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First report of natural infection of *Xerolenta obvia* (Pulmonata, Mollusca) by Dicrocoeliidae (Digenea) larval stages in Turkey

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Abstract

Aim of study: This study aimed to investigate the prevalence and morphological features of Dicrocoeliid larval stages (sporocysts and cercariae) in the terrestrial snail *Xerolenta obvia* (Menke, 1828) inhabiting Kastamonu.

Material and Methods: *X. obvia* samples were gathered after rainfall in the surrounding areas of the Kastamonu province over the spring (i.e. April and May) and autumn (i.e. October and November) of 2016. The samples were dissected alive, and their hepatopancreata were removed and placed into glass tubes containing a 0.6% NaCl solution. The hepatopancreas was dissected using a needle and liquid was spread on a slide as a thin layer, to investigate the parasites' live larval stages. Dicrocoeliid larval stages (sporocysts and cercariae) were examined under a compound microscope, fixed in 5% formalin and stained with Semichon's acetocarmine. The samples were also prepared for observation under a scanning electron microscope.

Main results: This was the first report of *X. obvia* as an intermediate host in the life cycle of the Dicrocoeliidae family in Turkey. In Kastamonu, the infection prevalence was 0.78%, with its highest occurrence in April.

Research highlights: Since the snails were gathered mainly from pastures surrounding the villages and farms, where herbivorous animals graze, it was concluded that the larval stages found in the snails might belong to *Dicrocoelium dendriticum*. The presence of larval stages of this trematode parasite in the snails gathered from the pastures poses a risk to the definitive host animals.

Keywords: *Xerolenta obvia*, Dicrocoeliidae, *Dicrocoelium dendriticum*, Larval stages, Kastamonu, Turkey

Xerolenta obvia (Pulmonata, Mollusca)'nın Dicrocoeliidae (Digenea) larval safhalarıyla doğal enfeksiyonunun Türkiye'den ilk raporu

Öz

Çalışmanın amacı: Bu çalışmada, Kastamonu'da dağılış gösteren kara salyangozu *Xerolenta obvia* (Menke, 1828)'daki Dicrocoeliid larval safhalarının yaygınlığının tespit edilmesi ve bu safhaların (sporokist ve serkarya) morfolojik özelliklerinin araştırılması amaçlanmıştır.

Materyal ve Yöntem: X. obvia örnekleri 2016 yılının ilkbahar (Nisan ve Mayıs) ve sonbahar (Ekim ve Kasım) aylarında yağmurdan sonra Kastamonu ili çevresinden toplanmıştır. Canlı iken diseke edilip, hepatopankreasları çıkarılmış ve %0.6'lık NaCl solüsyonu içeren temiz küçük cam tüplere yerleştirilmiştir. Bir iğne kullanılarak hepatopankreasları diseke edilmiş ve parazitin canlı haldeki larval safhalarını araştırmak için sıvı bir lamın üzerine ince bir tabaka şeklinde yayılmıştır. Dicrocoeliid larval safhaları (sporokist ve serkarya) ışık mikroskobuyla gözlemlenerek %5'lik formalinle tespit edilmiş ve Semichon's acetocarmine ile boyanmıştır. Örneklerin bir kısmı scanning elektron mikroskobu araştırmaları için hazırlanmıştır.

Sonuçlar: X. obvia'nın Dicrocoeliidae familyasının yaşam döngüsüne ara konaklık yaptığı Türkiye'den ilk kez rapor edilmiştir. Kastamonu'da enfeksiyonun yaygınlığı %0.78'dir ve en yüksek olduğu ay Nisan'dır.

Araştırma vurguları: Salyangozlar, özellikle otçul hayvanların otladığı köyler ve çiftliklerin civarındaki meralardan toplandığı için tespit edilen larval safhaların özellikle *Dicrocoelium dendriticum*'a ait olabileceği kanaatine varılmıştır. Meralardan toplanan salyangozlarda bu trematod parazitinin larval dönemlerinin varlığı, kesin konak hayvanlar için bir risk teşkil etmektedir.

Anahtar kelimeler: *Xerolenta obvia*, Dicrocoeliidae, *Dicrocoelium dendriticum*, Larval safhalar, Kastamonu, Türkiye

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Introduction

Dicrocoeliid species are parasitic endosymbionts of the intestine, gall bladder, liver and pancreas of mammals, birds, amphibians and reptiles. *Dicrocoelium dendriticum* that occurs in Europe, Asia and North America is the best investigated liver fluke of the Dicrocoeliidae family. It infects mainly herbivorous and omnivorous mammals including humans (Olsen, 1974; Cheng, 1986; Schuster, 1993; Otranto and Traversa, 2002; Gürelli and Göçmen, 2007). The life cycle of *D. dendriticum* was fully disclosed by Krull and Mapes (1952). Terrestrial snails assist as first and ants as second intermediate hosts (Krull & Mapes, 1952; Alunda and Rojo-Vazquez, 1984; Ducháček and Lamka, 2003; Murvanidze et al., 2010). The terrestrial snails that inhabit this parasite as first intermediate host in Turkey are *Cernuella virgata*, *Helicella candicans*, *Helix aspersa*, *H. lucorum Trochoidea pyramidata*, *Helicopsis derbentina*, *H. protea*, *Cochlicella acuta*, *Monacha cartusiana* and *Chondrus tournefortianus* (Kalkan, 1971; Gürelli & Göçmen, 2007; Gürelli, Alay & Koymalı, 2014; Kartal, Köse & Eser, 2015; Köse, Eser, Kartal & Bozkurt, 2015; Gürelli and Alay, 2016; Gürelli, 2017; Ünlü, Bilgiç, Eren & Karagenç, 2017).

The hygromiid snail *Xerolenta obvia* (Mollusca: Pulmonata) is a terrestrial snail species that can be naturally found in Anatolia, the Carpathians, the Balkans, along the Baltic coast, Mediterranean region and southeast France (Lazaridou and Chatziioannou, 2005). To our knowledge, there are currently no reports regarding the larval trematodes of this terrestrial snail in Turkey. Therefore, we investigated the prevalence of Dicrocoeliid larval stages in *X. obvia* found in Kastamonu and examined the morphological features of the parasite (sporocysts and cercariae).

Material and Method

X. obvia samples were gathered after rainfall in the surrounding areas of the Kastamonu province during the spring (i.e. April and May) and autumn (October and November) of 2016. The collection sites were located near villages and farms where goats, sheep, cattle, horses, and donkeys grazed and crops were grown.

A total of 383 terrestrial snails were gathered and dissected alive for removal of their hepatopancreata, which was placed into clean small glass tubes containing a 0.6% NaCl solution. The hepatopancreas was dissected using a needle and liquid was spread on a slide as a thin layer, to investigate the parasites' live larval stages. Dicrocoeliid larval stages (sporocysts and cercariae) were examined under a Zeiss compound microscope with an imaging system, and photographs were taken. The parasites were fixed with 5% formalin, covered with an adhesive mixture and stained with Semichon's acetocarmine.

Samples were also prepared for observations under a scanning electron microscope (SEM), by washing the formalin-fixed specimens with distilled water overnight and post-fixing them in 2% (w/v) osmium tetroxide aqueous solution for 4 h, at room temperature. After five washes with distilled water, the specimens were dehydrated through a series of ethanol concentrations for 20 min at each concentration and dried in a CO_2 critical-point dryer (Imai, Tsutsumi, Yumura & Mulenga, 1992). The dried specimens were sputter-coated with gold and examined under a FEG 250 SEM (FEI-Quanta, Hillsboro, OR, USA).

Results

A total of 383 specimens were examined, among which 3 (0.78%) presented second-generation Dicrocoeliid sporocysts and cercariae in hepatopancreata. The highest prevalence (2.17%) of sporocysts and cercariae was detected in April 2016 (Table 1).

Table 1. Prevalence of *Xerolenta obvia* infected with Dicrocoeliid sporocyts and cercariae according to the month of collection.

Count of dissected *X. obvia* according to collection month	Count of infected *X. obvia*	Prevalence of infection (%)
0 (March 2016)	-	-
46 (April 2016)	1	2.17
52 (May 2016)	-	-
0 (September 2016)	-	-
129 (October 2016)	2	1.55
156 (November 2016)	-	-
383 Total	3	0.78

Second-Generation Sporocyst

Second-generation sporocysts in the last stage present cercariae and develop the shape of a large elongated sac. The oral and ventral sucker and tail of cercariae become visible, and each sporocyst comprises 4–26 cercariae, which are released from a birth pore (Figs. 1a–b and 2a–b).

Cercaria

Cercariae possess two suckers, one ventral and one oral, which presents an anterior stylet. The stylet's pore is clearly visible in SEM images, and hair-like structures surround both the oral and ventral suckers. The excretory bladder is positioned posterior to the ventral sucker. The bifurcated intestine is positioned posterior to the oral sucker and ends in proximity to the ventral sucker. Cercariae possess long simple tails, although shorter tails may at times be present, which are known as Xiphidiocercariae (Figs. 1c–d and 2c–d).

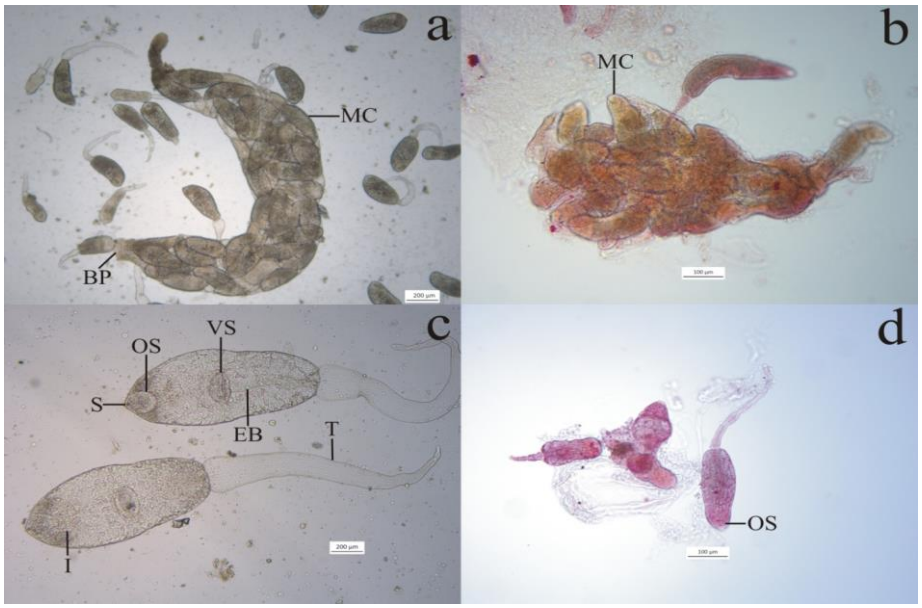

Figure 1. Photomicrographs of larval trematodes at different stages. a–b. Second-generation sporocyst in the last stage. c. Cercaria. d. Cercaria stained with Semichon's acetocarmine. MC, mature cercaria; BP, birth pore; OS, oral sucker; VS, ventral sucker; EB, excretory bladder; I, intestine; S, stylet; T, tail.

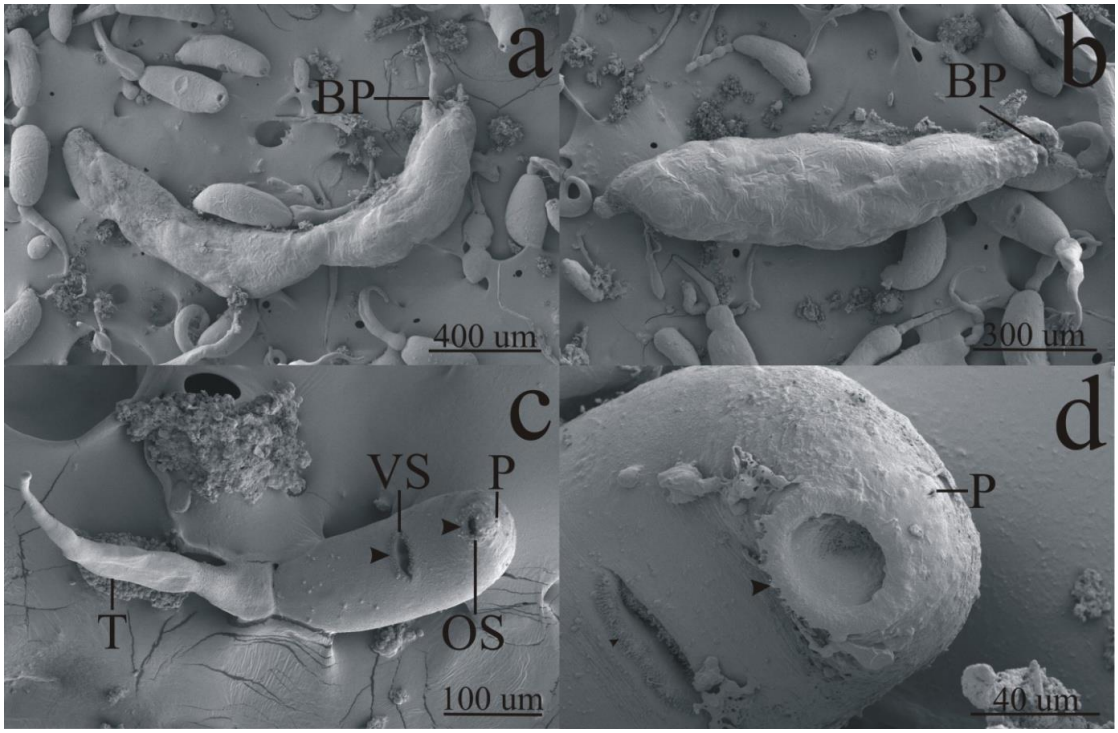

Figure 2. SEM images of larval trematodes at different stages. a–b. Second-generation sporocyst in the last stage. c–d. Cercaria. BP, birth pore; OS, oral sucker; VS, ventral sucker; P, pore of stylet; T, tail; hair-like structures (arrowheads).

Discussion

In the present study, *X. obvia* was reported for the first time as the intermediate host in the life cycle of the Dicrocoeliidae family, in Turkey. Within the studied area, the infection prevalence was 0.78%, with its highest occurrence in April, indicating that the risk of infection by the second intermediate hosts (arthropods) is high during that month. Given its wide distribution and abundance in Europe and Anatolia, the infection transmission of this parasite to the second intermediate host could be higher.

Second-generation sporocysts can be seperated into 3 stages according to their morphological features: the first stage comprises undifferentiated germinal masses, which resemble thin long sacs; the second stage comprises immature cercariae or germinal masses presenting the outline of cercariae; the last stage comprises developed cercariae (González-Lanza, Manga-González, Campo & Del-Pozo, 1997; Gürelli, 2006; Gürelli & Alay, 2016). In the present study, only the last stage of second-generation sporocysts was deteced. Otranto & Traversa (2002) and Ducháček & Lamka (2003) have expressed that the larval stages in snails are developed in approximately three or four months from the miracidia, which pass into snails through eggs, to first- and second-generation sporocysts, indicative of asexual reproduction. Hence, the *X. obvia* that carried infection in April, may have been infected during the former year. Following infection, *X. obvia* undergoes winter hibernation, awakening during spring, thus allowing the larvae in their hepatopancreata to resume development.

X. obvia was gathered near farms and villages since herbivores are known to graze on these sites. The larval stages could therefore be of *Dicrocoelium dendriticum*, which presents a high risk of infection transmission.

The life history of the mollusc intermediate hosts is of great epidemiological interest, regarding both the ingestion periods of Dicrocoeliidae eggs, dependent on the mollusc's activity and the survival of the parasites inside them. Mollusc age, species and ambient temperature, relative humidity, nutritional state, and infective dose, among

other aspects, affect the larval development of this digenean in the first intermediate hosts (Manga-González, González-Lanza, Cabanas & Campo, 2001).

In the present study, spring and autumn were selected according to the terrestrial snails' activity times; terrestrial snails go into hibernation during the winter and are awaken by the warmth and rain of the spring. Active terrestrial snails can therefore be infected by ingesting trematode eggs containing a miracidium; thus, the risk of infection is higher during these periods. During the summer, terrestrial snails could be in an aestivation time and may be active again in autumn because of the wet weather.

A large variation was found among different terrestrial snail species and across different countries, regarding the prevalence of infection. Such variation was also present within the same species of terrestrial snails inhabiting different sites in Turkey (Table 2).

Table 2. Prevalence of infection with Dicrocoeliid sporocysts and cercariae among different terrestrial snail species.

Prevalence (%)	Terrestrial snail species	Reference	Country
4.30	*Helicella candicans*	Kalkan, 1971	Turkey, Bursa
4.00	*Helicopsis derbentina*	Kalkan, 1971	Turkey, Bursa
2.60	*Helicopsis krynickii*	Kalkan, 1971	Turkey, Bursa
0.20	*Trochoidea pyramidata*	Kalkan, 1971	Turkey, Bursa
2.80	*Monacha cartusiana*	Kalkan, 1971	Turkey, Bursa
1.00	*Cernuella virgata*	Kalkan, 1971	Turkey, Bursa
0.80	*Helicopsis protea*	Kalkan, 1971	Turkey, Bursa
0.40	*Cochlicella acuta*	Kalkan, 1971	Turkey, Bursa
5.68	*Helicella itala*	Manga-González, 1987	Spain
26.80	*Helicella obvia*	Schuster, 1993	Germany
2.98	*Helicella itala*	Manga-González et al., 2001	Spain
1.06	*Helicella corderoi*	Manga-González et al., 2001	Spain
0.97	*Helix aspersa*	Gürelli & Göçmen, 2007	Turkey, Izmir
27.60	*Helix lucorum*	Gürelli et al., 2014	Turkey, Kastamonu
4.90	*Helix lucorum*	Kartal et al., 2015	Turkey, Afyonkarahisar
2.40	*Helix aspersa*	Köse et al., 2015	Turkey, Mersin
2.27	*Chondrus tournefortianus*	Gürelli & Alay, 2016	Turkey, Kastamonu
22.00	*Helix lucorum*	Ünlü et al., 2017	Turkey, Van
0.78	*Xerolenta obvia*	Present study	Turkey, Kastamonu

In conclusion, *X. obvia* is a noteworthy first intermediate host for transmission of Dicrocoeliid species to second intermediate hosts in Turkey and in Europe, due of its widespread distribution.

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