The prevalence of Dirofilaria immitis and D. repens in the Old World

The prevalence of *Dirofilaria immitis* and *D. repens* in the Old World

Claudio Genchi[1*], Laura Helen Kramer[2]

[1]Department of Veterinary Medicine, Università degli Studi di Milano, Italy; [2]Department Veterinary Medical Sciences, Parma, Italy

* Corresponding author: Claudio Genchi claudio.genchi@unimi.it

Abstract

Dirofilaria immitis and *D. repens* are endemic throughout Europe and southern eastern regions of Asia and reported with increasing frequency in Africa. Nevertheless, the increased awareness of veterinary practitioners, even in countries where the prevalence is low, has led to a decrease *D. immitis* prevalence in dogs, especially in previously endemic/hyper-endemic areas. Prevalence has significantly increased, however, in areas where heartworm has apparently spread more recently, such as Central and North Eastern Europe. Furthermore, autochthonous cases have been observed in Siberia. Low seroprevalence has been reported in Croatia, while in Romania it has reached 14%. In Greece, the prevalence ranges 0.7%-25% and in Turkey 0-18%. Data for canine dirofilariosis in Africa is scarce, and most are case reports. Overall, the dominant species is *Achanthocheilonema dracunculoides*, although both *D. immitis* and *D. repens* have been reported from some country. In the Far East, the prevalence ranges from 2%-15% in northeastern of China. In Hong Kong a novel species has been found in dogs and humans (*Candidatus* Dirofilaria hongkongensis). In India, the prevalence ranges from 4.7%-29.5% in Northeastern states.

The main factors that have influenced the spreading of *Dirofilaria* infections are the climate changes and the introduction of new, invasive, competent mosquito species such as *Aedes albopictus* and *Ae. koreicus.* Other factors include relocation and insufficient prevention in dogs, manly in the new areas of colonization.

Feline heartworm infection has been diagnosed in every European country when diagnosed either by the Knott test or by serology for circulating antibodies and antigens of the parasite. However, prevalence is much lower than in dogs.

In spite of the continuing spreading of heartworm infection, *D. repens* is the main concern in Europe, mainly for physicians, while the infection is nearly always asyptomatic in dogs. The infection is spreading from Portugal to the Southeastern regions of Finland and Siberia, and in some areas its prevalence overlaps that of *D. immitis*. Many reasons make more difficult the control of *D. repens* than *D*. *immitis*: further the frequent lack of clinical symptoms of suspicion, the specific diagnosis being possible only by blood examination and the inefficacy of some macrocyclic lactones.

Key Words: *Dirofilaria immitis*, *Dirofilaria repens*, prevalence, Old World

Europe and Russia

Dirofilaria immitis (canine and feline heartworm disease) and *D. repens* (subcutaneous infections) (Spirurida, Onchocercidae) are endemic throughout European countries and in the southern eastern regions of Asia, and reported with increasing frequency in Africa (McCall et al., 2008). Nonetheless, the increased awareness of veterinary practitioners and owners even in countries where the infection prevalence is low or the heartworm (HW) cases are sporadic (such as Germany, Netherlands, France) (Genchi et al., 2014) has decreased *D. immitis* prevalence mostly in the past endemic, hyper-endemic areas. For instance, in Northern Italy the prevalence decreased in three decades from >40% in dogs

living in lower Po River course (Guerrero et al., 1989) to about 8% in owned dogs not treated with preventive drugs (Genchi et al., 2009), and most veterinary practitioners currently surveyed have reported diagnosing no more than 5-20 yearly clinical cases of canine HW and *D*. *repens* infections in previous hyperendemic areas (Genchi et al., 2019). In the Canary Islands (Spain) the decrease was from 30% to 19% (Montoya-Alonso et al., 2010) and Japan from 46% in 1999-2001 to 23% in 2009-2011 (Oi et al, 2014).

In France data from a nationwide serological survey of *D. immitis* and tick borne infections carried out in 2009 (Panchev et al., 2009) showed a prevalence of 0.22% in healthy dogs and 6.87% in dogs with suspected infection. The global annual prevalence (including Corsica Island) of *D. repens* is 0.02%-0.12% (Capelli et al., 2018). In Spain, (Madrid area) the prevalence of canine and feline HW infection is 3% and 0.2%, respectively (Montoya-Alonso et al., 2017). In western regions the mean prevalence is 5.8% (Diosdado et al., 2018), and 2.8% in the southeast of the country (Catalonia and Barcelona area; Montoya-Alonso et al., 2015). In continental Portugal, canine HW infection is endemic and ranges between 4% in apparently healthy dogs and 9% in clinically suspected dogs (Alho et al., 2018). Furthermore, wild carnivores have been identified as a possible reservoir (prevalence 3%-12% in foxes by necropsy) (Alho et al., 2018). The highest prevalence of canine HW has been observed on Madeira Island (40%).

Data from a large serological survey (>80,000 serum samples) carried out in Germany has shown 1.4% of positive results for *D. immitis*, but all the dogs were from endemic countries such as Spain, Portugal and Greece (Vrhovec et al., 2017). *D. immitis* appears not to be endemic in Austria, probably because most dogs are kept indoors, but with regard to the data from neighboring countries (particularly Hungary, where the prevalence increase from 0.7% in the years 2006-2010, to 11.3% in 2015; Bacsadi et al., 2016), it will probably become established in the near future (Fueher et al, 2016). Low prevalence has been found by serology in Croatia in apparently healthy dogs (0.4%) (Jurković et al., 2019). In

78 Greece, the prevalence of canine HW infection ranges 0.7%-25%, with the higher values
79 in northern areas, where a high prevalence was recently found also in cats (9.4%)
80 (Angelou et al., 2019; Diaku et al, 2019).
81 In Romania, prevalences of 3.6%-14%, depending from the surveyed area, were found in
82 dogs (Mircean et al., 2012) and 18.5% in golden jackals (Ionică et al, 2016), although
83 prevalence as high as 42% was found in stray dogs in Southeast part of the country (Ciucâ
84 et al, 2016). In Poland, *D. immitis* prevalence is very low (< 1%) (Krämer et al., 2014)
85 while *D. repens* prevalence is 12% (Demiaszkiewicz et al, 2014). In Turkey, overall *D.*
86 *immitis* prevalence rages 0-18% (Köse and Erdoğan, 2012).
87 Regarding those countries where the parasite has more recently been observed, such as
88 the Eastern European countries, in some cases the prevalence has apparently significantly
89 increased in a short time. It is difficult to clarify if the parasite was present previously in the
90 dog population but not properly diagnosed or if the prevalence has actually increased. In
91 fact, it is also possible that the increasingly frequent cases of human infections (see as an
92 example Sałamentin et al., 2013; Moskvina and Ermolenko, 2018) have prompted
93 practioners to carry out several surveys in dogs. However, in Central Europe (Slovakia), *D.*
94 *immitis* prevalence has increased up to 64% (Miterpáková et al, 2018) in some South
95 Western areas, and in some regions of Bulgaria from 15% in 2013-2014 (Radev, et al.,
96 2016) to 34% (Iliev et al., 2017). In Russia, HW prevalence ranges 3.6% (Moscow region)
97 to 36%-55% in the southern (Rostov region15%) and central areas of the country (near the
98 course of large rivers such as the Volga and Amur) and 43.6% in far eastern regions, not
99 far from Vladivostok (Kartashev et al., 2011; Tumolskaya et al., 2016). Furthermore, the
100 prevalence of HW infection in wild carnivores examined by necropsy from the
101 Southwestern areas of the country was 20% for *D. immitis* in foxes; jackals were found
102 infected both by *D. immitis* and *D. repens* (31% and 10%, respectively), 10% of badgers
103 were found infected by *D. repens* only and 31% of raccoon dogs by *D. immitis* only

(Kravchenko et al., 2016). Autochthonous cases of canine *D. immitis* and D. *repens* infections have been observed in Siberia (Yakutsk, 62°02′N 129°44′E). In spite of extremely cold winters (until -36 °C), the parasite has homoeothermic conditions within the host. During the summers, when transmission potentially occurs, the mean temperature in Yakutsk is 18.7 °C (July 1961– 1990) (Pietikäinen et al., 2017). Therefore, the 130 *Dirofilaria* Development Units degree-days above 14 °C proposed to be required for the extrinsic development into the infective stage in mosquitoes, can likely be reached within the mosquitos' lifespan. In Ukraine, although data from dogs is scanty, an isolate of *D. immitis* from human ocular cases has shown molecular characteristic different from *D. immitis* reference stains, including those from dogs in the same area (Rossi et al, 2015).

One of the main factors that has influenced the spreading of *Dirofilaria* infection is the changing climate that has caused an increase in abundance of mosquito populations, has shortened the extrinsic development of infective stages and has lengthened the transmission season. Other critical factors are the introduction of the Pet Travel Scheme in 2000, which has allowed easier movement of companion animals throughout the European Union (Trotz-Williams and Trees 2003), the introduction of new, invasive, competent mosquitoes species such as *Aedes albopictus* and *Ae. koreicus* (Montasi et al., 2015), the presence of stray dogs with high prevalence of HW infection (e.g. 40% prevalence in Sofia, Bulgaria; Metralova et al., 2018), and an insufficient prevention in dogs, manly in the new areas of colonization.

It should be mentioned that there is no nationwide survey activity in Europe able to give a complete picture of HW prevalence, such as for North America (AHS, CAPC; Bowman et al., 2016), although forecasting maps have been published for *Dirofilaria* infections (Genchi et al., 2005; Genchi et al., 2009). So, the above data are from surveys carried out with different scopes (e.g. assessing prevalence in different age groups, sex or breed), in

different areas of a country and with different diagnostic methods (antigen serology, blood examination or molecular methods).

Asia

In the Far East, the reports of heartworm infections are scarce. In China, the prevalence ranges 2%-15% in northeastern areas of the country, with an increasing trend from 2009 to 2012 (13%-15%) (Liu et al., 2013). In Hong Kong and India a novel species of *Dirofilaria* has been also found in dogs and humans (*Candidatus* Dirofilaria hongkongenis) (Nazar et al., 2017; Yilmiz et al., 2019). Interestingly, the sera from dogs infected by this novel *Dirofilaria* species were negative when tested with the SNAP 4Dx *D. immitis* detection kit, except in the case of dogs with a mixed infection with *D. immitis* (To et al., 2012). In South Korea, the HW prevalence assessed by serology, was 17% in spite that 79% of examined dogs were in preventive medication, probably as a consequence of too short seasonal treatment (Suh et al., 2017)

In India, the prevalence ranges 4.7%-29.5% in Northeastern states (Borthakur et al., 2015) and in Thailand the prevalence revealed by molecular methods was 18% in dogs (Boonyapakorn et al., 2008) and 4.6% in cats (Sukhumavasi et al., 2012). In Sri Lanka no *D. immitis* was found but *D. repens* prevalence reaches 69% in dogs and 48% in cats (Mallawarachchi et al., 2018).

Africa

Information of the occurrence of canine dirofilariosis in sub-Saharan Africa is scarce, and most are case reports. Overall, the dominant species is *Achanthocheilonema dracunculoides* (Albtectova et al., 2011; Siwilla et al., 2015), although both *D. immitis* and *D. repens* have been reported from Tunisia (Rjeibi et al., 2017), Algeria (Tahir et al., 2017), Tanzania (Mukendi et al., 2016) and Mozambique (Schwan and Durand, 2000) with

prevalence ranging 1.4%-14.5%. In Madagascar *D. immitis* DNA and microfilariae were found in wild lemurs, although no data is available for dogs (Zohdy et al., 2019).

Feline heartworm infection

Feline HW infection has been observed in every European country endemic for canine infection when diagnosed either by Knott test or serology for circulating antibodies or antigens of the parasite. However, its prevalence is a much lower than in dogs.It has been reported that *D. immitis* prevalence in cats ranges from 9%-18% to that of dogs (Venco et al., 2011). In endemic areas of canine HW, such as Northern Italy, the prevalence assessed by echography was 6.7%, but the number of prepatent infections (aborted infections) shows higher prevalence (9.5%-18.5%) confirming the infection risk for cats (Venco et al., 2008). Recently, 9.4% of cats in Greece have been found infected in areas endemic for canine infection (Diaku et al, 2019).

In Northeastern China a significant difference of prevalence was observed between feral (8.4%) and domestic cats (1.4%) by serological methods (Hou et al., 2017). The prevalence was higher in older cats and in free-ranging animals ((Hou et al., 2017).

Conclusion

The current canine HW data shows a decreasing or stable situation in Western Europe, including previously hyperendemic areas. The prevalence is instead significantly increasing in Eastern countries and in the Asian areas of Russia. In fact, while the practitioners' and owners' awareness of the disease and the compliance for preventative treatment has decreased the risk in the Western countries, several factors have influenced the increase in Eastern countries. Along with the changing climate (Genchi et al., 2009), factors that that have facilitated the spreading of the infection include the underestimation of the risk (lack of educational campaigns), misdiagnosis (not always carried out according

to the current recommendations of international guidelines even in endemic areas Genchi et al.,), lack of prevention either because no drugs are available or for economic reasons, and the high number of untreated stray dogs and wild hosts (Otranto et al., 2015), mainly golden jackals (as an example Kravchenko et al., 2016; Ionică et al., 2016). The role of these alternative hosts as active reservoirs is, however, still questionable because data for the presence of microfilaremia are currently lacking.

Regarding Asia (excluding Japan) and Africa, the lack in many cases of historical data does not allow any considerations about the trend of the infection. However, it must be noted that when HW infection is properly looked for, it is found in dogs and cats everywhere.

In spite of the continuing spreading of the HW infection, also as a consequence of the movement of untreated and rescue dogs, *D. repens* is the main concern in Europe, mainly for physicians. In fact, the infection is frequently asyptomatic in dogs, and only a few reports of clinically manifest diseasehave been reported (Genchi and Kramer, 2017). Currently, *D. repens* is showing a faster and more intense spreading through Europe (Tasić-Otašević et al., 2015; Genchi and Kramer, 2017; Capelli et al., 2018). The infection has been diagnosed from Portugal to Southeastern regions of Finland, Northern of Russia and Estonia (Jokelainen et al., 2016; Pietikäinen et al, 2017) and in some areas its prevalence exceeds that of *D. immitis*, with an increasing number of human infections (Genchi and Kramer, 2017) and incidence values reaching 9.79 cases for 100,000 inhabitants (Sałamentin et al., 2013). Many reasons make more difficult an effective the control of *D. repens* than *D*. *immitis*: the frequent lack of clinical symptoms of suspicion, the species-specific diagnosis possible only by identification of circulating microfilariae, the difficulties in the control and the limited options for adulticide treatment (only moxidectin has shown a complete efficacy, Petry et al., 2015). To note that both parasites have the same mosquito species as intermediate hosts and vectors and, in experimental studies,

similar needs of temperature and time of extrinsic incubation to infective stage have been shown (Genchi and Kramer, 2017; Capelli et al., 2017).

In conclusion, both *D. immitis* and *D. repens* are endemic in the Old World. Although cases both of severe canine HW infection and zoonotic *D. immitis* infection are still reported (Simón et al., 2012), the spreading of *D. repens* and the recent observation of new or cryptic species (*Candidatus Dirofilaria* hongkongensis and *Dirofilaria* sp. "Thailand II" (Yilmaz et al., 2019), both zoonotic, is of increasing concern.

Declaration of interest

None

References

Albrechtová, K., Sedlák, K., Petrželková, K.J., Hlaváč, J., Mihalca, A.D., Lesingirian, A., Kanyari, P.W., Modrý, D., 2011. Occurrence of filaria in domestic dogs of Samburu pastoralists in Northern Kenya and its associations with canine distemper. Vet. Parasitol. 182, 230-238. doi: 10.1016/j.vetpar.2011.05.042..

Alho, A.M., Meireles, J., Schnyder, M., Cardoso, L., Belo, S., Deplazes, P., de Carvalho, L.M., Dirofilaria immitis and Angiostrongylus vasorum: The current situation of two major canine heartworms in Portugal, 2018. Vet. Parasitol. 252, 120-126. doi: 10.1016/j.vetpar.2018.01.008.

American Heartworm Society https://www.heartwormsociety.org

Angelou, A., Gelasakis, A.I., Verde, N., Pantchev, N., Schaper, R., Chandrashekar, R., Papadopoulos, E., 2019. Prevalence and risk factors for selected canine vector-borne diseases in Greece. Parasit Vectors. 12(1): 283. doi: 10.1186/s13071-019-3543-3.

Bacsadi, Á., Papp, A., Szeredi, L., Tóth, G., Nemes, C., Imre, V., Tolnai, Z., Széll, Z., Sréter, T., 2016. Retrospective study on the distribution of *Dirofilaria immitis* in dogs in Hungary. 220, 83-6. doi: 10.1016/j.vetpar.2016.03.001.

Boonyapakorn, C., Srikitjakarn, L., Morakote, N., Hoerchner, F., 2008. The epidemiology of *Dirofilaria immitis* infection in outpatient dogs at Chiang Mai University Small Animal Hospital, Thailand. Southeast Asian J. Trop. Med. Public Health. 39, 33-38.

Borthakur, S.K., Deka, D.K., Islam, S., Sarma, D.K., Sarmah, P.C., 2015. Prevalence and molecular epidemiological data on *Dirofilaria immitis* in dogs from Northeastern States of India. Scient. World J. 2015:265385. doi: 10.1155/2015/265385.

Bowman, D.D., Liu, Y., McMahan, C.S., Nordone, S.K., Yabsley, M.J., Lund, R.B., 2016. Forecasting United States heartworm *Dirofilaria immitis* prevalence in dogs. Parasit Vectors. 9(1):540 DOI: 10.1186/s13071-016-1804-y.

CAPC/Heartworm, Companion Animal Parasite Council
https://capcvet.org/guidelines/heartworm/

Capelli, G., Genchi, C., Baneth, G., Bourdeau, P., Brianti, E., Cardoso, L., Danesi, P., Fuehrer, H.P., Giannelli, A., Ionică, A.M., Maia, C., Modrý, D., Montarsi, F., Krücken, J., Papadopoulos, E., Petrić, D., Pfeffer, M., Savić, S., Otranto, D., Poppert, S., Silaghi, C., 2018. Recent advances on *Dirofilaria repens* in dogs and humans in Europe. Parasit Vectors. 19;11:663. doi: 10.1186/s13071-018-3205-x.

Ciucă, L., Musella, V., Miron, L.D., Maurelli, M.P., Cringoli, G., Bosco, A., Rinaldi, L., 2016. Geographic distribution of canine heartworm (*Dirofilaria immitis*) infection in stray dogs of eastern Romania. Geospat. Health. 11, 499. doi: 10.4081/gh.2016.499.

Demiaszkiewicz, A.W., Polańczyk, G., Osińska, B., Pyziel, A.M., Kuligowska, I., Lachowicz, J,, Sikorski, A., 2014. Prevalence and distribution of *Dirofilaria repens* Railliet et Henry, 1911 in dogs in Poland. Pol. J. Vet. Sci. 17, 515-517.

Diakou, A., Soubasis, N., Chochlios, T., Oikonomidis, I.L., Tselekis, D., Koutinas, C., Karaiosif, R., Psaralexi, E., Tsouloufi, T.K., Brellou, G., Kritsepi-Konstantinou, M., Rallis, T. 2019. Canine and feline dirofilariosis in a highly enzootic area: first report of feline dirofilariosis in Greece. Parasitol. Res. 118, 677-682. doi: 10.1007/s00436-018-6135-9.

Diosdado, A., Gómez, P.J., González-Miguel, J., Simón, F., Morchón, R., 2018. Current status of canine dirofilariosis in an endemic area of western Spain. J. Helminthol. 92, 520-523. doi: 10.1017/S0022149X17000591.

Fuehrer, H.P., Auer, H., Leschnik, M., Silbermayr, K., Duscher, G., Joachim, A., 2016. *Dirofilaria* in humans, dogs, and vectors in Austria (1978-2014)-from imported pathogens to the endemicity of *Dirofilaria repens*. PLoS Negl. Trop. Dis. 10(5):e0004547. doi: 10.1371/journal.pntd.0004547.

Genchi, C., Bowman, D., Drake, J., 2014. Canine heartworm disease (*Dirofilaria immitis*) in Western Europe: survey of veterinary awareness and perceptions. Parasit. Vectors 7:206. doi: 10.1186/1756-3305-7-206.

Genchi, C., Kramer, L., 2017. Subcutaneous dirofilariosis (*Dirofilaria repens*): an infection spreading throughout the old world. Parasit. Vectors. 10(Suppl 2):517. doi: 10.1186/s13071-017-2434-8.

Genchi, C., Rinaldi, L., Cascone, C., Mortarino, M., Cringoli, G., 2005. Is heartworm disease really spreading in Europe? Vet. Parasitol. 133, 137-148. doi: 10.1016/j.vetpar.2005.04.009

Genchi, C., Rinaldi, L., Mortarino, M., Genchi, M., Cringoli, G., 2009. Climate and *Dirofilaria* infection in Europe. Vet. Parasitol. 163, 286-292. doi: 10.1016/j.vetpar.2009.03.026

Genchi, M., Rinaldi, L., Venco, L., Cringoli, G., Vismarra, A., Kramer, L., 2019. *Dirofilaria immitis* and *D. repens* in dog and cat: A questionnaire study in Italy. Vet. Parasitol. 267, 26-31. doi: 10.1016/j.vetpar.2019.01.014.

Guerrero, J., Genchi, C., Vezzoni, A., Ducos de Lahitte, J., Bussieras, J., Rojo, F.A., Ortega, L.M., Rodenas, A., Bulman, G.M., Lorson, M.H., Labarthe, N.V., Charles, T., Bordin, E.L., 1989. Distribution of *Dirofilaria immitis* in selected areas of Europe and South America. In: Otto, G.F. (Ed.), Proceedings of the Heartworm Symposium '89, American Heartworm Society, Washington DC, 13-18.

Hou, H., Cao, L., Ren, W., Wang, D., Ding, H., You, J., Yao, X., Dong, H., Guo, Y., Yuan, S., Zhang, X., Gong, P, 2017. Seroprevalence of *Dirofilaria immitis* in cats from Liaoning Province, Northeastern China. Korean J. Parasitol. 55, 673-677. doi: 10.3347/kjp.2017.55.6.673.

Iliev, P., Kirkova, Z., Ivanov, A., Kalkanov, I., 2017. Retrospective analysis on helminthic and protozoan infections in dogs and cats in Bulgaria. Bulg. J. Vet. Med. 20, 389-393.

Ionică, A.M., Matei, I.A., D'Amico, G., Daskalaki, A.A., Juránková, J., Ionescu, D.T., Mihalca, A.D., Modrý, D., Gherman, C.M., 2016. Role of golden jackals (*Canis aureus*) as natural reservoirs of *Dirofilaria* spp. in Romania. Parasit. Vectors 9:240. doi: 10.1186/s13071-016-1524-3.

Jokelainen, P., Mõtsküla, P.F., Heikkinen, P., Ülevaino, E., Oksanen, A., Lassen, B., 2014. *Dirofilaria repens* microfilaremia in three dogs in Estonia. Vector Borne Zoonotic Dis. 16, 136-138. doi: 10.1089/vbz.2015.1833.

Jurković, D., Beck, A., Huber, D., Mihaljević, Ž., Polkinghorne, A., Martinković, F., Lukačević, D., Pilat, M., Brezak, R., Bosnić, S., Beck, R., 2019. Seroprevalence of vector-borne pathogens in dogs from Croatia. Parasitol. Res. 118, 347-352. doi: 10.1007/s00436-018-6129-7.

Kartashev, V., Batashova, I., Kartashov, S., Ermakov, A., Mironova, A., Kuleshova, Y., Ilyasov, B., Kolodiy, I., Klyuchnikov, A., Ryabikina, E., Babicheva, M., Levchenko, Y., Pavlova, R., Pantchev, N., Morchón, R., Simón, F., 2011. Canine and human dirofilariosis in the rostov region (southern Russia). Vet. Med. Int., 2011:685713. doi: 10.4061/2011/685713.

Köse, M., Erdoğan, M. 2012. Serological screening of canine heartworm (*Dirofilaria immitis*) infections in Turkey. Berl. Munch. Tierarztl. Wochenschr. 125, 503-508.

Krämer, F., Schaper, R., Schunack, B., Połozowski, A., Piekarska, J., Szwedko, A., Jodies, R., Kowalska, D., Schüpbach, D., Pantchev, N., 2014. Serological detection of *Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu lato and *Ehrlichia canis* antibodies and *Dirofilaria immitis* antigen in a countrywide survey in dogs in Poland. Parasitol. Res. 113, 3229-3239. doi: 10.1007/s00436-014-3985-7.

Kravchenko, V., Itin, G., Kartashev, V., Ermakov, A., Kartashov, S., Diosdado, A., González-Miguel, J., Simón, F., 2016. *Dirofilaria immitis* and *D. repens* in sylvatic reservoirs of Krasnodar Krai (Russian Federation). Vet. Parasitol. Reg. Stud. Reports 6,

35-38. doi: 10.1016/j.vprsr.2016.08.004.

Liu, C., Yang, N., He, J., Yang, M., Sun, M., 2013. Prevalence of *Dirofilaria immitis* in dogs in Shenyang, Northeastern China. Korean J. Parasitol. 51, 375-377. doi: 10.3347/kjp.2013.51.3.375.

Mallawarachchi, C.H., Chandrasena, N.T.G.A., Wickramasinghe, S., Premaratna, R., Gunawardane, N.Y.I.S., Mallawarachchi, N.S.M.S.M., de Silva, N.R., 2018. A preliminary survey of filarial parasites in dogs and cats in Sri Lanka. PLoS One. 13(11):e0206633. doi: 10.1371/journal.pone.0206633.

McCall, J.W., Genchi, C., Kramer, L.H., Guerrero, J., Venco, L., 2008. Heartworm disease in animals and humans. Adv. Parasitol. 66,193-285. doi: 10.1016/S0065-308X(08)00204-2.

Metlarova, H., Carretón, E., Falcón-Cordón, Y., Falcón-Cordón, S., Montoya-Alonso, J. A., 2018. Current prevalence of heartworm in stray dogs from Sofia (Bulgaria). Parasit. Vectors P12,11(Suppl 1), 623. doi: 10.1186/s13071-018-3183-z.

Mircean, V., Dumitrache, M.O., Györke, A., Pantchev, N., Jodies, R., Mihalca, A.D., Cozma, V., 2012. Seroprevalence and geographic distribution of *Dirofilaria immitis* and tick-borne infections (*Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu lato, and Ehrlichia canis) in dogs from Romania. Vector Borne Zoonotic. Dis. 12, 595-604. doi: 10.1089/vbz.2011.0915.

Miterpáková, M., Valentová, D., Čabanová, V., Berešíková Ľ., 2018. Heartworm on the rise-new insights into *Dirofilaria immitis* epiidemiology. Parasitol. Res., 117, 2347-2350. doi: 10.1007/s00436-018-5912-9.

Montarsi, F., Ciocchetta, S., Devine, G., Ravagnan, S., Mutinelli, F., Frangipane di Regalbono, A., Otranto, D., Capelli, G., 2015. Development of *Dirofilaria immitis* within the mosquito *Aedes* (*Finlaya*) *koreicus*, a new invasive species for Europe. Parasit. Vectors, 8:177. doi: 10.1186/s13071-015-0800-y.

Montoya-Alonso, J.A., Carretón, E., Juste, M.C., Mellado, I., Morchón, R., Simón. F., 2010. Epidemiological survey of canine heartworm disease on the island of Gran Canaria (Canary Islands - Spain) between 2000 and 2008. Vet. Parasitol. 173, 165-168. doi: 10.1016/j.vetpar.2010.06.008.

Montoya-Alonso, J.A., Carretón, E., Simón, L., González-Miguel, J., García-Guasch, L., Morchón, R,, Simón, F, 2015. Prevalence of *Dirofilaria immitis* in dogs from Barcelona: Validation of a geospatial prediction model. Vet. Parasitol. 212, 456-459. doi: 10.1016/j.vetpar.2015.06.025.

Montoya-Alonso, J.A., Morchón, R., Falcón-Cordón, Y, Falcón-Cordón, S., Simón, F., Carretón, E., 2017. Prevalence of heartworm in dogs and cats of Madrid, Spain. Parasit. Vectors 10(1):354. doi: 10.1186/s13071-017-2299-x.

Moskvina, T.V., Ermolenko, A.V., 2018. Dirofilariasis in Russian Federation. A big problem with large distribution. Russian Open Med. J., 7, CID e0102. doi:10.15275/rusomj.2018.0102.

Mukendi, J.P., Kimbita, E., Mbanzulu, K.M., Maindo, P.P., Misinzo, G., 2016. Morphological and molecular detection of canine dirofilarial species of veterinary and medical importance in Morogoro municipality, Tanzania. Vet. Parasitol. 220, 1-3. doi: 10.1016/j.vetpar.2016.02.005.

Nazar, N., Lakshmanan, B., Jayavardhanan, K.K., 2017. Molecular characterization of human *Dirofilaria* isolates from Kerala. Indian J. Med. Res. 146, 528-533. doi: 10.4103/ijmr.IJMR_1425_15.

Oi, M., Yoshikawa, S., Ichikawa, Y., Nakagaki, K., Matsumoto, J., Nogami, S, 2014. Prevalence of *Dirofilaria immitis* among shelter dogs in Tokyo, Japan, after a decade: comparison of 1999-2001 and 2009-2011. Parasite 2014, 21-10. doi:10.1051/parasite/2014008.

Otranto, D., Cantacessi, C., Dantas-Torres, F., Brianti, E., Pfeffer, M., Genchi, C., Guberti, V., Capelli, G., Deplazes, P., 2015. The role of wild canids and felids in spreading parasites to dogs and cats in Europe. Part II: Helminths and arthropods. Vet. Parasitol. 213, 24-37. doi: 10.1016/j.vetpar.2015.04.020.

Pantchev, N., Schaper, R., Limousin, S., Norden, N., Weise, M., Lorentzen, L., 2009. Occurrence of *Dirofilaria immitis* and tick-borne infections caused by *Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu lato and *Ehrlichia canis* in domestic dogs in France: results of a countrywide serologic survey. Parasitol. Res. 105 Suppl 1, S101-114. doi: 10.1007/s00436-009-1501-2.

Petry, G., Genchi, M., Schmidt, H., Schaper, R., Lawrenz, B., Genchi, C, 2015. Evaluation of the adulticidal efficacy of Imidacloprid 10 %/Moxidectin 2.5 % (w/v) Spot-on (Advocate®, Advantage® Multi) against *Dirofilaria repens* in experimentally infected dogs. Parasitol. 114 Suppl 1:S131-44. doi: 10.1007/s00436-015-4519-7.

Pietikäinen, R., Nordling, S., Jokiranta, S., Saari, S., Heikkinen, P., Gardiner, C, Kerttula, A.M., Kantanen, T., Nikanorova, A., Laaksonen, S., Lavikainen, A., Oksanen, A. 2017. *Dirofilaria repens* transmission in southeastern Finland. Parasit. Vectors10 (1), 561. doi: 10.1186/s13071-017-2499-4.

Radev, V., Lalkovski, N., Zhelyazkov, P,, Kostova, T., Sabev, P., Nedelchev, N., Vassileva, R. 2016. Prevalence of gastrointestinal parasites and *Dirofilaria* spp. in stray dogs from some 215 regions in Bulgaria. Bulg. J. Vet. Med. 19, 57-62.

Rjeibi, M.R., Rouatbi, M., Mabrouk, M., Tabib, I., Rekik, M., Gharbi, M., 2017. Molecular study of *Dirofilaria immitis* and *Dirofilaria repens* in dogs from Tunisia. Transbound Emerg. Dis. 64, 1505-1509. doi: 10.1111/tbed.12541.

Rossi, A., Peix, Á., Pavlikovskaya, T., Sagach, O., Nikolaenko, S., Chizh, N., Kartashev, V., Simón, F., Siles-Lucas, M. 2015. Genetic diversity of *Dirofilaria* spp. isolated from subcutaneous and ocular lesions of human patients in Ukraine. Acta Trop. 142, 1-4. doi: 10.1016/j.actatropica.2014.10.021.

Sałamatin, R.V., Pavlikovska, T.M., Sagach, O.S., Nikolayenko, S.M., Kornyushin, V.V., Kharchenko, V.O., Masny, A., Cielecka, D., Konieczna-Sałamatin, J., Conn, D.B., Golab,

E, 2013. Human dirofilariasis due to *Dirofilaria repens* in Ukraine, an emergent zoonosis: epidemiological report of 1465 cases. Acta Parasitol. 58, 592-398. doi: 10.2478/s11686-013-0187-x.

Schwan, E.V., Durand, D.T., 2002. Canine filariosis caused by *Dirofilaria immitis* in Mozambique: a small survey based on the identification of microfilariae. J. S. Afr. Vet. Assoc. 73, 124-126.

Simón, F., Siles-Lucas, M., Morchón, R., González-Miguel, J., Mellado, I., Carretón, E., Montoya-Alonso, J.A., 2012. Human and animal dirofilariasis: the emergence of a zoonotic mosaic. Clin. Microbiol. 25, 507-544. doi: 10.1128/CMR.00012-12.

Siwila, J., Mwase, E.T., Nejsum, P., Simonsen, P.E. 2015. Filarial infections in domestic dogs in Lusaka, Zambia. Vet. Parasitol. 210, 250-254. doi: 10.1016/j.vetpar.2015.04.009.

Suh, G.H., Ahn, K.S., Ahn, J.H., Kim, H.J., Leutenegger, C., Shin, S., 2017. Serological and molecular prevalence of canine vector-borne diseases (CVBDs) in Korea. Parasit. Vectors. 10(1):146. doi: 10.1186/s13071-017-2076-x.

Sukhumavasi, W., Bellosa, M.L., Lucio-Forster, A., Liotta, J.L., Lee, A.C., Pornmingmas, P., Chungpivat, S., Mohammed, H.O., Lorentzen, L., Dubey, J.P., Bowman, D.D., 2012. Serological survey of *Toxoplasma gondii*, *Dirofilaria immitis*, Feline Immunodeficiency Virus (FIV) and Feline Leukemia Virus (FeLV) infections in pet cats in Bangkok and vicinities, Thailand. Vet. Parasitol. 188, 25-30. doi: 10.1016/j.vetpar.2012.02.021.

Tahir, D., Damene, H., Davoust, B., Parola, P. 2017. First molecular detection of *Dirofilaria immitis* (Spirurida: Onchocercidae) infection in dogs from Northern Algeria. Comp. Immunol. Microbiol. Infect. Dis., 51,66-68. doi: 10.1016/j.cimid.2017.04.001.

Tasić-Otašević, S.A., Trenkić Božinović, M.S., Gabrielli, S.V., Genchi, C., 2015. Canine and human *Dirofilaria* infections in the Balkan Peninsula. Vet. Parasitol. 209, 151-156. doi: 10.1016/j.vetpar.2015.02.016.

To, K.K., Wong, S.S., Poon, R.W., Trendell-Smith, N.J., Ngan, A.H., Lam, J.W., Tang, T.H., AhChong, A.K., Kan, J.C., Chan, K.H., Yuen, K.Y., 2012. A novel *Dirofilaria* species causing human and canine infections in Hong Kong. J. Clin. Microbiol. 50, 3534-3541. doi: 10.1128/JCM.01590-12.

Trotz-Williams, L.A., Trees, A.J., 2003. Systematic review of the distribution of the major vector-borne parasitic infections in dogs and cats in Europe. Vet. Rec. 152, 97-105.

Tumolskaya, N.I., Pozio, E., Rakova, V.M., Supriaga, V.G., Sergiev, V.P., Morozov, E.N., Morozova, L.F., Rezza, G., Litvinov, S.K., 2016. *Dirofilaria immitis* in a child from the Russian Federation. Parasite 23, 37. doi: 10.1051/parasite/2016037.

Venco, L., Genchi, M., Genchi, C., Gatti, D., Kramer, L., 2011. Can heartworm prevalence in dogs be used as provisional data for assessing the prevalence of the infection in cats? Vet. Parasitol. 176, 300-303. doi: 10.1016/j.vetpar.2011.01.013.

Venco, L., Genchi, C., Genchi, M., Grandi, G., Kramer, L.H., 2008. Clinical evolution and radiographic findings of feline heartworm infection in asymptomatic cats. Vet. Parasitol.

158, 232-237. doi: 10.1016/j.vetpar.2008.09.011.

Vrhovec, M.G., Pantchev, N., Failing, K., Bauer, C., Travers-Martin, N., Zahner, H., 2017. Retrospective analysis of aanine vector-borne diseases (CVBD) in Germany with emphasis on the endemicity and risk factors of leishmaniosis. Parasitol. Res. 116 (Suppl 1):131-144. doi: 10.1007/s00436-017-5499-6.

Yilmaz, E., Wongkamchai, S., Ramünke, S., Koutsovoulos, G.D., Blaxter, M.L., Poppert S., Schaper, R., von Samson-Himmelstjerna, G., Krücken, J., 2019. High genetic diversity in the *Dirofilaria repens* species complex revealed by mitochondrial genomes of feline microfilaria samples from Narathiwat, Thailand. Transbound Emerg. Dis. 66, 389-399. doi: 10.1111/tbed.13033.

Zohdy, S., Valenta, K., Rabaoarivola, B, Karanewsky, C.J., Zaky, W., Pilotte, N., Williams, S.A., Chapman, C.A., Farris, Z.J., 2019. Causative agent of canine heartworm (*Dirofilaria immitis*) detected in wild lemurs. Int. J. Parasitol. Parasites Wildl. 9, 119-121. doi: 10.1016/j.ijppaw.2019.04.005.