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Molecular Detection of *Dirofilaria immitis* in Dogs

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Abstract

There are different species of nematodes which affect dogs health but the *Dirofilariaimmitis* is the dangerous one, in this study 2 ml of blood from one hundred four of samples, from dogs different breeds, breeding managements and age range from less than 6 months to more than 4 years old, then DNA extracted from this blood, PCR technique using a Primer designed as (5-AGTGTAGGTCAGCCCTGAGTTA-3) forward and (5-ACAGGCACTGACAATAC-CAAT-3) was used to amplify 203 bp in mitochondrial oxidase (COI), COI gene amplification results showed 203 bp of band for 15 blood samples. Not any of the negative samples for *D.immitis* give amplification of the DNA molecules, 15 samples from 104 animals showed positive using PCR (14.4%), high prevalence of infection in dogs 2-4 years old (40%), while the animals less than one year old not recorded any infection rates, Outdoor animals showed high prevalence of infection with *D.immitis* when compare with indoor once .

Key words: *Dirofilariaimmitis,* Dogs, Polymerase Chain Reaction, Mosul

One of the most important parasite which affect the dog health is *Dirofilariaimmitis*, the disease is also endemic areas which characterized by increase in body temperatures in high density arthropods vector regions. *Dirofilariaimmitis* also present in the pulmonary arteries of dogs. "This parasite infect canine, feline and different species of canidae (Albanese *et al*.,2013). It is one of affective nematodes parasite of dogs. Dirofilaria cause various symptoms ranged from cough to heart attack, hemolysis in vascular endothelium and thrombosis in pulmonary arteries which fatal if not treated (Otranto *et al*.,2013). This parasite can be investigated by conventicle methods of microfilariae in blood, investigation of antigens in bloodsmear, histochemical or immuno-histochemical staining or by Polymerase Chain Reaction. Conventicle investigation technique of microfilariae in blood is always difficult and is more misdiagnosed (Ionic *et al*.,2015).

Dirofilariaimmitis is transmitted by various arthropods of the genus Culex, Aedes, Ochlerotatus, Anopheles, Armigeres and Mansonia (Di Cesare *et al*.,2013). (Simsek *et al*.,2011) this study aims to use the polymerase chain reaction to detect the DNA of the parasite isolated from the dog's blood. Amplification used with custom primers on a small fragment of the mitochondrial small ribosomal RNA gene (12S rDNA)

Materials and Methods

Animals

Sample collection one hundred dogs in different breeds, breeding managements and age range from less than 6 months to more than 4 years old, 2 ml of Blood samples were collected from cephalic vein in EDTA tube and stored at 4ºC until molecular technique was applied.

Blood samples and DNA extraction

D. immitis DNA extracted from blood using

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a blood DNA miniprep system (Promega®), according to manual, DNA that extracted from blood purified by using Roche® High Pure PCR. (Alobaidii *et al*.,2019)

Polymerase Chain Reaction (PCR)

PCR technology using a Primer designed as 5-AGTGTAGGTCAGCCCTGAGTTA-3 forward and 5-ACAGGCACTGACAATACCAAT-3 was used to amplify 203 bp in mitochondrial oxidase (COI) (Rishniw *et al*.,2006 and Taíssa *et al*.,2020), a PCR presentation with a total volume of 20 l was presented. GoTaq Green Master Mix (Promega®) consists of 10 l, 0.5 l of each primer at a concentration of 250 μM, 1 l of DNA extracted and full volume using distilled water to final volume. The reactions were applied in a thermo cycler using the following program: an initial denaturation of 94°C in 1 minute, and 32 cycles that included: denaturation of 94°C in 30 seconds, an annealing cycle at 63°C in 30 seconds, and an extension cycle at 72° Celsius in 30 seconds. 30 sec and a final extension cycle at 72 °C in7 min (Bamorovat *et al*.,2017).

Results

COI gene amplification results showed 203 bp of band for 15 blood samples. Not any of the negative samples for D.*immitis* give amplification of the DNA molecules. Fig 1.

The results showed infection in 15 from 104 animals. According to age, the results showed high prevalence of infection with *D.Immitis* in dog's 2-4 years old while the animals less than one year old not recorded any infection rates. (Table I).

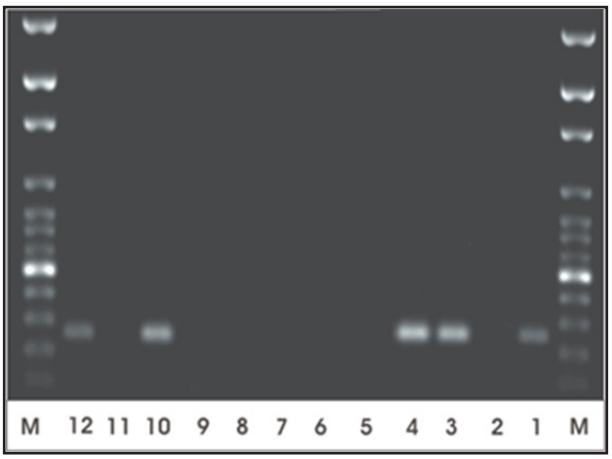

Fig 1. results of polymerase chain reaction , M=Marker , 1 positive control ,3,4,10,12 positive results , 2,5-9,11 negative results .

Outdooranimals showed high prevalence of infection with *D.immitis* when compare with indoor once Table II.

Discussion

D. immitis infection is world widespread and is a main parasitic disease in dogs. The present study recorded first speculation of this parasite infection in dogs. Recording of 14.4%, in 2020 *D.immitis* was recorded 10.91% in dogs worldwide (Chloe *et al*.,2016). The percentage of infection of *D. immitis* varies between different countries of the world , e.g., in Australia 22.68% (Mustafa *et al*.,2012), Turkey 1.6% (Claudio *et al*.,2012), in the USA11.60% (Claudio *et al*.,2020) in Europe 10.45% (Cilliers and Heidi ,1991), and 7.57% in Africa (Silva *et al*.,2019), in Brazil 15.5% (Sonjoy *et al*.,2015), 13.93% in India (Vieira *et al*.,2015), 13.7% in Portugal (Laidoudi *et al*.,2021). This study recorded percentage of infection10.6 % *D. immitis* infection in indoor household and 21% in

Table I. Percentage of infection with *D.immitis*according to the animal age

Animal Age	Number of samples	Number of positive samples	%
Less than 6 months	19	0	0
More than 6 months -12months	25	0	0
More than 1 year-2 years	32	5	15.6
2-4 years	15	6	40
More than 4 years	13	4	30.7
Total	104	15	14.4

Table II. Percentage of the infection with *D.immitis* according to breeding management of dogs

Breeding management	No of samples	No of positive samples	Percentage of infection
In door	66	7	10.6
Out door	38	8	21
Total	104	15	14.4

outdoor native dogs. These results are in agreement with what was mentioned by (Laidoudi *et al*.,2020) which recorded prevalence of infection 9.02% in household dogs and 25.90% in native dogs, other study conducted by (Tahir *et al*.,2017) which recorded similar results in India household and native dogs 4.7% ,29.5 respectively, (Simsek *et al*.,2008) showed high prevalence of *D immitis* in native dogs when compare with household dogs in Turkey, the reasons of high prevalence of *D.immitis* in native dogs is due to peregrination behavior which is free therefore it can easily exposed to biting by mosquito which increase intensity high rainfall and humidity seasons (Kemenesi *et al*.,2013). In addition, owners of dogs who manage them indoors are more concerned with health (Sulesco *et al*.,2016). In addition, there is routine health and programmed administration anthelmintic drugs like ivermectin, which microfilaricidal activity to decrease the number of circulating microfilariae. While native dogs are cared and administer any type of medications (Sulesco *et al*.,2016).

The results showed high prevalence in older dogs when compare with dogs less than 1 year which not recorded any infection with *D.immitis*, this result is similar to results of Simsekand Ayse,2016) which recorded that the infection varied with age: 0% in dogs less than 1year and 4.2% in dogs which aged between two-four years. Other researchers (Ceribasi *et al*.,2012) have showed the relationship between the ages and positivity of *D.immitis*, and showed the a risk factor to infection of this parasite in dogs three-seven years old when compared with other aged group. (Fan *et al*.,2001) found the lowest prevalence in up to one year old (6.3%) and following up to three years old (14.1%) while the highest rates has been found in up to six years old (23.7%). Ceribasi and Simsek (2017) showed highest percent in dogs up to three years old (17.5%) while there was no positivity in up to six years. While the highest was found in dogs up to 6 years old 17%, followed by the group up 3 years old 12 % and the lowest in the more than 6 months 6.%) (Tomazatos *et al*.,2018) other researchers recorded high prevalence in animals was less than three years of age,(Kurucz *et al*.,2016 and Bravo *et al*.,2016) recorded *D. immitis* occurred in younger animals several studies approved this differences which related with the time developing of filariids in this age, which best time of diagnosis due to the presence of microfilaremic, and to the longer time of exposure of the dogs to vector blood meals which favors the infection.

Conclusions

D. immitis was distributed in dogs in Mosul city and affected cats at different ages, and breeding management.

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Conflict of interest

The authors declare no conflict of interest in the manuscript.

References

Albanese F, Abramo F, Braglia C, Caporali C, Venco L, and Vercelli A. (2013) Nodular lesions due to infestation by Dirofilariarepens in dogs from Italy. *Vet Dermatol.* **24**: 255–6.doi: 10.1111/vde.12009.

Alobaidii,W.A. (2019) The serological diagnosis of canine Leishmaniasis by using ELISA in Nineveh province. *Iraqi .J.Vet.Sci.* **33**(2) : 11-114.doi:10.33899/IJVS.2019.163194

Bamorovat M, Sharifi I, FasihiHarandi M, Nasibi S, Sadeghi B, and Khedri J. (2017) Parasitological, Serological and Molecular Study of *Dirofilariaimmitis* in Domestic Dogs, Southeastern Iran. *Iran J Parasitol* **12**(2) :

260-266. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5527037/.

Bravo-Barriga D, Parreira R, Almeida APG, Calado M, Blanco-Ciudad J, and Serrano-Aguilera FJ,. (2016) *Culex-pipiens* as a potential vector for transmission of *Dirofilariaimmitis* and other unclassified Filarioidea in Southwest Spain. *Vet Parasitol*. **223**:173–80.doi: 10.1016/j.vetpar.2016.04.030.

Ceribasi A, and S Simsek. (2012) Histopathologic Effects of Dirofilariaimmitis Microfilaria on Internal Organs of Dog Confirming by PCR Technique. *Iranian J Parasitol*, **7**(2) : 103-107. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3469196/

Chloe Nguyen, Wei Ling Koh,AndreaCasteriano, NiekBeijerink, Christopher Godfrey, Graeme Brown, David Emery and Jan Šlapeta. (2016) Mosquito-borne heartworm *Dirofilariaimmitis* in dogs from Australia. *Parasites & Vectors* 9 : 535. DOI 10.1186/s13071-016-1821-x

Cilliers, W J and Heidi Schroeder. (1991) A case of heartworm (DirofilariaImmitis) in an imported dog and a report of the occurrence of Canine Microfilariae in the Republic of South Africa. *Journal of the South African Veterinary Association* **62**(1) : 33-4. https://pubmed.ncbi.nlm.nih.gov/2051447/

Claudio Genchi, Dwight Bowman and Jason Drake. (2014) Anine heartworm disease (*Dirofilariaimmitis*) in Western Europe: survey of veterinary awareness and perceptions. *Parasites & Vectors* 7 : 206 .doi: 10.1186/1756-3305-7-206.

Claudio Genchi and Laura Helen Kramer. (2020) The prevalence of Dirofilariaimmitis and D. repens in the Old World. *Vet Parasitol*. 280 :108995. DOI: 10.1016/j.vetpar.2019.108995

Di Cesare A, Otranto D, Di Giulio E, Simonato G, Latrofa MS, and La Torre F,. (2013) Microfilarial periodicity of Dirofilariarepens in naturally infested dogs. *Parasitol Res*. **112** : 4273–9. doi: 10.1007/s00436-013-3619-5

Fan C K , K E Su, Y H Lin, C W Liao, W Y Du, and H Y Chiou. (2001) Seroepidemiologic survey of Dirofilariaimmitis infection among domestic dogs in Taipei City and mountain aboriginal districts in Taiwan (1998-1999). *Vet Parasitol*. 3 :102(1-2) : 113-20. doi: 10.1016/s0304-4017(01)00511-8.

Ionică AM, Matei IA, Mircean V, Dumitrache MO, D'Amico G, and Győrke A. (2015) Current surveys on the prevalence and distribution of Dirofilaria spp. and Acanthocheilonemareconditum infections in dogs in Romania. *Parasitol Res*. **114** : 975–82.doi: 10.1007/s00436-014-4263-4.

Ionic AM, Zittra C, Wimmer V, Leitner N, Votýpka J, and Modrý D,. (2017) Mosquitoes in the Danube Delta: searching for vectors of filarioidhelminths and avian malaria. *Parasit Vectors*. **10** : 1–6. https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-017-2264-8

Kemenesi G, Kurucz K, Kepner A, Dallos B, Oldal M, and Herczeg R,. (2015) Circulation of *Dirofilariarepens*, *Setaria tundra*, and Onchocercidae species in Hungary during the period 2011–2013. *Vet Parasitol*. **214** : 108–13. DOI: 10.1016/j.vetpar.2015.09.010

Kurucz K, Kepner A, Krtinic B, Zana B, Földes F, and Bányai K,. (2016) First molecular identification of *Dirofilaria* spp. (Onchocercidae) in mosquitoes from Serbia. *Parasitol Res*. **115** : 3257–60.doi: 10.1007/s00436-016-5126-y.

-Laidoudi Y, Bedjaoui S, Medkour H, Latrofa MS, Mekroud A, and Bitam I,. (2020) Molecular approach for the diagnosis of blood and skin canine filarioids. *Microorganisms*. **8** : 1671.doi: 10.3390/microorganisms8111671.

LaidoudiYounes, Hélène Barré-Cardi, SamiaBedjaoui, NazliAyhan, Marie Varloud, Oleg Mediannikov, Domenico Otranto and Bernard Davoust. (2021) *Dirofilariaimmitis* and *Dirofilariarepens* in mosquitoes from Corsica Island, France .*Parasites & Vectors* , **14** : 427 doi: 10.1186/s13071-021-04931-y.

Mustafa Köse and Metin Erdoğan. (2012) Serological screening of canine heartworm (Dirofilariaimmitis) infections in Turkey.Berl Munch TierarztlWochenschr Nov-Dec **125**(11-12) : 503-8.DOI: https://doi.org/10.1017/S0022149X0896079X

Otranto D, Dantas-Torres F, Brianti E, Traversa D, Petrić D, Genchi C, and Capelli G. (2013) Vector-borne helminths of dogs and humans in Europe. *Parasit Vectors*. **6** : 16. doi: 10.1186/1756-3305-6-16.

Rishniw M, Barr SC, Simpson KW, Frongillo MF, Franz M, and Dominguez Alpizar JL. (2006) Discrimination between six species of canine microfilariae by a single polymerase chain reaction. *Vet Parasitol*, **135**(3-4) : 303-314. http://dx.doi.org/10.1016/j.vetpar.2005.10.013. PMid:16289566.

Silva M.S., G.Leles, A.P.Surdre, P.R.Millar, F.Uchoqa and B.Brener. (2019) Prevelance and molecular characterization of Dirofilariaimmitis (Filarioidea :onchocercerdia) in dogs from endemic areas of Rio De Janeiro state, *Brazil .J. of Parasitology*, **105**(2) : 387-390.

Simsek S, Utuk AE, Koroglu E, and Rishniw M. (2008) Serological and molecular studies on *Dirofilariaimmitis* in dogs from Turkey. *J Helminthol*. **82** : 181–186.doi: 10.1017/S0022149X0896079X

Simsek S, Ozkanlar Y, Balkaya I, and Aktas MS. (2011) Microscopic, serological and molecular surveys on Dirofilariaimmitis in strain dogs, *Turkey. Vet Parasitol* **183**(1-2) : 109-113. http://dx.doi.org/10.1016/j.vetpar.2011.06.012. PMid:21757293.

Simsek Sami and Ayse Turkan Ciftci . (2016) Serological and Molecular Detection of *Dirofilaria* Species in Stray Dogs and Investigation of *Wolbachia* DNA by PCR in Turkey. *J Arthropod Borne Dis*.**10**(4) : 445–453.http://jad.tums.ac.ir.

Sonjoy Kumar Borthakur, Dilip Kumar Deka, Saidul Islam, Dilip Kumar Sarma, and Prabhat Chandra

Sarmah. (2015) Prevalence and Molecular Epidemiological Data on *Dirofilariaimmitis* in Dogs from Northeastern States of India. *Scientific World Journal*. : 265385. doi: 10.1155/2015/265385.

Sulesco T, Von Thien H, Toderas L, Toderas I, Lühken R, and Tannich E. (2016) Circulation of *Dirofilariarepens* and *Dirofilariaimmitis* in Moldova. *Parasit Vectors.* **9** : 1–10.

TaíssaAngélicaLemos Trancoso; Nathália da Conceição Lima; Alynne Silva Barbosa; Daniela Leles; Ana Beatriz Monteiro Fonseca; Norma Vollmer Labarthe; Otilio Machado Pereira Bastos; and Claudia Maria AntunesUchôa. (2020) Detection of Dirofilariaimmitis using microscopic, serological and molecular techniques among dogs in Cabo Frio, RJ, Brazil. *Braz J Vet Parasitol*; **29**(1): e017219. http://doi.org/10.1590/S1984-29612020009.

Tahir D, Bittar F, Barré-Cardi H, Sow D, Dahmani M, and Mediannikov O,. (2017) Molecular survey of *Dirofilariaimmitis* and *Dirofilariarepens* by new real-time TaqMan®PCR assay in dogs and mosquitoes (Diptera: Culicidae) in Corsica (France). *Vet Parasitol*. **235** : 1–7. doi.org/10.1016/j.vetpar.2017.01.002

Tomazatos A, Cadar D, Török E, Maranda I, Horváth C, and Keresztes L,. (2018) Circulation of *Dirofilariaimmitis* and *Dirofilariarepens* in the Danube Delta Biosphere Reserve, Romania. *Parasites Vectors*. **11**:1–8. DOI: 10.1186/s13071-018-2980-8

VieiraL , A C Silvestre-Ferreira , A P Fontes-Sousa , A C Balreira , R Morchón , E Carretón , H Vilhena F Simón , and J A Montoya-Alonso. (2015) Seroprevalence of heartworm (Dirofilariaimmitis) in feline and canine hosts from central and northern Portugal. *J Helminthol*. 89(5) : 625-9. doi: 10.1017/S0022149X14000352. Epub 2014 May 14.

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Isolation and Identification of *Brucella Melitensis* in Diseased Nile Tilapia, *Oreochromis Niloticus* from a Commercial Fish Farm in Tamil Nadu, India

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Abstract

Brucellosis is a zoonotic bacterial disease affecting livestock with fewer reports in fishes. The present study reports the isolation and identification of *Brucella melitensis* from diseased Nile Tilapia, *Oreochromis niloticus* from a commercial fish farm. The diseased fish with clinical sings were collected and the organs *viz*., liver, kidney, spleen and intestine were aspectically dissected for isolation of bacterial pathogens. The bacteria isolate (H1/SRLAAH/2019) from the liver of the infected fish was characterised by the biochemical tests as *Brucella* sp. and confirmed by 16S rRNA gene sequencing analysis as *B.melitensis*. This is the first report on the isolation and identification of *B. melitensis* from *O. niloticus* in India.

Key words : Tilapia, zoonosis, *Brucella melitensis,* 16S rRNA gene sequencing

Brucellosis is a zoonotic disease caused by the bacterial pathogens of genus *Brucella* in mammals (Moreno *et al.,* 2002). *Brucella* spp. are facultative intracellular, gram-negative, cocco-

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