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# Helminth fauna in roe deer (*Capreolus capreolus* Linnaeus, 1758) in the province of Grosseto (central Italy)

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| Article info   | Summary   |
|--|---|
| Received September 19, 2022<br>Accepted March 24, 2023 | Helminth infection was analysed at necropsy and coprology in a total of 54 roe deer from the prov-<br>ince of Grosseto (central Italy) between 2018 – 2020. Age and sex data were recorded for each deer<br>for a total of 31 adults (23 females, 8 males) and 23 juveniles (11 females, 12 males).<br>The results on the small intestine (51 samples) highlighted that nematodes belonging to the species<br><i>Trichostrongylus colubriformis</i> were the most prevalent parasite (41.2 %), followed by the cestode<br><i>Moniezia expansa</i> (7.8 %). The large intestine results (52 samples) showed <i>Trichuris</i> spp. (53.8 %),<br><i>Oesophagostomum venulosum</i> (50 %) and <i>Chabertia ovina</i> (26.9 %). In the abomasum, only <i>Oster-<br/>tagia ostertagi</i> (17.9 %) was found. Of the 34 samples analysed by bronchopulmonary, only the lung<br>of an adult female was positive for <i>Dictyocaulus</i> spp. In two livers out of 33 samples analysed, nem-<br>atodes of the species <i>Setaria tundra</i> were found on the surface. Copropositivity was observed in 45<br>of the 52 faecal samples analysed. The results of the present study indicate that the roe deer is host<br>to several species of parasites, which are also common in other cervids and domestic ruminants.<br>Statistical testing highlighted a significant difference between mean intensities in males and females.<br><b>Keywords:</b> roe deer; helminth fauna; <i>Capreolus capreolus</i> endoparasites; central Italy; wild rumi-<br>nants |

#### Introduction

The roe deer (*Capreolus capreolus* L., 1758) is the most abundant and widespread species of deer in Italy and one of the most important species from a hunting perspective. After a historical population contraction over the last few decades, which affected Italy and most of Europe and led the species to near extinction, the roe deer is now widespread throughout the continent's forests (Lovari *et al.*, 2016). In Italy, it is found in two main sub-areas: the Alps, and Apennine chain with small, isolated populations in the south of the peninsula (Carnevali *et al.*, 2009). The causes of its recovery in Italy are thought to be the reduction in agricultural practices in hilly and mountainous areas, and the progressive increase in wooded and forest areas, as well as regulated hunting management (Perco, 2011).

Several studies have shown how the roe deer is host to numerous parasites, some of which are important not only in terms of its own health but also for domestic ruminants with which it is increasingly coming into contact (Vetyska, 1980; Ladini, 1989; Aguirre *et al.*, 1999; Rehbein *et al.*, 2000; Diaz *et al.*, 2010; Pato *et al.*, 2013; Beaumelle *et al.*, 2022).

Helminth infections have been documented in several studies in Europe, but data on the taxonomic composition of helminths in *C. capreolus* in Italy are out of date, not homogeneous, and not continuous (Genchi *et al.*, 1987; Poglayen *et al.*, 1990; Rossi *et al.*, 1997; Zaffaroni *et al.*, 2000).

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The most relevant investigations on the helminth fauna of roe deer in Italy have mostly been carried out in the Alpine region. Twenty-two species of gastrointestinal helminths have been found, of which the genus *Spiculopteragia, Ostertagia,* and *Nematodirus* were the most abundant (Rossi *et al.*, 1997). Sixteen species were detected in the abomasa of *C. capreolus,* many of which are shared with other wild ruminants (Zaffaroni *et al.,* 2000). An older investigation on gastrointestinal nematodes in Trento, showed a high prevalence of *Ostertagia leptospicularis* and *Spiculopteragia spiculoptera, Haemonchus contortus,* and the species *Nematodirus filicollis* and *Nematodirus rupicaprae,* the latter as a consequence of the promiscuity of receptive hosts (wild and domestic ruminants) in their habitat (Genchi *et al.,* 1987).

Although Tuscany hosts one of the most abundant Italian roe deer populations, epidemiological investigations carried out to date are scarce. A survey on the helminth fauna of 60 roe deer (*Capreolus capreolus*) was conducted in the Apennines in the province of Florence (Italy). Two helminth species were found in the lungs, and 16 in the digestive system (Poglayen *et al.*, 1996). Pacini *et al.*, 2020 found samples positive for gastrointestinal strongyle eggs, *Eimeria* spp. Oocysts, and numerous nematode larvae of the species *Varestrongylus capreoli*.

The aim of the current study is to provide first insights into the helminth fauna of roe deer in the province of Grosseto (the southernmost part of Tuscany, Italy), an area that has never been investigated before, and to analyse the data collected in order to test whether sex, age, or territory differences lead to statistically significant differences in these infections.

#### **Materials and Methods**

This survey was carried out in several municipalities in the province of Grosseto (Tuscany, Italy), an area of 4503km<sup>2</sup> which are primarily agro-silvopastoral.

During the 2018 – 2019 / 2019 – 2020 hunting seasons, the gastrointestinal and bronchopulmonary organs of 54 roe deer were collected and analysed at the Department of Veterinary Sciences of the University of Pisa, Italy. From the two hunting seasons, 31 adults (23 females, 8 males) and 23 juveniles (11 females, 12 males) were sampled. Samples were also collected in two different climatic areas of Grosseto: a coastal area characterized by an annual mean temperature of  $15 - 16^{\circ}$ C and annual mean precipitation of 600 - 700 mm; and a hilly-mountain area with an annual mean temperature of  $12 - 14^{\circ}$ C and annual mean precipitation of 800 - 1000mm (Sforzi & Ragni, 1997).

The samples were not always received complete with all the organs, and thus the total number of samples examined varied according to the type of analysis performed. In terms of the gastrointestinal tract, the abomasum, small intestine, and large intestine were analysed by the sedimentation and counting technique (SCT) (Eckert *et al.*, 2003). The liver and cardiorespiratory system tract, which includes the trachea and the lungs, were visually in-

spected under a stereomicroscope. The lungs were dissected and washed with tap water in a conical beaker to collect the parasites. Each nematode was clarified with lactophenol, and morphologically identified.

Coprological analyses were conducted on faeces extracted directly from the rectal ampulla of the sample using the Mini-Flotac technique (Cringoli *et al.*, 2017) with a solution of ZnCl<sub>2</sub> (p.s. 1.200).

For the adult parasites, the following epidemiological indices were calculated (Margolis *et al.*, 1982; Bush *et al.*, 1997): prevalence, mean intensity, intensity, and range.

Data were analysed using OpenOffice Calc 4.1.6 and R software version 3.1.2. (R Development Core Team, 2014).

We use the term prevalence (P) to indicate the ratio between the number of hosts infected and the sample size, mean intensity (MI) to indicate the ratio between the total number of parasites and the number of hosts infected.

The aim of the statistical analysis was to compare the prevalence and mean intensity between animals of different ages (young vs adults), sex (males vs females), and habitats (coast vs hilly-mountain). To better highlight any differences and understand whether these may be due to the single parasite taxon or are linked only to the difference between the three parameters mentioned above, it would be useful to extend the statistical comparisons to each parasite taxon. However, the high quantity of different parasite taxons infesting the animals analyzed, combined with a sample of about 50 units, would make it statistically irrelevant to test each taxon.

Before carrying out the tests, we thus put the individual parasites into groups linked by some affinities and tested the age, sex, habitat for each family. Any differences found in the single-family tests could therefore highlight a greater vulnerability of the animals to that particular family. On the other hand, any differences in the global tests (which include all parasites taxon at once) may highlight a particular vulnerability of animals of a certain age, sex, or habitat to infestations in general.

Based on the data collected, several tests (Pearson chi-squared and Mann-Whitney U test) were performed to check for differences between male and female specimens and between young and adult animals. Two-tailed Pearson's chi-square test was used for the prevalence, as all the hypotheses required were met. A median comparison test was used on mean intensity (namely, the Mann-Whitney, two-tailed U test: H0 = the distribution in the population is the same vs H1 = the distributions are different) since the distribution of values was far from normal, the sample sizes were relatively small (from 9 to 30 sample units) and no specific hypothesis was made regarding the distribution of the mean intensity values.

The different areas (coast or hilly-mountain) were also evaluated to investigate any statistical differences (H0 = no difference vs H1 = difference).

Since the number of non-parasitized animals was too low, a global (i.e. on the whole sample) check for prevalence on the entire sample would be statistically irrelevant. Only global tests on mean intensity values were thus run. According to our targets, the parasites found in the sample were then divided according to the families they belong to: Chabertiidae (Popova, 1952) (*Oesophagostomum venulosum* and *Chabertia ovina*), Trichostrongylidae (Leiper, 1912) (*Trichostrongylus colubriformis, Trichostrongylus* spp., and *Ostertagia ostertagi*), and Trichuridae (Ransom, 1911) (*Trichuris* spp.). A test for prevalence and mean intensity for each family was then run.

Since the number of total tests across all families was 18, Bonferroni's correction was applied to reduce the risk of false positives at the cost of increasing the chance of false negatives (reducing the tests' power).

# Ethical Approval and/or Informed Consent

All animal procedures used in this study agreed with the ethical and animal welfare concerns of the Committee on the Ethics of Animal Experiments of Minimally Invasive Surgery Centre and fully complied with recommendations outlined by Italian law. Animals were hunted following regional hunting laws (Regolamento di attuazione della legge regionale 12 gennaio 1994 n 3 DPGR 48/R/2017).

# Results

#### Sampling results

The results of the examination of the organs by SCT (summarized in Table 1) showed that the roe deer hosts a variety of parasites

which are also common in other cervids and domestic ruminants. Copropositivity was observed in 45 of the 52 faecal samples analyzed. The gastrointestinal Strongyles eggs were the most frequent with a prevalence of 71.2 %, followed by the eggs of *Trichuris* spp. with a prevalence of 42.3 % and the presence of oocysts in 36.5 % of cases.

A single infestation was found in 11 cases, but multiple infestations were also observed: double infestation in 16 cases, triple in 14, quadruple in 5, and fivefold in 3. Regarding the gastrointestinal tract, the large intestine showed the highest prevalence of parasites, followed by the small intestine and the abomasum.

#### Statistical results

The global test on mean intensity highlighted a statistically significant difference only between male and female roe deer (significance level 0.05, p-value = 0.02) with a higher mean intensity in males. Regarding the single families of gastrointestinal nematodes Trichostrongylidae, Trichuridae, and Chabertiidae) on mean intensity, no positives (significance level 0.05 / 18 due to Bonferroni's correction, 0.08 being the lowest p-value) were found. However male animals had a higher mean intensity for each family considered, although the result was not statistically significant.

There was no statistically significant difference between mean intensities in young and adult animals, or in animals living in coastal, or mountain areas (p-value > 0.05).

Regarding prevalence, the use of a test across the entire sample was not possible due to the low number of non-parasitized animals. When the individual families were tested (Trichostrongylidae,

| Table 1. Taxa (T), number of parasitised roe (NPR) | , the total number of parasites (TNP), prev | valence (P; %) with CI 95% | confidence interval, abundance (A) |
|--|---|----------------------------|------------------------------------|
|  | intensity (I) with range (min-max           | ax).                       |                                    |

|                                |     | , () | <b>e</b> ( )                                    |           |                        |
|--------------------------------|-----|------|---|-----------|------------------------|
| Т                              | NPR | TNP  | Prevalence %<br>(Cl 95% confidence<br>interval) | Abundance | Intensity<br>(min-max) |
| Small Intestine (N°=51)        |     |      |   |           |                        |
| Trichostrongylus colubriformis | 21  | 453  | 41.2 (27.8 – 55.8)                              | 8.40      | 21.6 (1 – 57)          |
| Trichostrongylus spp.          | 6   | 30   | 11.8 (4.9 – 24.5)                               | 0.60      | 5.0 (1 – 10)           |
| Moniezia expansa               | 4   | 39   | 7.8 (2.5 – 19.7)                                | 0.80      | 9.8 (1 – 22)           |
| Large Intestine (N°=52)        |     |      |   |           |                        |
| Trichuris spp.                 | 28  | 144  | 53.8 (39.6 - 67.5)                              | 2.77      | 6.9 (1 – 30)           |
| Oesophagostomum venulosum      | 26  | 174  | 50.0 (36.9 - 63.1)                              | 3.35      | 6.7 (1 – 17)           |
| Chabertia ovina                | 14  | 48   | 26.9 (15.6 – 41.2)                              | 0.92      | 3.4 (1 – 12)           |
| Abomasum (N°=39)               |     |      |   |           |                        |
| Ostertagia ostertagi           | 7   | 48   | 17.9 (8.1 – 41.1)                               | 1.23      | 6.9 (2 – 24)           |
| Liver (N°=33)                  |     |      |   |           |                        |
| Setaria tundra                 | 2   | 2    | 6.1 (1.0 – 21.6)                                | 0.06      | 1(1)                   |
| Bronchopulmonary tract         |     |      | 、   |           | . ,                    |
| (N°=34)                        |     |      |   |           |                        |
| Dyctiocaulus spp.              | 1   | 1    | 2.9 (0.1 – 17.0)                                | 0.03      | 1(1)                   |

|                                 | Trichostrongylus<br>colubriformis | Trichuris spp. | Chabertia<br>ovina | Oesophagostomum | Ostertagia | Dyctyocaulus spp. | Setaria | Moniezia |
|---------------------------------|-----------------------------------|----------------|--------------------|-----------------|------------|-------------------|---------|----------|
|                                 |                                   |                |                    | ACITATIONALI    | USICI LAY  | :                 | נמונחום | cypalisa |
| Italy                           | %                                 | %              | %                  | %               | %          | %                 | %       | %        |
| Poglayen <i>et al.</i> , 1990   | 14.0                              | 24.0           |                    | 9.5             | 7.0        |                   |         |          |
| Poglayen <i>et al.</i> , 1996   | 1.5                               |                | 71.0               | 32.3            | 4.6        |                   |         |          |
| Rossi <i>et al.</i> , 1997      | 16.4                              | 43.2           |                    |                 | 8.3        |                   | ·       |          |
| Zaffaroni <i>et al.</i> , 2000  | 1.0                               |                |                    |                 | 10         |                   |         |          |
| Favia <i>et al.</i> , 2003      |                                   |                |                    |                 |            |                   | OP      |          |
| Austria                         |                                   |                |                    |                 |            |                   |         |          |
| Schwarz <i>et al.</i> , 2011    | 5.0                               | 50.0           | 45.0               | 22.5            |            |                   |         |          |
| Czech Republic                  |                                   |                |                    |                 |            |                   |         |          |
| Vetyska <i>et al.</i> , 1980    |                                   |                | 42.9               | 0.9             | 52.7       |                   |         |          |
| Germany                         |                                   |                |                    |                 |            |                   |         |          |
| Rehbein <i>et al.</i> , 2000    | 1.6                               |                | 45.3               | 50.0            | 7.8        | 14.1              |         |          |
| Netherlands                     |                                   |                |                    |                 |            |                   |         |          |
| Borgsteede <i>et al.</i> , 1990 |                                   | 49.0           | 2.3                | 13.0            | 16.0       | 0.9               |         |          |
| Poland                          |                                   |                |                    |                 |            |                   |         |          |
| Tomczuk <i>et al.</i> , 2017    |                                   |                | ı                  | 15.0            |            | 11.3              | 5.66    | 7.4      |
| Balicka-Ramisz et al., 2003     |                                   |                | 9.3                | 10.5            |            |                   | ı       |          |
| Romania                         |                                   |                |                    |                 |            |                   |         |          |
| Hora <i>et al.</i> , 2017       |                                   | 27.4           | 12.3               | 11.0            |            | 26.0              |         | 17.8     |
| Spain                           |                                   |                |                    |                 |            |                   |         |          |
| Vazquez <i>et al.</i> , 2009    |                                   |                |                    |                 |            | 26.0              | ı       | ı        |
| Pato <i>et al.</i> , 2013       | 3.0                               |                | 2.3                | 50.5            |            |                   |         |          |
| Morrondo <i>et al.</i> , 2017   | ·                                 |                |                    |                 |            | 2.4               |         |          |
| Sweden                          |                                   |                |                    |                 |            |                   |         |          |
| Aguirre <i>et al.</i> , 1999    | 12.0                              |                | 3.0                |                 | 17.0       | 24.0              |         | 2.0      |
| Switzerland                     |                                   |                |                    |                 |            |                   |         |          |
| Andrews <i>et al.</i> , 1974    | 6.6                               |                | 73.3               |                 | 46.6       |                   |         | 26.6     |
| Turkey                          |                                   |                |                    |                 |            |                   |         |          |
| Bolukbas <i>et al.</i> , 2012   | 6.6                               | '              | 26.6               | 46.6            | ı          | ·                 |         | ·        |
| Umur <i>et al.</i> , 2011       | 16.6                              |                | 33.0               | 50.0            |            | ı                 |         |          |
| Ukraine                         |                                   |                |                    |                 |            |                   |         |          |
| Kuzmina <i>et al</i> ., 2010    |                                   |                | 28.3               |                 |            | 2.3/6.9*          | ·       | 1.1      |

Trichuridae, Chabertiidae), (significance level 0.05 / 18) on prevalence, no differences between males/females, young / adults, or coastal/mountain areas were shown. As all p-values were greater than 0.05, there was no difference between Bonferroni's correction and Holm-Bonferroni's method.

In conclusion, male animals showed a statistically significant higher mean infection value and this is not linked to a single family, but it is a general behavior.

# Discussion

In terms of gastrointestinal helminths in the small intestine, the nematode *Trichostrongylus colubriformis* (Trichostrongylidae) in our study (41.2 %) was found with a higher prevalence than in other studies carried out in Italy (Poglayen *et al.* 1990, 1996; Zaffaroni *et al.*, 2000; Rossi *et al.*, 1997) and in Europe (Table 2).

It is not easy to understand the reasons for the high prevalence found in our study compared to other surveys in Italy and Europe. One hypothesis is that our study area is a favorable environment for the spread of this parasitosis in roe deer and more generally for the genus Trichostrongylus spp. in other animal species. In Lepus europeus for example, in the same area as this survey, the species Trichostrongylus retortaeformis showed a higher prevalence than other parasites (Sergi et al., 2017 and Macchioni et al., 2022). A prevalence of 7.8 % (4/51) of the cestode Moniezia expansa was found in the present study which is, to the best of our knowledge, the first report of the species in roe deer in Italy, while it is common in domestic ruminants. A similar prevalence was found in roe deer in Poland (Tomczuk et al., 2017), while higher prevalences have been reported in Romania (Hora et al., 2017) and Switzerland (Andrews et al., 1974). Lower prevalences, however, have been found in Sweden (Aguirre et al., 1999), and Ukraine (Kuzmina et al., 2010) (Table 2).

To the best of our knowledge, the prevalence of the genus *Tri-churis* spp. (53.8 %), reported in our study is the highest among previous surveys carried out to date in Italy on roe deer (Table 2). *Oeosphagostomum venulosum* was the second most frequent parasite found in the large intestine (50 %). In previous studies, carried out in Italy on roe deer, lower prevalences have been found (Poglayen *et al.*, 1990; Poglayen *et al.*, 1996). In Europe, on the other hand, equal prevalences have been found in Germany (Rehbein *et al.*, 2000), Spain (Pato *et al.*, 2013), Turkey (Umur *et al.*, 2011), and slightly lower, again in Turkey (Bolukbas *et al.*, 2012). Lower prevalences have been found in the Czech Republic (Vetyska, 1980), Austria (Schwarz *et al.*, 2011), and Poland (Tomczuk *et al.*, 2017; Balicka-Ramisz *et al.*, 2003)

*Chabertia ovina* showed the lowest prevalence in the large intestine 26.9 % (41.2 %) in the present study. The only Italian report on roe deer showed a higher prevalence of 71 % (Poglayen *et al.* 1990), whereas, in Europe, the prevalence varies according to the region considered (Table 2).

In the present study, the only parasite found in the abomasum

was Ostertagia ostertagi, which has already been reported in Italy (Poglayen *et al.*, 1990; Poglayen *et al.*, 1996; Rossi *et al.*, 1997; Zaffaroni *et al.*, 2000) and also throughout Europe (Andrews *et al.*, 1974; Vetyska, 1980; Borgsteede *et al.*, 1990; Drozdz *et al.*, 1992; Korsholm *et al.*, 1992; Aguirre *et al.*, 1999; Rehbein *et al.*, 2000; Kusak *et al.*, 2012).

The liver examination was conducted on 33 samples. Nematodes belonging to *Setaria tundra* species (Onchocercidae) were found only on the surface of two livers. The first report in Italy for *Setaria tundra* was in the province of Turin (Piedmont region, Favia *et al.*, 2003) in the abdominal cavity of four roe deer. More recently *Setaria tundra* was reported in the province of Gorizia in an analysis conducted on roe deer with a prevalence of 47.6 % (Beraldo *et al.*, 2016), and its presence was associated with severe forms of peritonitis in the animals examined. Currently, *Setaria tundra* is widespread in 15 European countries, with Finland being the northernmost limit and Spain the westernmost and furthest from the site of the first report in Russia in 1928 (Oloś *et al.*, 2021). In Europe, *Setaria tundra* has recently been reported in roe deer in Croatia (Čurlík *et al.*, 2019) and Spain (Angelone-Alaasad *et al.*, 2016).

Of the 34 bronchopulmonary samples analysed, only the lung of an adult female was positive for the presence of *Dictyocaulus* spp. In Italy, reports regarding lung helminths in roe deer are scarce. A low prevalence of 6.6 %, but still higher than in our study, was found in the province of Florence by Poglayen *et al.* (1996).

As far as Europe is concerned, a prevalence not higher than 30 % has been reported for the genus *Dictyocaulus* (Vazquez *et al.*, 2009; Morrondo *et al.*, 2017) (Table 2).

Some studies have highlighted differences in prevalence and intensities of gastrointestinal helminths between adult roe deer and fawns (Borgsteede *et al.*, 1990; Segonds-Pichon *et al.*, 2000), while another study also reported no significant differences in both parameters (Pato *et al.*, 2013).

The susceptibility to parasites may be due to the influence of morphological, hormonal, and behavioral factors that characterize both sexes in certain periods of the year (Zuk & Mckean, 1996), which thus makes it difficult to highlight significant differences between the sexes. The differences between hunting policies, which can differ a lot from country to country, brings to different sampling types and makes even more difficult to compare different surveys. In our study, only the global test on mean intensity highlighted a statistically significant difference and only between male and female roe deer, while no statistically significant difference between mean intensities in young and adult animals, or in animals living in coastal or mountain areas (p-value > 0.05) were found. It is likely that the variations in temperature and rainfall in the two areas were not relevant to the parasite's spread and development. Pato et al., (2013) found statistically significant differences in different climatic areas in Spain only for the genera Trichuris, Oesophagostomum, and Spiculopteragia, which were more prevalent in the coastal area.

Our survey involved an exploratory study of the parasites in roe deer in the Grosseto area, for which there are no data in the literature. The results of the present study indicate that roe deer harbor several species of parasites.

In our study the parasite intensity found was relatively low. However, it should be highlighted that in the case of massive infection, these parasites play an important role in the health of roe deer and domestic ruminants. In fact, our results indicate that the roe deer hosts several species of parasites, which are also common in other cervids and domestic ruminants. It is thus crucial to address the problem within the One Health approach to prevent harm to farms and wild animals.

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# **Conflict of Interest**

The authors declare no competing interest.

# **Author Contributions Statement**

Conceived the study: FM, GR; Designed the experiment: FM, GR; Performed the field activities and sampling: FV, FM, GR; Performed the laboratory work: FV, CL, GM, FM; Analysed and interpreted the data: AM, FV, FM, FC; Wrote the original draft of the manuscript: FM, FV; Reviewed and edited the final version of the manuscript: FM, FV, CL, GR, FC, AM; Supervision: FM

All authors consent to participate. All authors consented to the publication. Data are available upon request.

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