

# Molecular investigation on *Echinococcus granulosus* and other *Taenia* spp. in grey wolves (*Canis lupus*) in Umbria region

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## INTRODUCTION

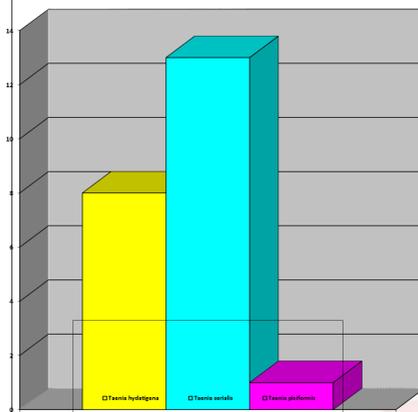
Human echinococcosis is a worldwide parasitic zoonosis caused by tapeworms of the genus *Echinococcus*, family *Taenidae*. Wolves (*Canis lupus*) and other wild canids (e.g. foxes, coyotes) are involved in a sylvatic cycle of *E. granulosus*, contributing to the transmission and the spread of the parasite. For *E. multilocularis*, red foxes (*Vulpes vulpes*) are the principal definitive hosts (DHs). DHs are infected through the consumption of viscera of infected intermediate hosts (IHs): domestic ruminants and swine in domestic cycle, wild boars and cervids in sylvatic cycle. Humans can become infected, but are considered as a dead-end host.

From an epidemiological point of view, it is crucial to identify potential hosts, because in addition to *E. granulosus*, more tapeworms can colonize the intestinal tract of animals, such as *E. multilocularis* and other members of *Taenia* genus.



## RESULTS

Among the 37 faeces sampled, 22 were positive for the 267-bp *Taenia* sp amplicon, while none of them were positive for *E. multilocularis* and *E. granulosus*. The results were confirmed by sequencing and data are shown in Table 2.



Thirteen samples (59.1%) result positive for *T. serialis*, 36.4% (n=8/22) are positive for *T. hydatigena* and 1 fecal sample (4.5%) is positive for *T. pisiformis*, with an identity between 99% and 100%.

## MATERIALS AND METHODS

### 1. Sampling

### 2. DNA extraction

### 3. Multiplex-PCR

### 4. Sequencing

1

From January 2014 to May 2019, faeces from 37 carcasses of wolves (*Canis lupus*) were collected and kept at -80°C for at least 3 days for safety reasons, before being processed.

2

DNA was extracted directly from 200 mg of faeces (QIamp® DNA Stool Mini Kit-Qiagen, Hilden, Germany)

3

Oligonucleotide primers for M-PCR for *E. multilocularis*, *Taenia* spp. and *E. granulosus*.

Primer	Sequence (5'-3')	Size of PCR product (bp)	Genus/Species
Cest1	TGCTGATTGTAAAGTTAGTGATC	395	<i>E. multilocularis</i>
Cest2	CATAAATCAATGGAAACAACAAG		
Cest4	YGAYTCITTTTAGGGGAAGGTGTG	267	<i>Taenia</i> sp.
Cest5	GCGGTGTGTACMTGAGCTAAC		
Cest3	GTTTTGTGTGTACATTAATAAGGGTG	117	<i>E. granulosus</i>
Cest5	GCGGTGTGTACMTGAGCTAAC		

4

Sequencing was performed in both direction by BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem®); reactions were separated through an 3500 Genetic Analyzer (Applied Biosystems). Consensus sequences were created by BioEdit Sequence Alignment Editor software v 7.0.9.0 and then aligned on Genbank database.

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*E. granulosus* was no detected, showing the importance to set up a surveillance plan that include also also domestic animals. In fact, rural dogs like shepherd dogs may represent a key point on the transmission of parasite. The absence of *E. multilocularis* confirms data available on literature; wolves are not the mainly definitive host of this parasite in Europe, represented instead of red foxes (Beck et al., 2018), but this wild species is not investigated in this paper.