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ON THE PARASITES OF GENUS *ANGIOSTRONGYLUS* (NEMATODA: ANGIOSTRONGYLIDAE) AND SOME CASES OF *ANGIOSTRONGYLUS DASKALOVI* IN BADGERS FROM BULGARIA

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Abstract

In the course of studies on filariid infections in wild carnivorous in Bulgaria nematodes of genus *Angiostrongylus* were found in the heart and pulmonary arteries of badgers. The detected specimens were examined morphologically and metrically, which allowed us to identify them as *Angiostrongylusdaskalovi*. A morphometrical description of the species is given in accordance with the present materials.

Key words: *Angiostrongylus daskalovi*, lungworms, badger, *Melesmeles*, Bulgaria

Реферат

В ходе исследования филариидных инвазий в Болгарии в сердце и легочных артериях барсуков обнаружены нематоды рода *Angiostrongylus*. Эти образцы исследовались морфологически и метрически, что позволило, с учетом также географического и гостального

распространения, идентифицировать их как *Angiostrongylus daskalovi* Janchev et Genov 1988. Приводится морфометрическое описание вида по данным текущих исследований.

Key words: *Angiostrongylus daskalovi*, барсук, Болгария.

Introduction

Angiostrongylids are nematodes of order Strongylida, superfamily Metastrongyloidea(Anderson et al., 2009). The representatives of this superfamily are also known as lungworms. The majority of species of *Angiostrongylus*genus parasitize in the pulmonary arteries and the heart of their host. There are species, like *Angiostrongyluscantonensis*, that affect the central nervous system (Wessmannetal., 2006), others can be found in the mesenteric blood vessels – *A. costaricensis* (Rebelloetal., 2013), or in the bronchioles – *A. michiganensis*(Ash, 1967). The *Angiostrongylus*species have an indirect life cycle. Definitive hosts are insectivores, rodents, felids and canids. The man can also be infected by some of those parasites, although he is a nonspecific host. Intermediate hosts are different species of mollusks. Most of the data about the damages done by those parasites are for human infections. Slom et al. (2002) have reported the development of eosinophilic meningitis in tourists returning from the Caribbean. Their infection with*A. cantonensis*has been linked with the consumption of native food. Lietal.(2008) have reported a serious cerebral damage and a severe illness with danger of fatality in a patient that has eaten raw mollusks infected with this species. Another species of the family - *A.costaricensis*might cause a heavy gastrointestinal disease among humans known as abdominal angiostrongylosis, it could be end fatally (Kramer et al., 1998; Rebelloetal., 2013). The present work describes some cases of parasitizing of badgers from Bulgaria with angiostrongylids.

Materials and Methods

Helminthological necropsies of hearts and lungs of 11 badgers(*Melesmeles* L.) from regions of Burgas, Pernik, Silistra, Sofia and VelikoTarnovo, and 8 martens (*Martesfiona* L.) from regions of Pazardjik, Plovdiv and Sofia were performed. During them nematodes were found in the heart and pulmonary arteries of two badgers. The parasites were collected in physiological salt and then preserved in Barbagallo's solution. The specimens used for identification were cleared with lactophenol. Pictures were taken using a light microscope "Leica DM5000 B", supplied with camera and software (LeicaApplication Suite LAS v.3.1).The measure of the parasite structures were done by the classic methods of parasitology as well as by the image analyzing computer program Image-Pro Plus - Version 6 as described byPanayotova-Pencheva and Alexandrov(2008). The collected helminths are found in Institute of experimental morphology, pathology and anthropology with museum of Bulgarian Academy of Sciences.

Results

Nematodes were found in two badgers from the region of Sofia. One of them was from sub-region of Pravets, and the adult parasites were situatedin the right atrium and ventricle of the heart, and another animal was from sub-region of Tran with parasites localized in the heart and pulmonary arteries.

At the beginning the specimens were identified as angiostrongylids. In order to find the exact species identification we have done morphological and metrical studies, which showed the following: The adult helminthes are spindle-shaped, with length of males 18-19 mm (mean 18.33) and of females 24-32 mm (mean 28).

The oral orifice is situated terminally and around it can be seen tree poorly developed lips, the oral orifice passes into the oesophagus, which is lightly widened in the distal part, the neural ring is difficult to detect and can be found in the first third of the oesophagus (Fig. 1).

The caudal end of the females is curved like an arc and the males have a spiral-shaped end. The bursa copulatrix is small but well-developed (Figures 2-3). The bursa has the following structure: The dorsal ray is medially situated it is the shortest and widest ray and has two growths. The extero-dorsal rays are separated, shorter and narrower then the lateral rays. The lateral rays are fused at the beginning and the antero-lateral ones are the first that split from the common trunk. The middle and postero-lateral rays remain together almost in half of their length. The antero-lateral rays are shorter then the middle-lateral and postero-lateral ones and don't reach the edge of the bursa copulatrix. The ventral rays are connected in the most part of their length.

The spicules (Fig. 3) are long and thin with a spongy-like structure and their proximal part is a little wider then the distal part. Almost immediately after the beginning of the spicules short transversally striated wings are observed which in their distal end become wider and nearly transparent (Fig. 4). A poorly developed gubernaculum (Fig. 5) from one part with elongated shape can beobserved in the area of the cloaca.

The vulva is situated in the caudal end of the body not to far from the anus. They are 4 cuticular elevations around its aperture (Fig. 6).

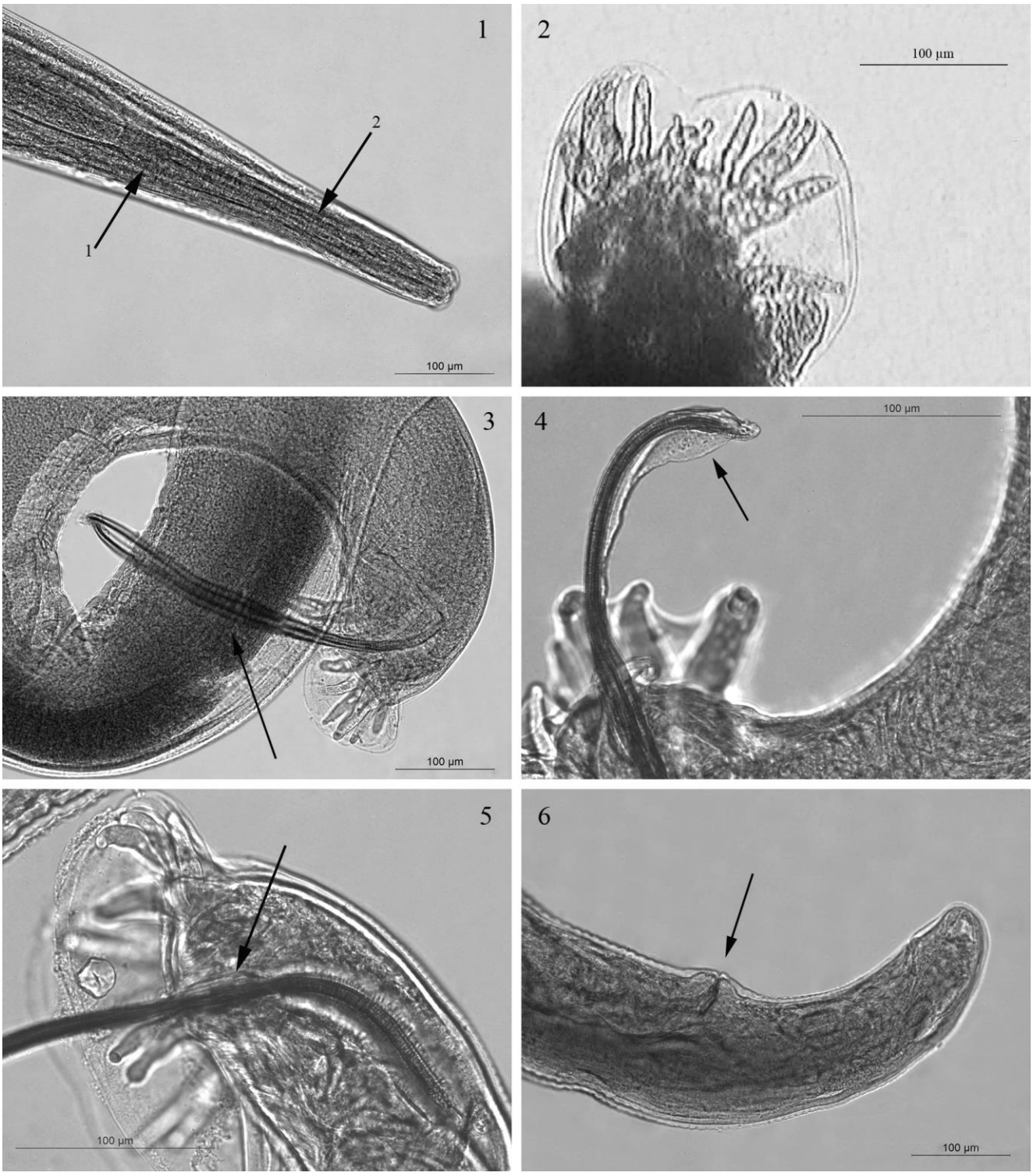
The metric data of the above-mentioned structures are given in Table 1.

Table 1.

Metric data of *Angiostrongylusdaskalovi* in materials from Bulgaria

Structure	Our data Min-Max (Mean)	Janchev and Genov 1988 Min-Max (Mean)
Body length of males - mm	18-19 (18.33)	13.36-21.31 (19.2)
Body width behind the oesophagus - µm	98-122 (110)	96-122 (106)
Body width before the bursa - µm	118-156 (137)	117-163 (146)
Oesophagus length of males - µm	293-362 (322.8)	336-366 (351).
Max Oesophagus width of males - µm	36-49 (40.7)	36---43 (40.3)
Spicule length - µm	386-410 (394.3)	336 - 409 (374)
Gubernaculum length - µm	46-69 (57)	43-56 (48.1)
Body length of females - mm	24-32 (28)	14.39--31.12 (26)
Body width behind the oesophagus - µm	129-184 (158.5)	126-219 (136)
Body width at vulvar region - µm	140-227 (186.9)	142-239 (225)
Oesophagus length of females - µm	365-488 (406)	356-556 (417 .9)
Max. Oesophagus width of females - µm	37-63 (49.4)	33-56 (44.6)

Distance from vulva to tail tip - µm	260-340 (288.4)	269-412 (337.3)
Distance from anus to tail tip - µm	68-88 (76.9)	76-115 (84.2)



Figures 1-6.

Angiostrongylus daskalovi in badgers from Bulgaria (original pictures), Scale bar-100 μm:1. Anterior end of the body:1-oesophagus, 2-neural ring. 2. Bursa copulatrix. 3. Spicules. 4. Distal spicule end. 5. Gubernaculum. 6. Vulva.

Discussion

The parasites of the genus*Angiostrongylus* are distributed all over the world.*A. cantonensis, A. costaricensis, A. dujardini, A.mackerrasae, A. malaysiensis,*and*A. vasorum*arewidespread, eithergloballyorregionally. Thesespeciesappear to bespeciesonthemove, spreadingintoregionswherepreviouslythey did notoccur (Spratt, 2015).

On the European territory *A. vasorum*has been established in domestic dogs in Denmark, France, Germany,Great Britain, Greece, Italy, Irland, Netherland, Spain, Sweden, Switzerland (Traversa et al., 2010), Poland (Schnyderet al. 2013), Slovakia (Miterpakova et al., 2015),Portugal (Alho et al., 2016), and the following species have been reported in wild animals:*A.chabaudi*in wild catsfrom Italy (Biocca, 1957), Greece (Diakou et al., 2016) and Romania (Gherman et al., 2016); *A. sciuri*in red squirrelfrom Turkey (Merdivenci, 1964); *A. vasorum* in foxes from Italy (Poli et al., 1984), Great Britain (Morgan et al., 2008), Iberian Peninsula (Gerrikagoitia et al., 2010) and badgers from Spain(Torres et al., 2001); *A. daskalovi* in badgers from Iberian Peninsula (Gerrikagoitia et al., 2010).

There are 3 reports about angiostrongylids in Bulgaria. The first is by Yanchev and Genov(1988) who have described *A. daskalovi*from badgers and martens as a new species. The next is by Kamenov et al. (1999) in which the authors have presented a clinical case of angiostrongylosis in domestic cats with larvoscopic diagnose of *A. vasorum.* The third report is by Pantchev et al. (2015) aboutsurveys of theseroprevalence of some parasites including*A.vasorum*indogsfrom Bulgaria.

In the course of species identification of angiostrongylids found by us we took into account the following: species of the hosts, geographical situation of the regions they came, morphological and metric characteristics of the parasites and their comparison with those of other angiostrongylid species established in Europe and reported in Bulgaria. The fact that parasites were found in badgers from Bulgaria, some morphological peculiarities of helminths as, for example, the shorter antero-lateral rays of bursa copulatrix then the rest of lateral rays and approach of metric data about different parasite structures to those pointed by Janchev and Genov (1988) about described by them *Angiostrongylusdaskalovi* sp. n. gave us ground to relate the nematodes found by us namely to this species.

The small number of investigated animals does not allow us to perform any conclusions about epidemiological features of this parasitosis. However, we can mention that in our studies we have established the species only in animals from mountain regions of West Bulgaria and not in those from plain and lowland parts of the country. For comparison, we can point the results of Janchev and Genov (1988), who before 30-40 years have found the bigger part of the materials in which have described the species namely in west Bulgaria.

The metric data found by us and JanchevandGenov (1988) according to the species are shown in Table 1. There are variations regarding some structures but they are small and we attribute them to population peculiarities.

The present study gives us grounds to put the question of Spratt (2015) again: if thedearth of reportsintheliterature of manyspecies of*Angiostrongylus*occurringin wildlife, *A. andersoni, A. chabaudi,A.daskalovi, A. felineus, A. gubernaculatus, A. lenzii, A. morerai, A. petrovi,A. raillieti, A. ryjikovi, A. sandarsae, A. schmidti, A. sciuri, A. siamensisand A. tateronae* is due to their ratherlimitedgeographicdistribution or itmayreflectlack of opportunityorinterestinexaminingnon-urbanandnon-agriculturalhosts.We also ask anotherquestionto us: do any of the species manifest bigger

specificity to their final host which could be a reason for their limited geographic distribution.Undoubtedly, further investigations in that area will contribute to clearing of arising questions.

References

1. Ash L.R. *Angiostrongylusmichiganensis*sp. n. (Nematoda: Metastrongyloidea), a LungwormOccurringintheShrew, Sorexcinereuscinereus, inMichigan. TheJournal of Parasitology, 1967,V. 53, №3, 625-629.
2. Alho A.M., Schnyder M., Schaper R., Meireles J., Belo S., Deplazes P., Madeira de Carvalho L.Seroprevalence of circulating *Angiostrongylusvasorum* antigen and parasite-specific antibodies in dogs from Portugal. Parasitol. Res., 2016, V. 115, 2567–2572.
3. Anderson R.C., Chabaud A.G., Willmott S. Keys to the nematode parasites of vertebrates. ArchivalVolume. Cab International, London, UK, 2009. 463 pp.
4. Biocca E. *Angiostrongyluschabaudi* n. sp. parassita del Cuore e deivasipolmonari del gattoselvatico (*Felissilvestris*). Atti. Accad. Naz. LinceiRc.,1957, V. 22, 526–532.
5. Diakou A., Psalla D., Migli D., Di Cesare A., Youlatos D., Marcer F., Traversa D. First evidence of the European wildcat (*Felissilvestrissilvestris*) as definitive host of *Angiostrongyluschabaudi*. Parasitol. Res.,2016, V.115, №3, 235-244.
6. Gerrikagoitia X., Barral M., Juste R.A.*Angiostrongylus*species in wild carnivores in the Iberian Peinsula. Vet. Parasitol.,2010, V. 174, 175–180.
7. Gherman C.M., Ionică A.M., D'Amico G., Otranto D., Mihalca A.D.*Angiostrongyluschabaudi* (Biocca, 1957) in wildcat (*Felissilvestrissilvestris,* S) from Romania. Parasitol. Res.,2016. DOI: 10.1007/s00436-016-5032-3 (In press).
8. Janchev J., Genov T.*Angiostrongylusdaskalovi* sp. n. (Nenatoda: Filaroididae) from Mustelidae in Bulgaria. Helminthologia,1988, V. 25, 8l-88.
9. Kamenov I., Radev V., Zlateva N.On diagnosis and clinic of angiostrongylosis of cats. Experimental pathology and parasitoilogy, 1999,№2, 51-54.
10. Kramer M.H., GreerG.J., QuiñonezJ.F., PadillaN.R., HernándezB., AranaB.A., LorenzanaR., MoreraP., HightowerA.W., EberhardM.L., HerwaldtB.L.First reported outbreak of abdominal angiostrongyliasis.ClinicalInfectiousDiseases, 1998, V. 26 №2, 365-372.
11. Li, H., Xu F., Gu J.B, Chen X.G. A severe eosinophilicmeningoencephalitis caused by infection of *Angiostrongyluscantonensis*. The American journal of tropical medicine and hygiene,2008, V. 79, №4, 568–570.
12. Merdivenci A. A new lungworm, *Angiostrongylussciuri* n. sp. parasitising in the venae pulmonales of the squirrel, *Sciurus vulgaris*. Istanb. Univ. Fen. Fak. Meim.,1964, V. 29, 155–158.
13. Miterpakova M., Schnyder M., Schaper R., Hurnikova Z., Cabanova V.Serological survey for canine angiostrongylosis in Slovakia. Helminthologia, 2015, V. 52, № 3, 205–210.
14. Morgan E.R., Tomlinson A., Hunter S., Nichols T., Roberts E., Fox M.T., Taylor M.A.*Angiostrongylusvasorum* and *Eucoleusaerophilus* in foxes (*Vulpesvulpes*) in Great Britain. Vet.Parasitol., 2008, V.154, 48-57.
15. Panayotova-PenchevaM.,Alexandrov M. Morphometric characteristics of first stage *Elaphostrongyluscervi* (Nematoda: Protostrongylidae) larvae from Bulgaria. European Journal of Wildlife Research, 2008, V. 54, 771-774.
16. Pantchev N., Schnyder M., GlobokarVrhovec M.,Schaper R., Tsachev I.Currentsurveys of theseroprevalence of *Borreliaburgdorferi, Ehrlichiacanis, Anaplasmaphagocytophilum, Leishmaniainfantum, Babesiacanis, Angiostrongylusvasorum*and*Dirofilaria immitis*indogsinBulgaria. Parasitology Research, 2015, V. 114 (Supl.1), 117 –130.

17. Poli A., Arispici M., Marconcini A., Mancianti F., de Monte D.*Angiostrongylusvasorum*(Baillet, 1866) in red foxes (*Vulpesvulpes* L.) in Italy. J.Wildl.Dis., 1984, V. 20, 345-346.

18. Rebello K.M., Menna-BarretoR.F.S., Chagas-MoutinhoV.A., MotaE.M., PeralesJ., Neves-FerreiraA.G.C., Oliveira-MenezesA., LenziH.Morphological aspects of *Angiostrongyluscostaricensis* by light and scanning electron microscopy.ActaTropica, 2013, V. 127, № 3, 191–198.

19. Slom T.J., Cortese M.M., Gerber S.I., Jones R.C., Holtz T.H., Lopez A.S., Zambrano C.H., Sufit R.L., Sakolvaree Y., Chaicumpa W., Herwaldt B.L., Johnson S.An outbreak of eosinophilic meningitis caused by *Angiostrongyluscantonensis* in Travelers Returning from the Caribbean. N. Engl. J. Med., 2002,V. 346, 668-675.

20. Schnyder M., Schaper R., Pantchev N., Kowalska D., Szwedko A., Deplazes P.Serological detection of circulating *Angiostrongylusvasorum*antigenand parasite-specific antibodies in dogs from Poland. Parasitol. Res., 2013,V. 112, 109–117.

21. Spratt D.M.Species of *Angiostrongylus*(Nematoda: Metastrongyloidea) in wildlife:A review. International Journal for Parasitology: Parasites and Wildlife,2015,V. 4, №2, 178–189.

22. Torres J., Miquel J., Motje M.Helminth parasites of the eurasian badger (*Melesmeles* L.) in Spain: a biogeographic approach. Parasitol. Res., 2001, V. 87, 259-263.

23. Traversa D., Di Cesare A., Conboy G. Canine and feline cardiopulmonary parasitic nematodes in Europe: emerging and underestimated. Parasites and Vectors, 2010, 3:62.

24. Wessmann A., Lu D., Lamb C.R., Smyth B., Mantis P., Chandler K., Boag A., Cherubini G.B., Cappello R.Brain and spinal cord haemorrhages associated with *Angiostrongylusvasorum* infection in four dogs. The Veterinary Record, 2006, V. 158, №25, 858-863.