Annals of Parasitology 2016, 62(1), 63–66
doi: 10.17420/ap6201.33

Original papers

Lungworm (Nematoda: Protostrongylidae) infection in wild and domestic ruminants from Małopolska region of Poland

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ABSTRACT. The conducted study has focused on domestic, as well as wild ruminant species. The *post mortem* examination was carried out on 68 animals, including three wild species: roe deer (*Capreolus capreolus*) (25 indyviduals), red deer (*Cervus elaphus*) (6), fallow deer (*Dama dama*) (5) and two domestic: sheep (*Ovis aries*) (14) and cattle (*Bos taurus*) (18). Some of the species have also been investigated in the field by the coproscopical analyses. The faecal samples from roe deer (27), fallow deer (20), red deer (36) and moose (*Alces alces*) (10) were collected from the environment, while from sheep (10) and goat (*Capra hircus*) (10) – per rectum. Based on the obtained results the following values were calculated: prevalence, mean intensity and intensity range. The *post mortem* examination did not reveal pulmonary nematodes neither in domestic nor in wild ungulates, however, the larvae of aforementioned parasites were often stated in the stool samples taken from the environment. All wild species, except fallow deer were infected. Consequently, six species of lungworms have been identified. The first stage larvae of *Varestrongylus capreoli* occurred in 11 samples of roe deer and *Varestrongylus alces* in one moose. The larvae of *Elaphostrongylus cervi* were found in 19 red deer and *Varestrongylus sagittatus* in 3. Furthermore, *Elaphostrongylus alces* larvae were noted in 6 moose. Within domestic ruminants only one sheep and two goats were infected by *Muellerius capillaris*.

Keywords: parasites, ungulate, post mortem examination, faecal analyses, Poland

Introduction

Lungworms belonging to the family Protostrongylidae (parasites of two ruminant families: Bovidae and Cervidae) are characterized by the high host specificity [1]. Some of them, however, can occur in the lungs of different hosts like *Varestrongylus sagittatus* in red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) [2]. Biology of nematodes of the genus *Elaphostrongylus* is even more surprising, due to their presence in the intermuscular tissue and central nervous system [3]. Except specific hosts (cervids), aforementioned parasites can also infect domestic animals as sheep, goats and cattle [4,5] or even Vietnam pigs (experimental infection) [6]. Nevertheless, in these hosts complete life cycle does not occur. Mixed infections (Protostrongylidae, Dictiocaulidae) were observed among such hosts as red deer and fallow deer (*Dictyocalus noerneri*, *V. sagittatus* and *E. cervi*) or roe deer and moose (*D. capreolus*) [7].

The aim of the study was to estimate the level of infection and biodiversity of lung nematodes in wild and domestic ungulates of southern Poland.

Materials and Methods

The study was carried out during the time period 2009–2013 on six districts of the Małopolska voivodeship (southern Poland) including agricultural areas and three large forest complexes (the Dulowa and Niepolomice Primeval Forests and forests located in the Beskid Sądecki Mountains).

Post mortem examination was carried out on wild (roe deer *Capreolus capreolus*, red deer *Cervus elaphus*, fallow deer *Dama dama*), as well as

domestic ungulates (sheep and cattle). The total number of analyzed animals was 68.

Roe deer and red deer have not been treated with any antiparasitic compound, whereas fallow deer from Dulowa Forest was annually de-wormed with an ivermectin product. In turn, domestic ruminants were de-wormed twice a year. Lung dissection was done by opening of bronchia, bronchioles and observed nodules with fine scissors then flushing their lumen with physiological saline. Arisen liquid was poured through 250 μm sieve. The material collected on the sieve was transferred into a Petri dish and carefully examined under stereo - microscope

A coproscopy survey was conducted on faecal samples collected from captive fallow deer in Dulowa Forest and wild ruminants in Dulowska and Niepołomicka Primeval Forests.

The faeces were distinguished on the basis of the shape and pellets' dimensions [8]. The faecal samples from wild ruminants (roe deer n=27, fallow deer n=20, red deer n=36 and moose n=10) were collected directly from the field (mainly from the vicinity of feeding racks), while from domestic (10 sheep and 10 goats) – per rectum. To isolate lungworm larvae from faecal material, the Baermann technique was used [9].

The first stage larvae of lung nematodes were identified using morphological criteria [10–13]. Larvae measurements were made by means of a microscope Motic BA210, in the Motic Image Plus 2.0 Program (magnification 40×, 100× and 600×).

Based on the obtained results the following values were calculated: prevalence of infection (P %), the average number of parasitic larvae per 5 g of faeces (I – intensity) and the minimal and maximal number of larvae (R – intensity range).

Results

Post mortem examination did not reveal pulmonary nematodes neither in domestic nor in wild ungulates, however, the larvae of aforementioned parasites were often stated in the stool samples taken from the environment. Additionally, larvae were found in samples from domestic sheep and goats. All wild species, except fallow deer were infected. Consequently, six species of lungworms have been identified. The first stage larvae of *Varestrongylus capreoli* occurred in 11 samples of roe deer (P=37.04%; I=265; R=1–933) and *Varestrongylus alces* in one moose (10%; I=3). The larvae of *Elaphostrongylus cervi* were found in 19 red deer (52.78%; 143; 1–833) and *Varestrongylus sagittatus* in 3 (8.33%; 9; 2–15). Furthermore, *Elaphostrongylus alces* larvae were noted in 6 moose (60%; 128; 3–365). Within domestic ruminants only one sheep and two goats were infected by *Muellerius capillaris* (1–8 larvae).

Discussion

Ruminants' lungworms belong to two families: Protostrongylidae (indirect life cycle involving intermediate hosts, mainly snails) and Dictiocaulidae (direct development cycle). In domestic animals (sheep, goat, cattle) infection with these parasites is widespread around the world [14]. In the present study in post mortem examination lung nematodes were not found in wild, as well as domestic ungulates.

The faecal samples collected from the field have shown the presence of *Elaphostrongylus* spp. larvae that occurred more frequently in red deer (about 52.78%) and moose (in 6 from 10 examined samples). Comparing the results of our study with those of other researchers, the prevalence of infection in red deer was the lowest. In other regions of Poland the prevalence ranged from 63.6% in the Świętokrzyski National Park (southern Poland) to 100% in the Białowieża National Park (east-western Poland) [15,16]. In addition, *E. cervi* in the red deer was found in the Borecka Forest (80%), the Bieszczady National Park (72.7–79.2%), the forests of Olsztyn (north-western Poland) and Silesia (west-southern Poland) (79.4 and 86.4%, respectively), the Bory Tucholskie National Park (100%), Piska Forest (80–92.3%) and in the Słowiński National Park (68%) and in eight voivodeships of Poland (66.7–100%) [15–21]. A part of cited research conducted in Małopolska have shown 75% *E. cervi* prevalence [21].

The second lungworm species found in red deer from the Dulowska and Niepołomicka Forest was *Varestrongylus sagittatus* (8.33%). The infection level was, however, lower than in the works of previously cited authors (from 14 to 50%) [15–20].

Our research did not confirm the presence of *Dictyocaulus* sp. in the studied area. Coproscopic analyses were conducted from February to April. During this period of the year the intensive *Elaphostrongylus* sp. and *Varestrongylus* sp. larvae excretion may be observed [22]. The peak of *Dictyocaulus* sp. larvae excretion can occur in

May–June and a new in October [23], so it could be unnoticed. The level of infection with these nematodes was high in various regions of our country and reached up to 25% in the red deer [16,19,20,23].

Despite the absence of adult forms of pulmonary nematodes in *post mortem* material in the examined roe deer, larvae of *Varestrongylus capreoli* were found in about 37.04% of stool samples collected from the environment. Nematode *V. alces* was also noted in one sample from moose (the Dulowska Primeval Forest), in addition to *Elaphostrongylus alces*.

Besides Małopolska region (the present study), this species was recorded in other region of Poland i.e., in Wielkopolska (12.7–51.4%), the forests of Olsztyn (north-western Poland) (48.6%) and Silesia (west-southern Poland) (62.5%), the Borecka Forest (25%), the Bory Tucholskie National Park (59%), the Białowieża National Park (43.5%), the Kampinoski National Park (44.4%) and the Świętokrzyski National Park (83.3%) [15,17, 24–26].

Another species, *Elaphostrongylus alces*, was recently found in Poland by means of larvoscopy in 37% of moose, with a maximum of up to 294 larvae excreted in 5 g of faeces [12].

Nematode *Elaphostrongylus cervi* is not a typical lung parasite and adults forms are localized mostly in the intermuscular tissue, therefore, the disease is most commonly subclinical. Sometimes they are located in the central nervous system – giving some abnormal symptoms [3,27] that can be observed within non-specific hosts like domestic ruminants [28]. Parasitic invasions pose a serious risk to domestic ruminants, mainly to sheep and goats, grazing in the same pastures with the cervids [4] and lesser to the cattle [5]. Pathogenicity for the cattle is lower due to the disease occurrence only after the digestion of large amount of larvae (above 20 thousands), however in the case of small domestic ruminants – 3000 infective larvae can cause mortality. In Italy, researchers have found out connection between elafostrongylidosis in sheep and goats, and the presence of this parasite in red deer pastured on the same area [29]. Infection with *Elaphostrongylus cervi* may also cause a serious disease also in cervids kept in enclosures [30]. Elaphostrogylidosis in captive red deer is difficult to recognize because the number of larvae excreted in faeces is variable and not valuable [31], therefore diagnosis should be based only on clinical symptoms.

The experimental research have shown that another species from this genus – *Elaphostrongylus alces* is nonpathogenic for sheep and goat [32], but can be dangerous for moose' calfs – a specific host [33].

There is also no direct evidence of the pathogenic effect of *Varestrongylus sagittatus* or *V. capreoli* invasion on the typical host (wild ungulates) [2], but according to Demiaszkiewicz et al. [26], they can be a source of fatal diseases for young red deer or roe deer.

Acknowledgements

This research was financed by the Ministry of Science and Higher Education of the Republic of Poland.

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Received 12 January 2016
Accepted 10 February 2016