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Research Note

The first description of *Setaria tundra* (Issaitshikoff & Rajewskaya, 1928) in roe deer from Croatia

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Summary

Genus *Setaria*, Viborg 1795, comprises 46 species that parasitize in the peritoneal cavity of Artiodactyla, Perissodactyla and Hyracoidea. The majority of these infections pass unnoticed, but occasionally they can induce severe peritonitis or neurological signs in aberrant hosts and, rarely, even in humans. In this paper we describe for the first time the finding of *Setaria tundra* in roe deer in Croatia. We examined 45 roe deer and determined the presence of *Setaria* nematodes in 24.4% of samples, which were subsequently diagnosed as *Setaria tundra* using molecular methods.
Keywords: roe deer; *Setaria tundra*; vector-borne disease; Croatia

Introduction

Filarioidea, the superfamily of nematodes comprises two families, *Filariidae* and *Onchocercidae*. The latter encompasses eight subfamilies: *Waltonellinae, Setariinae, Oswaldofilariinae, Icosellinae, Splendidofilariinae, Lemdaninae, Onchocercinae* and *Dirofilariinae* (Taylor *et al*., 2005). Presently, the genus *Setaria*, Viborg, 1795, contains 46 species that parasitize in the peritoneal cavity of Artiodactyla, Perissodactyla and Hyracoidea. The nematodes from *Setaria* genus have an indirect life cycle that includes mosquitoes (*Culicidae* family) and flies (*Haematobia* spp.) as vectors (vector-borne disease). Czajka *et al*. (2012) screened mosquitoes in Germany for filarial parasites using RT PCR, and found minimum prevalence rates of up to 24 infected per 1000 mosquitoes, which were attributed mainly to *Setaria tundra* infection. In the final hosts adult females that inhabit the peritoneal cavity produce large numbers of microfilariae each day (in thousands) which end up in the blood (Nelson, 1966), and are taken up by the vectors. Within 2 – 3 weeks in the vector, microfilariae become infective (L3) and are ready to be transferred to the final hosts (Anderson, 2000).
Usually *Setaria* sp. are not associated with clinical disease and therefore go undetected, unless the animals are submitted for necropsy. For example, Richter (1959) described findings of 43 nematodes in the peritoneal cavity of one roe deer (*Capreolus capreolus*), while Bednarski *et al*. (2010) reported finding 14 adult worms in the peritoneal cavity of a female roe deer, and in both cases the animals were without any notable signs of harmful effects. In rare circumstances, presence of adult worms in the peritoneal cavity can result in mild or even severe peritonitis. Such outbreaks of severe peritonitis were recorded in the ninety-seventies in reindeer (*Rangifer tarandus*) in Sweden and Norway, and in the period between 2003 to 2005 in reindeer and moose (*Alces alces*) in Finland, leading to significant economic losses (Rehbinder *et al*., 1975; Laaksonen *et al*., 2007). Occasionally, in aberrant hosts such as horses, goats or sheep, larvae have been known to migrate to the central nervous system, where they can induce severe neurological signs (Sundar and D'Souza, 2015). Panaitescu *et al*. (1999) reported four cases of human infection with *Setaria labiatopapillosa* in Romania.
In Croatia, the first scientific paper reporting the presence of *Setaria* sp. in different hosts dates back to as long ago as 1933 (Babić, 1933). Later, Mikačić (1941) described *S. labiatopapillosa* in cattle,

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and Richter (1959) pointed out the same species with a prevalence of 34 % in roe deer. However, due to the fact we have mentioned that these parasites rarely cause clinical disease, knowledge of their presence in Croatia is still limited. The goal of this research was to determine the present day presence of *Setaria* sp. in roe deer, using morphological and molecular methods.

Material and Methods

A total of 45 complete carcasses or whole digestive systems of roe deer were analysed at the University of Zagreb, Faculty of Veterinary Medicine, following the regular implementation of game management plans. The animals originated from two types of habitats: lowland (Zagreb County, n=28; Međimurska County, n=6; Bjelovarsko-bilogorska County, n=1; Sisačko-moslavačka County, n=1) and hilly/mountain (Nature Park Medvednica, n=9). Each sample was thoroughly analysed for free nematodes on the surface of the intestines, fore-stomachs or liver. The nematodes collected were counted, washed with physiological saline, and cleared with lactophenol to analyse their morphological characteristics according to recent morphological keys (Nikander *et al*., 2007), and then stored in 96 % alcohol. DNA isolation was performed using a commercial Genomic DNA Purification Kit (Wizard®, Promega) following the manufacturer's instructions. Subunit 1 of the cytochrome oxidase gene (Cox1) was amplified using cox1int F (5'-TGATTGGTGGTTTTGGTAA-'3) and cox1int R (5'-ATAAGTACGAGTATCAATATC-3') primers (Casiraghi, 2001). PCR reaction was executed in 25 µL suspension containing 2 µL of DNA, 0.25 µL of primers, 0.25 µL of nucleotides, 1.5 nM $MgCl_2$, and 1.25 U Promega GoTaq G2 Hot Start polymerase. Initial denaturation was performed at 95°C for 2 min, followed by 1 min cycles (n=35) at 94°C, and then 1 min at 52°C and 1 min at 72°C. Final extension was performed at 72°C for 5 min.
The PCR products obtained were sent to Macrogen Inc. (Amsterdam, Netherlands) for sequencing. The sequences were compared with those in GeneBank using a nucleotide blast tool.

Ethical Approval and/or Informed Consent

The research was performed under the approval of the Ethical Committee (Class: 640-01/14-305/16; No. 251-61-01/139-14-27)

Results and Discussion

Setaria nematodes were detected in 11 roe deer samples (prevalence 24.44 %) (Table 1) but we did not observe any gross lesions that could be attributable to *Setaria* sp. infection. According to the locality, the highest prevalence was in Zagrebačka County (P=25 %, n=7), followed by Medvednica Nature Park (P=22 %, n=2), and Međimurska County (P=17 %, n=1). Two other localities contributed with one sample each and should not be observed alone. The infection rate of nematodes per animal ranged from 1 to 24 (18 males, 77 females; 95 nematodes overall in 11 animals). Morphological analysis revealed typical *Setaria tundra* (Fig. 1) morphology (i.e. oval peribuccal crown with two elevations and without lateral lips; in femal's caudolateral appendages and the tip of the tail with a knob containing pores and grooves; caudal end containing 11 paired, one unpaired papillae and unequal spicules). DNA was successfully isolated from all samples and a 514 bp subunit of cox1 gene was amplified. Comparison with sequences archived in the GeneBank revealed that the analysed nematodes belong to the species *Setaria tundra*. Sequences from this research were deposited in the GeneBank under the accession number MH590581 – MH590586.
Recently, the number of reports describing this parasite in roe deer and mosquitoes in Europe has been increasing (Rehbein *et al*., 2001; Favia *et al*., 2003; Ferri *et al*., 2009; Laaksonen *et al*., 2009; Czajka *et al*., 2012; Kowal *et al*., 2013; Masny *et al*., 2013; Kemensei *et al*., 2015; Zittra *et al*., 2015; Angelone-Alasaad *et al*., 2016; Enemark *et al*., 2017). After the findings of Yanchev (1973), our results present the second confirmation of *S. tundra* in southeast Europe.The published prevalence in roe deer varies, from minimal, i.e. 9.4 % in Poland (Kowal *et al*., 2013), to as high as 40.1 % in Finland (Laaksonen *et al*., 2009.). Our findings of 24.4 % fit into this range. The fact that the prevalence of positive roe deer is rather high, with a wide area of distribution, and the fact that no gross lesions associated with *Setaria* infection were observed, means that roe deer are an important natural host and potential long-distance carrier of *S. tundra* (Laaksonen *et al*., 2009).
Another important question is whether the distribution of *S. tundra* started to spread due to climatic changes which favour the vectors, or due to the increase in the number of suitable hosts (namely roe deer), or was it simply previously mistakenly diagnosed as another species from the Setariinae subfamily? Proper species identifica-

Table 1. Number of samples positive on *Setaria* sp. according to location of sampling

Location/County	N	Positive	P%
Zagreb	9	2	22 %
Zagrebačka	28	7	25 %
Bjelovarsko – bilogorska	1	1	100 %
Sisačko – moslavačka	1	0	0 %
Međimurska	6	1	17 %

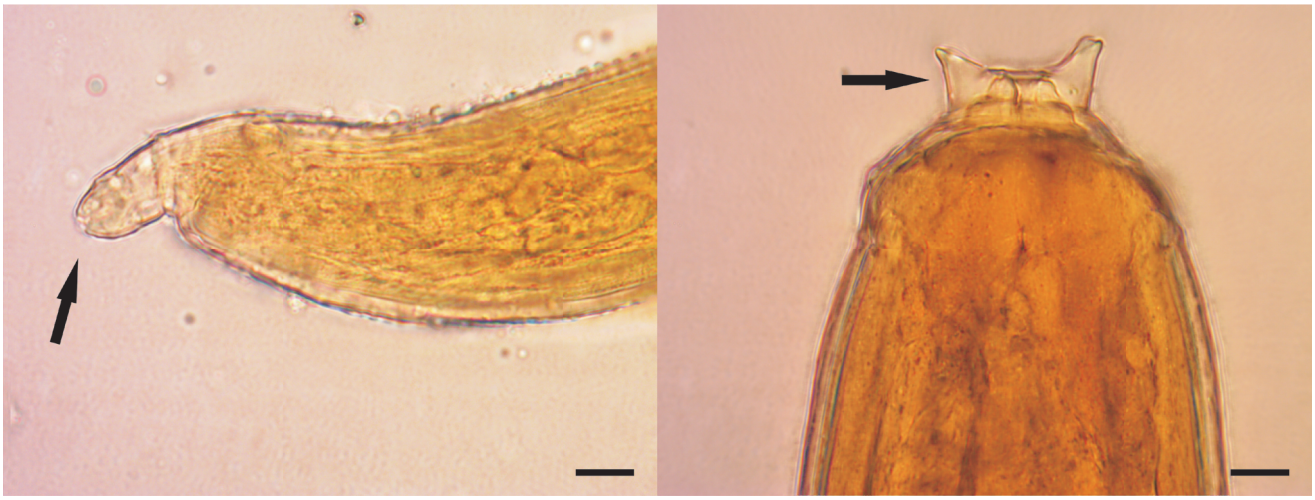

Fig. 1. *Setaria tundra*. The left part of the image shows tip of the tail with pores and grooves, without thorns (arrow). The right side points to an oval peribuccal crown with two elevations and without lateral lips (arrow). Scale bar – 10µm.

tion is of special importance for biologists and taxonomists, but also for medical and veterinary professionals due to the potential health implications. The traditional way to identify *Setaria* species was to use their morphological characteristics. However, the similar morphological keys of different *Setaria* lineages can weaken species identification, requiring application of molecular methods to confirm traditional tools (Yatawara *et al*., 2007; Ferri *et al*., 2009; Laaksonen *et al*., 2009). *S. tundra* differs from *S. labiatopapillosa* mainly in the appearance of the female peribuccal crown with the elongated mouth opening and cuticularized lateral lips (Yeh, 1959). However, according to Yeh (1959), the tails of female *S. tundra* and *S. labiatopapillosa* look very much alike, complicating proper identification. Later on, over history, many controversies about these two and other species have arisen, where authors described old species as new ones (reviewed in Nikander *et al*., 2007). On the molecular level, the difference between these two species is more pronounced and was shown in the phylogenetic study of *Setaria cervi* in Italy, which revealed that *S. labiatopapillosa* belong to one clade together with *S. cervi* and *S. digitata*, which is well separated from the one containing *S. tundra* and *S. equina* (Alasaad *et al*., 2012). In Croatia, from the first finding and over the past decades, the only previous detailed description available is from *S. labiatopapillosa* in cattle (Mikačić, 1941), where the author, besides the presence of lateral lips, clearly describes the thorn structures on the tail knob which were at that time also attributed as a characteristic of *S. tundra.* Interestingly, in our survey, among the 77 females, we did not find any thorned knobs (thorned morphotype). Eighteen years later, in his paper Richter recorded and described some parasitic fauna (without protozoa) in 47 roe deer. Among them, he pointed out findings of *Setaria labiatopapillosa* in 34 % of roe deer with one animal harbouring 43 nematodes in the abdominal cavity (Richter, 1959). Unfortunately, no detailed description or images were presented in the article, leaving us no possibility to re-examine his findings. A similar problem was encountered by Enemark Larsen *et al*. (2017) when discussing the findings of *S. transcaucausica* in Denmark (Korsholm, 1988). Therefore, the initial thought is that *S. tundra* might have been mistakenly diagnosed as *S. labiatopapillosa*. On the other hand, due to the controversial history and doubtful validity of setarian species in different and even the same mammalian species (Nikander *et al*., 2007), there is also a possibility that roe deer may harbour more than one species of *Setaria.* Since the description of *S. labiatopapillosa* in roe deer is indeed rare, this leaves us no other choice than to continue the survey of setarian species in roe deer and also cattle as type hosts for *S. labiatopapillosa*. Finally, there is also a possibility that *S. labiatopapillosa* was correctly diagnosed in roe deer, but now this species is very rare or even no longer exists in roe deer due to extrusion with *S. tundra* which seems to be increasingly prevalent in roe deer in Europe.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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