



# Experimental cerebral and non-cerebral coenurosis in goats: A comparative study on the morphological and molecular characteristics of the parasite



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## ARTICLE INFO

### Article history:

Received 6 May 2015

Received in revised form 7 June 2015

Accepted 8 June 2015

### Keywords:

*Taenia multiceps*

*Coenurus cerebralis*

Non-cerebral coenurosis

CO1

nad1

Experimental infection

Goats

## ABSTRACT

This experimental study was conducted to test whether cerebral and non-cerebral forms of *Coenurus cerebralis* belong to one origin or they are originated from two different tape worms. In the first step of the study, two groups of dogs were orally infected with the protoscoleces of cerebral and non-cerebral cysts and four months after infection, the adult worms were collected and morphologically characterized. Then the obtained eggs from two groups of adult worms were orally inoculated to two groups of goats to trace the predilection sites and also to compare the morphological and molecular characteristics of the larval stage of the parasites. The results showed that, both cerebral and non-cerebral coenuri, produced morphologically similar worms in the experimentally infected dogs. We observed only non-cerebral coenurus cysts in two groups of experimentally infected goats and the morphological characteristics of the cysts of two different groups were also similar. The molecular findings also showed that the cysts of two groups were 100% identical to each other based on the CO1 and nad1 sequences. In conclusion, our morphological and molecular findings reinforce the evidence that *Taenia multiceps* is the single origin of both cerebral and non-cerebral form of *C. cerebralis* but in goats, the cysts show more tendency to non-cerebral organs.

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## 1. Introduction

*Taenia multiceps* (Leske, 1780) is a taeniid cestode; its adult stage lives in the small intestine of dogs and other carnivores. The larval stage of *T. multiceps*, known as *C. cerebralis* causes coenurosis, in herbivore animals and has a worldwide distribution. *C. cerebralis* is usually found in the nervous system including brain and spinal cord in sheep and other ruminants (Scala and Varcasia, 2006; Sharma and Chauhan, 2006; Scala et al., 2007; Batista et al., 2010). Coenurosis has been also reported from horses, pigs and occasionally humans (Varcasia et al., 2012, 2013). Goats and sheep of all ages are susceptible to coenuriasis, and it presents a serious economic effect for the owners of these animals (Oryan et al., 2010). *Coenurus* cysts are frequently found in nervous system including brain and spinal cord. However, their occurrence, especially in

goats, in subcutaneous fascia, peritoneal areas, intramuscular tissues and other organs has been frequently reported (Sharma and Chauhan, 2006; Oryan et al., 1994, 2014). Intra-muscular coenuri have been described for the first time by Benkovskij (1899) and later by Sopikov (1931) and Boev and Mesjacev (1935) in sheep in Kazakhstan and by Shibaev (1937) in Uzbekistan while Gaiger (1907) found cases of coenuri in the connective tissues of goats in India (Schuster et al., 2010). Since non-cerebral coenurosis was seen for the first time in goats in India in 1907 by Gaiger, the species was named *M. gaigeri* in honor of Gaiger by Hall (1916) (Varcasia et al., 2012).

Acute cases of coenurosis are characterized by symptoms, such as fever, ataxia, muscle tremors and hemorrhagic retinal lesions. In chronic cases, major clinical signs in goats and sheep include dullness, circling, torticollis, loss of appetite, frequent bleating, pain response on pressure over the cystic area, paralysis, blindness, nystagmus, lack of coordination, lethargy and lack of response to stimuli. Infected animals tend to move away from the herd and press their head against objects. Acute coenurosis is a fatal disease

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and death occurs as a result of encephalitis on average 30 days after infection (Sharma and Chauhan, 2006; Batista et al., 2010)

The clinical signs and symptoms of coenurosis in sheep may be confounded with listeriosis, papilledema, polioencephalomalacia, head trauma or brain abscess (Batista et al., 2010). The mild to moderate forms of the non-cerebral coenurosis do not show specific clinical signs and are not clinically detectable and the cysts are found in the slaughterhouse. However, in severe cases lameness, paresis and paralysis together with outgrowing skin lumps, due to the subcutaneous cysts, are the major clinical manifestation of this disease. Location of the cysts in muscles may cause pain, muscular degeneration, necrosis and atrophy or result in impaired function of the involved organ particularly in severe infections (Oryan et al., 2014). Coenurosis is also zoonotic in nature and lead to serious pathological conditions in man (Sharma and Chauhan, 2006)

However, no experimental study has been conducted to elucidate whether cerebral and non-cerebral coenurosis belong to the same origin or they are originated from two different cestodes. In the present study, the dog as the definitive host of the parasite was experimentally infected with cerebral and non-cerebral coenurus cysts and the obtained eggs were experimentally ingested to goats as the intermediate hosts of the parasite. Thus, the present study was undertaken to compare the morphological characteristics of the adult worms resulting from both cerebral and non-cerebral cysts and also to compare the predilection sites and morphological and molecular characteristics of the larval stage of the parasite resulting from both types of the adult worms.

## 2. Materials and methods

### 2.1. Experimental design

In the first step of this study, two groups of dogs were orally infected with protoscoleces of coenurus cysts collected from the brain of sheep (cerebral coenuri) and muscles of goats (non-cerebral coenuri), respectively. The infected dogs were euthanized four months after infection and the small intestines were opened and the adult worms were collected for morphological comparison and preparation of the egg suspensions. Two groups of goats were then orally infected with the eggs of adult worms originated from the cerebral and non-cerebral cysts, respectively. Four, eight and 12 months after infection, the animals were humanely euthanized and the brain, spinal cord, muscles, liver, kidneys, lungs and spleen of the animals of the two groups were carefully examined for cyst formation. The cysts from the animals of the two groups were collected, counted and their morphological and molecular characteristics were compared.

### 2.2. Collection of cysts

The cerebral cysts were collected from brain of the naturally infected sheep with nervous manifestations such as circling and head-pressing behavior from different regions in Fars Province (southern Iran). Non-cerebral cysts were obtained from the naturally infected goats slaughtered at the Shiraz slaughterhouse in Fars Province, southern Iran.

### 2.3. Infection of dogs

Four puppies of mixed breed, aged 3–6 months were divided into groups A and B, two animals in each. Before infection, the feces of all dogs were examined for three consecutive days to ensure that they were free of tape worms especially taeniid species. The dogs in groups A and B were orally infected with 300–400 protoscoleces of cerebral and non-cerebral cysts respectively. The infected dogs were kept in separate cages, fed cooked food and had access to

water and food ad libitum. The gravid segments were first observed in the feces of infected dogs, 56 days after infection. The infected dogs were humanely euthanized 4 months after infection.

### 2.4. Collection of adult worms and morphological studies

After necropsy, the small intestines of the dogs of both groups were opened and the adult helminthes were collected separately and placed in a jar containing physiological saline. After relaxation, fixation, staining, dehydration and clearing of samples, measurements were done, using a calibrated eye piece micrometer and photographed under the microscope with a Canon digital camera. The main morphological features such as the diameter of scolex, rostellum and suckers, number of testes, length of testes and ovary, length and width of cirrus-pouch in both mature and gravid proglottids, the size of poral and aporal lobes of ovary, extension of cirrus-pouch, existence of vaginal sphincter, vaginal dilatation and vaginal loops (in cortex) and number of main uterine branches (unilateral) were evaluated, measured or counted and compared between the worms of the two groups. Twenty-three samples of mature and gravid segments of adult worms originated from the cerebral cysts were morphologically compared with 18 samples of mature and gravid segments of adult worms originated from the non-cerebral cysts.

### 2.5. Preparation of the egg suspensions

After necropsy and collection of the adult worms, a number of gravid proglottids obtained from the two groups of dogs were collected separately and washed several times with normal saline. The gravid proglottids were then teased in watch glasses and the eggs were collected. The eggs obtained from the dogs of two groups were stored separately in physiological saline at 4 °C until use (2–3 days).

### 2.6. Infection of goats

Twelve female weaned kids of mixed breed from a farm with no history of coenurosis were divided randomly into two groups 6 animals in each. The eggs were homogenized, diluted in PBS, and a calculated volume of egg suspension was prepared. The animals in groups 1 and 2 were orally inoculated with 80,000–100,000 eggs of adult worms originated from the cerebral and non-cerebral cysts respectively, using a stomach tube. The infected animals were housed under good conditions with free access to food and water. The animals were checked daily to detect clinical signs and behavioral changes.

### 2.7. Collection of coenuri from the experimentally infected goats

Two animals in each group were humanely euthanized at 4, 8 and 12 months after infection. Following necropsy the brain, spinal cord, entire musculature (thigh, biceps femoris, triceps, thorax, abdomen and diaphragm) and other organs such as brain, liver, kidneys, lungs, heart and spleen were sliced and examined carefully for coenuri. Viable and dead cysts were classified based on their macroscopic appearance. The cysts with clear fluid were considered as viable but the caseous or calcified cysts were considered as dead ones. The viable cysts were collected and washed with normal saline, placed in a Petri dish and used for further studies.

### 2.8. Morphological studies on the cysts

The number of coenuri in each animal and the number of protoscoleces in each coenuri were counted and recorded separately for the animals of both groups. The protoscoleces were kept in 70%

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