First Record of *Mesocestoides* spp. Vaillant, 1863 Tetrathyridia (Cestoidea: Cyclophyllidea) in Anatolian lizard, *Anatololacerta danfordi* (Günther, 1876) in Turkey

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Abstract. *Mesocestoides* spp. tetrathyridia were first recorded in Turkey in April and December 2006, from *Anatololacerta danfordi* individuals. Tetrathyridia were found encapsulated in the livers of the host animals. Although prevalence of infection was low, its intensity was very high. Both morphological and histological features of tehtrathyridia were determined. The morphological examination revealed the absence of buds, multiple scoleces, or any other evidence of asexual proliferation.

Key Words: *Mesocestoides*, tetrathyridia, *Anatololacerta danfordi*, cestodes, intermediate host, calcareous corpuscles.

Introduction

Mesocestoides spp. have been recognized for a long time as possessing several characteristics that make them distinct from all other cyclophyllidean tapeworms. For example, the median ventral position of the genital atrium and bipartite vitelline gland are unique within the Cyclophyllidea. Another characteristic feature may be the inferred requirement for three hosts in the life cycle (Crosbie et al. 2000). The life cycle of this tapeworm is indirect. It is generally thought that two intermediate hosts and one definitive host are involved in the life cycle, although the complete life cycle has never been worked out and some authors questioned the presence of a first intermediate host. The entire life cycle of *Mesocestoides* is still not clear (Bonfanti et al. 2004). The first intermediate host is a coprophagous arthropod which is ingested by the second intermediate host, usually a small rodent, a snake, a lizard, a toad, a bird or a frog, where the development of the second larval stage (tetrathyridium) occurs (Loos-Frank 1980, Hanson & Widmer 1985, McAllister 1988, McAllister & Conn 1990, McAllister et al. 1991a,b, Widmer et al. 1995, Bolette 1997, Gillilland III & Muzzall 2002, Millán et al. 2003, Bonfanti et al. 2004, Literák et al. 2004, Tantaleán & Chavez 2004, Muzzall 2005). The adult form of *Mesocestoides* develops in the intestine of the definitive host. Peritoneal infection occurs when ingested tetrathyridia penetrates the intestinal wall (Parker 2002, Caruso et al. 2003, Bonfanti et al. 2004, Hazıroğlu et al. 2005). Definitive hosts include humans, several wild and domestic carnivores, e.g. skunks, foxes, jackals, martens, coyotes, dogs and cats etc. (Schmidt & Todd 1978, Eom et al. 1992, Crosbie et al. 2000, Fuentes et al. 2003, Bonfanti et al. 2004, Ribas et al. 2004, Eguia-Aguilar et al. 2005, Dalimi et al. 2006, Saeed et al. 2006).

Thus, the aim of the present investigation was to evaluate the prevalence and morphological, histological characteristics of tetrathyridia of *Mesocestoides* spp. in *Anatololacerta danfordi*.

Materials and Methods

Anatololacerta danfordi (Günther, 1876) was collected in Spil Mountain National Park (Manisa), Yamanlar Mountain (Karşıyaka-Izmir) and Bozdag (Odemiş-Izmir) during April and December 2006.

A total of 17 lizards (*A. danfordi*) were collected. They were anaesthetized with ether and dissected. An undetermined number of encapsulated parasites were found in the livers removed from the hosts. These were placed on clean glass slide with a drop of 0.6% NaCl solution. The livers were incised with a mounted needle and a thin film of the liver fluid was drawn out on a slide for examination of living parasites. The tetrathyridia of *Mesocestoides spp.* Vaillant, 1863 were observed under a microscope light, and then fixated in 10% formaldehyde-alcohol solution. The fixed parasites were covered with adhesive mixture and stained with Borax-Carmine, Haematoxylin-Eosine and Ferric Haematoxylin methods (Mahoney 1966). For paraffin sections, small pieces of the liver were fixed in Bouin. Paraffin sections were cut to 5 µm thickness and stained with Haematoxylin-Eosine. Larval stages were measured with a calibrated ocular micrometer and photographs were taken with an Olympus CX51-Altra 20 Soft Imaging System.

SPSS (10.0) statistical package was used to get summarized statistics related to various morphological characteristics. Morphometric data are presented below (results section) as the range followed by the mean ± standard deviation and the number of measurements taken (n). In order to make easy comparisons with the references, measurements about length and width of larval stages were converted to mm from µm.

Results

Mesocestoides Vaillant, 1863 tetrathyridia were observed in the livers of six individuals (35.29%) out of the total 17 examined. The highest prevalence of larval stages related to the collection site of the individuals was observed in Spil Mountain National Park (Manisa) (50%) (tab.1). The intensities of infection by tetrathyridia in the lizards were very high (fig.1).

In the smear preparations, tetrathyridia were of variable shape, mostly longitudinally elongated, 0.3-1.3 mm (0.6 ± 0.2 mm, n= 50) long and 0.1-0.6 mm (0.3 ± 9.1 mm, n= 50) wide. Sometimes they were shorter and thicker. Their scoleces exhibited various degrees of invagination. The tetrathyridia started to invaginate in the midbody of the organism. Four suckers with a diameter of 67.50-187.50 µm (95.45 ± 20.92 µm, n= 100) and length of 75-400 µm (133.67 ± 39.77

μm, n= 100) were distinct on each scolex and without rostellum and hooks (fig.2). The cestode larvae were flat and nonsegmented. The pore of the osmoregulatory system (excretory pore) was frequently seen at the posterior end of the body (fig.3).

Table no.1 Prevalence of *Mesocestoides* spp. Tetrahyridia in *Anatololacerta danfordi* samples collected from the three different localities.

Localities	Number of dissected *A. danfordi*	Prevalence of infection (%)
Spil Mountain (Manisa province)	2	50
Yamanlar Mountain (Karşıyaka, İzmir province)	9	33.33
Bozdag Mountain (Odemiş-İzmir province)	6	33.33

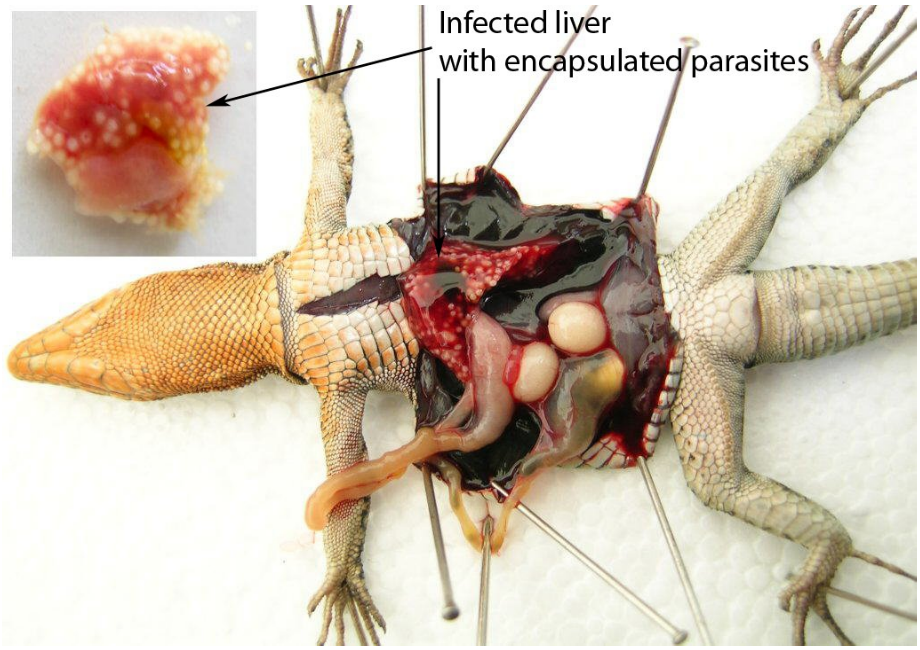

Figure no.1 Ventral aspect of a dissected and infected Anatolian Lizard, *Anatololacerta danfordi* with *Mesocestoides* tetrathyridia.

Figure no.2 A general aspect of a tetrathyridium of *Mesocestoides* spp. isolated from the infected liver of a Anatolian Lizard. (S) Tetra-acetabulate scolex.

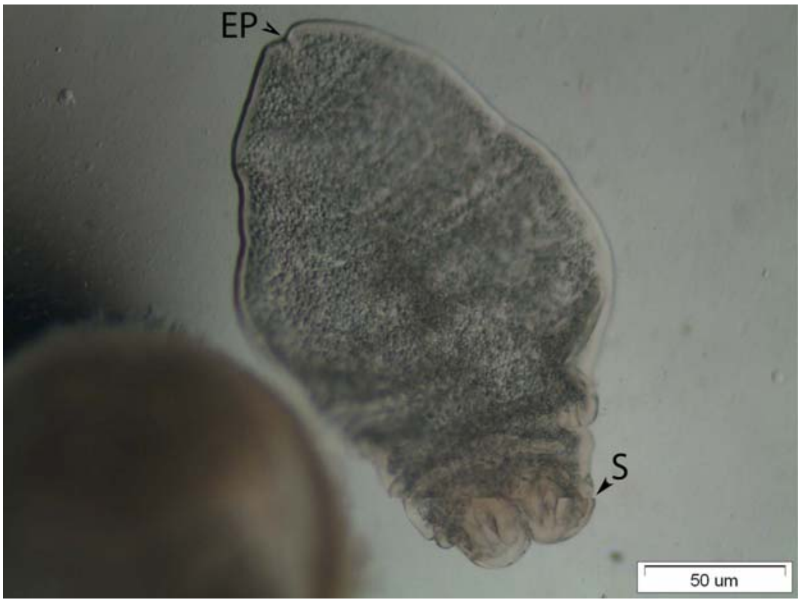

Figure no.3 A general aspect of a tetrathyridium of *Mesocestoides* spp. isolated from the infected liver of a Anatolian Lizard. (S) Tetra-acetabulate scolex, (EP) Excretory pore.

The paraffin sections of the larvae showed the presence of tetra-acetabulate scolex in the invaginated canal. *Mesocestoides* larvae were multiple, small, having lightly basophilic characteristic, large, empty, calcareous corpuscles (blue bodies with clear halos) within the mesenchymal network. Individual larvae were different in shape with convoluted borders. Larvae were lined by a syncytial tegument. The tegument had a thick eosinophilic, smooth surfaced cuticle. Beneath the cuticle was a single layer of cells. The excretory pore was in the hindbody. The remaining body of the parasite was composed of a loose mesenchymal network with widely scattered parenchymal and muscle cells. Numerous clear vesicles, i.e. calcareous corpuscles which are round to oval in shape, were observed within the matrix of the parasite (fig.4).

Discussion

This is the first record of tetrathyridia of *Mesocestoides* Vaillant, 1863 sp. from *A. danfordi*. Their identification to the species level has been difficult, due to the morphological uniformity of the tetrathyridia of *Mesocestoides* spp. Until now, the only reliable method has been the experimental infection of carnivores (Literák et al. 2004). According to Specht & Voge (1965), tetrathyridia are capable of asexual reproduction and can be maintained easily both *in vivo*, in experimental hosts (mouse and rat), and *in vitro* under appropriate culture conditions. Tetrathyridia are also capable of sexual differentiation *in vitro*. Many studies have been conducted to identify factors that are able to induce *in vitro* tetrathyridia differentiation (Markoski et al. 2003, Espinoza et al. 2005). The asexual reproduction of tetrathyridial metacestodes by longitudinal fission originally described by Specht & Voge (1965) in lizards is also unique but does not appear to be a universal characteristic of *Mesocestoides* spp. Similar proliferation of tetrathyridia representing other isolates of *Mesocestoides* spp. has not been unequivocally described and may be rare (Crosbie et al. 2000).

In the present study, none of the larval stages in the livers of lizards showed any morphological evidence for asexual proliferation (e.g. buds or multiple scoleces). In the studies conducted by McAllister & Conn (1990) and McAllister et al. (1991a,b), none of the *Mesocestoides* tetrathyridia exhibited any evidence of asexual proliferation such as multiple scoleces or buds in lizards, frogs and snakes. However, Hanson & Widmer (1985) reported observational evidence on the proliferation of tetrathyridia in the lizard *Sceloporus occidentalis*. This report is the first experimental evidence of asexual multiplication of tetrathyridia of a species of *Mesocestoides* in an ectothermic host. Later, Widmer et al. (1995) reported the first significant evidence for asexual replication of the proliferous tetrathyridia (*Mesocestoides* spp.) in an experimentally infected reptilian host.

In this study, the encapsulated tetratyridia of *Mesocestoides* spp. were found only in the livers of the lizards. According to Specht & Voge (1965), the liver is the principal organ for natural infections in lizards. *Mesocestoides* spp. can survive in low-oxygen environments.

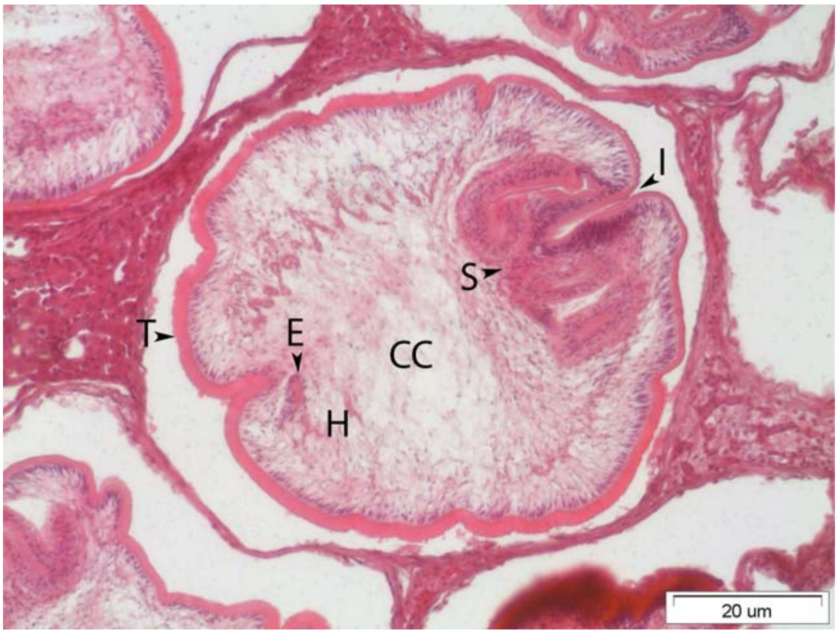

Figure no.4 A longitudinal section appearance of the tetrathyridium of *Mesocestoides* spp. encapsulated in the liver of *Anatololacerta danfordi*. Characteristic features include the epithelium of the excretory pore (E), solid cellular hindbody (H), deep vagination canal (I), tetra-acetabulate scolex (S), calcareous corpuscles (CC), and syncytial tegument (T). Note the absence of buds, multiple scoleces, or other evidence of asexual proliferation. Also note the thin host capsule, normal appearence of hepatic parenchyma and pigment deposition. (Fixed and stained with Bouin solution and Haematoxylin-Eosine, respectively).

We observed calcareous corpuscles in the matrix of larval stages. Calcareous corpuscles are specific to cestodes (Caruso et al. 2003). The function of calcareous bodies, commonly found in the parenchyma of cestodes, is relatively poorly understood (Etges & Marinakis 1991). Calcareous corpuscles are believed to be of cellular origin, possibly originnating from cytoplasmic vacuoles or golgi vesicles. Corpuscles originated within the mesenchymal cells of the metacestode and cestode consist of an organic matrix, usually organized in concentric rings and an inorganic matrix consisting mainly of calcium, phosphorus, zinc, silicon, magnesium and carbonate. They may serve as a buffer to protect the metacestodes as they pass through the host's stomach or as a means of calcium sequestration to protect the metacestodes/cestodes from calcification. More likely, calcareous corpuscles are excretory products of the metacestodes/ cestodes, because they can be excreted from the tegument of the larval and adult tapeworm stages (Etges & Marinakis 1991, Caruso et al. 2003).

To determine the first intermediate host of *Mesocestoides* spp., Padgett & Boyce (2005) have been used a PCR-based diagnostic assay method which can detect *Mesocestoides* DNA within pooled samples of ants. However, no mouse became infected with *Mesocestoides* metacestodes after ingesting these ants. Thus the life cycle is not clear.

When the percentage of infection by *Mesocestoides* spp. tetrathyridia in *A. danfordi* (35.29%) was compared with those given by other authors for different second intermediate host species from other countries (tab.2), a large degree of variability was observed.

The measurements of length and width of the Anatolian lizard tetrathyridia and the diameter values of the suckers were found very low compared with those for the European Starling, *Sturnus vulgaris* as a second intermediate host given by Literak et al. (2004) (tab..3). These variations in the measurements may indicate that the host type has an important effect on the sizes of larval stages.

Table no.2 Comparison of the prevalence in different intermediate hosts infected with tetrathyridia of *Mesocestoides* spp. and the present study.

Intermediate host species	Prevalence (%)	Country	Authors
Rana berlandieri	50	U.S.A.	McAllister and Conn (1990)
Rana pipiens	3	U.S.A.	McAllister and Conn (1990)
Cnemidophorus dixoni	5	U.S.A.	McAllister and Conn (1991)
C. gularis septemvittatus	9	U.S.A.	McAllister and Conn (1991)
C. marmoratus	3	U.S.A.	McAllister and Conn (1991)
C. tesselatus	3	U.S.A	McAllister and Conn (1991)
Crotalus atrox	67	U.S.A	Bolette (1997)
Alectoris rufa	2.7	Spain	Millán et al. (2003)
Anatololacerta danfordi	35.25	Turkey	Present study

Table no.3 Comparison of the measurements of tetrathyridia of *Mesocestoides* spp. obtained from a different study and the present study

Intermediate host species	Length of larval stages (mm)	Width of larval stages (mm)	Diameter of suckers (μm)	Authors
Sturnus vulgaris	2.30-8.53	1.31-2.84	118-206	Literák et al. (2004)
A. danfordi	0.32-1.31	0.17-0,6	67.50-187.50	Present study

In conclusion, *A. danfordi* seems to have an important role as second intermediate host of *Mesocestoides* spp., since it a high infection prevalence was found in the examined individuals (35.25%). Infected lizards could indicate that wild animals and humans in those regions are seriously at risk. Wild animals from Spil Mountain National Park are especially threatened, since here were found most of the second intermediate hosts (50%). Due to the high infection prevalence found there, the risk for definitive hosts is high.

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