## **Original papers**

# A further increase in the prevalence and intensity of infection with *Ashworthius sidemi* nematodes in red deer in the Lower Silesian Wilderness

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**ABSTRACT.** The nematode *Ashworthius sidemi* is a typical parasite of Asiatic deer, particularly sika deer (*Cervus nippon*). It was probably first carried into Poland by migrating red deer. The aim of the present study was to determine the prevalence and intensity of *A. sidemi* infection in roe deer and red deer in the Lower Silesian Wilderness in the winter season 2015/2016. Parasitological necropsy of 34 abomasa of red deer from Forest District Ruszów was performed. The prevalence of *A. sidemi* infection was found to be 91.1%, and the intensity to range from 10 to 2680 nematodes, with a mean intensity of 394 parasites. This prevalence of ashworthiosis and maximum intensity of infection was also slightly higher than reported previously.

Key words: Ashworthius sidemi, red deer, Cervus elaphus, Lower Silesian Wilderness

### Introduction

The nematode *Ashworthius sidemi* Schulz, 1933 (Trichostrongylidae: Haemonchinae) is a typical parasite of Asiatic deer, particularly sika deer (*Cervus nippon*). Both the parasite and the sika deer were introduced to the countries of the former Soviet Union, as well as Slovakia, Czech Republic and France [1–4]. It is belived that *A. sidemi* was then transmitted to a species of native ruminant, such as maral, red deer, roe deer, moose or mouflon. The nematode was probably transmitted to Poland by migrating red deer.

Following excretion with the feces, the eggs of *A. sidemi* develop fast. After only 48 hours, stage I larvae hatch, moult twice and after seven days, develop into their invasive stage. Wild ruminants become infected while grazing, eating invasive larvae of *A. sidemi* with grass and herbs. The larvae that infect ruminants in the summer and autumn

spend the winter with their development halted as IV stage larvae or as adult, but immature, nematodes. Although wild ruminants become infected from June to September, nematodes reach sexual maturity in the following June, and the nematodes of this generation are released three months later in September. Therefore, between June and autumn each year, wild ruminants are infected with two generations of nematodes: the sexually mature nematodes from the previous year, and the stage IV and V larvae and young adult parasites from the current year. In contrast, during winter and spring until June, the ruminants are infected with a single generation of nematodes comprising larvae whose development has been halted at stage IV and young, immature parasites [5].

The aim of this study was to determine the prevalence and intensity of *A. sidemi* infection in red deer from the Lower Silesian Wilderness two years after the first detection of ashworthiosis in this area.

#### **Materials and Methods**

Studies were conducted during the winter season in 2015/2016 in the Forest District Ruszów, Zgorzelecka Forest. The forest is located in the western part of the Lower Silesian Wilderness – the largest lowland forest complex in Europe.

Parasitological necropsy of abomasa of 34 red deer was performed. The abomasal sediment was decantated according to Dróżdż [6] and preserved in 1–2% formalin solution. In the laboratory, the preserved abomasal sediment was diluted with water and mixed thoroughly. A 1/10 sample was then taken from the whole volume of each abomasum. Small portions of samples were examined for the presence of nematodes under the stereoscopic microscope. *Ashworthius sidemi* was isolated and preserved in 75% ethanol with 5% glycerol. After evaporation of the alcohol, unstable preparations from nematodes in glycerol were made. Parasites were identified to species level on the basis of morphometrical features.

#### **Results and Discussion**

In the winter season 2015/2016, 91.1% of red deer examined in the Ruszów Forest District were infected with nematodes *A. sidemi*. Intensity of infection ranged from 10 to 2680 nematodes, with a maximal intensity of 394. Ashworthiosis was observed in 100% of bulls and calves and 85.7% of hinds. Among the examined red deer, the males showed the highest level of minimal, maximal and mean intensity of *A. sidemi* infection. This might be

a result of the high activity of the bulls, which penetrate large areas of territories contaminated with invasive larvae. In comparison to the bulls, the hinds demonstrated lower maximal intensity of infection and the calves the lowest. Similar mean intensities of ashworthiosis were observed in hinds and calves: almost three times lower than reported in bulls (Table 1).

In the Lower Silesian Wilderness, infection with A. sidemi nematodes was diagnosed for the first time in the 2012/2013 winter season, where it was observed in two of nine examined red deer. The low prevalence and intensity of ashworthiosis (only 60 and 80 nematodes in each red deer) observed in the season 2012/2013 indicated that the infection had appeared recently [7]. Studies performed during the next winter season (2013/2014) on red deer and roe deer from the same area revealed over a threefold increase of prevalence of A. sidemi infection, more than a fivefold increase of mean intensity of infection and more than a 24-fold increase of maximal intensity of ashworthiosis in red deer. Nematodes were also found in all three examined roe deer [8]. In the current studies (winter season 2015/2016), the prevalence of A. sidemi infection and maximal intensity increased by approximately 17.2% and 38%, whereas the mean intensity of infection was slightly higher in comparison with the previous season (2013/2014) (Table 2).

Nematode *A. sidemi* was identified for the first time in Poland in 1997 in the Bieszczady Mountains, where it was isolated from all examined European bison, red deer and roe deer [5,9,10]. In 2000, a few *A. sidemi* nematodes were observed in

Table 1. Infection of red deer with nematode Ashworthius sidemi in the winter season 2015/2016 in the Lower Silesian Wilderness

Group	Examined animals (n)	Infected animals (n)	Intensity range	Mean intensity
Calves	6	6	30–940	308
Hinds	21	18	10-1710	397
Bulls	7	7	120–2680	903

Table 2. Infection of red deer with nematode Ashworthius sidemi during three winter seasons in the Lower Silesian Wilderness

Season	Prevalence (%)	Intensity range	Mean intensity
2012/2013*	22	60–80	70
2013/2014*	74,2	10–1930	383
2015/2016	91,1	10–2680	394

\*Results were publicated in articles Demiaszkiewicz et. al. 2016, 2017 [7,8]

European bison in the Polish part of the Białowieża Primeval Forest. Infection with *A. sidemi* in European bison had been increasing over the years, finally reaching 100% prevalence in 2004 and maximal intensity of infection (77630 nematodes) in 2011. In the Białowieża Primeval Forest, *A. sidemi* nematodes were also observed in red deer and roe deer [11–13]. Since that time, ashworthiosis has also been diagnosed in European bison from the Knyszyńska Forest in 2009 [14], fallow deer, roe deer and red deer in Dulowska Forest, Małopolska voivodeship in 2011 [15,16], and moose in Biebrza Valley and the Augustowska Forest in 2013 [17] as well as European bison in Borecka Forest in 2016 [18].

Histopathological evaluation of the alimentary tracts of European bison infected with *A. sidemi* revealed massive inflammatory, necrotic and atrophic changes, mostly expressed in calves with intensive ashworthiosis [19]. Sobocińska [20] report that *A. sidemi* infection in European bison might induce a deterioration in blood parameters, leading to cachexia and death. Therefore, *A. sidemi* nematodes can arguably be classified as the most pathogenic gastrointestinal nematodes of wild ruminants. Experimental studies reveal that sheep have high susceptibility to *A. sidemi* infection [21]. Molecular studies have also confirmed the possibility of *A. sidemi* transmission from free-living animals to cattle using the same pastures [22].

The presented results indicate further growth in the prevalence and intensity of infection in this focus of ashworthiosis of red deer in the Lower Silesian Wilderness. Our studies confirm previous data about dynamics of this parasitosis in European bison in the Białowieża Primeval Forest [23]. It is necessary to monitor the infection in wild and domestic ruminants in the focus of ashworthiosis and to control the range and spread of the parasitosis.

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