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Original paper

Ashworthius sidemi in cattle and wild ruminants in Poland – the current state of play

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ABSTRACT. *Ashworthius sidemi*, a blood-sucking abomasal nematode, has been identified in various wild ruminants, including deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*) and moose (*Alces alces*). Although it has been observed throughout Poland, most sightings have been in the eastern part of the country. However, more recently, *A. sidemi* has been confirmed in the Ruszów Forest District (Lower Silesian Wilderness). It is now possible to test the faeces of cattle for the DNA of the third-stage infectious larvae (L3) of *A. sidemi*. The present paper describes such a molecular study of 120 faecal samples collected from cattle grazed in the Ruszów Forest District and Biebrza Marshland, where *A. sidemi* had previously been detected in wildlife. In this study, no *A. sidemi* DNA was identified in any of the examined samples.

Keywords: *Ashworthius sidemi*, cattle, faecal samples, DNA, PCR, Ruszów Forest District, Biebrza Marshland, Poland

Introduction

Ashworthius sidemi Schultz, 1933, a blood-sucking abomasal nematode, is considered to be as pathogenic as *Haemonchus* spp. [1–5]. However, it has been indicated that the intensity of histopathological change was determined by the number of nematode specimens identified in the walls of the abomasa and duodena of infected animals [2].

Ashworthius sidemi is mainly a parasite of sika deer (*Cervus nippon*), native Asian cervids that were introduced into many European countries: as Czech Republic in 1891 (Kluk area near Poděbrady), Germany in 1893 (Nenhaus area near Mőhnesee), or the countries of the former Soviet Union (Ukraine and Lituania). It is worth noting that in the monography on the history of introduction of sika deer to continental Europe by country Slovak Republic has not been mentioned [6]. Sika deer were introduced into present territory of Poland perhaps in 1895 in Kobiór (southern Poland) and next in 1910 in the Kadyny (northern Poland) [6]. Sika deer has the capability to migrate a long distance as a single animal, whole herd and together with the other red deer. In Kadyny sika were recorded to migrate 80–160 km [7].

It is believed that the parasite may have been introduced into the area of Poland by deer migrating from neighbouring countries. *A. sidemi* was reported for the first time in the country in European bison (*Bison bonasus*) in the Bieszczady Mountains in 1997 [8].

It has been well documented that the prevalence of *A. sidemi* infection has been gradually increasing over recent years in Poland, particularly among European bison and in the red and roe deer populations in the Ruszów Forest District (Lower Silesian Wilderness) [9–11]. The nematode has also been found amongst the helminthofauna of other

wild ruminants in various regions of Poland, such as the Bieszczady Mountains, Białowieża Forest, Knyszyńska Forest, Borecka Forest, Dulowska Forest, Augustowska Forest; lastly, in the Ruszów Forest District [9–16] and in Polesie National Park (Filip-Hutsch, personal communication). They have been identified in red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*) and moose (*Alces alces*) [9,12,13,15,16]. It has also been suggested that nematode may be able to infect cattle and sheep fed together on the same meadows or pastures. Therefore, it is not surprising that *A. sidemi* DNA was found to be present in cattle faecal samples collected in two areas: the Strzałowo Forest District, in the Mazurian lake district, and in the buffer zones of the Białowieża Forest [17].

Additionally, the direct evidence that invasions of *A. sidemi* may adversely affect the condition of wild animals, such as European bison, by worsening their blood parameters has been provided [18]. It is reasonable to assume that similar symptoms would be observed in cows.

Therefore, the aim of the present paper is to examine the prevalence of *A. sidemi* in faecal samples from cattle grazing in other areas of Poland, where the parasite was confirmed in wildlife and to summarize data about the presence of *A. sidemi* in Poland in over the past two decades.

Materials and Methods

Figure 1. Locations of cattle faecal samples collected for the presence of *A. sidemi* (1- Biebrza Marshland, 2 - Ruszów Forest District)

Material for the study was collected from cattle grazed in Ruszów Forest District (Lower Silesian Wilderness) and Biebrza Marshland, where *A. sidemi* has been previously detected in wildlife. Sampling locations are given in Figure 1. In total, 120 faecal samples were tested for the presence of A. *sidemi* DNA: 90 from the Ruszów Forest District and 30 from the Biebrza Marshland.

The faecal samples were collected directly from the rectum. Pellets weighing approximately 30g were placed in Petri dish culture and incubated at 25ºC for 20 days. The faecal cultures were examined every second day using a stereo-microscope. The mixtures with grown larvae were collected to Eppendorf tubes. These samples were then stored at -20ºC until molecular identification of the species.

A Nucleospin Tissue DNA Extraction Kit (Macherey-Nagel, Germany) was used to extract genomic DNA from the L3 mixtures. PCR was performed using a specific set of primers (R-specific 5'-ACA ACA TTA ACA CCT GTT GCA TGT-3'; F-specific 5'-ACT GTA TCC GAA TAT ATA TCG GAG-3') [19]. The DNA of adult *A. sidemi* was used as a positive control. PCR products were separated in 1.5% agarose gels; strains were confirmed by the presence of a band at approximately 406 bp.

Results and Discussion

After incubation, gastrointestinal nematode L3 larvae were found to be present in 78 examined samples; however, the numbers of obtained larvae were very low. No incidence of L3 *A. sidemi* DNA was confirmed molecularly in any of the examined samples. It is possible that *A. sidemi* had not yet parasitized the cattle in the studied area, or that the animals had been dewormed previously.

A previous study examined faecal samples from cattle living in different regions throughout Poland; however, the presence of *A. sidemi* DNA was confirmed in only two areas: the buffer zones of the Białowieża Forest and the Strzałowo Forest District [17]. It is entirely probable, though, that transmission of *A. sidemi* from wildlife to cattle will occur in both Ruszów Forest District and Biebrza Marshland in time: in the Białowieża Forest buffer zones, 14 years separated the first identification of *A. sidemi* DNA in cattle samples from its initial detection in wildlife in the area [8].

In the Ruszów Forest District, the first identification of *A. sidemi* infection was reported

after a full helminthological dissection of 14 red deer [10]; the study also noted an expansion of *A. sidemi* in red and roe deer in this region [11]. The researchers also reported a more than threefold increase of prevalence and fivefold increase of mean intensity of infection. In the present study, in spite of the significant increase in parasitological parameters observed in the examined free-living animals, no *A. sidemi* DNA was found in the faecal samples from cows grazing in this region. However, it is important to note that the present study was performed only five years since the first confirmation of *A. sidemi* in the Ruszów area.

Faecal samples have also been collected from cattle in the Biebrza Marshland area. In 2010, seven adult nematodes were found in one elk eliminated in this area [9]; these findings confirmed a new locus of ashworthiosis in Poland. However, in the presented paper, *A. sidemi* DNA was not detected in any of the examined samples.

The earlier studies did not confirm the presence of *A. sidemi* in any of 18 studied abomasa samples of red deer from the Strzałowo Forest District [15]. This may have been due to the small number of examined samples, or the fact that the study did not examine other cervids. Nevertheless, the helminth fauna of the Cervidae in Strzałowo Forest District should be updated following our identification of *A. sidemi* DNA in cattle faeces.

Until 2014, the presence of *A. sidemi* specimens in the abomasum and duodena of wildlife was confirmed during postmortem examination only. However, it has been demonstrated that DNA isolated from third stage infective larvae (L3) of *A. sidemi* can be used to diagnose ashworthiosis *in vivo* in European bison by PCR [17]. Later, a molecular study [19] confirmed the hypothesis that *A. sidemi* may infect domestic animals grazing on the same pastures used by wildlife infected with the parasite [13].

The faecal samples were also collected from cattle in the Borecka Forest area, where a small population of European bison are also living free in a fenced area [19]. Even in 2014, there was no sign of *A. sidemi* was in European bison, nor in the rest of the wildlife in this area, nor in cattle faeces; however, in 2016, anatomopathological and helminthological dissection of four European bison revealed *A. sidemi* infection in all animals [20]. Even so, the intensity of infection was not so high, and varied from 10 to 600 specimens per animal (mean intensity: 200 specimens).

Therefore, based on the available literature, and on our own experience, we can suspect that ashworthiosis may appear in new localities. Due to this increasing occurrence and the intensity of infection, further monitoring of *A. sidemi* is necessary in both wildlife and domestic animals.

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