformis* infections in domestic dogs in Cairo. J Egypt Soc Parasitol 21(3):597–610
20. Stancampiano L, Ravagnan S, Capelli G, Militerno G (2019) Cysticercosis by *Taenia pisiformis* in Brown Hare (*Lepus europaeus*) in Northern Italy: Epidemiologic and pathologic features. Int J Parasitol Parasites Wildl 9:139–143. https://doi.org/10.1016/j.ijppaw.2019.04.004
21. Sun X, Luo X, Wang X, Cai XP (2008) Observation of the organizational structure of *Cysticercus pisiformis* and *Taenia pisiformis* scolex. Chinese Veterinary Science 38(9):796–800
22. Szkucik K, Pyz-Łukasik R, Szczepaniak KO, Paszkiewicz W (2014) Occurrence of gastrointestinal parasites in slaughter rabbits. Parasitol Res 113(1):59–64. https://doi.org/10.1007/s00436-013-3625-7
23. Taylor MA, Coop RL, Wall RL (2016) Veterinary parasitology, 4th edn. Wiley-Blackwell, United States
24. Varga M (2014) Infectious Diseases of Domestic Rabbits. In: Varga M (ed) Textbook of Rabbit Medicine, 2nd edn. Butterworth-Heinemann, UK, pp 435-471. https://doi.org/10.1016/B978-0-7020-4979-8.00014-5
25. Wakelin D (1996) Helminths: Pathogenesis and Defenses. In: Baron S (ed) Medical Microbiology, 4th edn. Galveston (TX), University of Texas
26. Zhang L, Hu M, Jones A, Allsopp BA, Beveridge I, Schindler AR, Gasser RB (2007) Characterization of *Taenia madoquae* and *Taenia regis* from carnivores in Kenya using genetic markers in nuclear and mitochondrial DNA, and their relationships with other selected taeniids. Mol Cell Probes 21(5-6):379–385. https://doi.org/10.1016/j.mcp.2007.05.003

Tables

Table (1): Comparative measurements of Cysticercus pisiformis Bloch 1780 in the present study with those previously described.

Reference	Kang 1987	Pinto et al. 2004	Radwan et al. 2014	Present study
Length	7040 (4050–11660)	1053.86	------	3255.18 (2915.25-3595.11)
Width	4620 (3280–6840)	266.44	------	697.19 (641.56-752.83)
Scolex diameter	------	837.38	------	739.67 (243.09-1180.31)
Sucker diameter	------	178.88	1045	233.15 (115.96-370.71)
Hooks				
Number	44	------	42	40
Large hooks	250 (223–291)	1287	702.06	209.81 (100.93-366.49)
Small hooks	150 (134–178)	696.63	337.16	132.5 (76.4-253.32)
Host	Oryctolagus cuniculus	Sylvilagus brasiliensis	Oryctolagus cuniculus	Oryctolagus cuniculus
Locality	Korea	Rio de Janeiro	Egypt	Qena, Upper Egypt
Site of location	Liver, stomach, kidney, mesentery	Peritonium	Stomach and ileum	Liver, intestine, stomach, mesentery

Table (2): Partial 28S rDNA sequences of taennid species used in this study with their host species, locality and GenBank accession numbers. (*sequences collected from present study).

Species	Host	Locality	Accession No.	Reference
Taenia pisiformis	Panthera leo	Australia	AM503311	Zhang et al. 2007
Cysticercus pisiformis	Oryctolagus cuniculus	Qena Province, Egypt	MZ576207	Present study*
Taenia multiceps	Ovis aries	United Kingdom	AM503312	Zhang et al. 2007
Taenia saginata	Homo sapiens	Kenya	AM503308	Zhang et al. 2007
Taenia saginata	Homo sapiens	Republic of Korea	AF096224	Lee et al. 2007
Taenia saginata	Homo sapiens	Southeast Asia	S69004	Bowles and McManus 1994
Taenia solium	Homo sapiens	Mexico	AM503313	Zhang et al. 2007
Taenia hydatigena	Canis familiaris	Kenya	AM503305	Zhang et al. 2007
Taenia taeniaeformis	Rattus norvegicus	Argentina	JX419380	GenBank only
Taenia taeniaeformis	Rattus rattus	India	JN020349	GenBank only
Taenia taeniaeformis	Rattus norvegicus	India	JN020350	GenBank only
Taenia taeniaeformis	Felid	Australia	AM503314	Zhang et al. 2007
Uncibilocularis okei	Pastinachus atrus	Australia	KF685777	Caira et al. 2014

Table (3): Estimation of evolutionary divergence between the present sequences of Cysticercus pisiformis and the previously recorded sequences of Taeniidae												
	1	2	3	4	5	6	7	8	9	10	11	12
MZ576207 Taenia pisiformis												
AM503311 Taenia pisiformis	0.036											
AM503308 Taenia saginata	0.130	0.105										
AF096224 Taenia saginata	0.118	0.099	0.005									
S69004 Taenia saginata	0.127	0.106	0.015	0.016								
AM503313 Taenia solium	0.110	0.085	0.060	0.0555	0.069							
AM503312 Taenia multiceps	0.105	0.080	0.045	0.040	0.047	0.035						
AM503305.1 Taenia hydatigena	0.115	0.085	0.075	0.070	0.079	0.075	0.070					
JX419380 Taenia taeniaeformis	0.107	0.092	0.125	0.119	0.134	0.094	0.094	0.099				
AM503314 Taenia taeniaeformis	0.109	0.094	0.125	0.121	0.134	0.094	0.094	0.099	0			
JN020350 Taenia taeniaeformis	0.107	0.092	0.125	0.119	0.134	0.094	0.094	0.099	0	0		
JN020349 Taenia taeniaeformis	0.107	0.092	0.125	0.119	0.134	0.094	0.094	0.099	0	0	0	
KF685777 Uncibilocularis okei	0.161	0.140	0.148	0.136	0.164	0.132	0.143	0.122	0.119	0.122	0.119	0.119

Figures

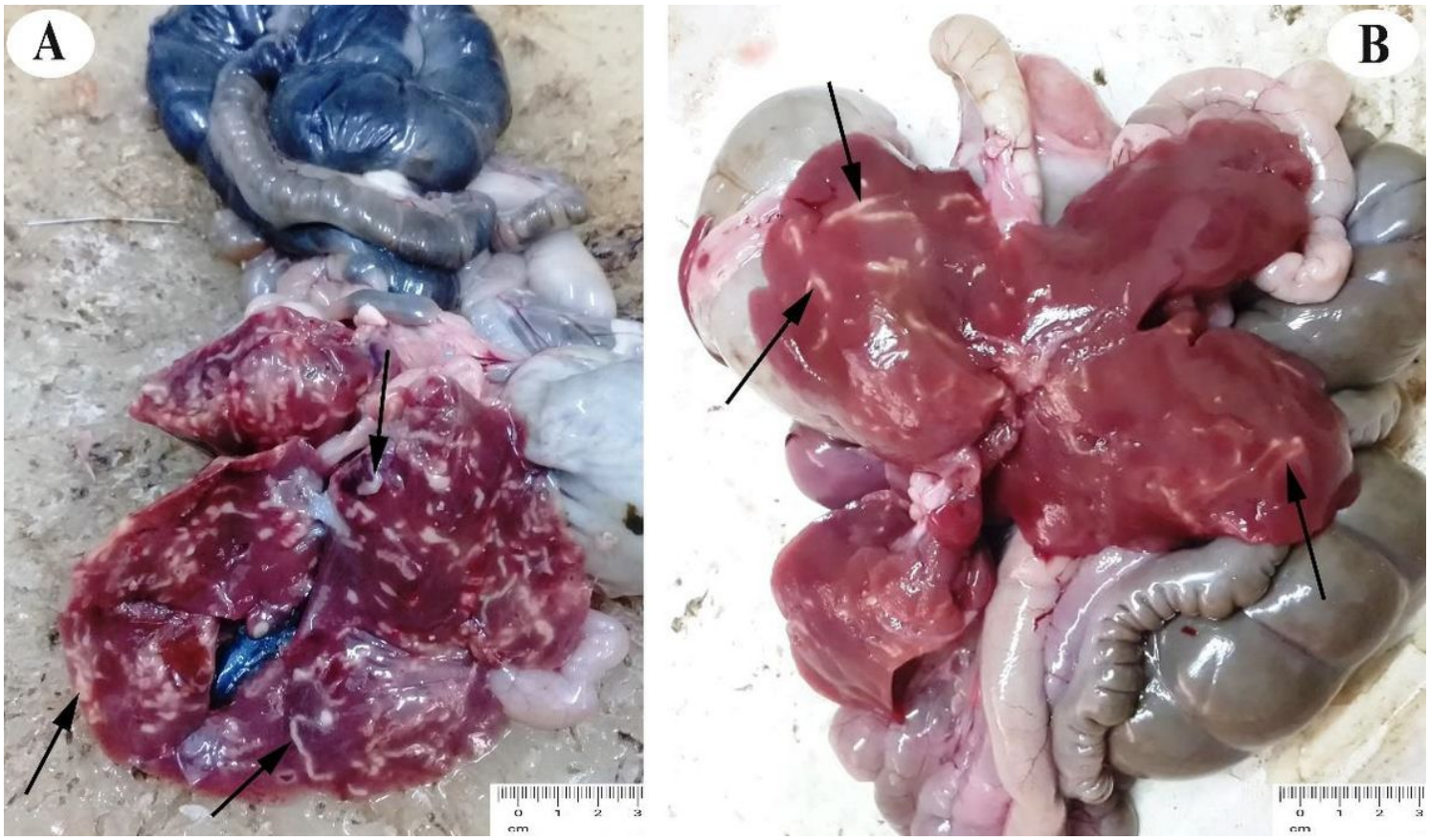

Figure 1

Photographs of *Cysticercus pisiformis* embedded into the liver of domestic rabbit, *Oryctolagus cuniculus*. **A, B** Heavy infection of *Cysticercus pisiformis* in the infected liver (black arrows)

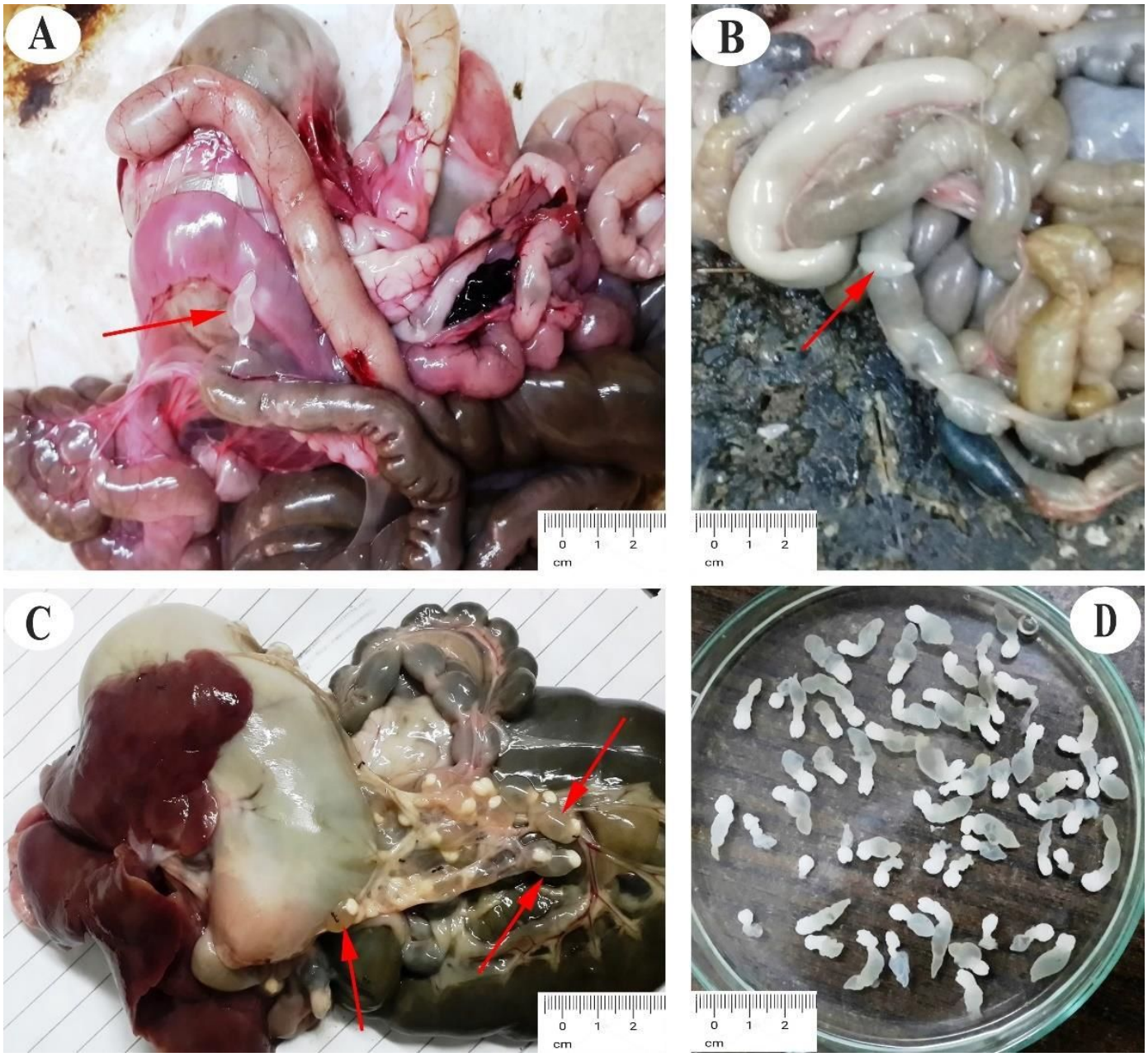

Figure 2

Photographs of *Cysticercus pisiformis* from domestic rabbit, *Oryctolagus cuniculus*. **A, B** *Cysticercus pisiformis* attached to the intestinal surface (red arrows). **C** Larvae attached to the mesenteries and stomach surface (red arrows). **D** Larvae after being removed from the infected sites

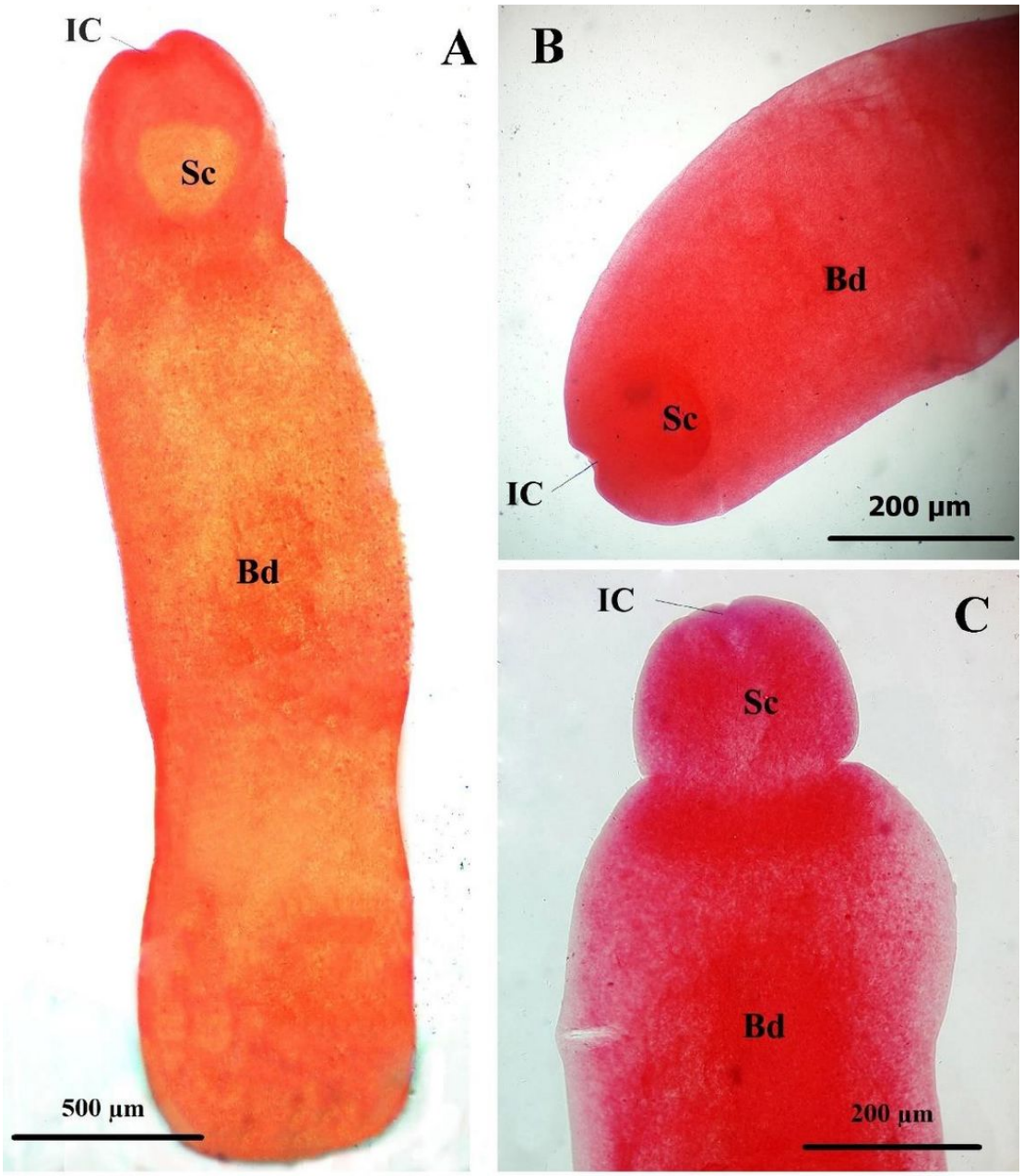

Figure 3

Light micrographs of *Cysticercus pisiformis* from the liver of domestic rabbit, *Oryctolagus cuniculus*stained with acetic alum-carmine. **A** A whole mount of *Cysticercus pisiformis* showing the invagination canal leading to scolex as well as the bladder. **B, C** High magnification of *Cysticercus pisiformis*. Scale bars; A, 500µm; B & C, 200µm. Bd, bladder; IC, invagination canal; Sc, scolex

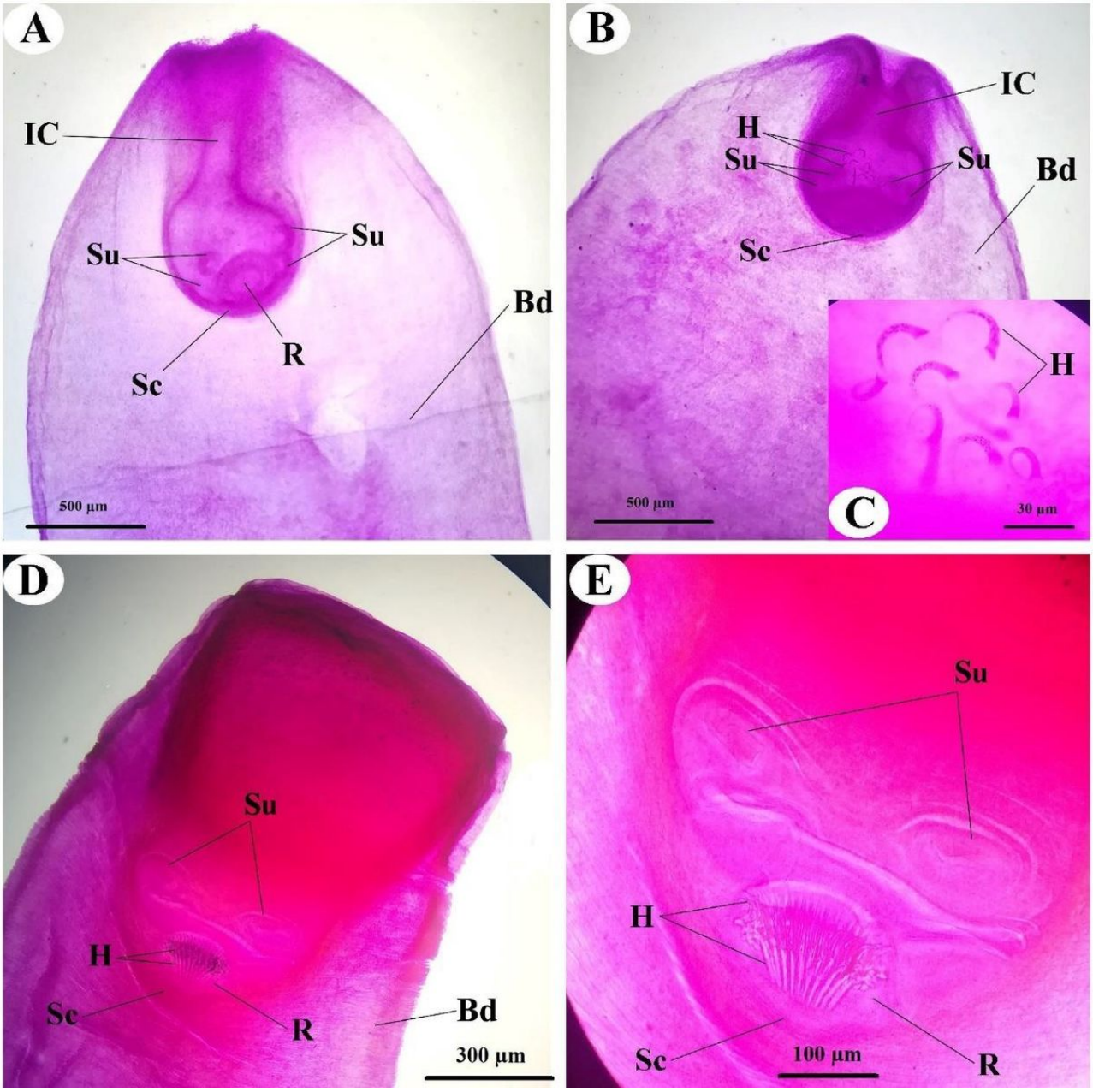

Figure 4

Light micrographs of a pre-fully developed stage of *Cysticercus pisiformis* from domestic rabbit, *Oryctolagus cuniculus* stained with acetic alum-carmine. **A, B, D** worms showing the invaginated scolex with suckers and rostellum with hooks, as well as the bladder. **C** High magnification of hooks. **E** High magnification of the invaginated scolex showing suckers and rostellum supported with hooks. Scale bars; A & B, 500µm; C & D, 300µm; E, 100µm. Bd, bladder; H, hooks; IC, invagination canal; Sc, scolex; Su, suckers; R, rostellum

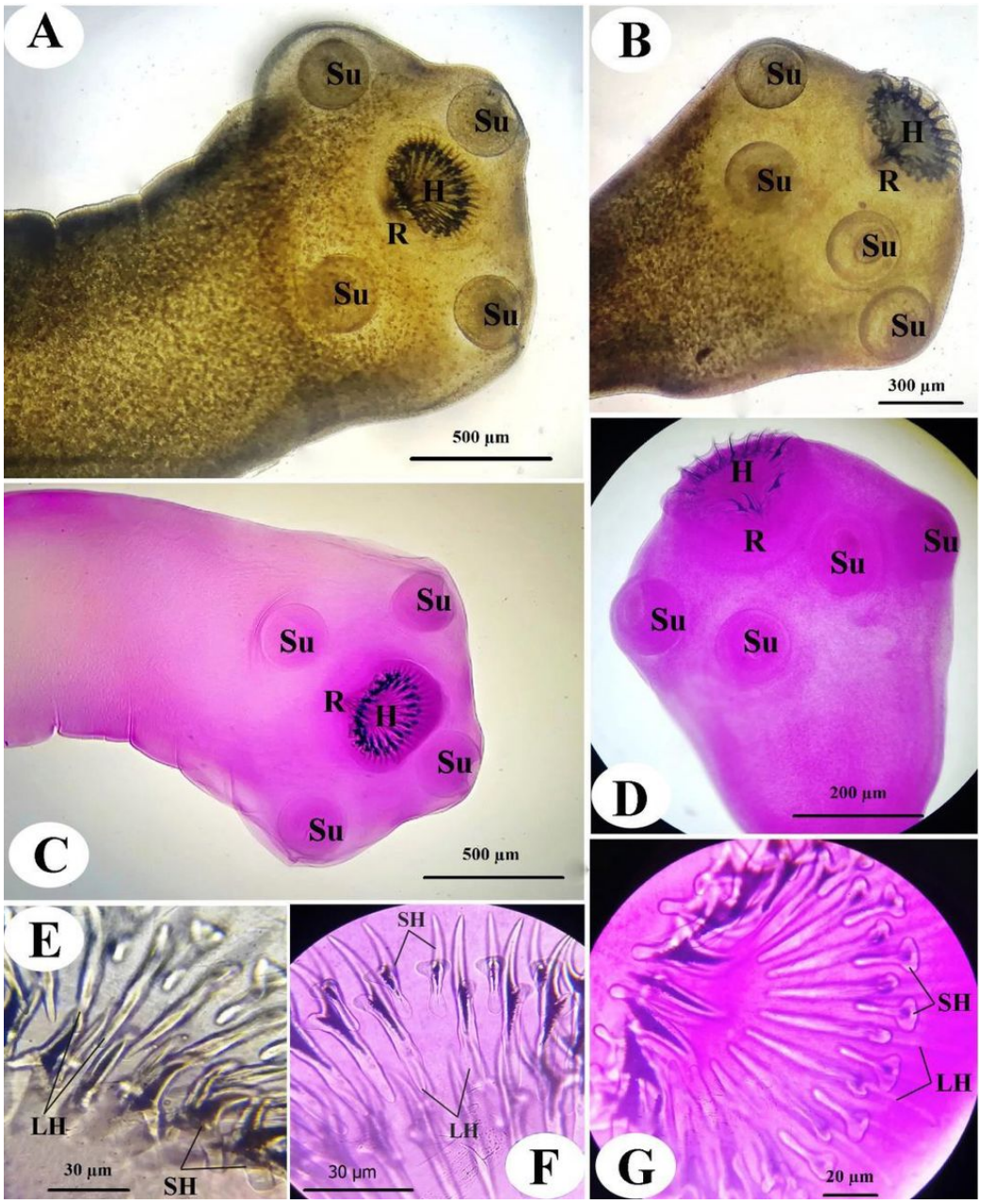

Figure 5

Light micrographs of a fully developed stage of *Cysticercus pisiformis* from domestic rabbit, *Oryctolagus cuniculus* stained with acetic alum-carmine. **A, B** Unstained worms showing the evaginated scolex with four well-developed suckers and rostellum supported with hooks. **C, D** Worms stained with alum-carmine. **E, F, G** High magnification of hooks arranged in two rows and divided into small and large hooks. Scale bars; A & C, 500µm; B, 300µm; D, 200µm; E, 30µm; F, 100µm; G, 200µm. H, hooks; LH, large hooks; R, rostellum; SH, small hooks; Su, sucker

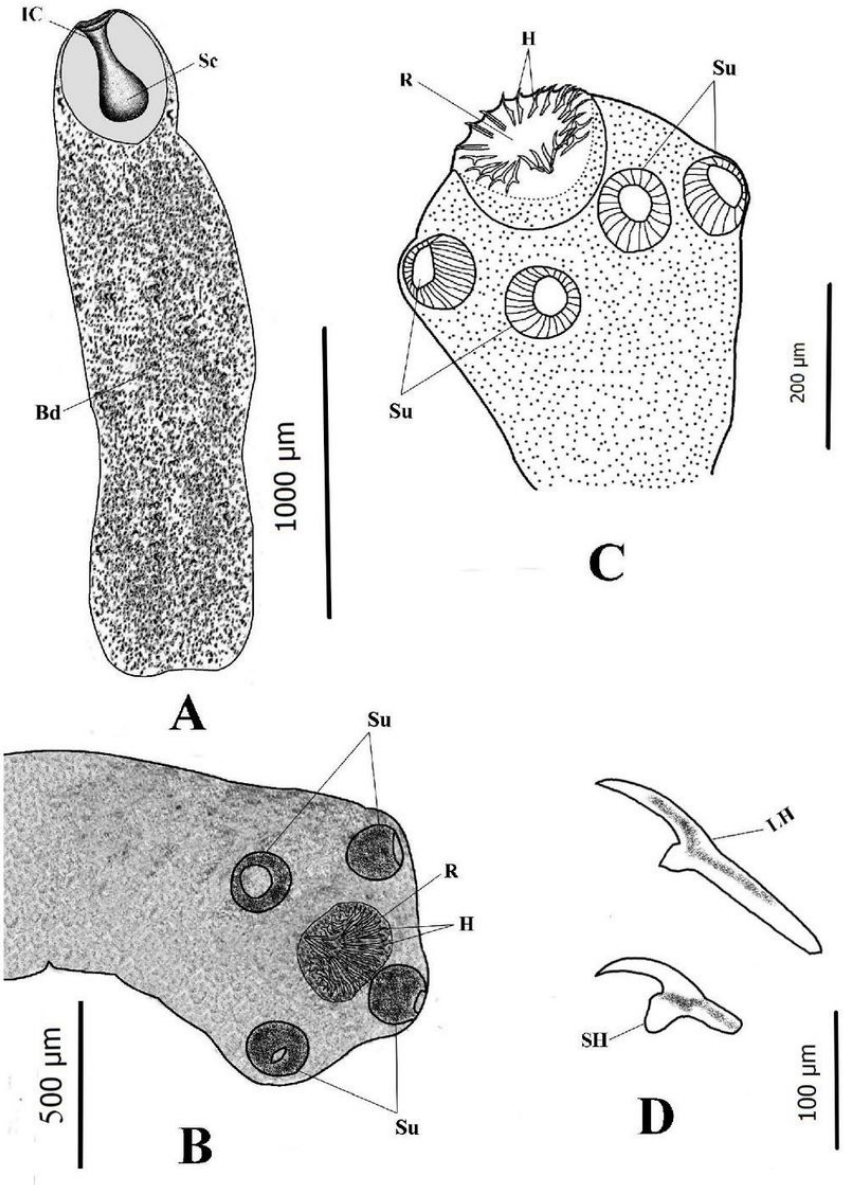

Figure 6

Line drawings of *Cysticercus pisiformis* from domestic rabbit, *Oryctolagus cuniculus*. **A** A worm from the liver. B, C. Fully developed cysticerci. **D** Small and large hooks. Scale bars; A, 1000µm; B, 500µm; C, 200µm; D, 100µm. Bd, bladder; H, hooks; LH, large hooks; IC, invagination canal; R, rostellum; Sc, scolex; SH, small hooks; Su, suckers

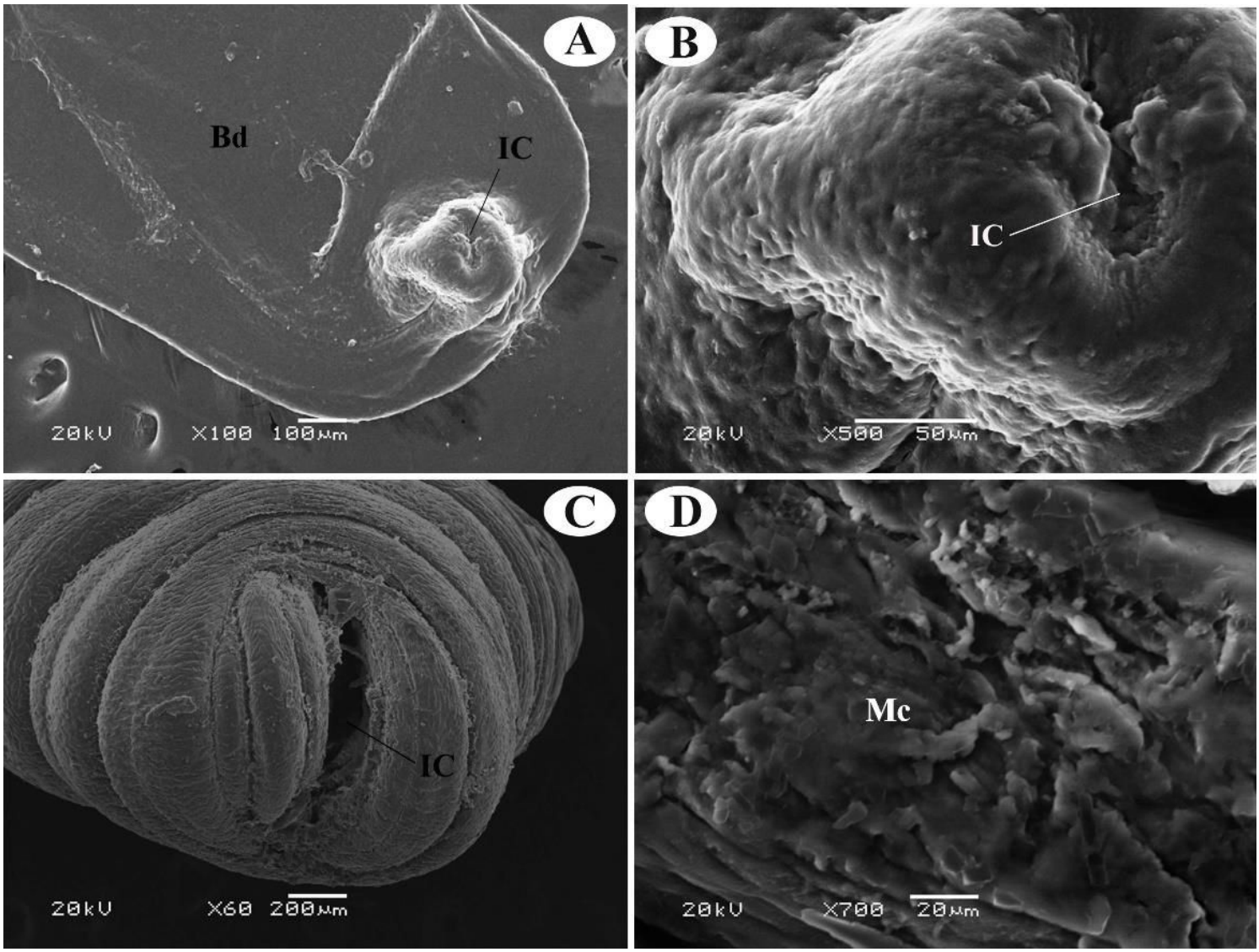

Figure 7

Scanning electron micrographs of *Cysticercus pisiformis* from domestic rabbit, *Oryctolagus cuniculus*. **A** A worm from the liver. **B** High magnification of the anterior region of cysticercus found in the liver. **C** A pre-fully developed stage of *Cysticercus pisiformis*. **D** High magnification of the tegument showing microtrichs. Scale bars; A, 100 µm; B, 50 µm; C, 200 µm; D, 20 µm. Bd, bladder; IC, invagination canal; Mc, microtrichs

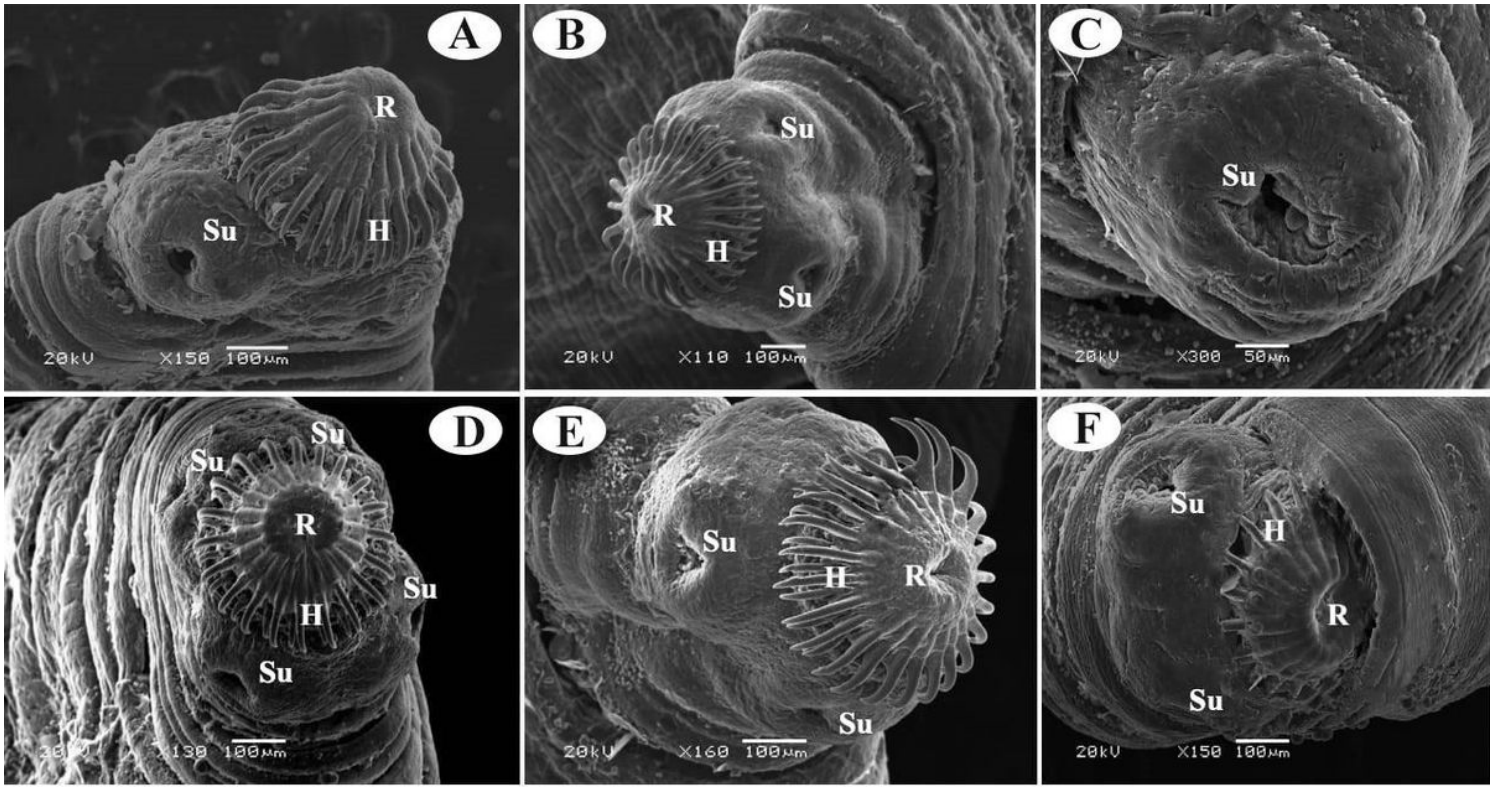

Figure 8

Scanning electron micrographs of *Cysticercus pisiformis* from domestic rabbit, *Oryctolagus cuniculus*. **A, B, D, E, F** fully developed cysticerci showing the arrangement and position of suckers and hooks in the rostellum. **C** High magnification of a sucker looked like a round cave. Scale bars; A, B, D & E, 100 µm; C, 50 µm; F, 150 µm. H, hooks; R, rostellum; Su, sucker

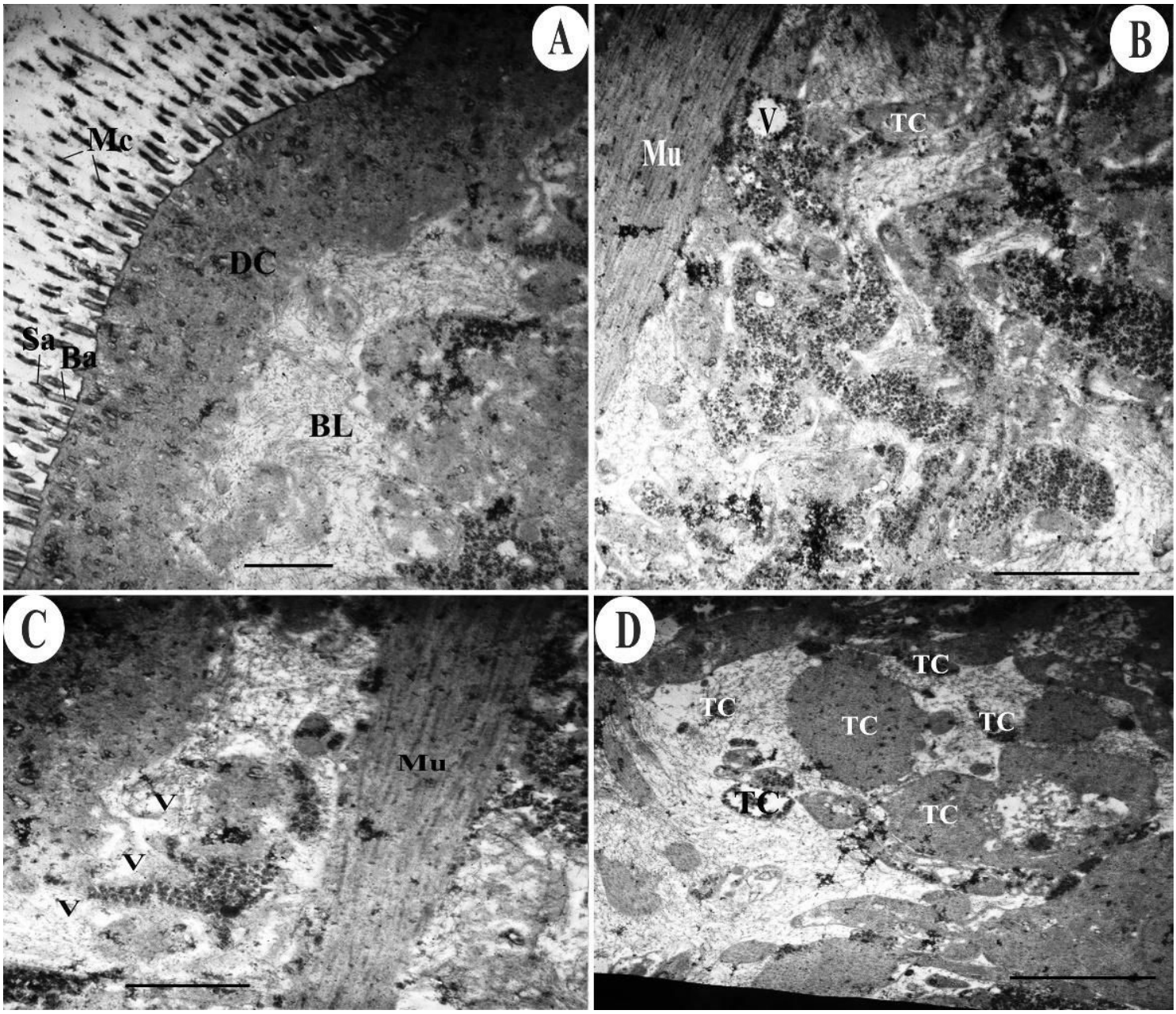

Figure 9

Transmission electron micrographs of *Cysticercus pisiformis* from domestic rabbit, *Oryctolagus cuniculus*. **A** The tegument consists of microtrichs and the distal cytoplasm. **B, C** The distal cytoplasm is made up of muscle bundles as well as secretory vesicles. **D** Tegumental cells embedded in the parenchymal matrix. Scale bars; A & C, 100 nm; B & D, 500 nm. Ba, base; BL, basal lamina; DC, distal cytoplasm; Mc, microtrichs; Mu, muscles; Sa, shaft; TC, tegumental cells; V, vesicle

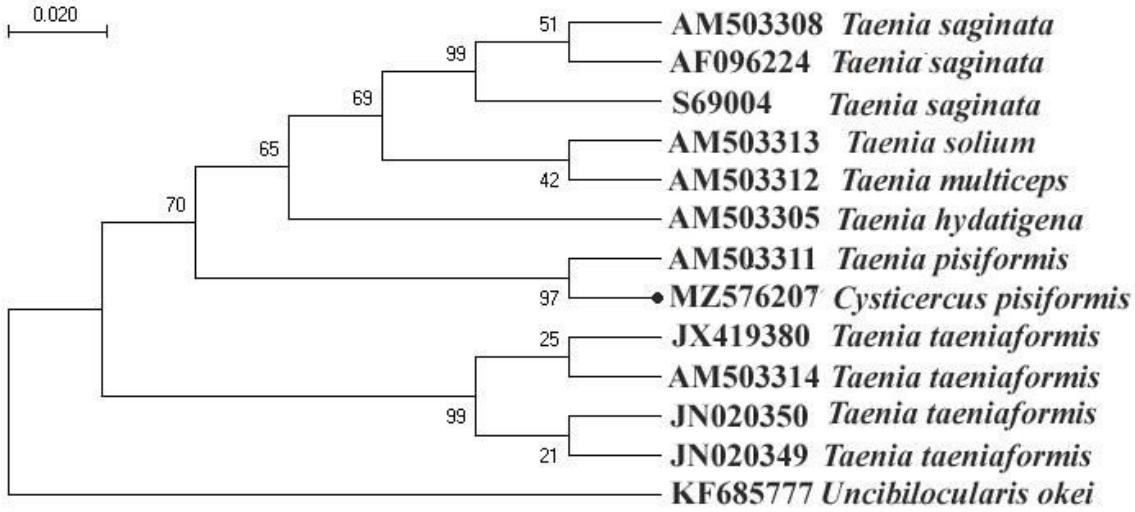

Figure 10

Maximum Likelihood (ML) tree based on 28S sequencing data displaying the phylogenetic relationships of representatives of Taeniidae. *Uncibilocularis okei* a member of the Onchobothriidae family, was chosen as an outgroup. The percentage of replicate trees in which the related taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.

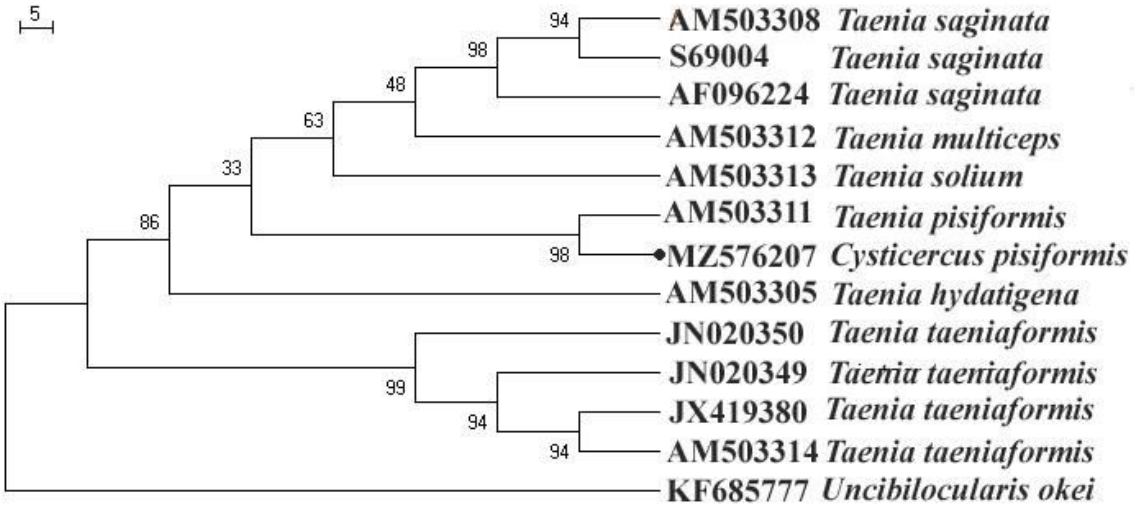

Figure 11

Light micrographs of transverse section of *Cysticercus pisiformis* from domestic rabbit, *Oryctolagus cuniculus*, stained with H&E, showing the structure of scolex and cyst wall. Abbreviations; CT, connective tissue; D, ducts; DC, distal cytoplasm; FS, fissure in sucker; H, hooks; IC, invagination canal; L, lipids; Mc, microtrichs; MF, muscular fibers; MFS, membrane of fissure in sucker; MIC, membrane of invagination canal; MS, membrane of sucker; OWC, outer wall of the cyst; Pr, parenchyma; R, rostellum; RFC, rostellum forming centre; Sc, scolex; Su, suckers; V, vesicle

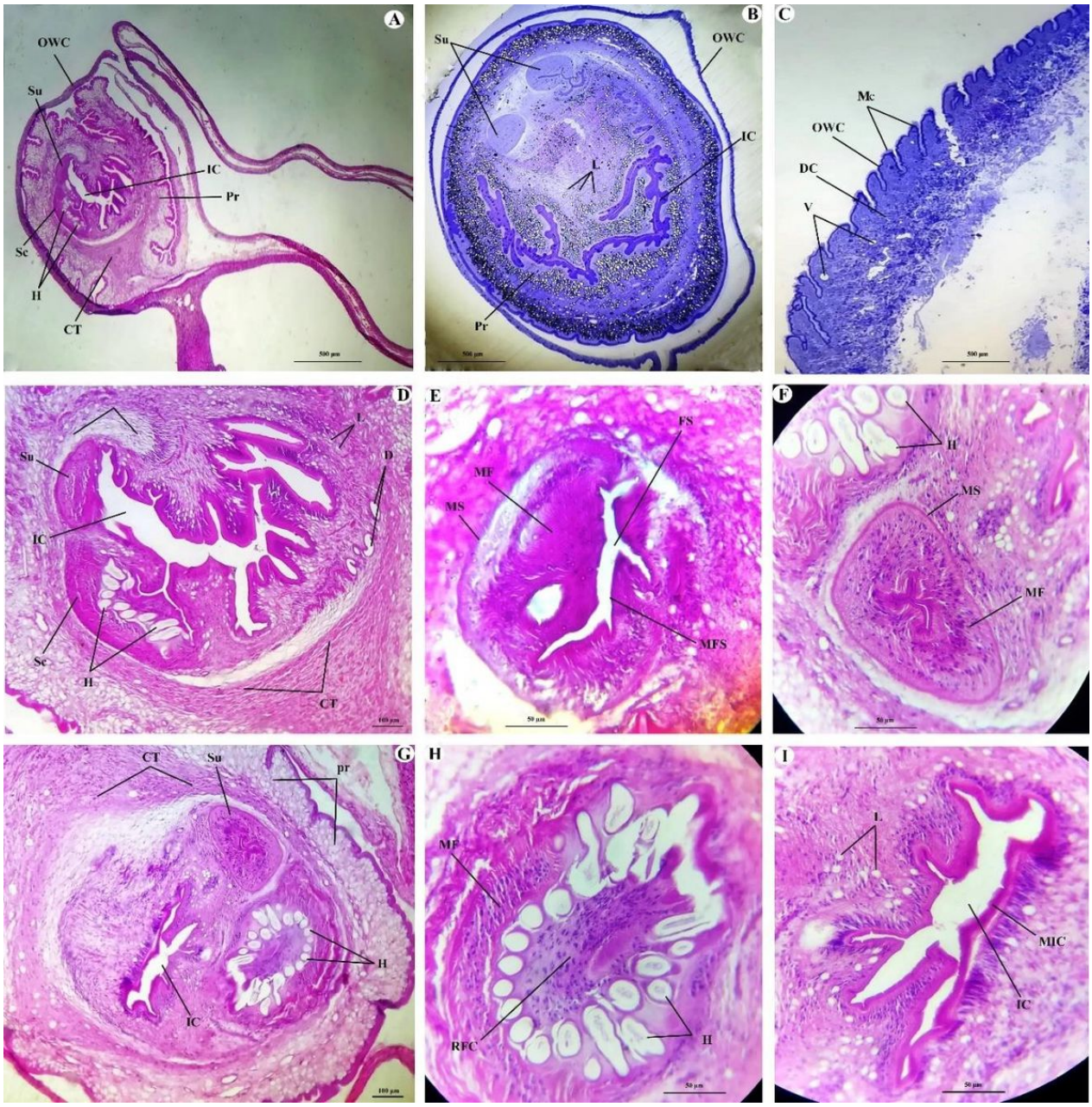

Figure 12

Light micrograph of transverse section of infected stomach of rabbit stained with H&E, showing gravid segments containing the eggs migrating among the gastric wall (arrows) (**A**), and high power to show the egg (Eg) enclosing *Taenia pisiformis*oncosphere (**B**) (H&E., x 60-160)

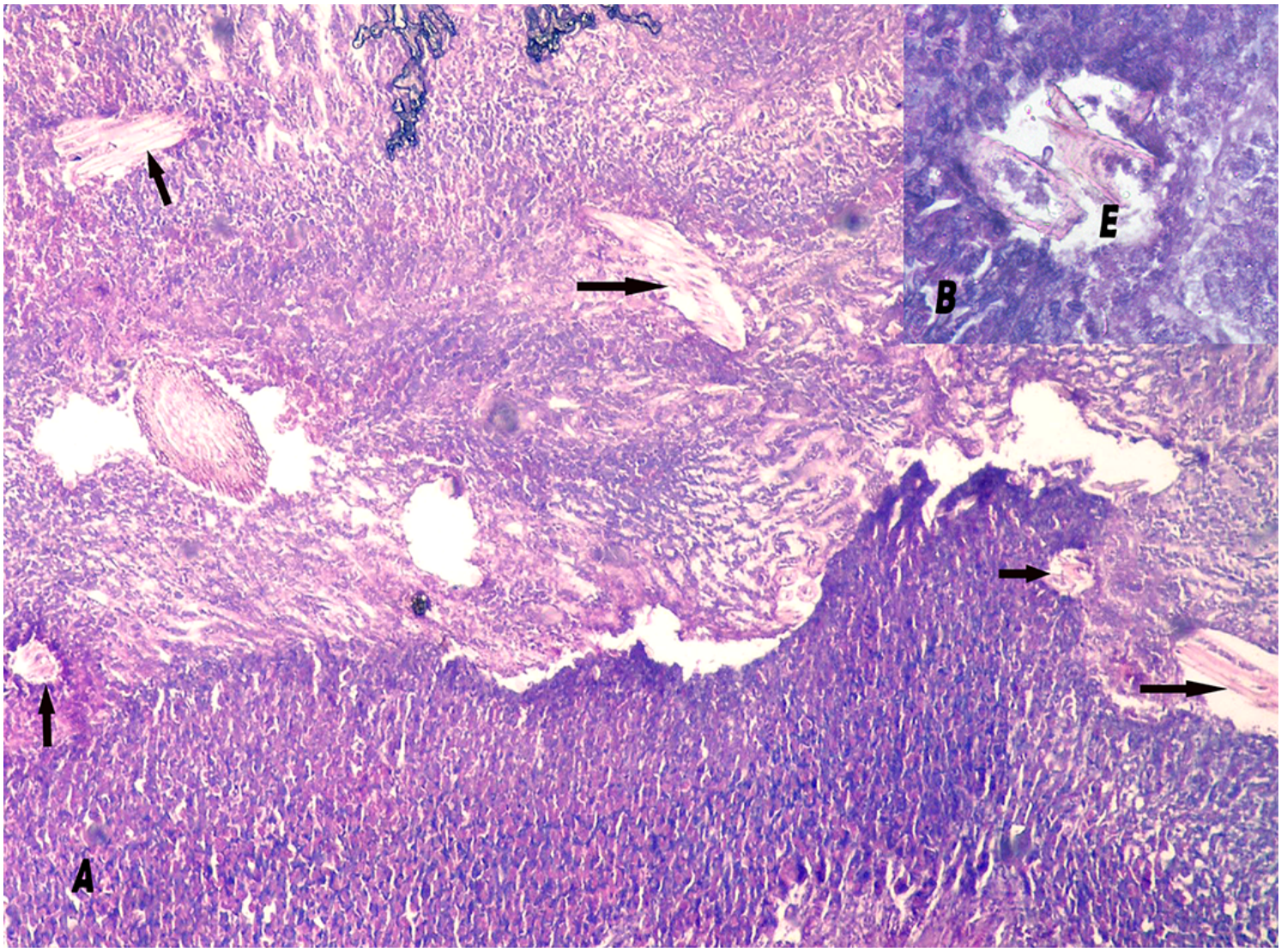

Figure 13

Light micrographs of transverse section of infected stomach of rabbit stained with H&E, showing mucinous degeneration in the epithelium lining the stomach (Ms) (**A**) and in the gastric gland (Ms) (**B**) (H&E., x 200)

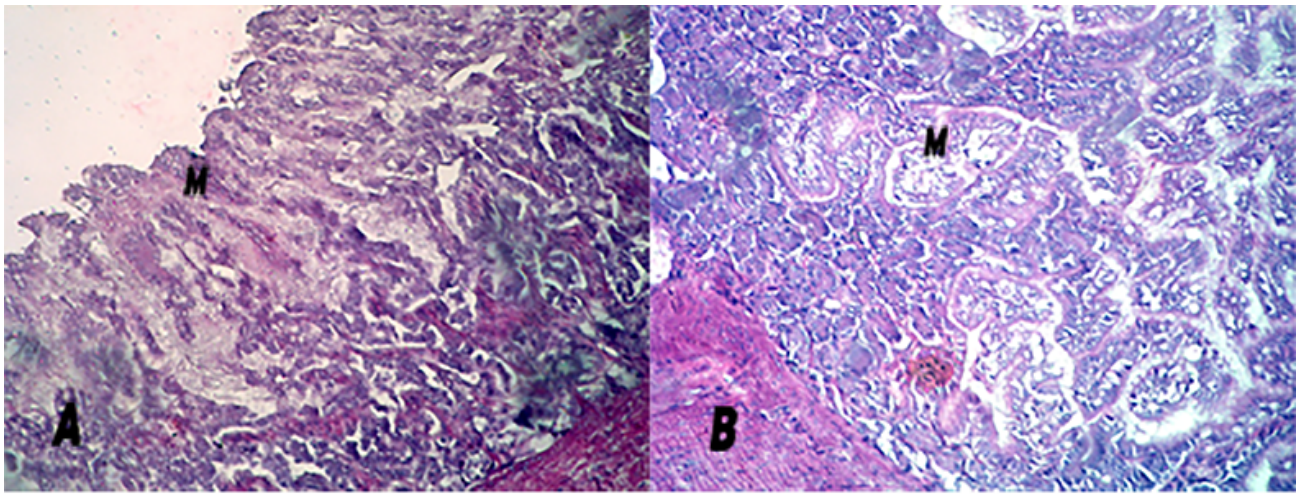

Figure 14

Light micrographs of transverse section of infected liver of rabbits stained with H&E, showing section of *Cysticercus pisiformis* penetrating the hepatic parenchyma (**A**), surrounded by a dense fibrous capsule compressing the hepatic cells (**B**), and the scolex with shallow depression in the anterior position (**C**) (H&E., x 60-120)

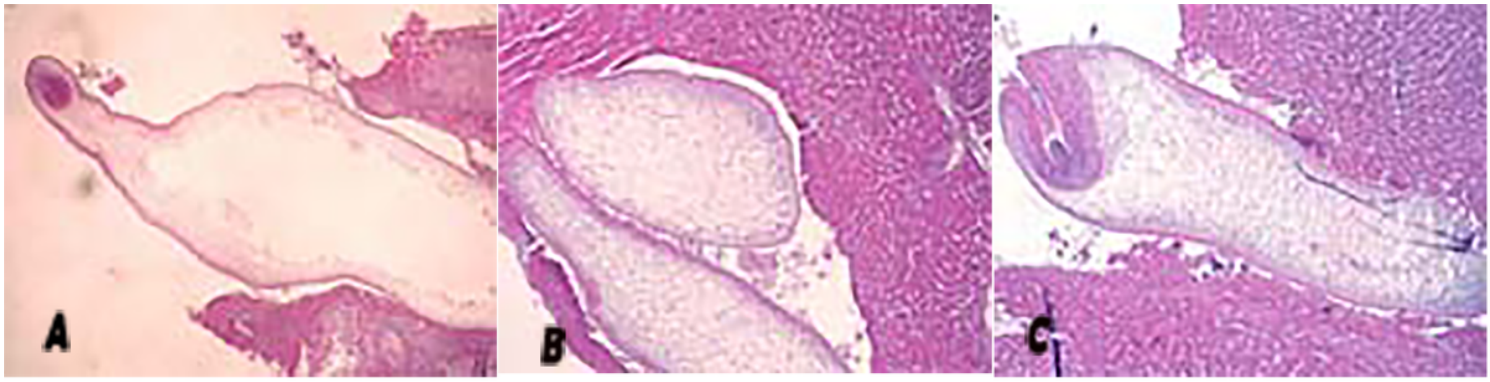

Figure 15

Light micrograph of transverse section of infected liver of rabbit stained with H&E, showing fibrinopurulent hepatitis with granuloma formation (**A**), characterized by severe necrosis (Ne) in the hepatic cells (**B**), with severe aggregation of polymorphonucleus (I) mainly neutrophils with few eosinophils and round cells as macrophages and lymphocytes surrounded thick wall of fibrous tissue proliferation forming granuloma (G) (**C**) (H&E., x 120-200)

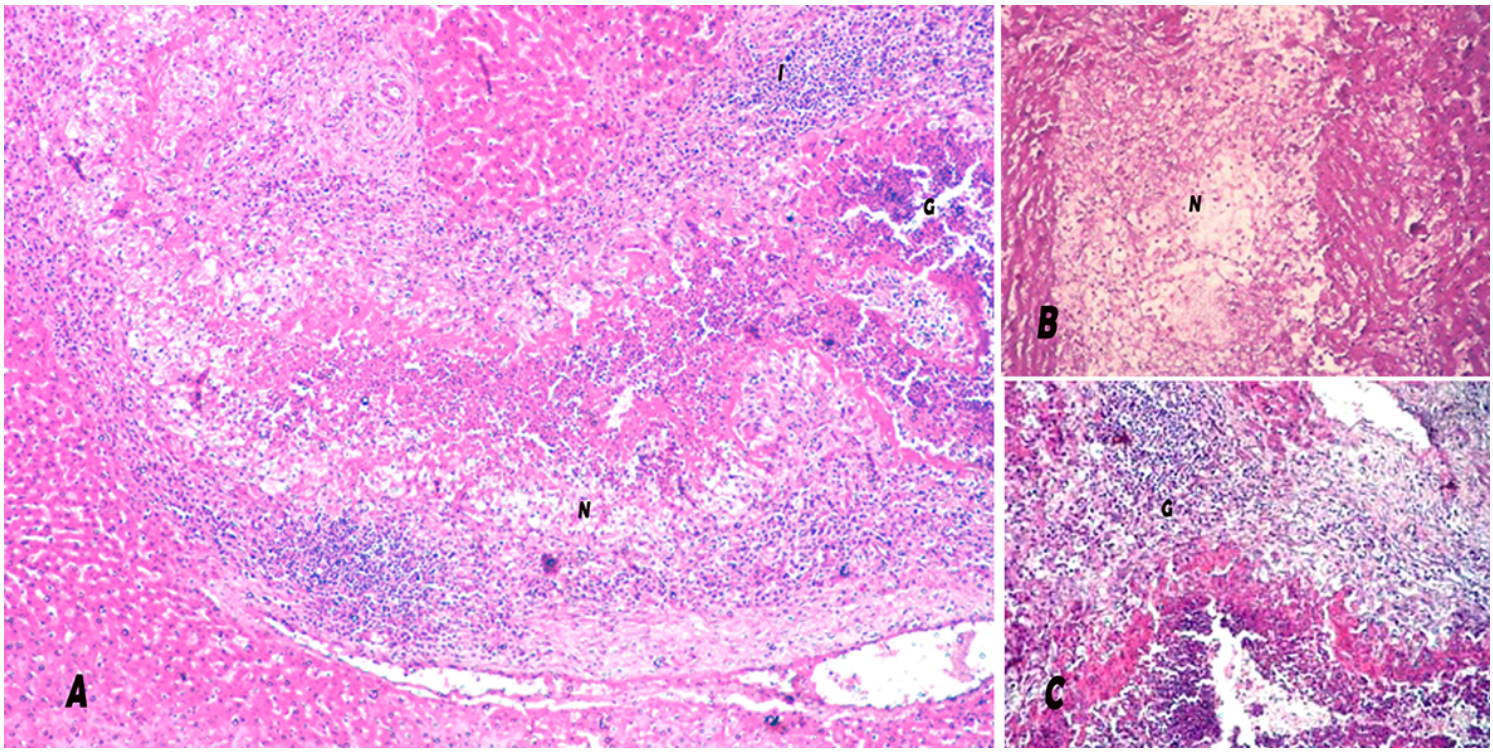

Figure 16

Light micrograph of transverse section of infected liver of rabbit stained with H&E, showing dead cyst replaced by necrotic area followed by mononuclear cells mainly macrophage, primarily macrophages, epithelioid cells, and fibrous tissue proliferation (H&E., x 160)

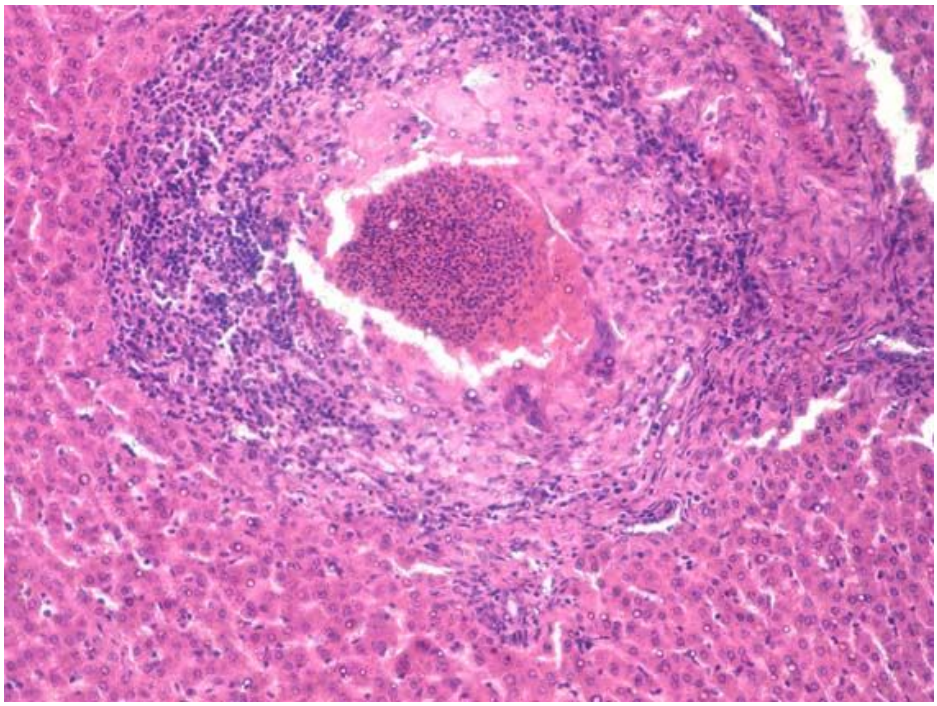

Figure 17

Light micrograph of transverse section of infected liver of rabbit stained with H&E, showing periportal fibrosis characterized by hyperplasia in the epithelial lining of the bile duct (Hp) causing lumen obstruction and fibrous tissue proliferation (FT) replaced the necrotic cells, surrounded the portal triad (H&E., x 200)

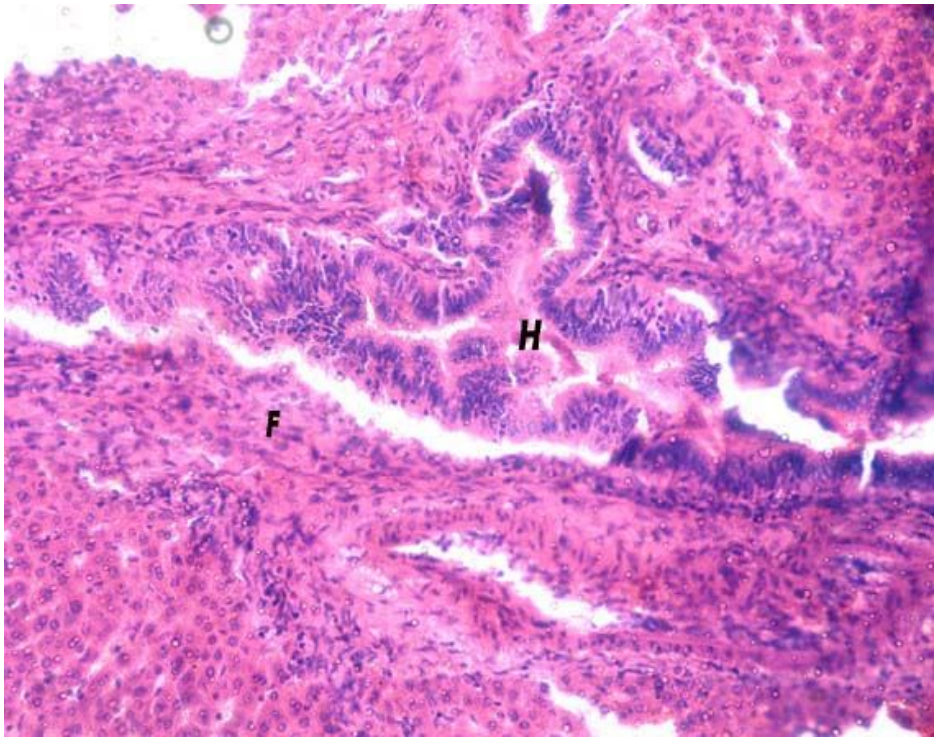

Figure 18

Legend not included with this version.