Veterinarski Glasnik 2023, 77(1), 1-15
UDC: 636.7.09:616.995.132
https://doi.org/10.2298/VETGL220307008J

Review

CLINICAL-PARASITOLOGICAL AND EPIDEMIOLOGICAL REVIEW OF THE NEMATODE *ACANTHOCHEILONEMA RECONDITUM*

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Received 07 March 2022; Accepted 23 May 2022
Published online: 27 July 2022

How to cite: Nemanja M. Jovanović, Darko Despotović, Predrag Stepanović, Milan Rajković, Tamara Ilić. Clinical-parasitological and epidemiological review of the nematode *Acanthocheilonema reconditum*. Veterinarski Glasnik, 2023. 77(1): 1-15. https://doi.org/10.2298/VETGL220307008J

Abstract

Acanthocheilonema (*Dipetalonema*) *reconditum* is a less pathogenic species of filaria from the superfamily Filarioidea, and which parasitizes in the subcutaneous connective tissue of dogs, hyenas and jackals. The results of epidemiological studies indicate the zoonotic potential of *A. reconditum*, bearing in mind it can cause infections with clinical disorders in humans. This filaria is spread globally and it is mostly described in geographical areas such as the Mediterranean Basin, the Middle East, South Africa, South America and Oceania, where it is the only or is the most common filaria that infects dogs. The prevalence and distribution of *A. reconditum* depend mainly on the vector population, impact of environmental factors, and lifestyle of pets and their owners. Apart from the standard parasitological techniques for differential diagnostics of filariae, more attention is being dedicated to the development of protocols that are based on the simultaneous detection of specific DNA regions in each type of individual filaria. Due to its importance for public health, effective vector control is required, as well as regular

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preventive examinations, reliable diagnostics and therapy for *A. reconditum* in dogs, and continuous cooperation between veterinary and medical surgeons.

Key Words: Acanthocheilonemosis, dog, diagnostics, geographical distribution, zoonosis

INTRODUCTION

Among parasitic filariae belonging to the superfamily Filarioidea, *Dirofilaria immitis*, *D. repens*, and *Acanthocheilonema* (*Dipetalonema*) *reconditum* are the most commonly diagnosed species in animals and humans in Europe (Eckert et al., 1992; Tasić et al., 2012; Brianti et al., 2012). *D. immitis* has the greatest pathological significance, causing cardiopulmonary dirofilariasis in dogs and wild canids, but also in less sensitive hosts – cats and ferrets − which leads to significant functional damage of the heart (Morchón et al., 2012). Because of their zoonotic potential, *D. immitis* and *D. repens* are the most researched parasites in dogs (Otranto et al., 2013).

A. reconditum is less pathogenic for dogs because its adult forms are localized in the subcutaneous tissue of the back, and less often in the subcutaneous tissue of the hind legs or the trunk (Nelson, 1962), as well as in perirenal (adipose) tissue (Grassi and Calandruccio, 1890; Korkejian and Edeson, 1978; Sonin, 1985). Although it is benign for dogs, this filaria is gaining in public health importance due to the numerous documented cases of human infections (Beaver et al., 1980; Stansfield, 1992; Orihel and Eberhard, 1998; Huynh et al., 2001; John et al., 2012).

In Serbia, several studies related to the presence of zoonotic nematodes in wild carnivores (Ilić et al., 2016a; 2016b; Hadži-Milić et al., 2016; Gajić et al., 2019), non-owner dogs (Ristić et al., 2020a; 2020b), shelters dogs (Ilić et al., 2021), and owned dogs and cats (Ilić et al., 2017; Ristić et al., 2020a; Stepanović et al., 2021) have been reported, while those related to carnivorous filariasis are of special importance (Tasić et al., 2008; Tasić et al., 2012; Penezić et al., 2014; Stepanović et al., 2015; Gavrilović et al., 2015; Kurucz et al., 2016).

In previous studies of dog filariasis in Serbia, not enough attention was paid to acanthocheilonemosis, which in combination with its zoonotic potential can have a major impact on differential diagnosis. Moreover, due to their morphological similarity, *A. reconditum* microfilaria can be easily confused with *Dirofilaria* microfilaria by inexperienced diagnosticians. This study suggests the need for additional research related to *A. reconditum*.

MORPHOLOGICAL CHARACTERISTICS OF *ACANTHOCHEILONEMA RECONDITUM*

A. reconditum belongs to the kingdom Animalia, phylum Nematoda, class Chromadorea, order Rhabditida, superfamily Filarioidea, family Onchocercidae and genus *Dipetalonema* (Chabaud and Anderson, 1959).

The head bulbous of the parasite is poorly expressed. The mouth is triangular, surrounded by four pairs of papillae arranged in two circles. The oesophagus is divided into muscular and glandular parts. The posterior end of the tail is usually twisted in a spiral dorsally and has three pairs of lateral preanal papillae (also called tubercles; one dorsal and two latero-ventral) and one pair of poorly visible, ventral subterminal lateral postanal papillae (nodules) (Brumpt, 1949; Sonin, 1975).

The length of males (Figure 1) is 9.3-17.1 mm with a maximum thickness of 92-100 μm. In many specimens, lateral odd papillae are visible and located between the second and third pair of papillae. The medial odd papilla is located in front of the anus (Flynn, 1973). The spicules are of unequal length. The left spicule is longer (220-300 μm) and consists of three parts: a thick cylindrical pedicle, a tortuous central part, and a long, narrow distal paddle. The right spicule is bent and has a length of 92-104 μm (Sonin, 1975; Kelly, 1977).

The female body (Figure 2) is 20.7-25.2 mm long and 146-168 μm thick. The distance from the top of the head to the vulva is 680-920 μm. The vulva is poorly visible while the anus is completely invisible. The tail length is 180-300 μm (Sonin, 1975). However, microfilariae located in peripheral blood are 213-270 μm long and 4.7-5.8 μm, and maximally up to 29 μm thick (Eckert et al., 1992). The tail is long, consisting of a terminal conical tubercle and two needle-shaped ventrolateral nodules, which is a characteristic of all species in this order (Simić and Petrović, 1962; Sonin, 1975).

THE LIFE CYCLE OF *ACANTHOCHEILONEMA RECONDITUM*

A. reconditum parasitizes in the subcutaneous connective tissue of dogs, hyenas, and jackals. Unlike other filariae that are transmitted to dogs by mosquitoes (e.g., *D. immitis* and *D. repens*) or ticks (e.g., *Cercopithifilaria* spp.), one part of *A. reconditum* development takes place in intermediate hosts, such as fleas (*Ctenocephalides canis*, *C. felis*, *Pulex iritans*, *P. simulans*, *Echidnophaga gallinae*) or lice (*Heterodoxus spiniger*, *Linognathus setosus*) (Newton and Wright, 1956; Nelson, 1962). Moreover, recent studies indicate a potential role of the brown dog tick (*Rhipicephalus sanguineus*) as a vector of this filaria species (Pennington and Phelps, 1969; Korkejian and Edeson, 1978; Cringoli et al., 2001).

At the moment when the dog is being stung/bitten by the infected intermediate host, inoculation of stage 3 infectious larvae occurs in the dog's subcutis, where (during several months) further development continues to the adult forms. After fertilization, adult females release live larvae (microfilariae) that end up in the blood circulation system. The next intermediate host becomes infected after it stings or bites the infected dog, allowing the infection to spread further. The prepatent period for this helminthiasis is 67-101 days (Brianti et al., 2012), and in exceptional circumstances, it can last as long as 795 days (Eckert et al., 1992). Outside temperature affects the rate of development of microfilariae in the flea, due to which it can take 6-15 days to form an infectious L3 stage larva (Hoseini et al., 2020).

Besides the usual localization in the subcutaneous tissue of dogs, a very low percentage of adult forms of *A. reconditum* can be found in the peritoneal cavity of dogs (Bowman, 2009). Adult forms of parasites cause clinical symptoms very rarely, but pruritus, alopecia, and dermal lesions can be found as a consequence of a very intense infection. Many of the symptoms attributed to this parasite solely could be the result of simultaneous parasitism with other pathogens (Hoseini et al., 2020).

GEOGRAPHICAL DISTRIBUTION OF DIPETALONEMOSIS IN DOGS

A. reconditum is globally spread, and in many geographical areas, such as the Mediterranean Basin, the Middle East, South Africa, South America and Oceania, it is the only (Mazzotti and Chabaud, 1962; Korkejian and Edeson, 1978; Minnaar and Krecek, 2001) or the most common filaria that affect dogs (Ortega-Moraet al. 1991; Alves et al., 1999; Cringoli et al., 2001; Reifur et al., 2004; Giannetto et al., 2007). At the end of the 1970s and the beginning of the 1980s, this filaria was diagnosed in 2% of wild dogs in central New York State (Todaro et al., 1977), and in 12% of coyotes in California (US) (Weinmann and Garcia, 1980). While examining the enzootic character of dirofilariasis and dipetalonemosis, *A. reconditum* microfilariae were found in 0.1% of dogs in Missouri (US) (Pratt et al., 1981), 6% of dogs in Virginia and North Carolina (US) (Falls and Platt, 1982), and 3.6% of greyhounds in New South Wales, Australia (Martin and Collins, 1985).

In Morocco, Pandey et al. (1987) found *A. dracunculoides* microfilariae in 10.5% of dogs older than 1 year. In the Netherlands Antilles, *A. reconditum* was diagnosed in 27.8% of dogs with no statistical difference in findings of this filaria between domestic and wild dogs (Saleh et al., 1988). Different prevalences of *A. reconditum* in dogs were proved in north-west Spain - 2.1% (Perez-Sanchez et al., 1989), in the east part of Tennessee, US - 5.37% (Patton and Faulkner, 1992), in the territory of Sydney, Australia - 3.7% (Bidgood and Collins, 1996), in northern Taiwan, China - 12.2% (Wang, 1997), in the vicinity of Barcelona, Spain - 3.7% (Aranda et al., 1998) and in Brazil - 6.9% (Alves et al., 1999).

A study of endoparasitosis in dogs at Oklahoma State University, US, conducted from 1981-1990, revealed a significant decline in the prevalence of infection caused by filariasis belonging to genus *Dipetalonema*, which decreased from 4% in 1981 to below 1% in 1991.

In Greece, *A. reconditum* was detected in 8% of dogs that had occasional symptoms of weakness and in 4% of healthy dogs (Papazahariadou et al., 1994). Three years later, Vakalis and Himonas (1997) found four species of this filaria (*D. immitis*, *D. repens*, *A. grassi* and *A. reconditum*) in 12-37% of dogs from this area.

In western Sicily, Giannetto et al. (1997) diagnosed *A. reconditum* in one dog (0.5%) and coinfection with *D. repens* and *A. reconditum* in five dogs (2.33%).

During 1993-1996, Zahler et al. (1997) reported the presence of filarial infections in dogs that were imported into Germany. They found 80 dogs had filarial infections, of which five dogs (originating from Italy, Greece, the Former Republic of Yugoslavia and Hungary) were infected with *D. repens* species, three dogs (originating from Spain) with *A. reconditum,* and one dog (originating from Corsica, France) was coinfected with *D. immitis*. Within the remaining 72 samples, 45 were infected with *D. immitis*, and in 27 samples, microfilariae that were not differentiated were detected, despite repeated examination of freshly collected blood samples. Zahler et al. (1997) point out the limitations of the acid phosphatase staining method in microfilaria diagnostics and the need for the introduction and application of more reliable diagnostic methods.

In a study conducted by Rishniw et al. (2006), in three out of five confirmed infections, based on diagnostics of morphological characteristics, additional confirmation by PCR was necessary, due to the risk of incorrect morphological diagnosis. Pantchev et al. (2011) used PCR for detection of the parasites in dogs imported into Germany during 2008-2010 and confirmed the presence of *A. reconditum* in 13 dogs originating from Spain, one dog from Hungary and one dog from Portugal. Recent studies of the occurrence of *A. reconditum* in the southern regions of Italy have shown that the prevalence of this parasite is as high as 13.3%, with an annual incidence rate of 5.9%, which was noted in naturally infected dogs (Napoli et al., 2014). During 2010-2011, *A. reconditum* was found in 2.05% of dogs from eight areas in Romania (Ionică et al., 2015). Diakou et al. (2016) examined the epizootiological situation of canine filariasis in the five largest urban centres in Greece. Using Knott's test, *A. reconditum* microfilariae were detected in 1.3% of animals. Serological examination for *A. reconditum* antibodies revealed seroprevalences ranged from 1-3% in the different municipalities.

Patel et al. (2018) confirmed the presence of microfilariae in 7.89% of dogs in India. The detection of *A. reconditum* microfilariae was more frequent (69.69%) than the detection of *D. immitis* (30.30%), with a total prevalence in the population of examined dogs of 5.50% and 2.39%, respectively. In the majority of cases, *A. reconditum* was registered in Pomeranian dogs (30.43%) or in adult dogs aged 4-14 years (39.39%). In Iran, which is an endemic region for dog heartworm disease, *A. reconditum* filaria was diagnosed with a prevalence of 9.55% in sheepdogs (Hoseini et al., 2020). The first systematic study of filariasis in dogs in Serbia was conducted by Tasić (2005), wherein dogs had a total filariasis prevalence of 47.90%, and the prevalence of *A. reconditum* was 1.68%. In following research, this filaria was proven in 2.1% of dogs from the same area (Tasić et al., 2008).

UP-TO-DATE DIAGNOSTIC APPROACHES IN THE DETECTION OF *ACANTHOCHEILONEMA RECONDITUM*

Problems in the diagnosis of different types of filariae that parasitize dogs can occur in cases of mixed infections or low level parasitaemia. Knott's test is used to detect microfilariae in the blood and analyse them morphometrically. It has proven to be a

very efficient, fast and inexpensive diagnostic method. When Knott's test is not used, morphological confusion can occur between the filariae *D. immitis*, *D. repens* and *A. reconditum*, as well as between two species of the genus *Acanthocheilonema* (*A. reconditum* and *A. dracunculoides*), which is why histochemical staining is recommended as a more reliable method of identification and determination (Magnis et al., 2013). Commercial serology test kits intended for antigen detection can produce cross-reactions between *D. immitis, D. repens* and *A. reconditum*. Accordingly, the use of such serological tests is not recommended in areas where these filariae are endemic, while molecular methods have a significant place in the diagnosis of *A. reconditum*. Considering the similarities of some microfilaria, the newer protocols for their detection are based on the simultaneous detection of specific DNA regions for each type of individual filaria. There are several described protocols available for the detection of *A. reconditum*. The most commonly used protocol was designed by Rishniw et al. (2006). In that study, pairs of primers for genotyping were designed to include the internal transcribed spacer region 2 (ITS2) of *D. immitis* and *A. reconditum* ribosomal DNA and for the 5S ribosomal DNA region of *D. repens*. Additionally, another primer pair (DIDR-F1 and DIDR-R1) was designed to amplify fragments of different lengths in the different filaria. In a study performed by Pantchev et al. (2011), three different PCR reactions were performed simultaneously. In the first and second reactions, primers confirmed the presence of *D. immitis* and *D. repens* DNA based on the research of Favia et al. (1996). If these first two reactions were negative, a third PCR test was performed using the protocols for genotyping and species identification following the research of Rishniw et al. (2006).

Recently, in a study conducted by Laidoudi et al. (2020), procedures and a triplex qPCR protocol were developed enabling rapid, specific and comparative diagnosis of *D. immitis*, *D. repens* and *A. reconditum*. In addition, this approach to molecular detection also enables the confirmation of the filaria symbiotes, bacteria of the genus *Wolbachia*. In this study, panfilarial primers and probe specific for the 28S rRNA (the large subunit ribosomal rRNA - LSU) gene, within the Pan-fil 28S qPCR protocol, were used to detect the presence of genomic DNA from most filaria species. In the case of a positive finding, the Triplex TaqMan cox1 qPCR protocol is further applied to confirm the filaria species. As a basis for the development of the Triplex TaqMan cox1 qPCR protocol, a gene encoding the formation of cytochrome c oxidase subunit 1 (cox1) was used as the target. The cox1 subunit is specific in each of three filaria species (*D. immitis, D. repens* and *A. reconditum*). In the case of negative findings with the Triplex TaqMan cox1 qPCR protocol, sequencing was performed to confirm the other filaria species. A similar principle was applied to confirm the presence of *Wolbachia* DNA using the All-Wol 16S protocol. In the case of a positive reaction, a further step is to confirm the presence of *Wolbachia*-specific for particular filariasis. Note that *Wolbachia* are not present in *A. reconditum* (Figure 1).

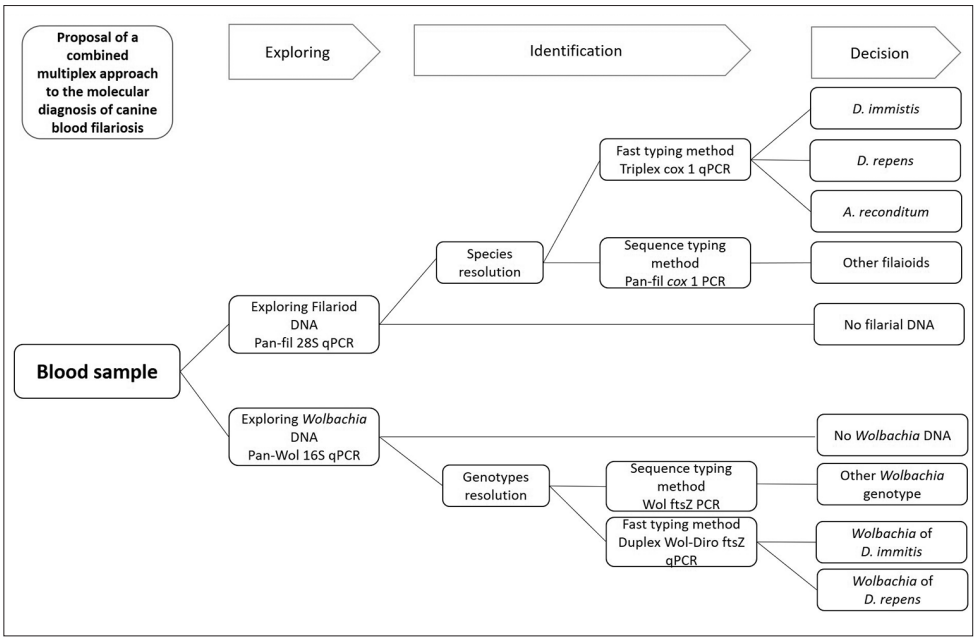

Figure 1. Diagnostic procedure for dog filariosis detection (Laidoudi et al., 2020)

SIGNIFICANCE OF CANINE DIPETALONEMOSIS FOR HUMAN MEDICINE

In the diagnosis of this parasitosis in humans, interference can often be produced by *A. perstans*, *A. streptocerca* and *Mansonella ozzardi*. These three species are often diagnosed in humans in Africa and South America. The species *A. perstans* and *A. streptocerca* have also been isolated from chimpanzees and gorillas in Africa. Adult forms of *A. perstans* parasitize in body cavities, while *A. perstans* and *M. ozzardi* microfilariae appear in the blood and *A. streptocerca* microfilariae appear in the skin. All of these species are considered nonpathogenic, and so they are not studied enough. However, studies suggest that although they do not cause more severe pathological lesions, they can still cause clinical disorders (Виноградова-Волжинского, 1977; Petrocheilou et al., 1998). One man from Oregon (US) had diagnosed Acanthocheilomatosis, and microfilariae were found in the anterior chamber of the eye (Beaver et al., 1980), as well as in a 62-year-old patient from Australia in whom microfilariae were found the subconjunctival space (Stansfield, 1992). John et al. (2012) report the finding of filariae of the genus *Dipetalonema* in a 74-year-old man from India, in whom severe irritation was observed in the area of the right eye, which increased gradually over the days. Detailed observation revealed localised conjunctival chemosis, erythema, pain and S-shaped subconjunctival oedema, about 2 cm long. Due to the noticeable mobility in the mentioned region, the presence of a nematode was suspected, and it was confirmed after the removal of the parasite. Body cavities and kidneys in humans are unusual sites for localization of this nematode, but microfilariae have been found in

infected people (Huynh et al., 2001). Treatment of this infection in humans is carried out with preparations based on ivermectin or diethylcarbamazine.

The prevalence and distribution of *A. reconditum* depend mainly on the vector population, the action of environmental factors, and the lifestyle of pets and their owners. There are reports of filariasis caused by *A. reconditum* in humans in Serbia and the former Yugoslavia (Kulišić et al., 1995).

Due to the greater clinical and pathological significance of *D. immitis* for dogs, their health controls are focused on this filariasis. However, due to the therapeutic protocol, it is necessary to differentiate this infection from *A. reconditum* infection, which is benign for dogs (Patel et al., 2018).

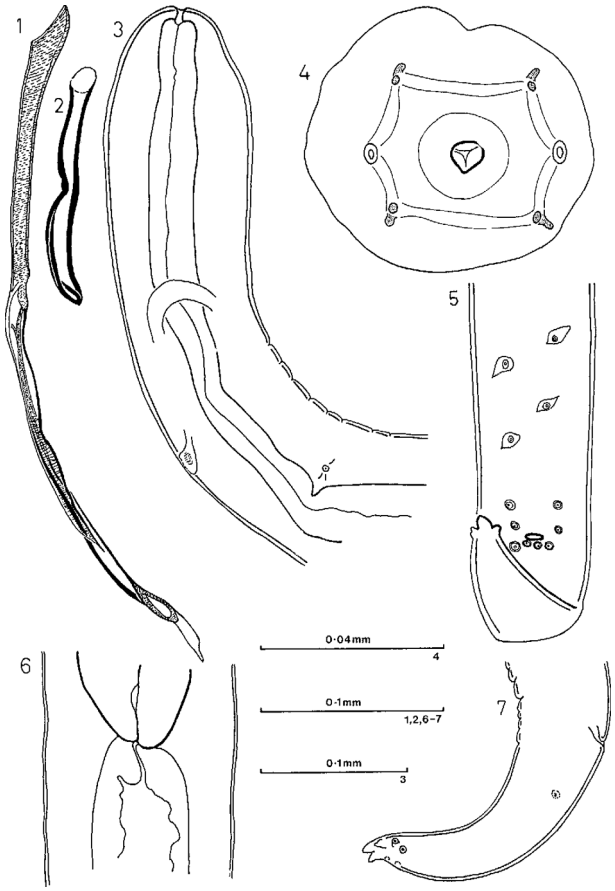

Figure 2. *Acanthocheilonema reconditum*, male. 1. Left spicule. 2. Right spicule. 3. Anterior end. 4. Face-on view. 5. Posterior end, ventral view. 6. Junction of oesophagus and intestine. 7. Tail lateral view (Gibbons and Jacobs 1991).

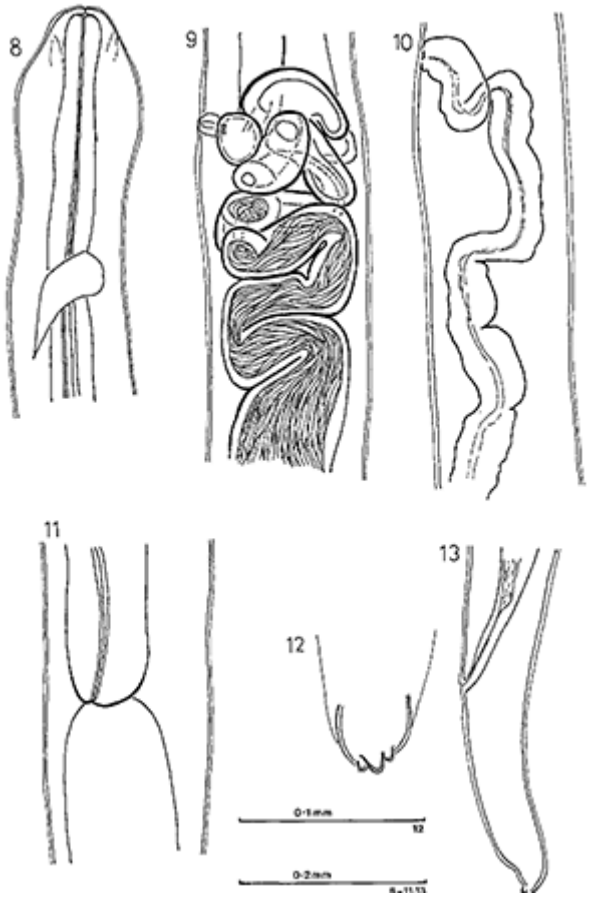

Figure 3. *Acanthocheilonema reconditum*, female. 8. Anterior end. 9. Vulvar region, mature worm. 10. Vulvar region, less mature worm. 11. Junction of oesophagus and intestine. 12. Distal end of tail, dorsal view. 13. Tail, lateral view. (Gibbons and Jacobs 1991).

CONCLUSION

Due to the proven zoonotic potential of both *Dirofilaria* spp. and *A. reconditum*, regular preventive examinations of dogs for the presence of microfilariae of these nematodes are of special importance. Having in mind the potential of these parasites to infect humans, it is necessary to raise the awareness of dog owners, veterinarians and physicians, to conduct reliable diagnostics of filariasis in dogs, and to instigate therapeutic and preventive treatments in companion animals and effective control measures against vectors.

Acknowledgements

The study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (contract no. 451-03-9/2021-14/200143).

Authors' contributions

All authors (JMN, DD, PS, RM, and IT) contributed to manuscript design, performed literature searches, wrote and revised the article, and approved the final manuscript

Competing interests

The authors declare that they have no competing interests

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KLINIČKO-PARAZITOLOŠKI I EPIDEMIOLOŠKI OSVRT NA NEMATODU *ACANTHOCHEILONEMA RECONDITUM*

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Kratak sardžaj

Acanthocheilonema (*Dipetalonema*) *reconditum* je manje patogena vrsta filarije iz superfamilije Filarioidea, koja parazitira u potkožnom vezivnom tkivu pasa, hijena i šakala. Rezultati epidemioloških istraživanja ukazuju na njen zoonozni potencijal, jer može izazvati infekcije sa kliničkim poremećajima i kod ljudi. Ova filarija je globalno rasprostranjena, a najviše je opisana u geografskim oblastima kao što su Mediteranski Basen, Bliski Istok, Južna Afrika, Južna Amerika i Okeanija, gde može biti jedina i najzastupljenija filarija koja inficira pse. Obrazac prevalencije i rasprostranjenosti *A. reconditum* zavisi uglavnom od populacije vektora, delovanja faktora sredine, načina života kućnih ljubimaca i njihovih vlasnika. U dijagnostici, pored standardnih parazitoloških tehnika diferencijalne dijagnostike za determinisanje filarija, sve više pažnje se posvećuje i razvojima protokola za njihovo dokazivanje, koji se baziraju na istovremenoj detekciji posebnih regiona DNK za svaku vrstu filarije pojedinačno. Zbog značaja za javno zdravlje važno je sprovođenje efikasne kontrole vektora, redovnih preventivnih pregleda, pouzdane dijagnostike i terapije *A. reconditum* kod pasa, kao i kontiniurana saradnja svih segmenata veterinarske i medicinske struke.

Ključne reči: Akantoheilonemoza, dijagnostika, pas, geografska distribucija, zoonoza