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Article

Molecular Detection of the Endosymbiont *Wolbachia* sp. in *Acanthocheilonema Reconditum* and Potential Vectors

Reinel Germán Rincón Hurtado[1†], Yineth Alexandra Palacios Erazo[1,†] and Angélica María Castellanos Sánchez[2]

[1] Universidad Santiago de Cali, Santiago de Cali, Colombia: reinel.rincon00@usc.edu.co (R.G.R.H.); yineth.palacios00@usc.edu.co (Y.A.P.E.)
[2] Department of Veterinary Medicine FUSM, Prosavez Research Group, Cali, Colombia; ancastellanosan@gmail.com (A.M.C.S.)
[3] Zoolavet Clinical Veterinary Laboratory, Santiago de Cali, Colombia
† Reinel Germán Rincón Hurtado and Yineth Alexandra Palacios Erazo contributed equally to this work.
* Correspondence:yineth.palacios00@usc.edu.co

Abstract: Background: *Wolbachia* sp. is an endosymbiotic bacterium that is widespread among arthropods and filarial nematodes. It affects the fertility and maturation of its arthropod hosts, which in some cases are vectors of infectious diseases of humans and animals. *Acanthocheilonema reconditum* is a canine filarial nematode, which can eventually infect humans through arthropods. An outbreak of *A. reconditum* has been reported recently in dogs in the southwest of Colombia, specifically in Cali. In addition, *Wolbachia* sp. is currently used for the biological control of the mosquito *Aedes egypti* in cities in the north and south-west of Colombia. *Aedes egypti* is a vector of dengue fever, a major public health disease in tropical and subtropical areas of the world. The objectives of this study were to detect *Wolbachia* sp. in mosquito and flea samples collected from dogs in southwestern Colombia and to determine the co-infection of *Wolbachia* sp. and *A. reconditum* in the recent outbreak of canine filariasis in Santiago de Cali.

Methods: A total of 18 mosquitoes of the genera *Aedes* and *Culex* and 18 *Ctenocephalides felis* fleas were collected in Santiago de Cali and nearby municipalities. DNA was extracted and 16S rRNA and *ftsZ* gene fragments were identified using conventional and nested polymerase chain reaction. In addition, the presence of *Wolbachia* sp. was analyzed in 55 cases positive for *A. reconditum* previously detected in the city, using the same molecular techniques.

Results: From the analysis of 18 mosquitoes and 18 fleas, using molecular techniques based on the 16S rRNA and *ftsZ* genes, *Wolbachia* sp. was detected in all the arthropods analyzed. *Wolbachia* sp. was not detected in the samples obtained from dogs infected with *A. reconditum*.

Conclusions: Molecular analyses were shown to be effective for the identification of *Wolbachia* sp. in mosquitoes and fleas but not in samples positive for *A. reconditum*.

Keywords: Wolbachia; Aedes sp., Culex sp., Ctenocephalides felis; Acanthocheilonema reconditum; polymerase chain reaction

Background

Wolbachia sp. is a gram-negative obligate endosymbiotic bacterium, which belongs to the family *Anaplasmataceae* and the order *Rickettsiales* [1] and infects a wide variety of arthropods and filarial nematodes [2, 3]. A frequency of infection by *Wolbachia* sp. of 40% to 66% has been reported in different arthropods [4, 5]. Because of its intracellular location in the gonads of arthropods [6], *Wolbachia* sp. can cause reproductive alterations and developmental changes in its host, such as cytoplasmic incompatibility, induction of

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parthenogenesis, male-killing, feminization of males, and manipulation of the meiotic process [7–10]. Till date, *Wolbachia pipientis* is the only species that has been formally described [11]; however, it can be classified in different clades. Eighteen monophyletic lineages or supergroups have been reported [1].

Wolbachia sp. infections can protect insects against a variety of pathogens, including bacteria, viruses (Zika, dengue), nematodes, and malaria-causing parasites, which has motivated its use in the biological control of vector-borne diseases [12-15]. In some arthropods, especially in some species of mosquitoes, the prevalence of *Wolbachia* sp. can be higher than 90%. For instance, in natural populations of *Aedes albopictus* in China, *Wolbachia* sp. was detected in 93.36 % of mosquitoes by analysis of the 16S rRNA and *wsp* genes [16].

Wolbachia sp. is a mutualistic symbiont of filarial nematodes of the family *Onchocercidae* [17]. *Acanthocheilonema reconditum* is a nematode of growing importance in veterinary medicine, which infects dogs and causes asymptomatic-to-mild pathologies and is mainly transmitted by *Ctenocephalides felis* [18]. In a recent outbreak of filariasis in southwestern Colombia, *A. reconditum* was detected using molecular analysis in most cases recorded in 2018–2019 [19]. *Wolbachia* sp. has been widely associated with canine filarial species such as *Dirofilaria immitis* [20]; however, few reports exist on the relationship between *Wolbachia* sp. and *A. reconditum*. *Wolbachia* sp. was found in up to 82.45% (47/57) of domestic dogs infected with filarial species, such as *D. immitis, Brugia pahangi, and B. malayi*, in the Bangkok Metropolitan Region, Thailand [21]. The objectives of this study were to detect *Wolbachia* sp. and to perform its molecular characterization in a cohort of cases positive for *A. reconditum* and in some potential vectors (fleas and mosquitoes) in the city of Cali.

Methods

Ethical statement

All procedures described in this study were previously approved by the Ethics Committee for Experimental Use and Animal Care of the Universidad ICESI (CIECUAE) with registration number 0019/2020.

Study location and sampling

Mosquito and flea sampling and DNA extraction

A total of 18 mosquitoes (56% *A. aegypti* and 44 % *Culex* spp.) and 18 fleas (100% *C. felis*) were collected in Santiago de Cali, Palmira, and Jamundí, in the Valle del Cauca Department, Colombia. The mosquitoes were collected randomly inside and outside households following instructions by El Servicio Nacional de Erradicación del Paludismo [22]. The fleas were collected from dogs older than 3 months of age; pregnant females were excluded. First, the animals were brushed, and the fleas were immobilized with 70% ethanol. The fleas were then placed in Eppendorf tubes and stored at −20°C until DNA extraction. Genomic DNA (gDNA) was extracted from arthropod whole bodies according to the Vanderbilt University protocol [23].

Positive cases for canine microfilariasis and DNA extraction

Fifty-five samples positive for *A. reconditum* had been previously collected from dog blood in an outbreak of canine filariasis throughout August 2018–August 2019. DNA was extracted using the Thermo Scientific™ GeneJET ™ DNA Purification Kit. DNA concentration and quality were assessed by measuring absorbance (A260/280 and A260/230) using Thermo Scientific NanoDrop One/Onec Microvolume UV-Vis Spectrophotometers.

Identification of Wolbachia sp. using polymerase chain reaction (PCR)

DNA samples from mosquitoes, fleas, and dogs positive for microfilariasis were used for the detection of *Wolbachia* sp. through PCR amplification of 16S rRNA and *ftsZ* gene fragments. A 438 bp fragment of the 16S rRNA gene was amplified with conventional PCR

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using the Wspec-F (5′-CATACCTATTCGAAGGGATAG-3′) and Wspec-R (5′-AGCTTCGAGTGAAACCAATTC-3′) primers [27]. Each 25-µL PCR reaction contained 2 µL DNA (20–200 ng/µL), 0.02X Taq 2X Master Mix (New England Biolabs), and 5 mM of each primer. The PCR conditions were 94°C for 2 min; 30 cycles of 94°C for 30 s, 55°C for 45 s, and 72°C for 1 min; and a final extension at 72°C for 10 min. A fragment of the *ftsZ* gene was amplified with nested PCR using two sets of primers. In the first amplification round, the Wol1-fwd (5′-CCTGTACTATATCCAAGAATTACTG-3′) and Wol1-R (5′-ACTATCCTTTATATGTTCCATAATTTC-3′) primers were used to amplify a 167-bp fragment. In the second round, a 147-bp fragment was amplified using the Wol7-fwd (5′-GGTGGAAATGCTGTGAATAAC-3′) and Wol7-R (5′-AGCACCGAGCCCTTTAG-3′) primer set [28]. The PCR mix for the *ftsZ* gene was the same as that described for the 16S rRNA gene, with the exception of the concentration of primers (0.1 mM each). The PCR temperature cycling conditions for the first round were as follows: 95°C for 4 min; 40 cycles of 94°C for 30 s, 51°C for 30 s, and 72°C for 30 s; and a final extension at 72°C for 5 min. The second round differed only in the annealing temperature (57°C). *Wolbachia* sp. DNA provided by collaborators at Vanderbilt University was used as a positive control; molecular biology-grade water was used as a negative control. PCR products were analyzed by electrophoresis on 1.5% agarose gels and stained with Diamond™ (Promega) for visualization.

Results

Molecular identification of Wolbachia sp. in DNA samples from mosquitoes and fleas using PCR

Wolbachia sp. was detected in all mosquito (18/18) and flea (18/18) samples using PCR amplification of 16S rRNA and *ftsZ* gene fragments (438 bp and 147 bp, respectively) (Figs. 1–4).

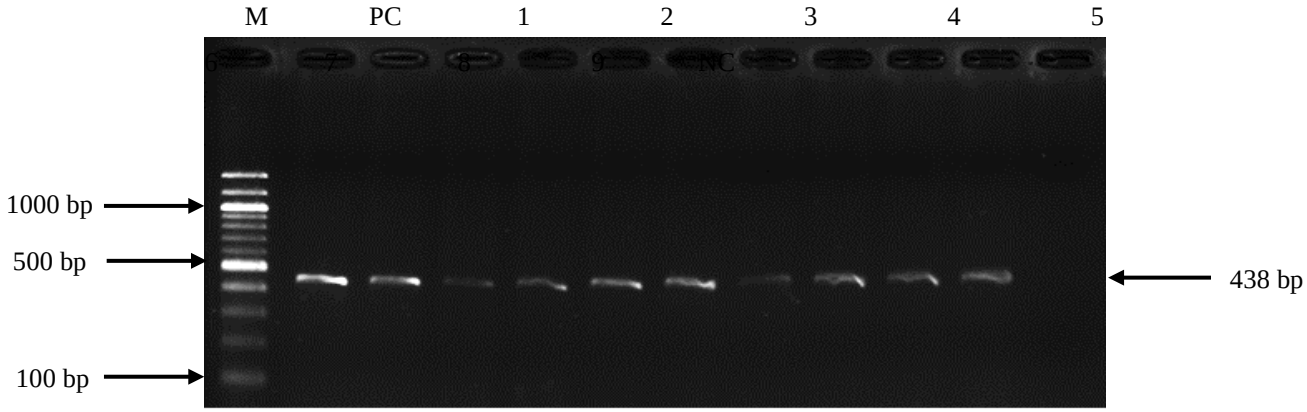

Figure 1. Electrophoresis gel for the molecular identification of *Wolbachia* sp. in mosquito DNA samples using PCR amplification of a 16S rRNA gene fragment. Size marker (M), positive control (PC), mosquito DNA samples (1–9), negative control (NC).

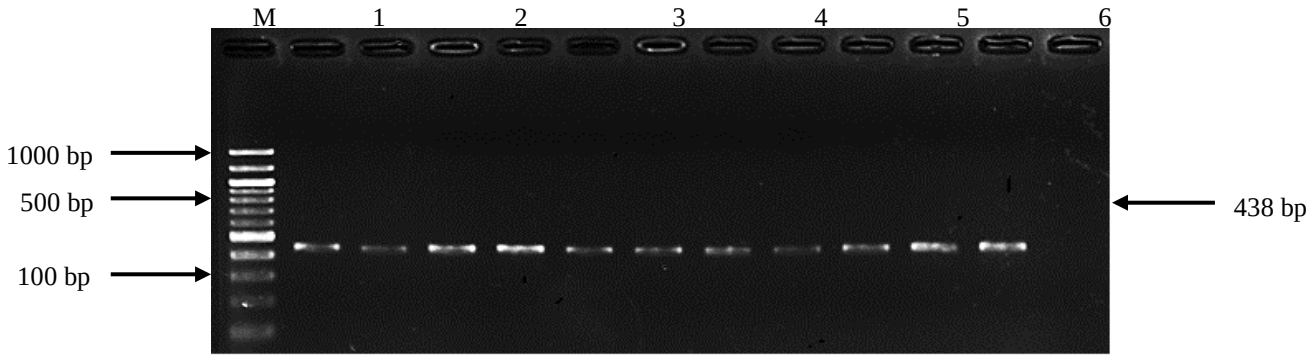

Figure 2. Electrophoresis gel for the molecular identification of *Wolbachia* sp. in flea DNA samples using PCR amplification of a 16S rRNA gene fragment. Size marker (M), flea DNA samples (1-10), positive control (PC), negative control (NC).

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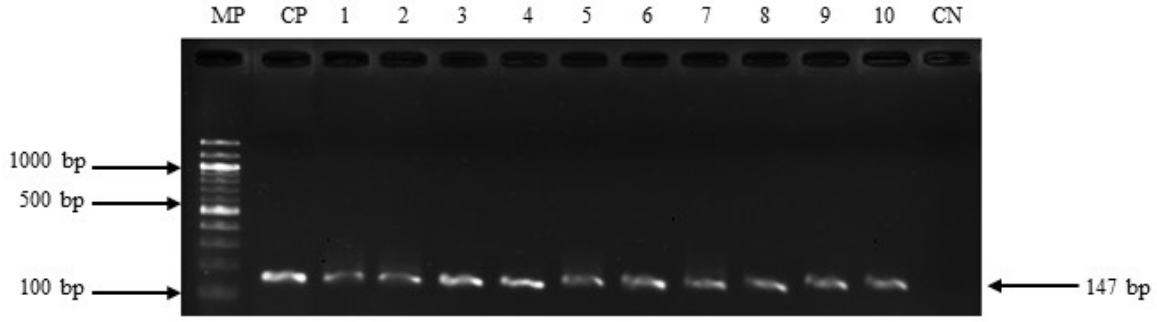

Figure 3. Electrophoresis gel for the molecular identification of *Wolbachia* sp. in mosquito DNA samples using nested PCR amplification of an *ftsZ* gene fragment. Size marker (M), positive control (PC), mosquito DNA samples (1-10), negative control (NC).

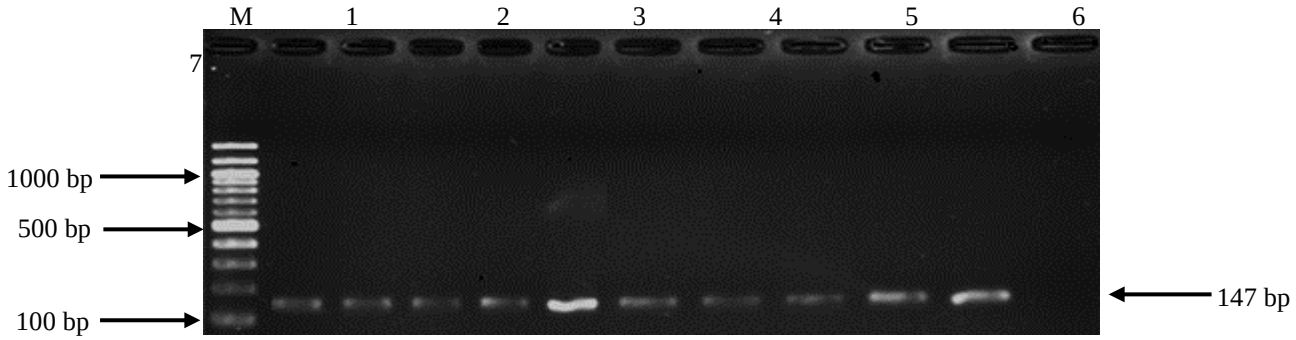

Figure 4. Electrophoresis gel for the molecular identification of *Wolbachia* sp. in flea DNA samples using nested PCR amplification of an *ftsZ* gene fragment. Size marker (M), flea DNA samples (1–9), positive control (PC), negative control (NC).

Molecular detection of Wolbachia sp. in DNA samples from positive cases for canine microfilariasis

The 16S rRNA and *ftsZ* genes were not detected in any of the 55 gDNA samples from dog's positive for *A. reconditum*. Therefore, co-infection of *A. reconditum* and *Wolbachia* sp. in the recent filariasis outbreak in Cali was discarded.

Discussion

Wolbachia sp. is an obligate endosymbiont of arthropods and nematodes of the family *Onchocercidae* [9, 24]. It can trigger mechanisms that allow for metabolic complementation, enhancement or increase of biochemical versatility and the pathways of one or both organisms [25-26]. This bacterium is naturally found in insects such as mosquitoes of the genus *Culex* [27] and has been recently identified in a population of *A. aegypti* in Coimbatore, India [28]. Likewise, it is an endosymbiont of fleas, which are essential for human and animal health because they are vectors of pathogens, such as *Rickettsii typhi*, *Rickettsia felis*, and *Bartonella* spp., around the world [29–33]. *Wolbachia pipientis* has been recently proposed as a biocontrol agent to mitigate the reproduction of the *A. aegypti* mosquito [28], one of the main vectors of diseases such as dengue, chikungunya, zika, and yellow fever [34-35]. The increased impact of these infectious diseases, especially dengue, in Latin America in the last two decades [36] has required the implementation of new control methodologies. *Aedes aegypti* mosquitoes carrying *Wolbachia* sp. were released in the cities of Bello and Medellín (northwestern Colombia) in 2018 [34] and in Cali in 2019. The purpose of the latter was to reduce the transmission of dengue because Cali accounted for approximately 20% of the total number of cases reported in Colombia in 2016 [37].

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In this study, *Wolbachia* sp. was identified in all DNA samples from *Culex* and *Aedes* mosquitoes and *C. felis* fleas using the 16S rRNA and *ftsZ* genes. *Wolbachia* sp. is a natural endosymbiont of arthropods and can be found in up to 66% of this phylum [5]. In addition, other studies using the same species have identified the bacterium through PCR amplification of the 16S rRNA, *ftsZ*, and *wsp* genes [27-28, 38]. It is important to note that prior to this study, *A. aegypti* mosquitoes carrying *Wolbachia* sp. laboratory methods had been released in the city of Cali, which could have contributed to the results observed, because is the first time this vector is reported.

Wolbachia sp. could not be detected by PCR amplification of the 16S rRNA and ftsZ genes in gDNA samples extracted from the blood of 55 *A. reconditum* positive cases. However, we found that, two blood sample from dogs with *Dirofilaria immitis* were amplified for *Wolbachia* by these two methods (16S rRNA and ftsZ). This result is in agreement with that of previous studies that failed to detect the bacterium in *A. reconditum* [20, 39]. The fact that the DNA was extracted from the blood of dogs with microfilariae, but not from adult (mature) forms of filariae, could explain the observed results. Furthermore, because the DNA samples were not purified, both canine and microfilaria DNA were present, which may have hindered the release of the intracellular bacterial molecule. Laidoudi *et al*. [39] also used canine blood samples to identify *Wolbachia* sp. and microfilariae and were able to detect the bacteria in cases positive for *D. immitis* and *Brugia* sp. but not in cases positive for *A. reconditum*. Casiraghi *et al*. [20] suggested that the absence of *Wolbachia* sp. in *A. reconditum* could be the result of the loss of the bacterium during evolution, along some lineages of filarial nematodes. We consider that to discard or confirm the endosymbiotic relationship between *Wolbachia* sp. and *A. reconditum*, the bacterial DNA purification strategy should be improved and adult specimens of the filariae should be evaluated to increase the probability of detection if present. Although *A. reconditum* is not considered pathogenic in dogs, three cases affecting the human eye have been reported, two of them in Turkey, and one in Australia [39].

Conclusions

Wolbachia sp. was detected in both flea and mosquito samples collected from natural environments in the city of Cali and some neighboring cities. *Wolbachia* sp. co-infection could not be detected in DNA samples positive for *A. reconditum* with the PCR techniques used. We believe that the methods to obtain DNA from intracellular bacteria should be optimized to accurately analyze the association between *Wolbachia* sp. and *A. reconditum*. Our research is an important contribution to human and veterinary public health because it provides information on the distribution of bacteria of great interest for the control of vectors that transmit tropical viral diseases, as well as for the distribution of potential infectious agents containing *Wolbachia* sp.

Abbreviation

PCR: polymerase chain reaction

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Authors' contributions：RGRH collected samples, performed laboratory procedures, analyzed PCR results, and wrote the manuscript. YAPE processed sequences, performed bioinformatic and phylogenetic analyses, and wrote the manuscript. AMCS reviewed and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate：All procedures described in this study were previously approved by the Ethics Committee for Experimental Use and Animal Care of the Universidad ICESI (CIECUAE) with registration number 0019/2020.

Consent for publication：Not applicable

References

[1] Khoo JJ, Kurtti TJ, Husin NA, Beliavskaia A, Lim FS, Zulkifli MMS, et al. Isolation and propagation of laboratory strains and a novel flea-derived field strain of wolbachia in tick cell lines. Microorganisms. 2020;8:928.
[2] Stouthamer R, Breeuwer JAJ, Hurst GDD. Wolbachia pipientis: microbial manipulator of arthropod reproduction. Annu. Rev. Microbiol. 1999;53:71–102.
[3] McLaren DJ, Worms MJ, Laurence BR, Simpson MG. Micro-organisms in filarial larvae (Nematoda). Trans R Soc Trop Med Hyg. 1975;69:509–514.
[4] Hilgenboecker K, Hammerstein P, Schlattmann P, Telschow A, Werren JH. How many species are infected with Wolbachia? A statistical analysis of current data. FEMS Microbiol Lett. 2008;281:215–220.
[5] Zug R, Hammerstein P. Still a host of hosts for Wolbachia: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. PLoS One. 2012;7(6):e38544.
[6] Werren JH, Zhang W, Guo, LR. Evolution and phylogeny of Wolbachia-reproductive parasites of arthropods. Proc Biol Sci. 1995;261:55-63.
[7] Weeks AR, Breeuwer JAJ. Wolbachia–induced parthenogenesis in a genus of phytophagous mites. Proc. R. Soc. Lond. B. 2001, 268;2245–2251.
[8] Hornett EA, Duplouy AMR, Davies N, Roderick GK, Wedell N, Hurst GDD, et al. You can't keep a good parasite down: Evolution of a male-killer suppressor uncovers cytoplasmic incompatibility. Evolution 2008;62:1258–1263.
[9] Werren JH, Baldo L, Clark ME. Wolbachia: Master manipulators of invertebrate biology. Nat. Rev. Microbiol. 2008;6:741–751.
[10] Kern P, Cook JM, Kageyama D, Riegler, M. Double trouble: Combined action of meiotic drive and Wolbachia feminization in Eurema butterflies. Biol. Lett. 2015;11:20150095.
[11] Hertig M. The rickettsia, Wolbachia pipientis (gen. et sp.n.) and associated inclusions of the mosquito, Culex pipiens. Parasitology 1936;28:53–486.
[12] Brelsfoard CL, Dobson, SL. Wolbachia-based strategies to control insect pests and disease vectors. Asia Pac. J. Mol. Biol. 2009;17(3): 55-63.
[13] Ye YH, Woolfit M, Rancés E, O'Neill SL, McGraw EA. Wolbachia-Associated Bacterial Protection in the Mosquito Aedes aegypti. PLoS Negl Trop Dis. 2013;7(8): e2362.
[14] Pan X, Pike A, Joshi D, Bian G, McFadden MJ, Lu P, et al. The bacterium Wolbachia exploits host innate immunity to establish a symbiotic relationship with the dengue vector mosquito Aedes aegypti. ISME J. 2018;12(1):277–288.
[15] Flatau R, Segoli M, Khokhlova I, Hawlena H. Wolbachia's role in mediating its flea's reproductive success differs according to flea origin. FEMS Microbiol Ecol. 2018;94(10).
[16] Hu Y, Xi Z, Liu X, Wang J, Guo Y, Ren D, et al. Identification and molecular characterization of Wolbachia strains in natural populations of Aedes albopictus in China. Parasites Vectors. 2020;13:28.
[17] Fenn K, Blaxter M. Are filarial nematode Wolbachia obligate mutualist symbionts?. Trends Ecol Evol. 2004;19(4):163-166.
[18] Brianti E, Gaglio G, Napoli E, Giannetto S, Dantas-Torres F, Bain O, Otranto D. New insights into the ecology and biology of Acanthocheilonema reconditum (Grassi, 1889) causing canine subcutaneous filariosis. Parasitology. 2012;139:530–536.
[19] Espinosa N, Rosero A, Villegas CL, Garcia IC, Gaviria-Cantin T, Peña A, Ferro BE, Nieto RLM. Canine filariasis outbreak in southwestern colombia: a molecular and epidemiological study. ResearchGate. 2020.
[20] Casiraghi M, Bain O, Guerrero R, Martin C, Pocacqua V, Gardner SL, et al. Mapping the presence of Wolbachia pipientis on the phylogeny of filarial nematodes: evidence for symbiont loss during evolution, Int. J. Parasitol. 2004;34(2):191-203.
[21] Satjawongvanit H, Phumee A, Tiawsirisup S, Sungpradit S, Brownell N, Siriyasatien, et al. Molecular Analysis of Canine Filaria and Its Wolbachia Endosymbionts in Domestic Dogs Collected from Two Animal University Hospitals in Bangkok Metropolitan Region, Thailand. Pathogens. 2019; 8:114.
[22] SENEPA. Manual de Campo para la Vigilancia Entomológica de Anopheles. Asunción, Paraguay; 2013. p. 14-16.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 16 February 2022 doi:10.20944/preprints202202.0191.v1

[23] Vanderbilt University. Lab 2: DNA extraction Molecular biology I. In: the Wolbachia Project. 2020.
[24] Bouchery T, Lefoulon E, Karadjian G, Nieguitsila A, Martin C. The symbiotic role of Wolbachia in Onchocercidae and its impact on filariasis. Clin. Microbiol. Infect. 2013;19(2):131-140.
[25] Hosokawa T, Koga R, Kikuchi Y, Meng XY, Fukatsu T. Wolbachia as a bacteriocyte-associated nutritional mutualist. Proc Natl Acad Sci USA. 2010;107:769–74.
[26] Nikoh N, Hosokawa T, Moriyama M, Oshima K, Hattori M, Fukatsu T. Evolutionary origin of insect–Wolbachia nutritional mutualism. Proc Natl Acad Sci. 2014;111:10257–62.
[27] Shaikevich E, Bogacheva A, Ganushkina L. Dirofilaria and Wolbachia in mosquitoes (Diptera: Culicidae) in central European Russia and on the Black Sea coast. Parasite. 2019;26:2.
[28] Balaji S, Jayachandran S, Ramatchandirane PS. Evidence for the natural occurrence of Wolbachia in Aedes aegypti mosquitoes. FEMS Microbiol Lett. 2019;366(6): fnz055.
[29] Rust MK, Dryden MW. The biology, ecology, and management of the cat flea. Annu Rev Entomol. 1997;42:451–73.
[30] Glickman LT, Moore GE, Glickman NW, Caldanaro RJ, Aucoin D, Lewis HB. Purdue University-Banfield National Companion Animal Surveillance Program for emerging and zoonotic diseases. Vector Borne Zoonotic Dis. 2006;6:14–23.
[31] Bouhsira E, Franc M, Boulouis H-J, Jacquiet P, Raymond-Letron I, Liénard E. Assessment of persistence of Bartonella henselae in Ctenocephalides felis. Appl Environ Microbiol. 2013;79:7439–44.
[32] Nogueras MM, Pons I, Ortuño A, Miret J, Pla J, Castellà J, et al. Molecular detection of Rickettsia typhi in cats and fleas. PLoS One. 2013;8:e71386.
[33] Angelakis E, Mediannikov O, Parola P, Raoult D. Rickettsia felis: the complex journey of an emergent human pathogen. Trends Parasitol. 2016;32:554–64.
[34] Vélez ID, Santacruz E, Kutcher SC, Duque SL, Uribe A, Barajas J, et al. The impact of city-wide deployment of Wolbachia-carrying mosquitoes on arboviral disease incidence in Medellín and Bello, Colombia: study protocol for an interrupted time-series analysis and a test-negative design study. F1000Res. 2020;8:1327.
[35] Weaver SC, Reisen WK. Present and future arboviral threaths. Antiviral Res. 2010;85:1–36.
[36] Stanaway JD, Shepard DS, Undurraga EA, Halasa YA, Coffeng LE, Brady OJ, et al. The global burden of dengue: an analysis from the Global Burden of Disease Study 2013. Lancet Infect Dis. 2016;16(6):712–23.
[37] World mosquito program. World Mosquito Program trabaja en Cali para proteger a las comunidades de las enfermedades transmitidas por mosquito, como el dengue, Zika, chikungunya y la fiebre amarilla.2019. https://www.worldmosquitoprogram.org. Accessed 20 Dic 2021.
[38] Gorham CH, Fang QQ, Durden LA. Wolbachia endosymbionts in fleas (Siphonaptera). J. Parasitol. 2003;89(2):283-289.
[39] Laidoudi Y, Marie JL, Tahir D, Watier-Grillot S, Mediannikov O, Davoust B. Detection of Canine Vector-Borne Filariasis and Their Wolbachia Endosymbionts in French Guiana. Microorganismos. 2020;8(5):770.