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1 **Comparative Immunology, Microbiology and Infectious Diseases**

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4

5 **Molecular and serological detection of animal and human vector-borne pathogens in the**
6 **blood of dogs from Côte d'Ivoire**

7

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25 **Abstract**

26 In Côte d'Ivoire, limited information are available on vector-borne pathogens, their
27 prevalence and distribution. Here, we assess the occurrence and diversity of canine vector-
28 borne diseases (CVBDs) in Abidjan and Yamoussoukro cities. Blood from a total of 123 dogs
29 were tested for *Leishmania infantum* and *Ehrlichia canis* antibodies and screened for
30 *Leishmania* and *Trypanosoma* spp., Piroplasmida, Filariidae and Anaplasmataceae by PCR
31 and sequencing. Among dogs, 39% were positive for at least one pathogen. Seroprevalences
32 were: 15.4% and 12.2% for *L. infantum* and *E. canis*, respectively. DNA of *L. infantum* and *T.*
33 *congolense* (4.1%), *Babesia vogeli* (1.6%), Filariidae (*Dirofilaria immitis*, *D. repens* and
34 *Acanthocheilonema reconditum*) (10.6%) has been detected. Anaplasmataceae were detected
35 in (17.1%) and *E. canis* was the only identified specie. Co-infections were observed in 13.8 %
36 of dogs: *E. canis-L. infantum* co-infection was the most prevalent (4.9%). Age, breed and sex
37 of dogs do not seem to influence infections. Village dogs were more susceptible to CVBDs
38 than kennel dogs (PV= 0.0000008). This study reports for the first time the presence of *L.*
39 *infantum*, *B. vogeli*, *A. reconditum*, *D. immitis* and *D. repens* in dogs from Côte d'Ivoire and
40 determines the prevalence and diversity of CVBD pathogens. The results indicate that human
41 and animal pathogens are abundant in Ivoirian dogs which requires attention of veterinarians,
42 physicians and authorities against these diseases, especially against major zoonosis such as
43 visceral leishmaniasis (*L. infantum*).

44 **Keywords:** Vector-borne pathogens, Côte d'Ivoire, Dog, PCR, *Leishmania infantum*,
45 *Trypanosoma congolense*, *Babesia vogeli*, Filariidae, *Ehrlichia canis*

46

47 **1. Introduction**

48 Canine vector-borne diseases (CVBD) are a group of rapidly spreading, globally distributed
49 diseases caused by a range of arthropod-borne pathogens, including ticks, fleas, mosquitoes
50 and phlebotomine sandflies which are the vectors of a rank of bacteria, viruses, protozoa and
51 helminths. In addition to their veterinary importance, some CVBD-causing pathogens are of
52 major zoonotic concern [1]. Dogs are sentinels and reservoirs of diseases that are a risk for
53 human population, therefore, the interest to follow CBVD in dog populations remains of
54 greater importance. *Ehrlichia canis*, *Hepatozoon* spp., *Anaplasma* spp., *Leishmania infantum*,
55 *Trypanosoma* spp., *Babesia* spp. and filariae are the most vector-borne pathogens detected in
56 dogs in multiple countries [2- 6].

57 Bacteria from the Anaplasmataceae family include numerous dog-associated pathogens
58 vectored by ticks [4]. Canine monocytic ehrlichiosis (CME) caused by *E. canis* and
59 transmitted by the tick *Rhipicephalus sanguineus*, is the most recognized, and is responsible
60 for severe clinical signs and a high mortality rate in infected dogs. The infection is common in
61 Sub-Saharan Africa, such as Côte d'Ivoire and Gabon [5]. Native African dogs are often
62 asymptomatic (immunotolerance). Warm climates seem to be favorable for the infectious
63 agents propagation [6].

64 Canine leishmaniosis, caused by *L. infantum* and transmitted by a phlebotomine sand fly
65 vector, is the most serious CVBD considering its large distribution (in Central and South
66 America, Asia and several countries of the Mediterranean Basin) and high pathogenicity [7-
67 8]. In Sub-Saharan Africa, data on CanL are rare, except some reports in Senegal [9],
68 Burkina-Faso [10]. Anecdotal *L. infantum* infections in human were also reported in Côte
69 d'Ivoire [11] and Gambia [12].

70 Canine trypanosomosis is a disease caused by haemoprotozoan parasites *Trypanosoma brucei*
71 *brucei* and *T. congolense* in Africa [13]. This disease can also be caused by *T. cruzi*, the agent
72 of American trypanosomosis known as the Chagas disease in humans. Dogs are also infected
73 by *T. b. rhodesiense* and *T. b. gambiense* pathogens for humans [14].

74 Babesiosis is a potentially serious illness caused by intraerythrocytic protozoan parasites of
75 the genus *Babesia*. *B. vogeli*, *B. gibsoni* and *B. rossi* are the main causative agents of the
76 canine babesiosis vectored by ticks. They may also be transmitted vertically [15] or by blood
77 exchange in fighting dogs [16]. *Babesia* species have a global distribution and have been
78 identified in African dogs [17].

79 Among the vector-borne pathogenic helminths belonging to the order Spiruridae, the suborder
80 *Spirurina* and the families Filariidae and Onchocercidae: *Dirofilaria immitis* and *D. repens*
81 are probably the best known to cause infestations in dogs and humans [1, 18], followed by
82 *Acanthocheilonema reconditum* (*Spirurida*, Onchocercidae) often infects dogs [19].
83 Differently from other filarioids transmitted by mosquitoes (e.g., *D. immitis* and *D. repens*) or
84 ticks (e.g., *Cercopithifilaria* spp.) to dogs, *A. reconditum* is vectored by fleas (i.e.,
85 *Ctenocephalides canis*, *C. felis*, *Pulex irritans*, *P. simulans*, *Echidnophaga gallinae*) or lice
86 (i.e., *Heterodoxus spiniger*, *Linognathus setosus*) with a rate of infestation in fleas of about
87 5% [19]. Globally, vector-borne diseases account for about 17% of the burden of all
88 infectious diseases and cause millions of dollars in losses to the livestock industry each year,
89 with implications for human and animal health and the global economy [20]. Information on
90 filarial infections in dogs in sub-Saharan Africa is scarce. From a review of available
91 literature by Schwan (2009) it appeared that all the above mentioned species have been
92 reported from various places on the continent [21]. survey for filariae in dogs in northern
93 Kenya and in Zambia have later been documented [22][23].

94 In Côte d'Ivoire, few information is available on the prevalence and the distribution of
95 CVBD. The purpose of this study was to evaluate the occurrence of these pathogens of
96 veterinary and public (for some of them) health importance and their diversity in dogs from
97 the city of Abidjan (kennel dogs) and in a city near Yamoussoukro (village dogs) by using
98 serological and molecular tools including new PCR assays.

99 **2. Methods**

100 **2.1. Study Sample collection and preparation**

101 In April 2018, blood samples were collected from 123 dogs, living in three kennels in Abidjan
102 (N=95) and Kongouanou town (N=28), (7°00'42.0"N 5°22'04.0"W) localized 24 km from
103 Yamoussoukro and 241 km from Abidjan. The largest kennel is located (5°16'54.9"N
104 3°56'46.0"W) in Koumassi, district of Abidjan and the other two are between five (Cocody)
105 and six (Treichville) kilometers away. Kennel dogs are working dogs used for protection,
106 security and defense. They belong to adapted breeds (ridgeback, Belgian shepherds, etc.).
107 They receive veterinary care and sometimes external pest control. The village dogs from
108 Kongouanou are medium-sized dogs mostly used for hunting. They live outdoors freely in the
109 village and its surroundings. They do not benefit from preventive veterinary care (32% carried
110 ticks). Data about sanitary status or possible prophylaxis for kennels dogs were collected. A
111 volume of 4 to 5 mL of blood was collected from the radial vein of each dog, into dry and
112 EDTA tubes; serum was recovered after centrifugation (10 min, 3000g) and frozen at -20 °C.
113 EDTA tubes were directly frozen at -20 °C. Each animal sampled was examined and
114 information about gender, breed and age were collected.

115 The animals were examined and sampled after obtaining the verbal consent of the persons
116 (owners) responsible for the dogs. The blood samples were taken from radial vein by
117 veterinarians (French and Ivorian) with the ethical responsibility to ensure the animal care in
118 accordance with Articles 433-434; Chapter 2 of the Ivoirian Penal Code.

119 **2.2. Serological assay to detect *E. canis* and *L. infantum* specific antibodies**

120 The presence of anti-*E. canis* and anti-*L. infantum* promastigote specific antibodies was
121 revealed by indirect fluorescent antibody test (IFAT). Plate wells were sensitized by 10 µL
122 and 20 µL of the commercial antigens of *E. canis* and *L. infantum* (Zoetis, France)

123 respectively and assays were assessed following manufacturer's instructions. Both anti-
124 *Ehrlichia* and anti-*Leishmania* antibodies were detected by secondary antibodies against
125 rabbits, anti-dog immunoglobulins G conjugated with fluorescein isothiocyanate (Sigma-
126 Aldrich, St Louis, MO, USA). Sera were considered positive when antibodies titers were
127 higher than 1:100 and 1:80 dilutions for *Ehrlichia* and *Leishmania* respectively.

128 **2.3.Morphological identification of filariae species**

129 Blood samples were examined for the presence of microfilaria after concentration by a
130 modified Knott test (Knott, 1939) which is the gold standard [24]. Microfilariae were
131 quantified and identified when existed. When co-infections were found, we performed a serial
132 dilution until we obtained 1 microfilariae/ 200 mL of blood which was used to extract the
133 DNA. The objective was to concentrate only one species of microfilariae and extract its DNA
134 for a possible future molecular identification.

135

136 **2.4.Molecular assays**

137 **2.4.1. DNA extraction**

138 DNA was extracted from 200 µL of blood after digestion with proteinase K at +56°C
139 overnight using a commercial DNA extraction kit (QIAamp DNA Mini Kit®, [Qiagen,
140 Courtaboeuf, France]) and performed on BIOROBOT EZ1 (Qiagen, Qiagen, Courtaboeuf,
141 France) per the manufacturer's instructions. In order to achieve maximum yield, digestion
142 was performed overnight with proteinase K in Qiagen lysis buffer before the QIAamp DNA
143 Mini Kit® was used. DNA was eluted in 200 µl of distilled water and stored at -20°C.

144 **2.4.2. PCR tools design for *Leishmania* spp. and *Trypanosoma* spp. screening**

145 We designed primers and probe using the conserved region of encoding ribosomal RNA
146 genes 18S to detect DNA from *Leishmania* species: a first, qPCR for the screening of all
147 *Leishmania* targeting a conserved region of the 18S gene; a second, conventional PCR
148 targeting the same gene that produce the 550-bp amplicon for the following confirmation and
149 species identification. In order to detect DNA from trypanosomes, we designed a qPCR
150 system targeting the ribosomal RNA genes 5.8S. To design the group-specific primers and
151 probes, the corresponding genetic sequences of all *Leishmania*/*Trypanosoma* species
152 available in the GenBank were multiple aligned. All conserved motifs were visualized using
153 BioEdit software version 7.0.5.3 (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). The free
154 web-based Primer3 software, version 4.0 (<http://frodo.wi.mit.edu/primer3/>) was used to
155 design the primers and probes based on chosen conserved motifs (Table 1).

156 The specificity and sensitivity of all PCR assays were tested *in silico* using primer-BLAST
157 (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and validated *in vitro* using a DNA panel of
158 cultivated *Leishmania* species: *L. infantum*, *L. donovani*, *L. major* and DNA of *L. guyanensis*
159 for *Leishmania* PCR systems, a DNA panel of cultivated *Trypanosoma* species: *T. congolense*
160 *IL 3000*, *T. evansi*, *T. vivax*, *T. brucei brucei*, *T. brucei gambiense* and *T. brucei gambiense*
161 *biyamina* groupe II for *Trypanosoma* qPCR system, and several arthropods of laboratory-
162 maintained colonies as well as human, monkey, donkey, horse, cattle, mouse and dog DNAs
163 for all the PCR systems (Table S1).

164 **2.4.3. Molecular screening for the presence of pathogens DNA**

165 All the DNA samples were screened for *Leishmania* spp. using two qPCR assays: i) the 18S-
166 based qPCR was used for the initial screening and the detection of DNA *Leishmania* spp., ii)
167 the specific *L. infantum* qPCR assay based on the amplification of kinetoplast minicircle DNA
168 (kDNA), recognized for its high sensitivity which allows the quantification of the parasite

169 load [25]. qPCR assays were performed to screen DNA samples using primers and probes for
170 Anaplasmataceae, *Trypanosoma* spp. and *Piroplasmida* (Table 1). Sequences of primers and
171 probes and their respective sources used in this study are presented in Table 1. Concerning
172 Filariidae, we used a triplex qPCR to detect *D. immitis*, *D. repens* and *A. reconditum* targeting
173 the *Cox1* gene. In order to detect the occult filariosis due to *D. immitis* we screened the samples
174 using a qPCR targeting *ftsZ* gene specific to *Wolbachia* endosymbiont of *D. immitis* (Laidoudi
175 et al. unpublished).

176 Reaction mix for qPCR assays contained 5 µl of the DNA template, 10 µl of Eurogentec
177 Master Mix Roche, 0.5 µl of each reverse and forward primers and UDG, 0.5 µl of the FAM-
178 labeled probe (Table 1) and 3 µl of distilled water DNase and RNase free, for a final volume
179 of 20 µl. The qPCR amplification was performed in a CFX96 Real-Time system (Bio-rad
180 Laboratories, Foster City, CA, USA) with the following thermal profile: The first step is an
181 incubation at 50°C for two minutes and an initial denaturation step at 95°C for five minutes,
182 followed by 40 cycles of denaturation at 95°C for 5 seconds and annealing-extension at 60°C
183 for 30 seconds. We included the DNA of the target parasites or bacteria as positive controls
184 and master mixtures as a negative control for each test. Samples were considered positive
185 when the cycle threshold (Ct) was lower than 35 Ct for all qPCR assays and lower than 38 Ct
186 for *Leishmania* 18S-based qPCR.

187 **2.4.4. PCR amplification, sequencing and phylogenetic analysis**

188 Positive samples by qPCR for vector borne pathogens were confirmed by standard PCR
189 assays and sequencing for the fragments of the genes 23S for Anaplasmataceae, kinetoplast
190 DNA, ITS II and 18S for *Leishmania* spp., ITS I for *Trypanosoma* spp. and 18S for
191 *Piroplasmida*. Primer pairs are listed in the (Table 1). Positive samples by the triplex qPCR

192 for Filariidae were also confirmed by standard PCR amplifying a 1230 bp from 18S gene of
193 Filariidae species (Laidoudi et al. unpublished).

194 The standard PCR assays consisted of a volume of 25 µl, including 12.5 µl of Amplitaq Gold
195 Master Mix, 0.75 µl of each primer, 5 µl of DNA model and water. The thermal cycling
196 conditions were: incubation step at 95°C for 15 minutes, 40 cycles of 1 minute at 95°C, 30
197 annealing at a different hybridization temperature for each PCR assays and one minute at
198 72°C followed by a final extension for five minutes at 72°C (Table 1). PCR amplification was
199 performed in a Peltier PTC-200 model thermal cycler (MJ Research Inc., Watertown, MA,
200 USA). The results of amplification were visualized by electrophoresis on 2% agarose gel. The
201 purification of PCR products was performed using NucleoFast 96 PCR plates (Macherey
202 Nagel EURL, Hoerd, France) according to the manufacturer's instructions. The amplicons
203 were sequenced using the Big Dye Terminator Cycle Sequencing Kit (Perkin Elmer Applied
204 Biosystems, Foster City, CA, USA) with an ABI automated sequencer (Applied Biosystems).
205 The obtained electropherograms were assembled and edited using ChromasPro software
206 (ChromasPro 1.7, Technelysium Pty Ltd., Tewantin, Australia) and compared with those
207 available in the GenBank database by NCBI BLAST
208 (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The DNA sequences obtained for each gene, for
209 each pathogen, from positive samples were aligned with those available in the GenBank
210 database for the same gene. A maximum-likelihood method was used to infer the
211 phylogenetic analyses and tree reconstruction was performed using MEGA software version 7
212 (<https://www.megasoftware.net/>). Bootstrap analyses were conducted using 100 replicates.

213

214 **2.5. Statistical analysis**

215 Statistical analysis was conducted using IBM SPSS statistics version 23. After the databases
216 were established, the association between infections and age, sex, breeds and origin (Kennels
217 vs village) of dogs was assessed using the X^2 test. The Fischer's exact test was used when the
218 percentages were low. Statistically significant was consider at P-value <0.05.

219 3. Results

220 3.1.Sensitivity and specificity of PCR assays

221 Designed PCR assays listed in Table 1 were tested. First, we tested the *Leishmania* genus-
222 specific 18S rRNA-based qPCR system by screening of positive controls (Table S1). As
223 expected *in silico*, we amplified all available positive controls: *L. infantum*, *L. donovani*, *L.*
224 *major* and *L. guyanensis*. These controls were also amplified by the *Leishmania* 18S rRNA-
225 based standard PCR system. In order to validate these news PCR systems and to reproduce
226 the results, *Leishmania*-positive samples were tested simultaneously by *Leishmania* genus-
227 specific 18S, *L. infantum*-specific kDNA qPCR assays and 18S, ITS1, ITS2 standard PCR
228 tools. All of them gave the same result. Secondly, we tested the *Trypanosoma* 5.8S rRNA-
229 based qPCR system by screening the controls. As tested *in silico*, it amplified all positive
230 controls (Table S1). The same results were obtained by using *Trypanosoma* ITS1- based
231 standard PCR. None of the DNA negative controls were amplified by any of the PCR tools.

232

233 3.2.Serological and molecular results

234 A total of 123 dogs were sampled: 91 males and 32 females. Ages were available for all dogs.
235 The average age was three years old [4 month–10 years] with 49 juveniles [4 month -2 years],
236 33 young dogs [2-4 years], 26 adults [4-6 years] and 15 oldies (>6years) dogs. Prevalence of
237 the vector-borne pathogens detected by serology or PCR is presented in the Table 2. Results
238 showed that 39% (48/123) of dogs were positive for at least one of the pathogens by either
239 PCR or serology (Table 3). Screening using qPCR assays showed a prevalence of 17.1%
240 (21/123) for Anaplasmatidae infections (*E. canis* was the only species identified), followed
241 by Filariidae infections 10.6% (13/123) with at least one filaria species, *L. infantum* in 4.1%
242 (5/123) with 28.9 *Leishmania*/mL of blood (Min: 2.1; Max: 117.8) as an average of the

243 parasite load found by the kDNA qPCR, *Trypanosoma* spp. in 4.1% (5/123) and finally
244 *Babesia* spp. with 1.6% (2/123). Prevalence by IFAT was of 15.4% (19/123) for *L. infantum*
245 and 12.2% (15/123) for *E. canis* (Table 2).

246 Regarding mono-infections, *E. canis* infection was the most prevalent with 9.7% (12/123).
247 We found a prevalence 8.9% (11/123) for *L. infantum*, *A. reconditum* 4.1% (5/123).
248 *Trypanosoma* spp. 1.6% (2/123) and finally *Babesia* spp. with 0.8% (1/123).

249 Mixed infections 13.8% (17/123) were also found in this study (Fig. 2). Mixed infection of *E.*
250 *canis-L. infantum* 4.9% (6/123) was more frequent than *E. canis-A. reconditum* with 3.3%
251 (4/123), *L. infantum-Trypanosoma* spp. and *L. infantum-A. reconditum* with 1.6% (2/123) for
252 each of them and finally, co-infection with *E. canis* and *B. vogeli* with only one case (0.8%).
253 Triple infection with *A. reconditum-D. immitis-D. repens* were observed in two dogs. One of
254 them was positive by modified Knott's test and the triplex qPCR for the three species. The
255 other one was positive for *A. reconditum* and *D. repens* but negative for *D. immitis* for both
256 tests. By contrast, it was positive by qPCR *ftsZ* for *Wolbachia* endosymbiont of *D. immitis*,
257 suggesting an occult infection by *D. immitis*.

258 There was no significant association between sex, age, breed and the presence of vector-borne
259 dog infections in this study ($PV > 0.05$), except for filariasis for which mongrel dogs were
260 more susceptible ($PV = 0.02$). By contrast, dogs from Kongouanou (village dogs) were found
261 to be more infected 60.7% (17/28) than dogs from Abidjan (kennel dogs) 32.6% (31/95) for at
262 least one pathogen ($PV = 0.003$). Also, Filariidae infections were more frequent in the village
263 dogs 35.7% (10/28) than the kennel dogs 3.2% (3/95) ($PV = 0.0000008$) (Table 3).

264 **3.3. Phylogenetic analysis results**

265 Positive samples for vector-borne pathogens were sequenced. DNA sequences of all positive
266 PCR products were ≥ 99 – 100 % homologous to those available in the GenBank database for

267 all CVB pathogens identified, except the obtained sequences for *Leishmania* kDNA. For
268 Anaplasmataceae, after sequencing the 485 bp-long amplicons of the 23S rRNA gene portion,
269 BLAST analysis was conclusive for *E. canis* for all sequences obtained with 99-100% identity
270 with *E. canis* (KM021429) from Algeria and (NR 076375) from the USA. *L. infantum* ITS 2
271 obtained sequences (420 bp) were 100% identical to published sequences (KU680956,
272 KU680957, KU680958, KU680959 and KU680960). *Leishmania* kDNA sequence (126-128
273 bp) exhibited 96-97% identity with *L. infantum* isolate MHOM/CN/80/D2 from China
274 (Z35292). In addition, *T. congolense* ITS1 sequences (620-630 bp) exhibited 99% identity
275 with published sequence MG283145. *B. vogeli* 18S rRNA obtained sequences (865 bp), in
276 this study, were 99% identical to published sequence (LC331058, MG586234). For Filariidae,
277 18S sequences for *A. reconditum* is not available in the GenBank database. By contrast, the
278 sequences found for *D. repens* had 100% of identity with (AB973229) published sequence.
279 We could not sequence the positive sample for *D. immitis* because it was co-infected with
280 other species. Phylogenetic trees are built on the basis of these genes and show the position of
281 the identified species (Fig. 3).

282 Sequences were submitted in the GenBank database under accession numbers: MK494932-
283 MK494941 for *E. canis*, MK481041- MK481044 for *L. infantum* ITS2 sequences,
284 MK770156- MK770158 for *L. infantum* kDNA sequences, MK495745- MK495747 for *T.*
285 *congolense*, MK495836-MK495837 for *Babesia vogeli*, MK495727-MK495733 for *A.*
286 *reconditum*, MK495734 and MK495735 for *D. repens*.

287

288 4. Discussion

289 This study describes canine vector-borne infections in dogs from Côte d'Ivoire. Our results
290 showed that pathogens such as *E. canis*, *L. infantum*, *T. congolense*, *B. canis vogeli*, *A.*
291 *reconditum*, *D. repens* and *D. immitis* are endemic in this area.

292 The Anaplasmataceae family includes several pathogens associated with dogs; they are
293 reported worldwide and transmitted by *Ixodidae* ticks. [1]. In this study, we found a
294 prevalence of 17.1% (21/123) by qPCR 23S. This is not new, as this region is known to be
295 endemic [5]. Sequencing analysis reported the presence of one species, *E. canis*, the causative
296 agent of monocytic ehrlichiosis. We also noted a seroprevalence of 12.2% (15/123) for *E.*
297 *canis* by IFAT. It was not possible to obtain sequences for all qPCR-positive samples.
298 qPCR-positive samples not sequenced and not positive by IFAT for *E. canis* could be another
299 Anaplasmataceae sp. not *E. canis*. In 2003, about 74% of the 76 dogs in Abidjan and 84%
300 (93/111) of the dogs in Gabon were seropositive [26]. Also, 27% of *R. sanguineus* s.l. ticks
301 collected (16/60) from dogs in Abidjan, were positive for *E. canis* using quantitative real-time
302 PCR [27]. Since then, we believe that there is a decreasing in the prevalence due to the
303 awareness of veterinarians and dog owners in the. But the area remains endemic because the
304 dog's state of life promotes transmission and persistence of infection (presence of ticks) and
305 not all dog owners are able to treat and protect their dogs. We did not find *Anaplasma platys*,
306 the agent of canine cyclic thrombocytopenia. However, prevalence of 8.5 and 56.2% has
307 already been reported in Ivorian dogs and ticks collected on these dogs, respectively [28].

308 Data about canine leishmaniosis in Côte d'Ivoire are absent. To the best of our knowledge,
309 our study is the first to report *L. infantum* in dogs from this area. The prevalence observed is
310 15.4% (19/123) by IFAT. We have developed a qPCR test with a wide specificity
311 (*Leishmania* spp.) capable of detecting the main known pathogens of *Leishmania* species such

312 as *L. donovani*, *L. infantum*, *L. major*, *L. guyanensis* as well as probable new species. Only
313 4% (5/123) were positive for PCR, which differs significantly (PV=0.0026) with serological
314 results. This is not surprising since PCR assays on blood samples often provide less positive
315 results for leishmaniosis than for other sites (bone marrow and lymph node) [27, 28], hence
316 the choice of IFAT as gold standard in this study. We achieved species identification by the
317 amplification from the qPCR-positive samples ITS2 gene (Table1) and kinetoplast gene. This
318 was conclusive for *L. infantum*. All positive dogs were apparently healthy. The level of
319 parasite load found is not important (amount 29 parasite/ mL blood), which may explain an
320 asymptomatic carriage stage. Cases of human visceral leishmaniasis were reported in sub-
321 Saharan countries such as Côte d'Ivoire and Gambia [29, 30], but these reports remain
322 anecdotal with no evidence of an autochthonous transmission cycle. Three human cases of
323 visceral leishmaniasis were observed in a cohort of 528 patients in Abidjan (2001–2002)
324 [11]. The detection of *L. infantum* in local dogs (15.4%) provides evidence that this region is
325 endemic for CanL and indicates the possible involvement of domestic dogs in the human
326 infection. Classically, sand flies involved in the *Leishmania* transmission belong either to the
327 *Phlebotomus* genus (Old World) or to the *Lutzomyia* genus (New World). The sand fly
328 species involved in *L. infantum* transmission cycle in Côte d'Ivoire are still unknown and
329 need further investigations. The results obtained in Senegal, with the molecular confirmation
330 of *L. infantum* species, high seropositivity in 160 dogs (46.3%) and 133 humans (33.3%) [9],
331 the high rate of *L. infantum* on female sand fly species (5.4% of *Sergentomyia dubia*, 4.2% of
332 *S. schwetzi* and 3.6% of *S. magna*) [31] strongly suggests that the *L. infantum* life cycle is
333 well established in this area. In addition, this raises further questions about vectors in sub-
334 Saharan regions and challenges once again the dogma that leishmaniasis is exclusively
335 transmitted by the genus *Phlebotomus* in the Old World. In Burkina-Faso, CanL prevalence is
336 about 5.9% (5/85) [10]. The disease has mainly been described in the north of the African

337 continent, range of results reported for some countries in the Mediterranean Basin using
338 IFAT: Algeria: 27-45% [32], Tunisia 18-53% [35, 36], Morocco 9-19% [37, 38].

339 To perform molecular screening of *Trypanosoma* spp. we have developed a new qPCR
340 system to detect all *Trypanosoma* species. This molecular tool seems to be sensitive and
341 specific and allowed us to found a prevalence of 5.3% (5/95) in dogs from Abidjan. In 2003,
342 Keck et al. reported a prevalence of 30 % in 123 dogs from kennels of Abidjan by using PCR
343 specific primers for *T. congolense* "forest type" with no difference between age, class, breed
344 and sex [13]. The fact that we found fewer infected dogs may be due to the sensitization of
345 local veterinarians against this disease. *T. congolense* "forest type" has been found in other
346 host animals (sheep, goats, cattle, pigs) in Côte d'Ivoire and other forest areas [37]. However,
347 we did not detect this trypanosome. ITS 1 gene sequencing showed 99% of identity with *T.*
348 *congolense* isolate 237-51-00095-1-60-10 (GenBank: MG283145) identified in *Glossina*
349 *palpalis palpalis* in Cameroon (Fig. 2). In Nigeria, nineteen dogs presenting with canine
350 African trypanosomosis were infected with trypanosomes of the *T. brucei* group [38]. *T.*
351 *congolense* is cyclically transmitted by tsetse flies (*Diptera: Glossinidae*). Trypanosomosis
352 caused by *T. congolense* is a major threat to livestock production in Sub-Saharan Africa.
353 Infected dogs can revealed pale mucous membranes, weak pulse, enlarged lymph nodes,
354 rough hair coat and loss of skin elasticity [39].

355 We found a prevalence of 1.6% (2/123) infected by *B. canis vogeli*. To our knowledge, this is
356 the first detection of this pathogen in Côte d'Ivoire. This species has a global distribution [40]
357 and has been identified in Africa [17][41]. The presence of ticks *Rhipicephalus sanguineus*
358 s.l., the main vector of *B. canis vogeli* [42] on dogs in Côte d'Ivoire [27] may explain our
359 results.

360 Concerning Filariidae, 10.6% (13/123) of dogs were infected by at least one species. *A.*
361 *reconditum* was the most detected by modified Knott's test and the triplex qPCR (13/123).
362 Obtained sequences for 18S rRNA gene showed 99% identity with *Acanthocheilonema viteae*
363 and *Loa loa* (DQ094171, XR_002251421). No sequence for the 18S rRNA gene of *A.*
364 *reconditum* is available in GenBank. *A. reconditum* has a worldwide distribution [19][21]. In
365 Liberia, 56 of 137 dogs (40.9%) and about 9.2% of Nigerian dogs were found positive for
366 microfilaria of *A. reconditum* from morphological identification, 8.8% in South Africa and
367 Maputo province, Mozambique [21]. *A. reconditum* is generally considered non-pathogenic
368 for dogs, it lives in the peritoneal cavity and adipose tissue [43]. Recently, it has been reported
369 to induce skin lesions with atopic allergy in a dog [44]. This parasite is transmitted by fleas
370 and chewing lice [22].

371 We found 13.8% (17/123) of dogs with two or three pathogens and *E. canis*-*L. infantum*
372 association was the most observed 4.9% (6/123) co-infection. Co-infections in dogs is
373 common. It is known that infection of the dog with more than one pathogen is associated with
374 high pathogenicity [45]. The main cases observed are co-infections of dogs with tick-borne
375 pathogens, including *E. canis*, *B. vogeli*, *A. platys* and *Hepatozoon canis*, that increase anemia
376 for example, more than mono-infections [46]. Co-infection *L. infantum*-*A. reconditum* has
377 been found in two dogs (1.6%). Co-infections *L. infantum* with filariidae are known. In
378 Portugal, 8.3 % of 230 dogs were infected with both *L. infantum* and *D. immitis* [47]. This co-
379 infection has also been observed in dogs from Greece [48]. This is relatively observed in dogs
380 living in areas where vectors competent for different pathogens coexist, which can complicate
381 the diagnosis, treatment and prognosis of CVBD [47].

382 In addition to these classical co-infections, we also noted co-infections of *E. canis*-*A.*
383 *reconditum* in 3.3% (4/123) and *L. infantum*-*T. congolense* in two dogs. Two dogs had triple
384 infection, one of them was positive for the three filaroid species by modified Knott's test and

385 the triplex Cox1-based qPCR, the other was positive for *A. reconditum* and *D. repens* by these
386 same tools and negative for *D. immitis*, but it was positive on qPCR targeting *ftsZ* gene for
387 *Wolbachia* endosymbiont for *D. immitis*, thus suggesting an occult infection. *D. immitis* has a
388 severe impact on veterinary medicine, because of the heartworm disease threatening dogs and
389 cats, whereas *D. repens*, causing subcutaneous infestation in dogs, is the main agent of the
390 human dirofilariosis [49]. *D. immitis* has been reported from Guinea-Bissau, Nigeria, Senegal,
391 Sierra-Leone and in Central Africa (Angola, Cameroon and Gabon). In addition, it has been
392 reported that *D. repens* is the most common filarial worm in Nigerian dogs and cats. This
393 species is also endemic in Central and Eastern Africa [21]. All these co-infections can lead to
394 overlaps or atypical clinical signs. They should be expected in dogs living in areas that are
395 highly endemic for several vector-borne pathogens, mainly in dogs kept predominantly
396 outdoors and not regularly treated with ectoparasitides [50]. The prevalence of CVBD varied
397 according to the dog's origin and Kongouanou dogs were more infected than those in Abidjan.
398 They also had more filaroid infections. These dogs live freely in the open air in the village
399 and its surroundings. They do not receive preventive veterinary care and most of them were
400 tick carriers, compared to kennel dogs, which receive veterinary care and sometimes external
401 pest control.

402 **5. Conclusion**

403 To the best of our knowledge, this study is the first to report the prevalence and diversity of
404 vector-borne pathogens of humans and animals in dogs from Côte d'Ivoire. Co-infections
405 with more than one pathogen were found to be common. For the first time, we highlighted the
406 presence of *L. infantum*, *B. vogeli*, *A. reconditum*, *D. immitis* and *D. repens* in this region.
407 This last remains endemic for *E. canis* and *T. congolense*. Ivorian veterinarians should be
408 informed of these results in order to be able to detect clinical cases of canine leishmaniasis
409 and make diagnoses using laboratory tests. This epidemiological situation also requires the
410 implementation of a national program to control vector-borne diseases, especially for zoonotic
411 agent such *Leishmania infantum*, the agent of human visceral leishmaniasis.

412

413 **Abbreviations**

414 Bp: Base pair; CanL: Canine leishmaniosis; CME: Canine monocytic ehrlichiosis; Ct: Cycle
415 threshold; CVBD: Canine vector-borne diseases; EDTA: Ethylenediaminetetraacetic acid;
416 IFAT: Immunofluorescence antibody test; PCR: Polymerase chain reaction; PV: P-value;
417 qPCR: Quantitative PCR.

418 **Supporting Information**

419 **Table S1. Positive and negative** control DNA used for sensitivity and specificity
420 determination of designed oligonucleotides.

421 **Conflict interests**

422 The authors declare that they have no competing interests.

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598 **Legends:**

599 **Fig 1.** Location of the study: down Abidjan kennel dogs, up dogs from Kongouanou village

600 (A. Côte d'Ivoire map,

601 https://commons.wikimedia.org/wiki/Atlas_of_C%C3%B4te_d%27Ivoire#/media/File:C%C3

602 [%B4te_d%27Ivoire_Map.jpg](https://commons.wikimedia.org/wiki/Atlas_of_C%C3%B4te_d%27Ivoire#/media/File:C%C3%B4te_d%27Ivoire_Map.jpg); B. Copyright pictures: B. Davoust)

603 **Fig. 2** Mixed infections found in dogs surveyed in the present study. The legends represent:

604 Coinfection and its prevalence; number of dogs presented this coinfection; frequency of the

605 coinfection according to the sum of coinfections (N= 17).

606 **Fig. 3** Phylogenetic trees highlighting the position of *Ehrlichia* spp. (A), *Leishmania* spp. (B

607 and C), *Trypanosoma* spp. (D) and *Babesia* spp. (E) and filaroid species (F) identified in the

608 present study compared to other sequences available on GenBank. The sequences for 23S (A),

609 ITSII and kDNA (B and C), ITSI (D) and 18S (E) and 18S (F) genes, for each of the six trees,

610 were aligned using CLUSTALW, and phylogenetic inferences was conducted in MEGA 7

611 using the maximum likelihood method based on the Tamura-Nei model for nucleotide

612 sequences. . Initial tree(s) for the heuristic search were obtained automatically by applying

613 Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the

614 Maximum Composite Likelihood (MCL) approach, and then selecting the topology with

615 superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the

616 number of substitutions per site. All positions containing gaps and missing data were

617 eliminated. The GenBank accession numbers are indicated at the beginning. Statistical

618 support for the internal branches of the trees was evaluated by bootstrapping with 1,00

619 iterations. Bootstrapping under 40 were removed.

620 **Table 1** Sequences of primers used for species detection and identification in this study

Targeted microorganisms	Target gene	Name	Primers (5'-3') and probe	Tm	References	
<i>Anaplasmataceae</i>	23S	TtAna-F	TGACAGCGTACCTTTTGCAT	-	[4]	
		TtAna-R	GTAACAGGTTTCGGTCCTCCA			
		TtAna-S*	FAM-CTTGGTTTTCGGTCTAATCC-TAMRA			
		Ana23S-212f	GTTGAAAARACTGATGGTATGCA	55°C		
		Ana23S-753	TGCAAAAAGGTACGCTGTCAC			
<i>Leishmania</i> spp.	18S	Leish. F	GGTTTAGTGCCTCCGGTG	-	This study	
		Leish. R	CGGCCATAAGATCC CCAA			
		Leish. P*	FAM-CGGCCGTAACGCCTTTTCAACTCA -TAMRA			
		Leish. F1	CTGTGACTAAAGAAGCGTGAC	52°C		This study
		Leish. R1	AGGCCGAATAGAAAAGATACGT			
	kDNA	RV1	CTTTTCTGGTCTCCGGGTAGG	-	[25]	
		RV2	CCACCCGGCCCTATTTTACACCAA			
		Probe. Leish*	FAM-TTTTCGCAGAACGCCCTACCCGC-TAMRA			
	ITS II	LGITSF2	GCATGCCATATTCTCAGTGTC	60°C	[52]	
		LGITSR2	GGCCAACGCGAAGTTGAATTC			
	kDNA	RV1	CTTTTCTGGTCTCCGGGTAGG	59°C	[54]	
		RV2	CCACCCGGCCCTATTTTACACCAA			
	<i>Trypanosoma</i> spp.	5.8S	Tryp 5.8SF	CAACGTGTCGCGATGGATGA	-	This study
Tryp 5.8SR			ATTCTGCAATTGATACCACTTATC			
Tryp 5.8S P*			FAM-GTTGAAGAACGCAGCAAAGGCGAT-TAMRA			
ITS I		ITS1-CF	CCGGAAGTTCACCGATATTG	58°C	[51]	
		ITS1-BR	TTGCTGCGTCTTCAACGAA			
<i>Piroplasmida</i>	5.8S	5.8S-F5	AYYKTYAGCGRTGGATGTC		[53]	
		5.8S-R	TCGCAGRAGTCTKCAAGTC			
		5.8S-S*	FAM-TTYGCTGCGTCCTTCATCGTTGT-MGB			
	18S	piro18S-F1	GCGAATGGCTCATTAAACA	58°C		
		piro18S-R4	TTTCAGMCTTGCGACCATACT			

621 Abbreviations: Tm: Annealing temperature; *: Probe.

622 **Table 2** Prevalence of the vector-borne pathogens detected by serology or PCR and risk factors

Risk Factor		No.	<i>Anaplasmataceae</i>				<i>Leishmania</i> spp.				<i>Trypanosoma</i> spp.			<i>Piroplasma</i>		<i>Filariidae</i>						
			IFAT <i>Ehrlichia</i>	<i>PV</i>	qPCR 23S	<i>PV</i>	IFAT	<i>PV</i>	qPCR 18S and kDNA	qPCR 5.8S	<i>PV</i>	qPCR 5.8S	<i>PV</i>	qPCR Cox1	<i>PV</i>							
Region	Abidjan	95	14	14.7	*0.18	18	18.9	*0.4	15	15.8	*1.00	4	4.2	5	5.3	*0.58	1	1.0	*0.4	3	3.2	* 0.0000008
	Kongouanou	28	1	3.6		3	10.7		4	14.3		1	3.6	0	0		1	3.6		10	35.7	
	Total	123	15	12.2		21	17.1		19	15.4		5	4.1	5	4.1		2	1.6		13	10.6	
Age (year)	[0-2]	49	4	8.2	0.47	9	18.4	0.58	7	14.3	0.36	2	4.1	0	0	0.12	2	4.1	0.38	6	12.2	0.72
	[2-4]	33	6	18.2		7	21.2		8	24.2		2	6.1	2	6.1		0	0		3	9.1	
	[4-6]	26	4	15.4		7	26.9		3	11.5		0	0	1	3.8		0	0		3	11.5	
	>6	15	1	6.7		1	6.7		1	6.7		1	6.7	2	13.3		0	0		1	6.7	
Gender	Female	32	2	6.2	*0.35	9	28.1	0.16	8	25	0.08	0	0	0	0	*0.32	1	3.1	*0.45	4	12.5	* 0.45
	Male	91	13	14.3		15	16.5		11	12.1		5	5.5	5	5.5		1	1.1		9	9.9	
Breed	Berger	33	3	9.1	0.36	4	12.1	0.9	5	15.1	0.86	2	6.1	3	9.1	0.34	0	0	0.82	0	0	0.02
	Beauceron	9	2	22.2		2	22.2		1	11.1		1	11.1	0	0		0	0		0	0	
	Ridgeback	11	2	18.2		2	18.2		1	9.1		1	9.1	2	18.2		0	0		1	9.1	
	Rot	9	3	33.3		2	22.2		3	33.3		0	0	0	0		0	0		0	0	
	Labrador	4	0	0		1	25		1	25		0	0	0	0		0	0		0	0	
	Boer Bull	8	0	0		2	25		1	12.5		0	0	0	0		0	0		0	0	
	Dogue	5	1	20		1	20		1	20		0	0	0	0		0	0		0	0	
	Mongrel	44	4	9.1		10	22.7		6	13.6		1	2.3	0	0		2	4.5		12	27.3	

623 **Abbreviations**

624 IFAT: immunofluorescence antibody test; *PV*: P-value, *PV*> 0.05 means that the observed differences between prevalence is not statically
 625 significant; *: Fisher’s exact test.

626 **Table 3** Single and mixed infection of canine vector-borne pathogens

Infection	Kennel dogs n=95 (Abidjan)		Village dogs n=28 (Kongouanou)		Total N=123	
	No. positive	%	No. positive	%	No. positive	%
Single infection	20	21.1	11	39.3	31	25.2
<i>Ehrlichia canis</i>	10	10.5	2	7.1	12	9.8
<i>Leishmania infantum</i>	7	7.4	4	14.3	11	8.9
<i>Trypanosoma congolense</i>	2	2.1	0	0.0	2	1.6
<i>Acanthocheilonema reconditum</i>	1	10.5	4	14.3	5	4.1
<i>Babesia vogeli</i>	0	0.0	1	3.6	1	0.8
Mixed infection	11	11.6	6	21.4	17	13.8
<i>E. canis</i> + <i>L. infantum</i>	6	6.3	0	0.0	6	4.9
<i>E. canis</i> + <i>B. vogeli</i>	1	1.1	0	0.0	1	0.8
<i>E. canis</i> + <i>A. reconditum</i>	0	0.0	4	14.3	4	3.3
<i>L. infantum</i> + <i>T. congolense</i>	2	2.1	0	0.0	2	1.6
<i>L. infantum</i> + <i>A. reconditum</i>	0	0.0	2	7.1	2	1.6
<i>A. reconditum</i> + <i>D. immitis</i> + <i>D. repens</i>	2	2.10	0	0.0	2	1.6
Total positive	31	32.6	17	60.7	48	39
Total negative	64	67.4	11	39.3	75	61

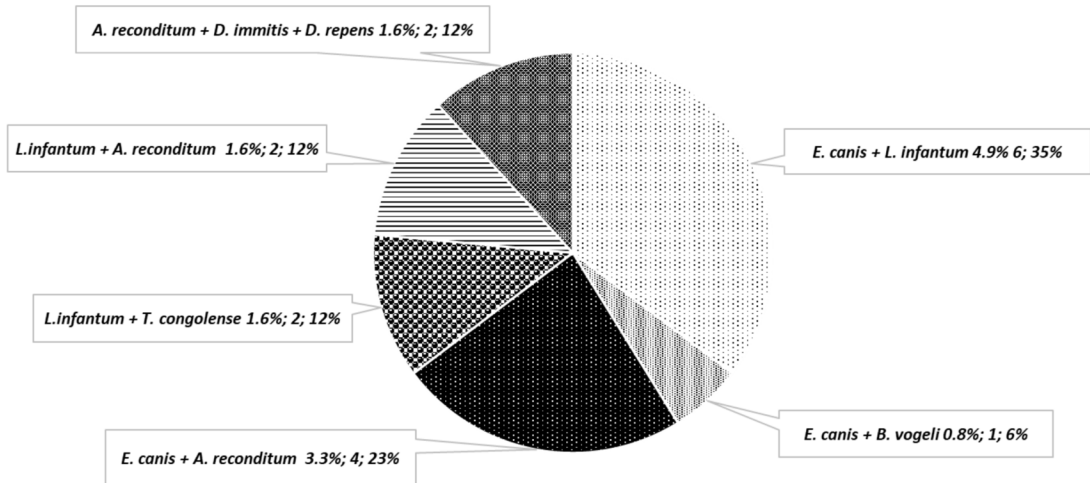
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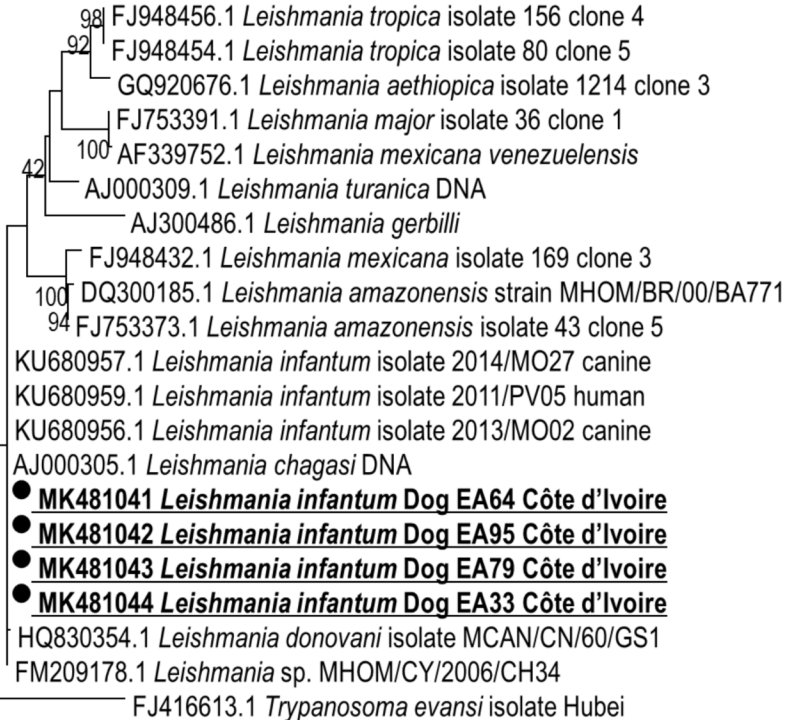
A

B

Mixed infection

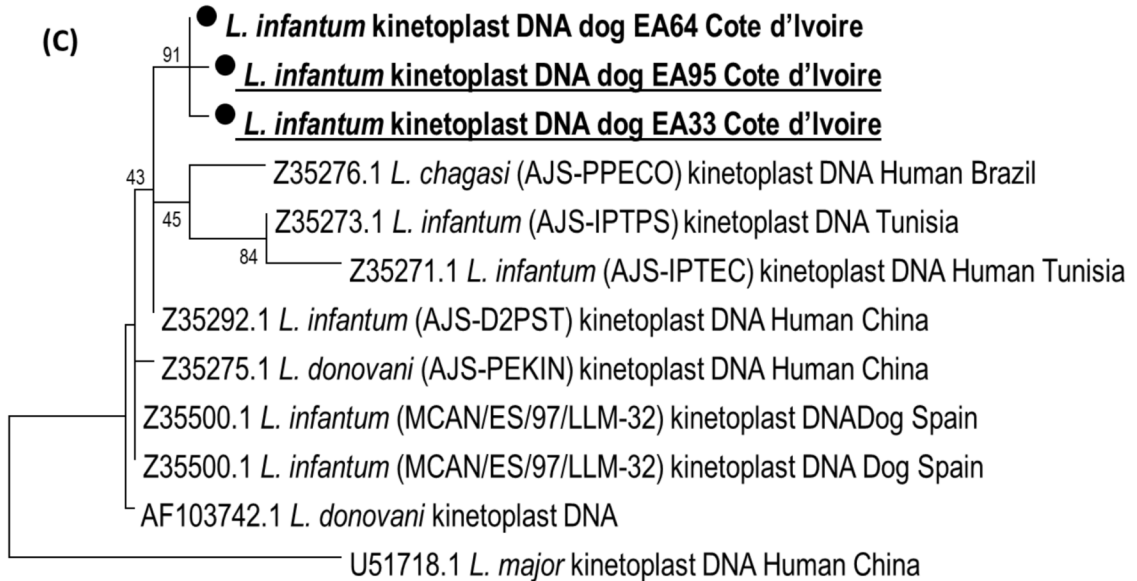


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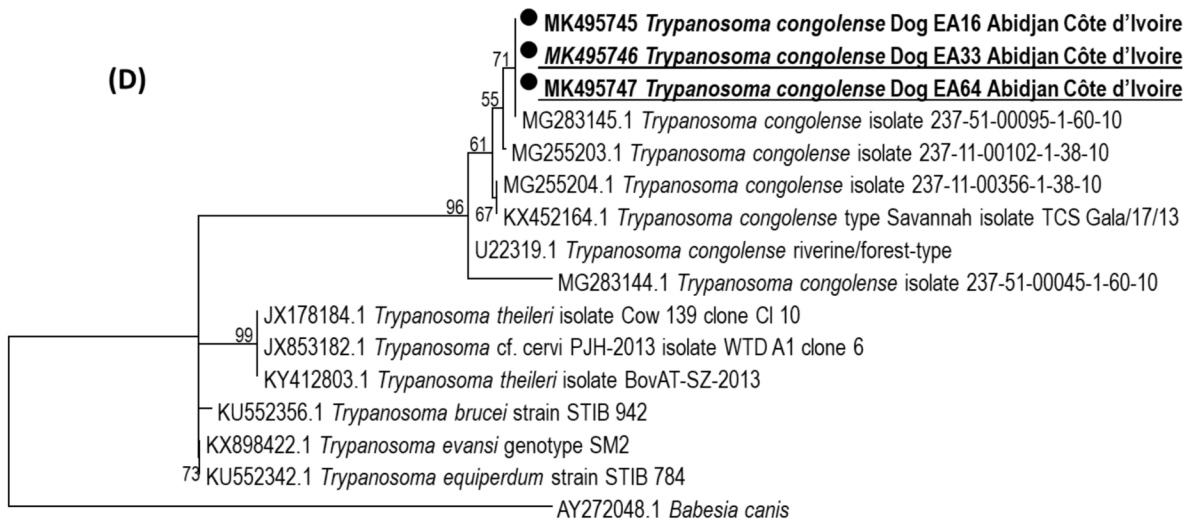
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(c)



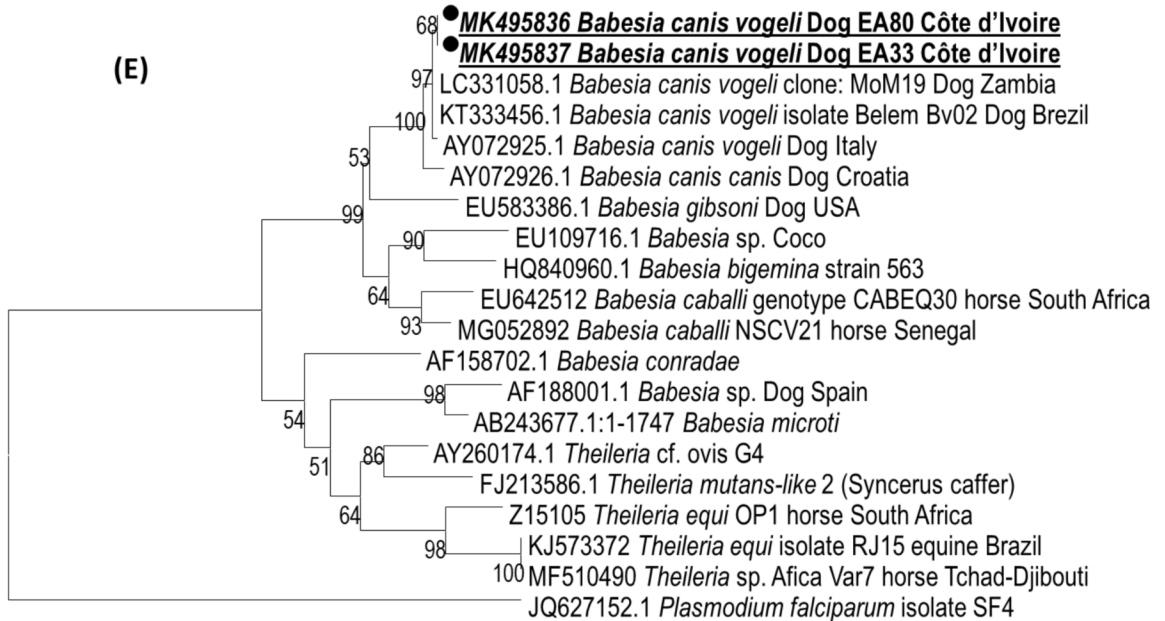
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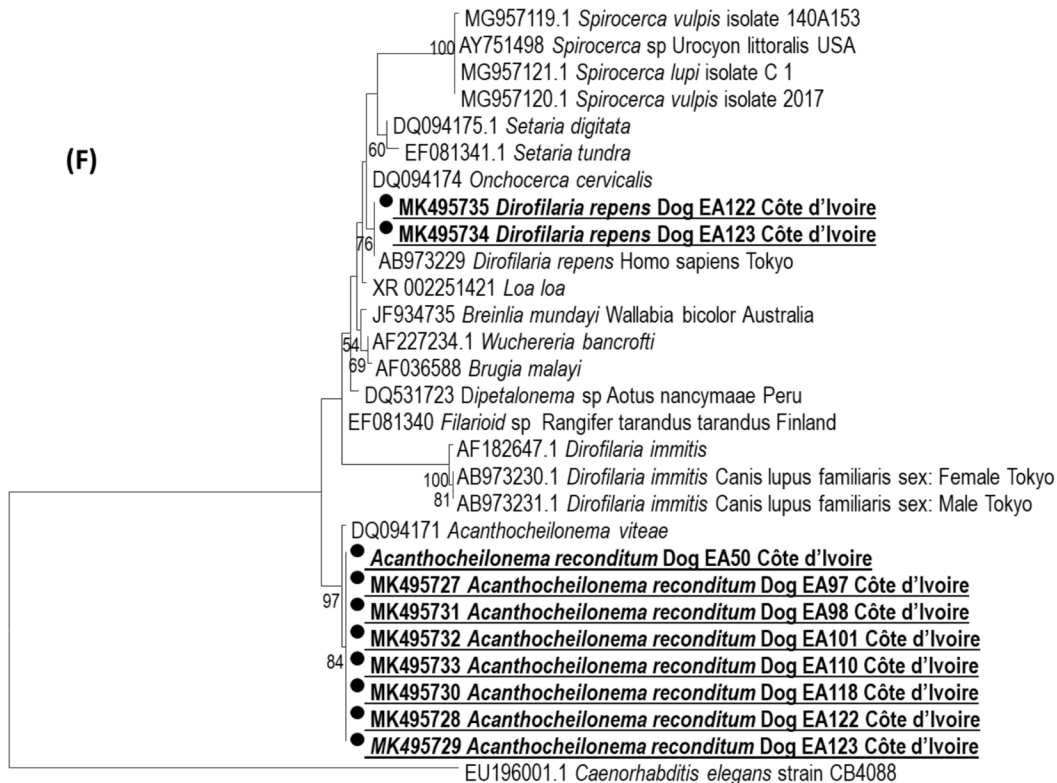
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(E)



0.02

(F)



0.02