Shiraz University

IJVR

ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol. 27 No. 2 Ser. No. 95 2026

IRANIAN JOURNAL OF VETERINARY RESEARCH

Original Article

Experimental cerebral and non-cerebral coenurosis caused by *Taenia multiceps* in laboratory animals

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10.22099/ijvr.2026.53744.8047

(Received 21 Jul 2025; revised version 17 Jul 2026; accepted 28 Jul 2026)

Abstract

Background: Coenurosis, caused by the larval stage of *Taenia multiceps*, is a parasitic disease of considerable importance in veterinary medicine. **Aims:** This study aimed to evaluate whether rabbits, rats, and mice are susceptible to experimental infection with *T. multiceps* and can support the migration and/or development of its larval stage following oral inoculation with eggs obtained from adult worms derived from cerebral- and muscular-origin coenuri. **Methods:** The eggs derived from adult worms originating from cerebral and muscular coenuri, were orally inoculated to two groups of rabbits, rats and mice to trace the predilection sites of the larval stage of parasites. In addition, histopathological examinations were performed on brain and liver tissues from the experimentally infected animals. To identify the parasite in affected tissues based on mitochondrial DNA markers, PCR was performed, using primers targeting the *CO1* and *ND1* genes, followed by sequencing of the amplified products. **Results:** Necropsy and histopathological evaluation revealed no coenurus cyst formation in any of the examined tissues. However, eight infected rabbits displayed migratory tracks in the liver, together with histopathological lesions, including cell swelling and focal hemorrhages. Molecular analysis of liver samples confirmed the presence of *T. multiceps* DNA (*CO1* and *ND1* mitochondrial markers) in affected tissues. No histopathological changes or parasite remnants were detected in the tissues of infected rats and mice. **Conclusion:** Rabbits (*Oryctolagus cuniculus*) may serve as a potential animal model for studying certain aspects of coenurosis pathogenesis. However, further studies are necessary to clarify the role of host-specific factors, immune status, parasite strains, and infection methods in establishing experimental coenurosis.

Key words: Coenurosis, Experimental infection, Mouse, Rabbit, Rat

Introduction

Cerebral coenurosis, which is caused by the larval form of *Taenia multiceps* (*Coenurus cerebralis*), is a parasitic disease that mainly occurs in the central nervous system, most commonly in sheep and goats, and results in heavy economic losses in the animal husbandry industry (Liu *et al*., 2019; Mohammadi *et al*., 2021). *Taenia multiceps* is also a parasite of public health significance (Haq *et al*., 2024). The adult tapeworm is found in the small intestine of canids particularly domestic dogs, foxes, and wolves (Gunyakti Kilinc *et al*., 2020; Varcasia *et al*., 2022). Intermediate hosts become infected by ingesting *T. multiceps* eggs excreted in the feces of definitive hosts and the oncospheres then migrate into the brain or spinal cord to form a fluid-filled cyst (*Coenurus cerebralis*) (Varcasia *et al*., 2022). These cysts typically result in clinical neurological signs including ataxia, circling, blindness and head pressing and subsequent morbidity and mortality in the affected animals (Mohammadi *et al*., 2021; Varcasia *et al*., 2022).

Although coenurosis is extensively recorded in domestic animals, the utility of laboratory animals as experimental models to investigate the pathogenesis and immunology of *Coenurus cerebralis* has been scarcely addressed (Tas *et al*., 2024). It is important to develop a well-characterized laboratory animal model to enable detailed studies on the life cycle, host-parasite interaction and the development of efficient diagnostic and therapeutic techniques (Li *et al*., 2018, 2021). The relatively poor development of such models has prevented progress in the elucidation of the complexities

of coenurosis and the design of control measures (Philipo *et al*., 2024). Recent studies have indicated the necessity of investigating the genetic diversity, and molecular features of *T. multiceps* for diagnosis, and control measures (Liu *et al*., 2019; Gunyakti Kilinc *et al*., 2020; Li *et al*., 2021). Molecular tools, including PCR-based assays targeting mitochondrial genes such as cytochrome c oxidase subunit 1 (*CO1*) and NADH dehydrogenase subunit 1 (*ND1*), have been used as powerful methods for parasite genotyping and characterization (Oryan *et al*., 2015). In addition, transcriptome analysis has provided information on the gene expression profiles of various developmental stages of *T. multiceps*, and provided targets for drug and vaccine development (Li *et al*., 2021).

Another important field of study is the host immune response in the control of *Coenurus cerebralis* infection. Work on other cestode infections, including cystic echinococcosis (*Echinococcus granulosus*), has shown how the development of Th1 and Th2 responses plays a key role in the outcome of infection (Tamarozzi *et al*., 2022). The study of the immune response in various laboratory animal models might offer useful insights into parasite escape mechanisms and the possibilities of immunotherapeutic applications (Hidifira *et al*., 2023). Experimental studies have investigated a series of hypotheses regarding non-cerebral coenurosis, and showed that *Coenurus cerebralis* develops at sites other than the brain (Christodoulopoulos *et al*., 2016; Gururaj *et al*., 2019). More studies are required to elucidate the biological importance of non-cerebral coenurosis and its role in disease epidemiology and control.

Due to the constraints of the currently available control strategies, new approaches such as the application of vaccines and anthelmintic drugs need to be explored (Varcasia *et al*., 2022; Tas *et al*., 2024). The expression of proteins, for example heat shock proteins (HSPs) and acidic ribosomal protein P2, in *T. multiceps* has been reported to be closely related to the identification of promising vaccine and diagnostic targets (Huang *et al*., 2015, 2016; Liu *et al*., 2019). Recombinant DNA technology and immunoinformatics may make it possible to develop effective vaccines for coenurosis (Yu *et al*., 2024). Acute disease may be successfully treated with chemotherapy (Abbas *et al*., 2024). Surgical excision of *Coenurus cerebralis* cysts has been performed in sheep, however, it is not always possible especially for cysts located deeply in the brain (Tas *et al*., 2024).

The aim of this work was to help fill a gap in the knowledge regarding the use of laboratory animals for experimental coenurosis. Through experimental infection of rabbits, rats, and mice with *T. multiceps* eggs, this study also aimed to identify potential experimental animal models for studies of the pathogenesis, immune response, and control of this economically significant parasitic disease. Insights into the host-parasite relationships in these animal models may support the development of more efficient tools to prevent coenurosis in livestock and, eventually, in humans (Labuschagne *et al*., 2022).

Materials and Methods

Ethics approval and consent to participate

This experiment was performed under the approval of the state committee on animal ethics, Shiraz University, Shiraz, Iran. In addition, the recommendations of European Council Directive (86/609/EC) of November 24, 1986, regarding the protection of animals used for experimental purposes, was also considered. The dogs used in this study were stray dogs that dewormed one month before the experimental infection. The laboratory animals were purchased from Shiraz Branch of Razi Vaccine and Serum Research Institute (Shiraz, Iran).

Collection of the cysts

The cerebral cysts were obtained from the brains of infected sheep showing nervous clinical signs such as head-pressing behavior and circling. These naturally infected animals were from different areas of Fars Province, southern Iran. Non-cerebral cysts were obtained from naturally infected goats slaughtered at the Shiraz slaughterhouse, Fars Province, southern Iran.

Infection of dogs

Four dogs of mixed breed aged 3 to 6 months were used. The dogs were injected with a single subcutaneous dose of praziquantel (5 mg/kg). The Faeces of each dog were examined regularly for three successive days to confirm that they were free from cestodes particularly Taeniidae. The dogs were then divided into two groups (A and B), with two animals in each. The dogs of groups A and B were orally infected with cerebral and non-cerebral cysts respectively (300-400 protoscolices for each animal). The infected dogs were kept in isolated cages and fed with cooked food while they had free access to water. Four months after infection, the dogs were euthanized, the small intestines were opened and the adult worms were obtained for collection of parasite eggs.

Preparation of egg suspensions

At necropsy, the intestines of dogs from groups A and B were longitudinally incised and carefully inspected. The parasites originating from non-cerebral and cerebral coenuri were collected from the intestinal lumen of infected dogs and were washed three times with normal saline according to the method described by Flatt and Campbell (1974). A number of gravid segments were crushed in watch glasses then the eggs collected from the dogs of the two groups were stored separately in normal saline at 4°C until use.

Infection of laboratory animals

Fourteen adult female *Oryctolagus cuniculus* rabbits 180-185 days old, with an average body weight of 2.0±0.2 kg, were randomly divided into groups A (n=6),

B (n=6) and control (n=2) groups. After a period of adaptation, each animal of groups A and B was orally infected with 10,000 eggs (Worley, 1974; Flatt and Moses, 1975) of *T. multiceps* originating from cerebral and non-cerebral cysts, respectively. The eggs were diluted in 5 ml of PBS and inoculated, using a stomach tube. Fourteen female *Rattus norvegicus* rats weighing 260 to 280 g of 90-100 days old and 14 female *Mus musculus* mice weighing 80 to 120 g of 80-120 days old were similarly divided into A, B and control groups. The animals in groups A and B were orally infected with the eggs of *T. multiceps* originating from the cerebral and non-cerebral cysts, respectively. Each rat and mouse was infected with 6000 and 4000 eggs, respectively. Animals of the control groups received no eggs. Subsequently, the animals were kept under standard conditions while they had free access to water and food during the experimental period. The animals were checked daily for clinical signs and behavioral changes. At three- and five-months post-infection (the end of the experiment), the animals were euthanized. Euthanasia was carried out by intravenous injection of pentobarbitone sodium. Following necropsy, different organs including the brain, spinal cord, muscles, liver, lungs, kidneys and spleen were sliced and examined for pathological changes and coenuri cysts.

Molecular assay and sequence analysis

Total DNA was extracted from all cysts originating from both cerebral and non-cerebral sources, adult worms and damaged liver tissue of animals in the experimental groups using a DNA extraction kit (Qiagen DNAeasy; Qiagen, Valencia, California). The sequences of NADH dehydrogenase 1 (*ND1*) and cytochrome c oxidase subunit 1 (*CO1*) were amplified as previously described (Zhang *et al*., 2007; Lavikainen *et al*., 2008; Oryan *et al*., 2010). For amplification of ND1 JB11/JB12 primers (5´-AGA TTC GTA AGG GGC CTA ATA-3´/5´ ACC ACT AAC TAA TTC ACT TTC-3´) were used. For amplification of CO1 JB3/JB4.5 primers (5´-TTT TTT GGG CAT CCT GAG GTT TAT-3´/5´-TAA AGA AAG AAC ATA ATG AAA ATG-3´) were employed (Bowles *et al*., 1992; Bowles and McManus, 1994). All PCR products were sequenced, using a capillary DNA analyzer (ABI 3730; Applied Biosystems, Foster City, California). Forward and reverse nucleic acid sequences were assembled with BioEdit software version 7.0.5 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html).

Further comparison of the continuous sequences obtained from the infected animals was made with the original sequences of the parasite used for inoculation.

Histopathological studies

The infected and suspicious tissues were fixed in 10% solution of neutral buffered formalin, dehydrated in 70, 80, 90, 95 and 100% ethanol, cleared in xylol, and embedded in paraffin wax. Sections 5 µm thick were then stained with hematoxylin and eosin for light microscopic examination.

Results

Twelve experimentally infected and two control rabbits, rats and mice were assessed for a period of 90 (three animals in the test and one animal in the control groups) and 150 days (three animals in the test and one animal in the control groups), during which none showed any clinical signs of the disease. At necropsy, no coenuri were found in the subcutaneous tissues, muscles, viscera or central nervous system of the experimentally infected or control laboratory animals. Macroscopic furrows, possibly produced by the migrating oncospheres were seen in the liver parenchyma of only eight rabbits (five rabbits in group A and three in group B). Sections from the brains and livers were examined histopathologically. No evidence of parasitic invasion or development was detected in the brains of any of the animals but histopathological examination revealed cell swelling and focal hemorrhages in the liver parenchyma of eight

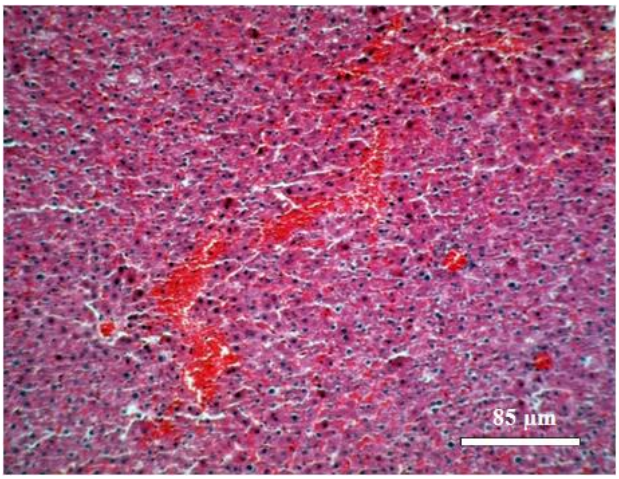

Fig. 1: Section from the rabbit liver tissue showing focal hemorrhage with cell swelling caused by the invasion of migrating oncospheres (H&E staining, scale bar, 85 µm)

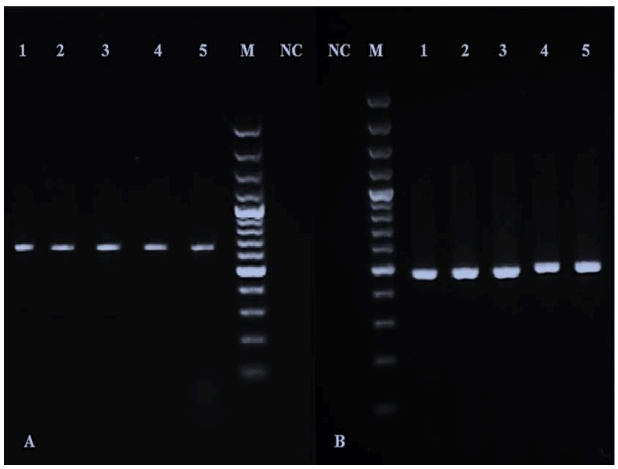

Fig. 2: Agarose gel electrophoresis of the PCR products amplified with DNA samples of *Taenia multiceps* by *ND1* primers (**A**) and *CO1* primers (**B**). Lane M: Marker ladder (100 bp). Lane NC: Negative control, and Lanes 1-5: Positive samples from hepatic lesions of rabbits

Table 1: The results of oral inoculation of the eggs of *Taenia multiceps* in different laboratory animals, three to five months after infection

Laboratory animal	Source of infection	Number of eggs	Number of animals	Euthanization Time (mounth)	Animals with migratory tracks or coenuri in cerebral organs	Animals with migratory tracks or coenuri in non-cerebral (liver) organs
Mouse	Cerebral (A)	4000	3	3	0	0
	Non-cerebral (B)	4000	3	5	0	0
	Cerebral (A)	4000	3	3	0	0
	Non-cerebral (B)	4000	3	5	0	0
	Control	0	1	3	0	0
	Control	0	1	5	0	0
Rat	Cerebral (A)	6000	3	3	0	0
	Non-cerebral (B)	6000	3	5	0	0
	Cerebral (A)	6000	3	3	0	0
	Non-cerebral (B)	6000	3	5	0	0
	Control	0	1	3	0	0
	Control	0	1	5	0	0
Rabbit	Cerebral (A)	10000	3	3	0	3
	Non-cerebral (B)	10000	3	5	0	2
	Cerebral (A)	10000	3	3	0	2
	Non-cerebral (B)	10000	3	5	0	1
	Control	0	1	3	0	0
	Control	0	1	5	0	0

infected rabbits (Fig. 1). In addition, the PCR assay and sequencing results revealed the presence of mitochondrial genome (*CO1* and *ND1*) of the parasite in the damaged livers of these eight rabbits (Figs. 2A and B).

The sequences (*CO1* and *ND1*) obtained from experimentally infected rabbits in both groups were 100% identical to each other as well as to the corresponding original parasite sequences. Finally, accession numbers from NCBI were provided for *CO1* (KT253933, KT253934) and *ND1* (KT253935 and KT253936) for sequences related to the cerebral and non-cerebral cysts, respectively. No pathological changes or coenuri were found in any of the examined organs of rats and mice in both groups (Table 1).

Discussion

Mice, rats, rabbits are common intermediate hosts for *Taenia pisiformis*, whereas rodents can serve as intermediate hosts for *T. crassiceps* and T. *taeniaeformis* (Alvi *et al*., 2021; Remesar *et al*., 2021; Rosa *et al*., 2022). The larval stages of these parasites are *Cysticercus pisiformis*, *Cysticercus crassiceps* and *Cysticercus fasciolaris*, respectively (Toral-Bastida *et al*., 2011; Alvi *et al*., 2021; Remesar *et al*., 2021; Rosa *et al*., 2022; Gomez-Puerta *et al*., 2023).

In the present study, we infected female *Mus musculus* mice with 4000 eggs of *T. multiceps* from both cerebral and muscular sources, but we did not observe the development of the larval stage of the parasite in cerebral or non-cerebral organs of the experimentally infected mice. Murine models of cysticercosis show that BALB/c mice (Th2-biased) allow Taenia larval growth, whereas C57BL/6 mice (Th1-biased) mount a sustained immune response that limits infection (Jiménez *et al*., 2023). In a study, female BALB/c mice were used as experimental animals to develop cysticerci following intraperitoneal inoculation with 5,000 *T. saginata* oncospheres. Viable cysticerci were observed in the organs of the animals from the 8th to the 20th week post-infection (Kandil *et al*., 2013). They confirmed the identification of cysticerci by polymerase chain reaction assay of the tumor-like cysts, at this stage of post-infection (Kandil *et al*., 2013). When the female normal C3H/HeN mice were subcutaneously infected with 5000 active oncospheres of *T. saginata asiatica*, viable cysticerci were observed in the infected animals at five weeks post infection (Peng *et al*., 2009). Mature cysticerci were recovered within the tumor-like cysts, in the inoculation sites, at seven to nine weeks post-infection (Peng *et al*., 2009). Mice have also been frequently used for establishment of secondary hydatid cysts, after intraperitoneal injection of live protoscolices (Mamuti *et al*., 2002; Ahmadnia *et al*., 2014; Moazeni *et al*., 2014a, b).

The mechanisms of host specificity or sex differences in susceptibility to experimental infections with taeniid cestodes are still unknown (Ito *et al*., 2001). The difference between the results might be due to genetic differences between the mice used in different studies. Although the mouse is a normal intermediate host for *T. crassiceps*, genetic factors play an important role in the susceptibility of this animal to the larval stage of the parasite (Kandil *et al*., 2013; Adalid-Peralta *et al*., 2021).

Rattus norvegicus rats were infected with 6000 eggs of *T. multiceps* from with both cerebral and muscular origins in the present study, but we did not observe the development of *Coenurus cerebralis* in cerebral or non-cerebral organs of the experimentally infected animals. To the best of our knowledge, there is no published report showing the successful establishment of the larval stage of taeniid tapeworms in rats following oral inoculation with the taeniid eggs.

We observed no apparent pathological lesions in any

external or internal organs of the experimentally infected mice and rats and our results indicated that female *Mus musculus* mice and *Rattus norvegicus* rats are resistant to oral infection with eggs of *T. multiceps*. One possible reason for this resistance could be the rapid excretion of ingested eggs so that the eggs do not remain for an optimum period in the small intestine and cannot complete their hatching process. However, the oncospheres hatch and gain access to the target tissues, some special requirements may be necessary for the development of the parasite. The factors that make mice and rats unsuitable hosts for infection are unknown. However, further studies are needed to evaluate the mechanism underlying this particular host parasite relationship.

The female *Oryctolagus cuniculus* rabbits were inoculated with 10000 eggs of *T. multiceps* from both cerebral and muscular sources, in the present study. Migration tracks of the oncospheres were observed in the liver parenchyma of experimentally infected rabbits infected with eggs from both cerebral and muscular sources. The pathological findings were confirmed by molecular investigation based on the *CO1* and *ND1* mitochondrial markers. *Taenia pisiformis* occurs in the small intestine of carnivores as definitive hosts (Bariselli *et al*., 2023). The larval stages of *Cysticercus pisiformis* are found in the liver and peritoneal cavity of rodents, including rabbits worldwide (Zhu *et al*., 2019). Rabbits display individual variation in immune competence because of genetic polymorphisms. Sex-associated susceptibility has also been documented, as female rabbits develop higher cyst burden than males, which may be hormonally mediated through immune function (Domínguez-Roldan *et al*., 2018).

An impairment of female fecundity and immune polarization in response to *T. pisiformis* infection has also been observed in the context of elevated progesterone levels (Hallal-Calleros *et al*., 2016; Rosa *et al*., 2022). On the other hand, numerous cysts may develop after inoculation of an animal with few eggs and conversely a low number of cysts may be produced after infection with high number of eggs (Betancourt *et al*., 2012; Wang *et al*., 2021). Immune protection has been induced by three different forms of the porcine S3Pvac anticysticercosis vaccine in a rabbit experimental model of *Taenia pisiformis* cysticercosis (Wang *et al*., 2021). The genetic background of the rabbits used, their sex, as well as the infectivity of the particular strains isolated in some areas may underlie this phenomenon (Betancourt *et al*., 2012). The incidence of cysticercosis due to *T. pisiformis* has been reported to be 3 to 93%, among wild rabbits, depending on age, intermediate and definitive host interactions, and environmental factors (Owiny, 2001; Tsotetsi-Khambule *et al*., 2018; Remesar *et al*., 2021).

The adult *Taenia serialis* inhabits the small intestine of canids such as wild and domestic dogs, foxes, and occasionally other carnivores as definitive hosts (Jenkins *et al*., 2014; Thakre *et al*., 2019). The larva, *Coenurus serialis*, occurs in rabbits, as intermediate hosts, forming fluid-filled cysts in subcutaneous tissue, intermuscular connective tissue, and sometimes visceral organs or behind the eyes (O'Reilly *et al*., 2002; Zhang *et al*., 2018). This tapeworm is regarded by some authors to be synonymous with *T. multiceps* (Soulsby, 1968). In a study, New Zealand White Rabbits were orally infected with 2000 eggs of *Taenia hydatigena* and *Taenia ovis.* Six weeks after infection, the larval stages of these parasites were found in the liver of the experimentally infected animals (Rickard and Coman, 1977).

One of the limitations of our study was the lack of independent positive control in the present study. However, it should be noted that the parasite eggs used in the present study were also used in other studies conducted in sheep and goats and cysts were observed in the organs of both animal species (Akbari *et al*., 2015; Oryan *et al*., 2015; Amrabadi *et al*., 2019). Considering that these studies were conducted by the same research group, the results of the present study could be linked to the above studies and their positive control groups may be considered as historical positive controls for the present work.

In the present study, the migration tracks of *T. multiceps* were observed in the liver of infected rabbits. It is evident that the larval stage of *T. multiceps* may show a particular tendency toward some internal organs, depending on the animal species of the intermediate host. It has been reported that *Coenurus cerebralis* may have a greater tendency to involve cerebral organs in sheep, while it has a greater tendency to involve non-cerebral organs, in goats (Akbari *et al*., 2015). There is limited knowledge concerning the direct or indirect effects of different factors involved in the susceptibility or resistance of laboratory animals to experimental infections with the larval stages of taeniid tapeworms. The species and strain, genetic factors, sex, enzymes, hormones and immunity of laboratory animals in addition to parasite burden, parasite species or strain virulence, infection frequency as well as the route of infection (oral inoculation or intragastric injection of eggs, intraperitoneal injection of scolices, or intraperitoneal, subcutaneous or intravenous injection of activated oncospheres) may play prominent roles in the intensity of infection. However, further studies are needed to evaluate the particular role and effects of these factors. Therefore, with regard to the presence of migratory tracks of *T. multiceps* and the *CO1* and *ND1* mitochondrial markers in the liver of experimentally infected rabbits, female *Oryctolagus cuniculus* rabbits may be introduced as an animal model for studying the larval stage of *T. multiceps*. However, further studies are required to more precisely determine the role of rabbit characteristics; parasite features, particularly the state of the immune system as well as the method of infection in the successful establishment of infection.

Acknowledgement

The authors are thankful to Shiraz University for

providing financial assistance.

Conflict of interest

The authors declare no competing interests.

Research funding

There was no funding.

Use of generative AI and AI-assisted technologies statement

The authors declare that artificial intelligence was not used in the preparation of this manuscript.

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