


1. Introduction



The wolf plays a key role in the maintenance and transmission of several taeniids species, some of which are relevant from a one health perspective.

In this study was evaluated the presence of taeniids in a wolf pack living in a highly anthropic area of Pisan hills characterized by an interaction between wildlife, livestock, and recreational human activities

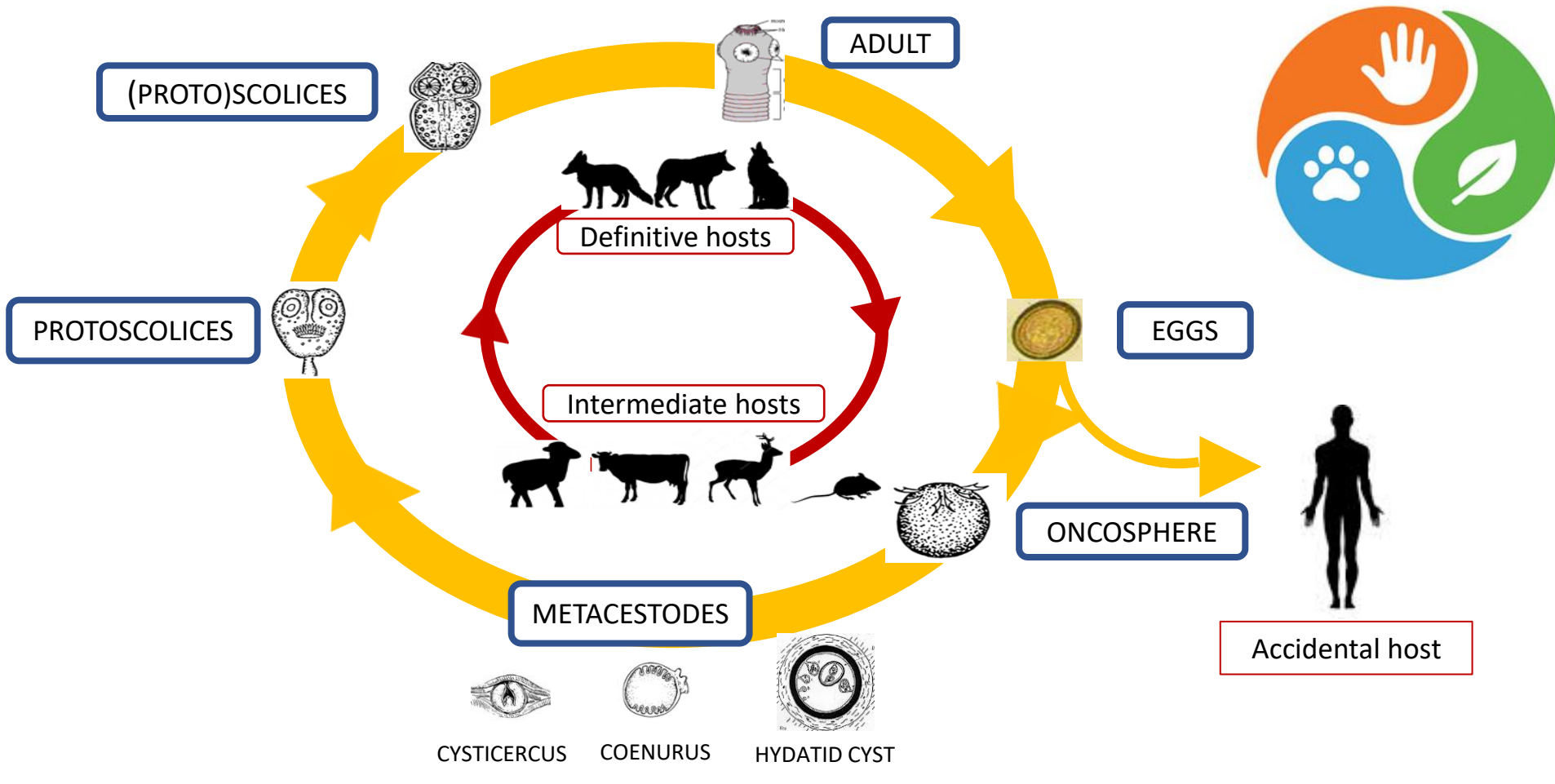


Figure 1: Life cycle of Taeniids

2. Materials and methods

a. Sample collection and parasitological analysis

Fecal samples collection: October 2018 - December 2019
Deep-frozen at - 80 °C for 3-5 days
Placing of 2 g of each fecal sample in 15 mL tubes
Taeniid eggs isolation by flotation with zinc chloride (specific gravity 1.350 solution) and the sieving method morphological identification under a light microscope

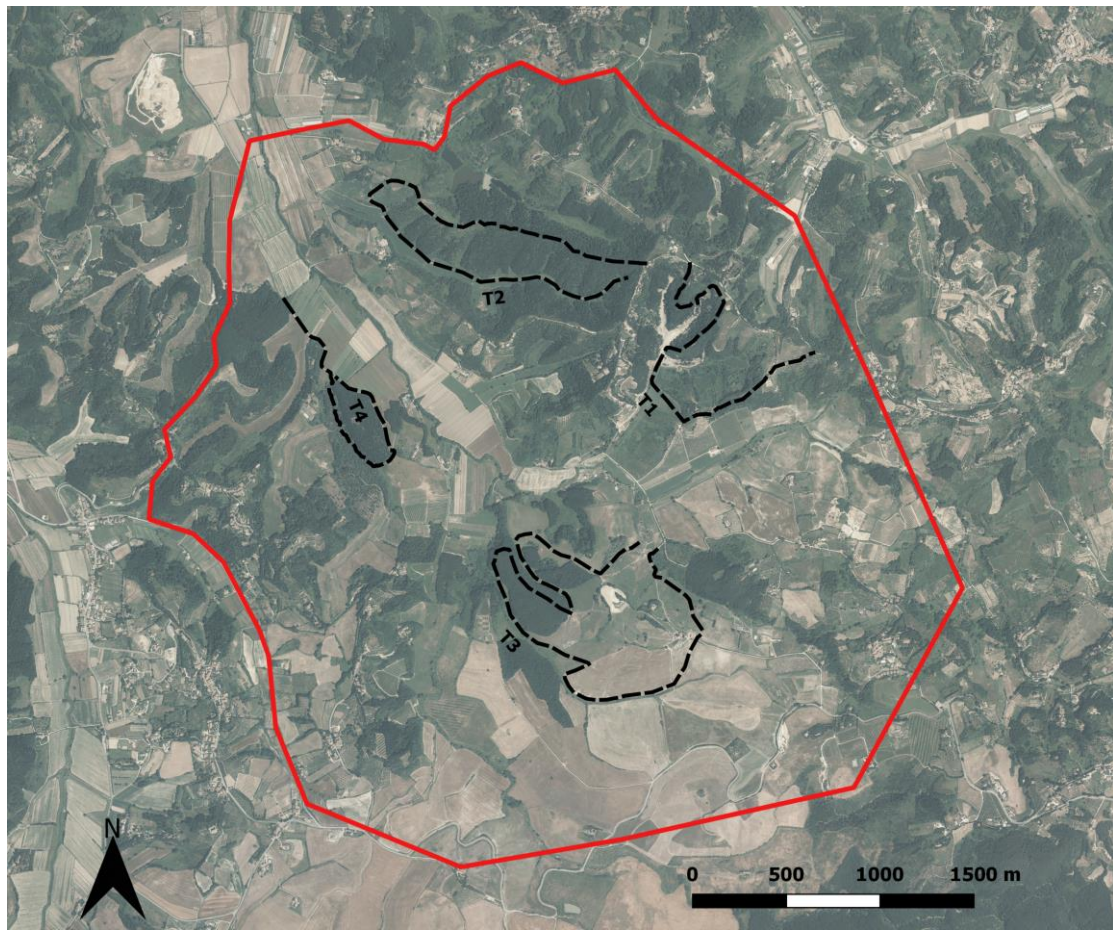


Figure 2: Study area (red line) and sampling transects (black lines)




Figure 3: Sampling

b. Molecular analysis (DNA extraction and PCR amplification)

Suspension of fecal samples in PBS 1X, filtration and centrifugation at 13,000 rpm for 10 min
Digestion of pellet using 15 mg/mL proteinase K solution at 58 °C overnight
DNA extraction using a Fecal DNA kit (Bioline, United Kingdom)
Amplification of partial sequences from the cox1 and nad1 gene following the PCR protocols
Purification and sequencing of amplicons by Sanger automated sequencing by Bio-Fab Research
Analyzation and editating of chromatogram using Chromas v. 2.33
Alignment of readable sequences of partial cox1 and nad1 using MEGA7
Comparison to GenBank retrieved homologous sequences

c. Diet analysis

Samples collection;
Storage for 5 days at -80 °C;
Washing through two sieves with different meshes size;
Observation through an optical microscope




Figure 4: Wash of samples

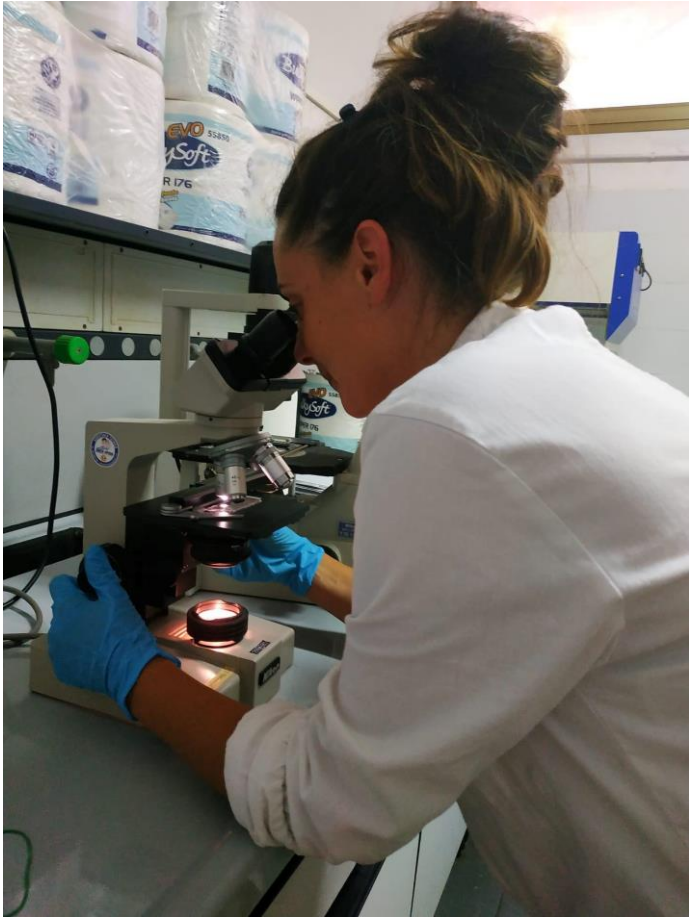


Figure 5: Hairs identification by optical microscope




Figure 6: Roe deer (*Capreolus capreolus*) hair at optical microscope (20X)

3. Results

Parasite	No. positive (%)
<i>Capillaria</i> spp.	21/38 (55.26%)
Ancylostomatidae eggs	7/38 (18.42%)
<i>Crenosoma vulpis</i> larvae	5/38 (13.15%)
Taeniidae eggs	13/38 (34.21%)
<i>Toxocara canis</i>	2/38 (5.26%)
Coccidian oocysts	1/38 (2.63%)

Table 1. Number of positive fecal samples for each identified parasite taxon.

Taeniid DNA was successfully amplified with at least one target (cox1 or nad1) in 10 out of 13 faecal samples positive to taeniid eggs.

Molecular analysis	
<i>Echinococcus granulosus</i> s.s.	10/38 (26.3%)
<i>Taenia hydatigena</i>	4/38 (10.5%)

Table 2. Frequencies of Taeniids identified at molecular analysis




Figure 7: Wolves eating on Massese sheeps in the study area

Wolf diet

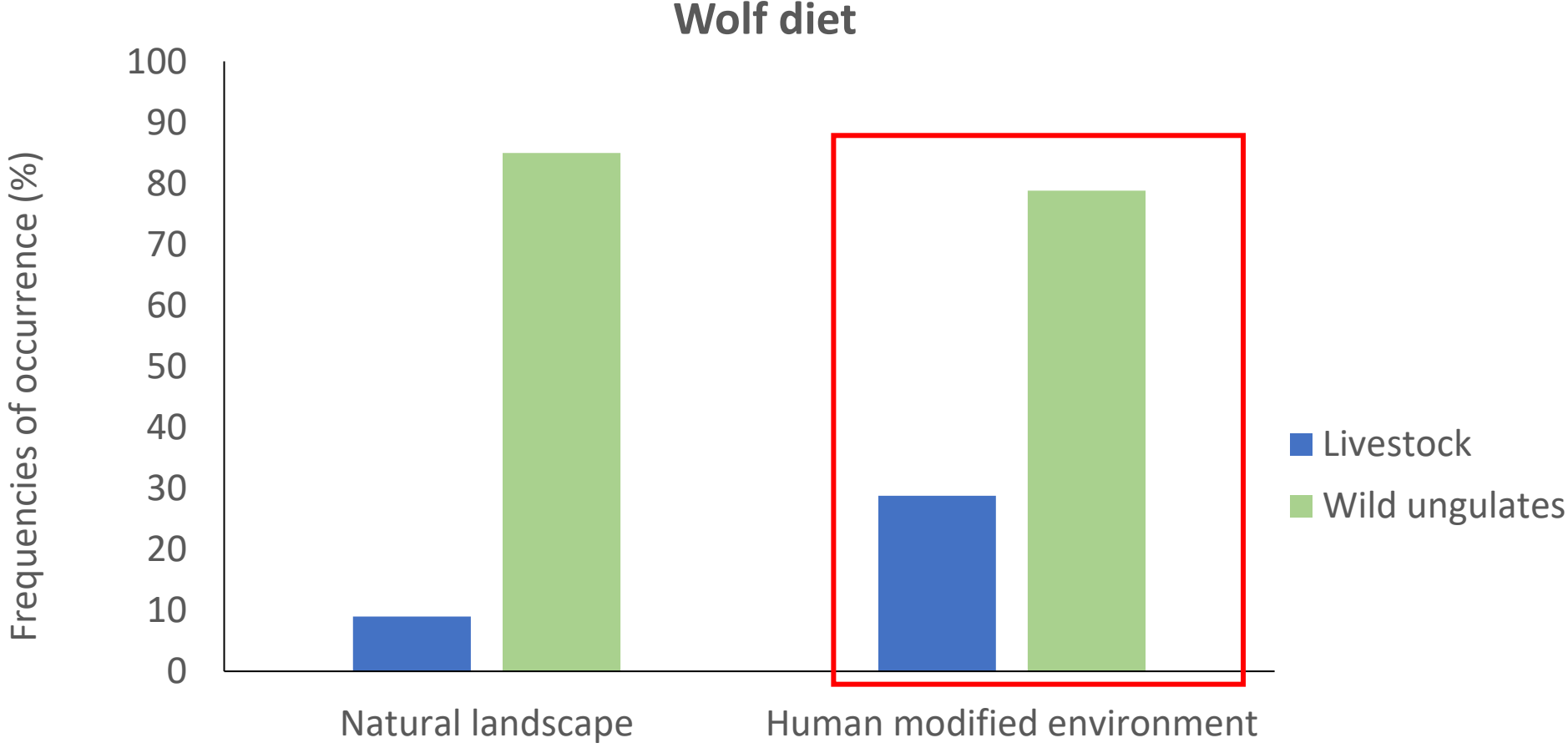


Figure 8. Frequencies of occurrence of livestock and wild ungulates in wolf diet obtained in the study period 2018-2019 (red square) compared to those reported in literature regarding wolf living in wild environment

4. Conclusion

- The high frequency of *E. granulosus* should be ascribed to the highly anthropized environment with a high livestock density and proximity to farms with infected dogs and sheep.
- The low biodiversity of taeniids detected in wolves could be due to low parasite biodiversity in wild prey in this restricted anthropized area.