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Structure of Metastrongylidae in wild boars from southern Poland

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Summary

Of 25 wild boars (Sus scrofa) collected in southern Poland during the winter season of 2009/2010 and examined for lung nematodes, 20 (80.0 %) were concominantly infected, and the mean \pm SD intensity reached 84.8 \pm 67.6 (range 7 - 250) parasites. From the whole of 1695 gathered Metastrongylidae specimens, 1121 (66.1 %) were distinguished to five species: Metastrongylus pudendotectus, M. salmi, M. asymmetricus, M. elongatus and M. confusus. The species ratios were 3.4:2.7:1.5:1.1:1.0, respectively, with the average male to female worms proportion of 1:2.7. M. pudendotectus and M. confusus affected most (76.0 %) of animals, followed by M. salmi (72.0 %), M. elongatus (64.0 %) and M. asymmetricus (40.0 %). Compared to juveniles under 1 year and females, adults and male hosts tended to be more infected, and wild boars inhabiting primeval forest were more affected by lung nematodes than those living in the arable land, all the differences being however not significant. Possible factors structuring Metastrongylidae communities are discussed.

Keywords: Metastrongylus spp., wild boars, southern Poland

Introduction

The wild boar (*Sus scrofa* L.) constitutes in Poland one of the main large game species, with its current population of approximately 200 000 individuals, following the rapid increase registered over the last decades (Kamieniarz & Panek, 2008). Lungworms of the genus *Metastrongylus* Molin 1861 (Nematoda: Strongylida) are considered as one of the important selective factors affecting wild boar populations and increasing the mortality of young animals (Frączak, 1974; Humbert & Henry, 1989; Houszka, 2001). The following species of lung nematodes could be incorporated in Europe: *M. elongatus* (Dujardin, 1846) (syn. *M. apri* (Gmelin, 1790)), *M. pudendotectus* Wostokow, 1905, M. salmi Gedoelst, 1923, M. confusus Jansen, 1964 and M. asymmetricus (Noda, 1973). In Poland, M. elongatus and M. pudentodectus were hitherto considered to be the dominants, followed by M. salmi (Tarczyński, 1956; Gadomska, 1981). The other species, M. confusus (Dróżdż & Zalewska-Schönthaler, 1987) and M. asymmetricus (Nosal et al., 2009b), were recorded in the country later. Since the differences of both geohraphical and temporal origin are observed in Metastrongylus spp. composition, the study was performed to assess the quantitative and qualitative appearance of lung nematodes in wild boars inhabiting southern Poland. The aim of the research was also to assess the differences in infection level related to host age and sex, or habitat occupied by wild boars. Furthermore, lungworms structure description could be of value for successive comparisons, as well as in studies aimed at recognition of existing species interactions that might influence the pathogenicity of different Metastrongylidae guilds.

Material and methods

The survey was conducted from November 2009 to February 2010, in two regions of southern Poland (Małopolska province, north and west of Kraków, altitude about 300 m), situated at no great distance of approximately 50 km each other. The first area, of less humid conditions (fecund loess soil), is under cultivation, and faintly wooded (oak and pine prevails), whereas the second one occupies mostly wetlands (marshy clay grounds) overgrown with primeval forest predominated by pine and birch.

A total of 25 hunted wild boars were examined, 16 originating from the arable land and 9 from primeval forest. Data about the site of shooting, as well as the age and sex of animals, were collected at sampling time. Host age was estimated based on tooth development, and two groups of wild boars were established: juveniles under 1 year and adults. Lungs were evaluated according to Roepstorff and Nansen (1998).

Collected parasites were fixed in 70 % ethanol with 5 % glycerine and identified according to the descriptions by Tarczyński (1956), Jansen (1964), Holló (1965b), Noda

(1973) and Morita *et al.* (2007). Depending on the intensity of infection, at least 30 % of nematodes gathered from any host were differentiated. Measurements and photographs were made in Motic Images Plus 2.0 program, under 100 and $400 \times$ magnifications.



Fig. 1. Morphological features of the caudal end of five Metastrongylidae species collected. a, f: *M. elongatus*; b, g: *M. confusus*; c, h: *M. salmi*; d, i: *M. pudendotectus*; e, j: *M. asymmetricus*. a – e: Male bursa ventral/dorsal view, and f – j: female lateral view. Magnification: a – c and f – i: 400×: d – e: 100×

The species of helminths were defined as common, after Pence *et al.* (1988), if occurred at least at 20 % prevalence in the host population. Quantitative Parasitology 3.0 (Rózsa *et al.*, 2000) was used to reveal any association between helminth infections and wild boar site of collection, sex, and age-group.

Results

The prevalence of Metastrongylus spp. infection reached 80.0 %, and the mean \pm SD intensity was 84.8 \pm 67.6 (range 7 - 250) parasite specimens. Out of 1695 isolated lungworms, 1121 (66.1 %) were allocated to the species. All of the five Metastrongylidae species identified (Fig. 1, Table 1) occurred commonly (≥ 20 %), with the most prevalent M. pudendotectus and M. confusus (76.0 %), predominating over M. salmi and the remaining species - M. elongatus and M. asymmetricus (Table 2). Wild boars were infected concurrently: 7 animals harboured all 5 nematode species; 9 were affected by 4 species (7 without M. asymmetricus, and single hosts without M. elongatus or M. confusus); 3 were infected with 3 species (M. elongatus, M. salmi and M. asymmetricus were all lacking twice), and one with 2 species: M. salmi and M. confusus. Within the parasite community, in proportional species share, M. pudendotectus (34.7 %) and *M. salmi* (28.2 %) appeared to be the most common

(Table 3). The proportion of males to females was the highest (1:1.9) for *M. pudendotectus* and smallest (1:6.2) for *M. confusus*, reaching the average of 1:2.7.

The infection level tended to increase with the age of wild boars, and also male hosts were more infected than females (Table 2). The animals inhabiting primeval forest tended to be more affected by parasites than those living in agricultural environment. There was no statistically significant difference between the allotted groups of animals, as regards the helminth infection levels.

Discussion

In European countries, the infection of wild boars with lung nematodes is prevalent and affects 85.1 to 100 % of animals (Humbert & Henry, 1989; Epe *et al.*, 1997; de-la-Muela *et al.*, 2001). Similarly in Poland, according to Gadomska (1981) and Houszka (2001), *Metastrongylus* spp. infects 85 – 92 % of wild boars, though Houszka (2001) claims that depending on habitat and nourishment conditions, the prevalence of infection could attain 100 % in humid woodlands, while in dry areas does not exceed 30 %. Natural peculiarities of the area, including sufficient availability of earthworms as the intermediate hosts of Metas-

trongylidae, are important factors affecting the infection of

wild boars (Järvis et al., 2007). There is, however, a dis-

Table 1. Measurements (min – max. and mean \pm SD in brackets) of collected parasite specimens (n = 20 females and 10 males of each species)

Characteristic	M. pudendotectus	M. elongatus	M. salmi	M. confusus	M. asymmetricus			
Dimensions of females								
Padu longth [mm]	23.4 - 35.8	34.2 - 50.5	27.7 - 41.0	25.3 - 44.1	21.0 - 28.4			
Body lengui [inin]	(28.4 ± 2.75)	(42.4 ± 4.80)	(36.0 ± 3.57)	(34.9 ± 5.39)	(25.2 ± 2.13)			
I enoth of tail [um]	126.1 - 171.7	72.0 - 106.0	60.6 - 100.1	68.9 - 94.4	147.8 - 219.8			
Length of tan [µm]	(147.2 ± 16.30)	(86.9 ± 9.82)	(85.2 ± 12.71)	(79.6 ± 8.03)	(182.7 ± 22.42)			
Dimensions of eggs								
I an oth []	56.3 - 61.1	41.8 - 54.7	45.1 - 53.8	41.9 - 58.9	49.2 - 55.9			
Length [µm]	(59.1 ± 1.83)	(50.3 ± 2.71)	(49.1 ± 2.21)	(50.9 ± 3.68)	(52.8 ± 2.00)			
Width [um]	38.2 - 43.3	31.2 - 41.8	32.3 - 41.6	26.0 - 41.0	34.4 - 41.4			
widui [µiii]	(40.7 ± 1.80)	(36.8 ± 2.83)	(35.5 ± 2.70)	(33.9 ± 3.53)	(37.6 ± 2.37)			
Dimensions of males								
Rody length [mm]	14.6 - 19.5	13.8 - 19.4	12.5 - 18.5	14.6 - 17.9	14.3 - 20.1			
body lengui [mm]	(16.7 ± 1.78)	(17.5 ± 1.87)	(15.7 ± 1.93)	(16.4 ± 1.07)	(17.2 ± 1.77)			
Length of spicules [um]	1362 - 1502	3712 - 4731	1713 - 2760	2019 - 2913	520 - 688			
Length of spicules [µm]	(1437 ± 50.2)	(4206 ± 332.9)	(2146 ± 345.2)	(2574 ± 352.1)	(632 ± 55.4)			
Length of copulatory	496.4 - 617.5	334.6 - 462.4	376.2 - 586.5	326.8 - 468.7	486.2 - 621.5			
bursa [µm]	(553.7 ± 51.88)	(402.4 ± 47.59)	(494.0 ± 70.30)	(396.5 ± 43.01)	(578.7 ± 41.61)			
Width of copulatory bursa	440.8 - 610.3	333.1 - 407.1	446.9 - 527.5	287.5 - 383.9	578.4 - 623.8			
[µm]	(517.5 ± 81.00)	(365.0 ± 27.51)	(495.0 ± 25.49)	(328.6 ± 33.48)	(599.1 ± 17.44)			

Will boons data	No	M. J	pudendotectus	M	elongatus	7	M. salmi	N	1. confusus	M. i	asymmetricus
W 11U DUALS UALA	.0N	Р	Ι	Р	Ι	Р	Ι	Р	Ι	Р	I
Host age											
<1 year	10	70.0	8.9 (2 – 19)	50.0	3.8(1-13)	50.0	4.2(1-7)	60.09	2.5(1-6)	30.0	12.7 (1 – 34)
adults	15	80.0	27.3 (1 – 131)	73.3	9.6 (2 – 26)	86.7	22.7 (1 – 58)	86.7	7.7 (1 – 33)	46.7	19.7 (1 – 72)
Host sex											
females	14	64.3	11.7 (2 – 52)	42.9	2.0(1-3)	64.3	7.2 (1 – 27)	64.3	3.7 (1 – 11)	28.6	16.3 (2 – 34)
males	11	90.9	28.4 (1 – 131)	90.9	11.3 (1-26)	81.8	27.9 (5 – 58)	90.9	8.2 (1 – 33)	54.5	18.5 (1 – 72)
Shooting site											
primeval forest	6	77.8	26.6 (4 – 93)	55.6	10.6 (3 – 19)	77.8	26.1 (1 – 58)	88.9	9.8 (2 – 33)	77.8	22.6 (1 – 72)
arable land	16	75.0	16.9 (1 – 131)	68.8	6.5 (1 – 26)	68.8	12.1 (1 – 50)	68.8	3.4(1-10)	18.8	6.0(1-14)
Total	25	76.0	20.5(1-131)	64.0	7.8 (1 – 26)	72.0	17.6 (1 – 58)	76.0	6.1 (1 – 33)	40.0	17.6 (1 – 72)

tinct site dependence in the transmission of the parasites. Studies of Humbert and Henry (1989) reveal that the intensity of lungworm infection depends highly on frequenting the areas at high risk for acquiring the infection, such as the feeding centers, since no parasited earthworms the authors could find in the forest.

Previous studies made in Poland (Tarczyński, 1956; Sobieszewski, 1969) informed of low infection of domestic swine mainly with *M. elongatus*, the species affecting 2.2 to 3.3 % of pigs. In swine housed in the areas presently investigated, Metastrongylidae are extinct. Consequently, the invertebrates of arable lands should stay uninfected, as the result of breaking the chain leading from definite to intermediate hosts of the nematodes. Wild boars collected in agricultural site were less infected (Table 2), and this might also be the consequence of different feed structure, because the alternative vegetable fodder is easily accessible there. The exception was, however, the higher *M. elongatus* infection. The question arises, whether the ploughland is inhabited by earthworm species favoured by *M. elongatus*.

According to some authors, significant differences in the intensities of lungworm infection occurr across both wild boar ages and sexes. Humbert and Henry (1989) found in France significantly greater number of *Metastrongylus* spp. in juveniles, and male hosts were significantly heavily infected with *M. elongatus* – constituting the main American species – in the study of Forrester *et al.* (1982). Also in German investigation (Epe *et al.*, 1997) wild boars under

one country area (north-east of Poland, presumably imported from Belorussia). Of the total number of lungworms, M. elongatus represented 65.8 %, M. pudendotectus 33.1 %, and M. salmi 1.1 %, thus the proportional share of particular species equalled 59.8:30.1:1.0. Also Gadomska (1981) found *M. elongatus* as the dominant species in wild boar population from Kampinos National Park (neighbourhood of Warsaw, central Poland), with the subdominant M. pudendotectus and M. salmi occupying the position of accessory species. The nematode species proportion already differed, varying respectively in consecutive years from 6.3:3.8:1.0 to 3.0:2.7:1.0. In wild boars living in enclosures of the same area, the position of dominants even balanced, and the quantitative proportions of M. elongatus to M. pudendotectus and M. salmi were the following in alternate years: 5.3:6.1:1.0 and 17.2:6.7:1.0. The conclusion of Gadomska (1981) was, that the domination structure of Metastrongylus community is blurred, and there is no distinct dominant and accessory species observed. The results of Polish reserchers were conformable with other studies conducted in Europe those years (Jansen, 1964; Hollo, 1965a).

Similar species makeup to these obtained in the present investigation found Humbert and Henry (1989). They detected *M. pudendotectus*, *M. salmi* and *M. confusus* in all of the infected animals, whereas *M. elongatus* only in some regions investigated and *M. asymmetricus* in one alone closed game reserve, with the mean infection intensity of 167 parasites. In the enclosures of southern Germany, even

Characteristic	M. pudendotectus	M. elongatus	M. salmi	M. confusus	M. asymmetricus	Metastrongylus spp.
Species relevant						
No. of parasites	389	125	316	115	176	1 121
Species share (%)	34.7	11.1	28.2	10.3	15.7	100.0
Species ratio	3.4	1.1	2.7	1.0	1.5	_
Sex relevant						
No. of females	254	84	244	99	133	814
No. of males	135	41	72	16	43	307
$\mathcal{F}\mathcal{F}: \mathcal{G} \ proportion$	1:1.9	1:2.0	1:3.4	1:6.2	1:3.1	1:2.7

Table 3. Species and sex structure of identified Metastrongylidae specimens

1-year-old and males tended to be more infected, and in Spain (de-la-Muela *et al.*, 2001) the intensity of infection was greater in juveniles, although the prevalence tended to increase with age. Statistically however, the differences were not confirmed, which is consistent with the results of most other researches (Pence *et al.*, 1988; Barutzki & Richter, 1990; Barutzki *et al.*, 1991; Rajković-Janje *et al.*, 2002; Biddau *et al.*, 2003; Järvis *et al.*, 2007), and also with the present study.

In Poland, Tarczyński (1956) found three species of lungworms in wild boars, with the prevalences of 52.5, 44.3 and 11.5 % for *M. elongatus*, *M. pudendotectus* and *M. salmi*, accordingly – the last species being found only in 216 *M. salmi* prevailed (91.9 %) in lungs of wild boars, followed by *M. elongatus* (88.7 %) and *M. pudendotectus* (87.1 %) (Barutzki *et al.*, 1990), and the intensity equalled 176.9, 56.9 and 254.2 worms of the species (Barutzki *et al.*, 1991). However, in the open areas, 82.2% of differentiated lungworms constituted *M. pudendotectus*, and only 14.1% *M. salmi*, 2.5% *M. elongatus* and 1.2 % *M. confusus* (Barutzki & Richter, 1990). Later, Epe *et al.* (1997) found *M. pudendotectus* (93.3 %) and *M. elongatus* (91.1 %) being predominat in wild boars of German population, followed by *M. salmi* (80.0 %) and *M. confusus* (24.5 %), with respectively 28.1, 24.0, 9.8 and 4.3 parasite species specimens. *M. pudendotectus* (prevalence of 78 %, 58.5

nematodes per animal) was also the most prevalent and numerous, together with *M. salmi* (77 %, 40.8 worms), as against the third detected species *M. elongatus* in Estonian island (41 %, 4.6 nematodes) (Järvis *et al.*, 2007), the composition of the species being partly different from that observed on the mainland of Estonia (92, 30 and 89 %, respectively). In southern Europe, in Sardinia island, four of the five lungworm species recorded in Italy were identified, and the highest prevalence were found again for *M. salmi* (81.8 %) and *M. pudendotectus* (80 %), whereas *M. elongatus* infected only 50.9 % animals, and *M. confusus* 46.4 % (Biddau *et al.*, 2003).

On the other continents, *M. salmi* was dominant in Asiatic wild boar *S. s. leucomystax* (100 %) over *M. elongatus* (92.9 %), *M. asymmetricus* (88.1 %) and *M. pudendotectus* (71.4 %) (Morita *et al.*, 2007). The species composition (equalling 3.4:1.3:1.0:1.4, respectively) was however drastically different from the previous reports made in Japan, which showed *M. elongatus* as the most frequent species and no presence of *M. pudendotectus* (Noda, 1973). In feral swine of the USA, *M. elongatus*, with its 75 – 94% prevalence, still dominates over *M. salmi* (76 %) and *M. pudendotectus* (35 – 64 %) (Forrester *et al.*, 1982; Pence *et al.*, 1988).

In our studies, any species interaction did not seem to cover the ecological or host factors determining the distribution of the parasites. However, as lugworm species mainly co-occur in mixed infections, it would be important to recognize in detail their noninteractive coexistence, or antagonistic and synergistic interactions, which might essentially influence the community pathogenicity. The structure of lung nematodes may be very complex, as Ewing *et al.* (1982) suggested mutualism between *M. elongatus* and *M. pudendotectus*, although Holz and Lian (1968) never found simultaneouos occurrence of the two lungworm species in swine.

The differences in *Metastrongylus* spp. composition are difficult to any explanation. The dominant species vary depending on the countries, as well as within them, over the regions. The structure of Metastrongylidae is changing also with years, and the species diversity seem to increase. Järvis *et al.* (2007) state that wild boars from different regions of Europe have quite similar helminthofana, but the species diversity together with infection level is increasing from north to south. Growing host population densities are of essential consequence in structuring parasite communities (Pence *et al.*, 1988, Fernandez-de-Mera *et al.*, 2003), but their structure can also change with introductions. Fernandez-de-Mera *et al.* (2003) warn of the risk of importing new parasites into naïve populations.

In Poland, over a span of a half-century, particularly in the last few decades, not only the domination structure within *Metastrongylus* spp. community changed (*M. pudendotectus* and *M. salmi* instead of *M. elongatus* and *M. pudendotectus*), but the diversity increased with the recognition of two new for the country species: *M. confusus* and *M. asymmetricus*. If *M. confusus*, was discovered in European wild boar, and could be acknowledged as an indigenuous,

the last species seems to be an alien, translocated with Asian suids. To mention, *M. asymmetricus* was found in the investigated area together with *Bourgelatia diducta* Railliet, Henry et Bauche, 1919 – the nematode species foreign for European wild boar (Nosal *et al.*, 2009a; article in preparation).

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