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Abstract

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

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

1. Introduction

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

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

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

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

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

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

In Côte d'Ivoire, few information is available on the prevalence and the distribution of CVBD. The purpose of this study was to evaluate the occurrence of these pathogens of veterinary and public (for some of them) health importance and their diversity in dogs from the city of Abidjan (kennel dogs) and in a city near Yamoussoukro (village dogs) by using 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 isothiacyanate (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***

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

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

2.4.3. Molecular screening for the presence of pathogens DNA

All the DNA samples were screened for *Leishmania* spp. using two qPCR assays: i) the 18S-based qPCR was used for the initial screening and the detection of DNA *Leishmania* spp., ii) the specific *L. infantum* qPCR assay based on the amplification of kinetoplast minicircle DNA (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 du 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 phylogenic 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, Hoerdt, 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.

3. Results

3.1.Sensitivity and specificity of PCR assays

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

3.2.Serological and molecular results

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

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

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

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

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

3.3.Phylogenetic analysis results

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

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

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

4. Discussion

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

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

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

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

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

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

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

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

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

In addition to these classical co-infections, we also noted co-infections of *E.canis-A. reconditum* in 3.3% (4/123) and *L. infantum–T. congolense* in two dogs. Two dogs had triple 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 ectoparacitides [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.

5. Conclusion

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

Abbreviations

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

Supporting Information

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

Conflict interests

The authors declare that they have no competing interests.

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Legends:

Fig 1. Location of the study: down Abidjan kennel dogs, up dogs from Kongouanou village (**A.** Côte d'Ivoire map, 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)

Fig. 2 Mixed infections found in dogs surveyed in the present study. The legends represent: Coinfection and its prevalence; number of dogs presented this coinfection; frequency of the coinfection according to the sum of coinfections (N= 17).

Fig. 3 Phylogenetic trees highlighting the position of *Ehrlichia* spp. (A), *Leishmania* spp. (B and C), *Trypanosoma* spp. (D) and *Babesia* spp. (E) and filaroid species (F) identified in the present study compared to other sequences available on GenBank. The sequences for 23S (A), ITSII and kDNA (B and C), ITSI (D) and 18S (E) and 18S (F) genes, for each of the six trees, were aligned using CLUSTALW, and phylogenetic inferences was conducted in MEGA 7 using the maximum likelihood method based on the Tamura-Nei model for nucleotide sequences. . Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. The GenBank accession numbers are indicated at the beginning. Statistical support for the internal branches of the trees was evaluated by bootstrapping with 1,00 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
Anaplasmataceae	23S	TtAna-F	TGACAGCGTACCTTTTGCAT	-	[4]
		TtAna-R	GTAACAGGTTCGGTCCTCCA		
		TtAna-S*	FAM-CTTGGTTTCGGGTCTAATCC-TAMRA		
		Ana23S-212f	GTTGAAAARACTGATGGTATGCA	55°C	
		Ana23S-753	TGCAAAAGGTACGCTGTCAC		
Leishmania spp.	18S	Leish. F	GGTTTAGTGCGTCCGGTG	-	This study
		Leish. R	CGGCCCATAAGATCC CCAA		
		Leish. P*	FAM-CGGCCGTAACGCCTTTTCAACTCA -TAMRA		
		Leish. F1	CTGTGACTAAAGAAGCGTGAC	52°C	This study
		Leish. R1	AGGCCGAATAGAAAAGATACGT		
	kDNA	RV1	CTTTTCTGGTCCTCCGGGTAGG	-	[25]
		RV2	CCACCCGGCCCTATTTTACACCAA		
		Probe. Leish*	FAM-TTTTCGCAGAACGCCCCTACCCGC-TAMRA		
	ITS II	LGITSF2	GCATGCCATATTCTCAGTGTC	60°C	[52]
		LGITSR2	GGCCAACGCGAAGTTGAATTC		
	kDNA	RV1	CTTTTCTGGTCCTCCGGGTAGG	59°C	[54]
		RV2	CCACCCGGCCCTATTTTACACCAA		
Trypanosoma 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	TTGCTGCGTTCTTCAACGAA		
Piroplasmida	5.8S	5.8S-F5	AYYKTYAGCGRTGGATGTC		[53]
		5.8S-R	TCGCAGRAGTCTKCAAGTC		
		5.8S-S*	FAM-TTYGCTGCGTCCTTCATCGTTGT-MGB		
	18S	piro18S-F1	GCGAATGGCTCATTAIAACA	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.	*Anaplasmataceae*						*Leishmania* spp.						*Trypanosoma* spp.			*Piroplasma*			*Filariidae*		
			IFAT*Ehrlichia*		*PV*	qPCR 23S		PV	IFAT		*PV*	qPCR 18S and kDNA		*PV*	qPCR 5.8S		*PV*	qPCR 5.8S		*PV*	qPCR Cox1		*PV*
Region	Abidjan	95	14	14.7	*0.18	18	18.9	*0.4	15	15.8	*1.00	4	4.2	*0.58	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	0.12	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	*0.32	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	0.34	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: immunoflurescence 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

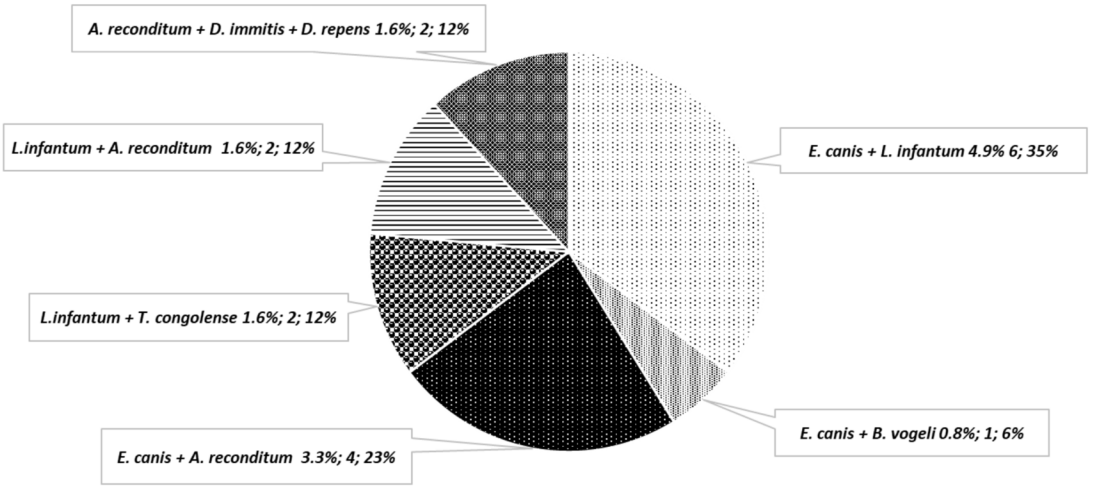
Infection	Kennel dogs (Abidjan) n=95		Village dogs (Kongouanou) n=28		Total N=123	
	No. positive	%	No. positive	%	No. positive	%
Single infection	**20**	**21.1**	**11**	**39.3**	**31**	**25.2**
Ehrlichia canis	10	10.5	2	7.1	12	9.8
Leishmania infantum	7	7.4	4	14.3	11	8.9
Trypanosoma congolense	2	2.1	0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

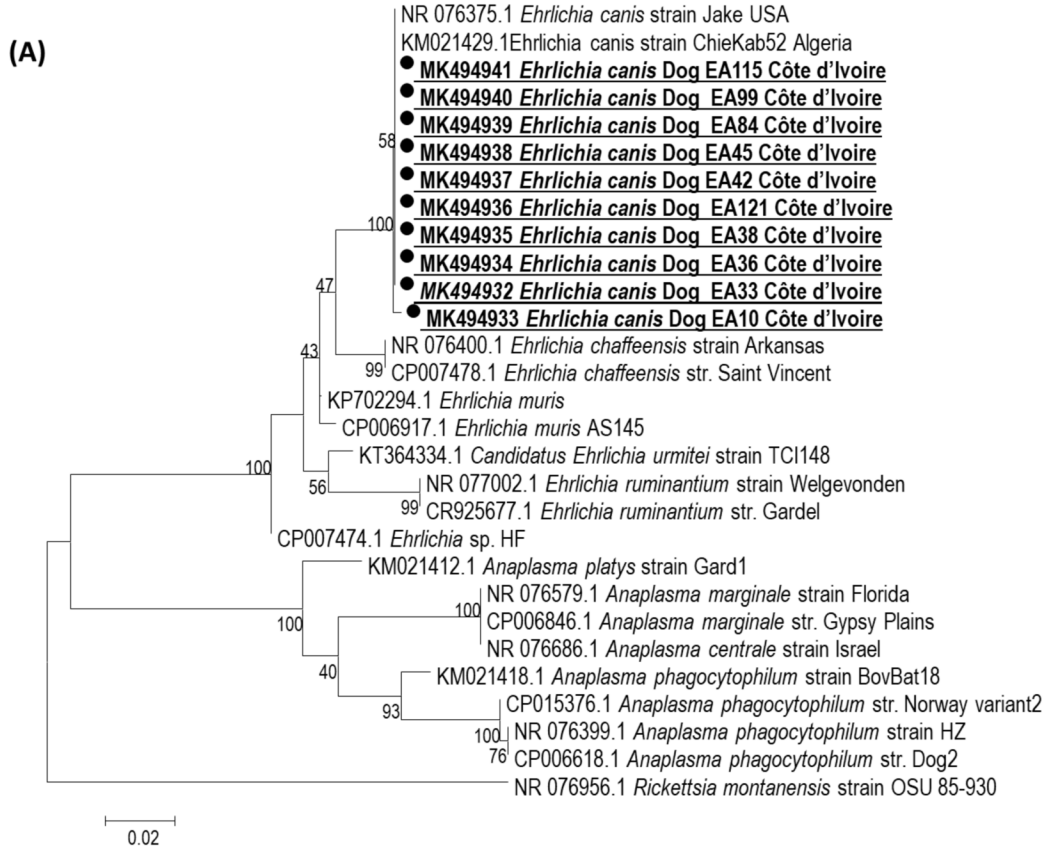
627


MALI
BURKINA FASO
GUINEA
LIBERIA
GHANA
SAVANES
DENGUELE
ZANZAN
VALLÉE DU BANDAMA
WORODOUGOU
BAFING
DIX-HUIT MONTAGNES
HAUT-SASSANDRA
MARAHOUÉ
FROMAGER
MOYEN-CAVALLY
SUD-BANDAMA
BAS-SASSANDRA
LACS
COMOE
MOYEN-COMOE
N'ZI
LAGUNES
AGNEBY
Gulf of Guinea
Côte d'Ivoire
A


B

Mixed infection

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KM021429.1Ehrlichia canis strain ChieKab52 Algeria
MK494941 Ehrlichia canis Dog EA115 Côte d'Ivoire
MK494940 Ehrlichia canis Dog EA99 Côte d'Ivoire
MK494939 Ehrlichia canis Dog EA84 Côte d'Ivoire
MK494938 Ehrlichia canis Dog EA45 Côte d'Ivoire
MK494937 Ehrlichia canis Dog EA42 Côte d'Ivoire
MK494936 Ehrlichia canis Dog EA121 Côte d'Ivoire
MK494935 Ehrlichia canis Dog EA38 Côte d'Ivoire
MK494934 Ehrlichia canis Dog EA36 Côte d'Ivoire
MK494932 Ehrlichia canis Dog EA33 Côte d'Ivoire
MK494933 Ehrlichia canis Dog EA10 Côte d'Ivoire
NR 076400.1 Ehrlichia chaffeensis strain Arkansas
CP007478.1 Ehrlichia chaffeensis str. Saint Vincent
KP702294.1 Ehrlichia muris
CP006917.1 Ehrlichia muris AS145
KT364334.1 Candidatus Ehrlichia urmitei strain TCI148
NR 077002.1 Ehrlichia ruminantium strain Welgevonden
CR925677.1 Ehrlichia ruminantium str. Gardel
CP007474.1 Ehrlichia sp. HF
KM021412.1 Anaplasma platys strain Gard1
NR 076579.1 Anaplasma marginale strain Florida
CP006846.1 Anaplasma marginale str. Gypsy Plains
NR 076686.1 Anaplasma centrale strain Israel
KM021418.1 Anaplasma phagocytophilum strain BovBat18
CP015376.1 Anaplasma phagocytophilum str. Norway variant2
NR 076399.1 Anaplasma phagocytophilum strain HZ
CP006618.1 Anaplasma phagocytophilum str. Dog2
NR 076956.1 Rickettsia montanensis strain OSU 85-930
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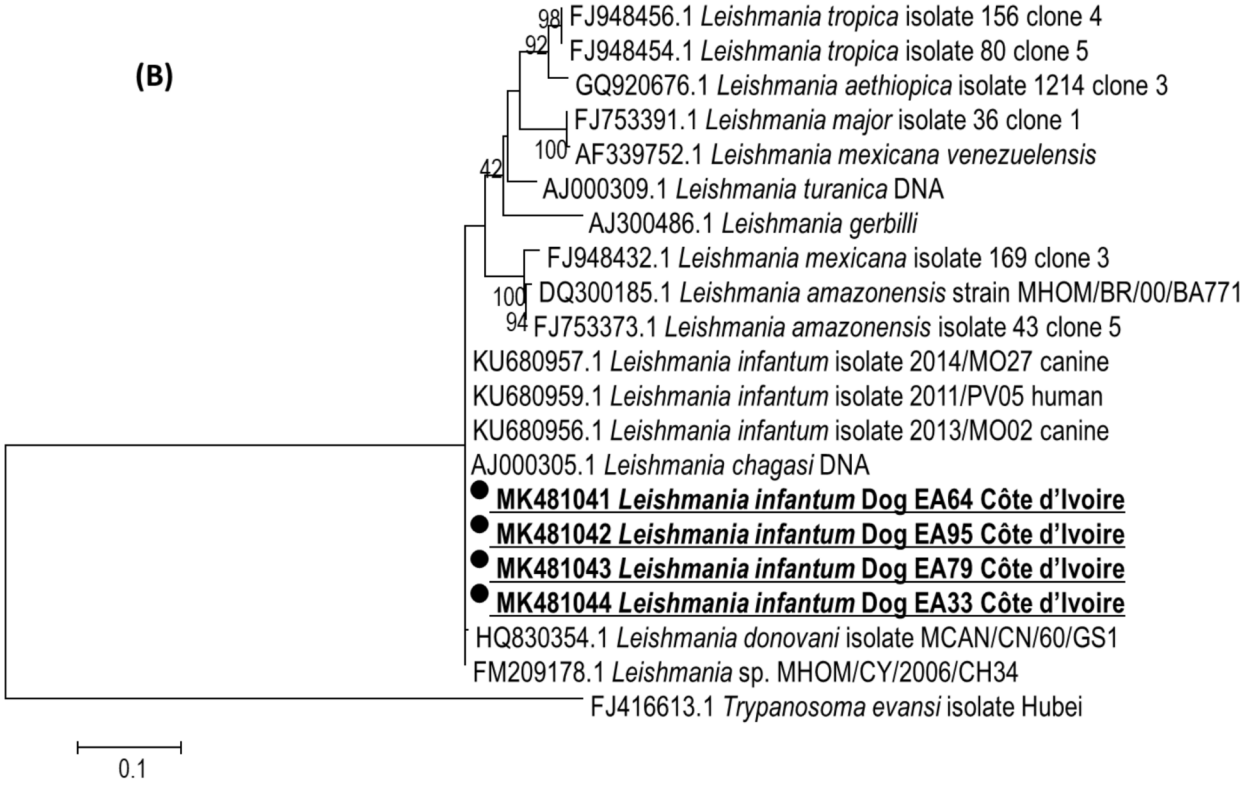
76
0.02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GQ920676.1 Leishmania aethiopica isolate 1214 clone 3
FJ753391.1 Leishmania major isolate 36 clone 1
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AF339752.1 Leishmania mexicana venezuelensis
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AJ000309.1 Leishmania turanica DNA
AJ300486.1 Leishmania gerbilli
FJ948432.1 Leishmania mexicana isolate 169 clone 3
100
DQ300185.1 Leishmania amazonensis strain MHOM/BR/00/BA771
94
FJ753373.1 Leishmania amazonensis isolate 43 clone 5
KU680957.1 Leishmania infantum isolate 2014/MO27 canine
KU680959.1 Leishmania infantum isolate 2011/PV05 human
KU680956.1 Leishmania infantum isolate 2013/MO02 canine
AJ000305.1 Leishmania chagasi DNA
MK481041 Leishmania infantum Dog EA64 Côte d'Ivoire
MK481042 Leishmania infantum Dog EA95 Côte d'Ivoire
MK481043 Leishmania infantum Dog EA79 Côte d'Ivoire
MK481044 Leishmania infantum Dog EA33 Côte d'Ivoire
HQ830354.1 Leishmania donovani isolate MCAN/CN/60/GS1
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FJ416613.1 Trypanosoma evansi isolate Hubei
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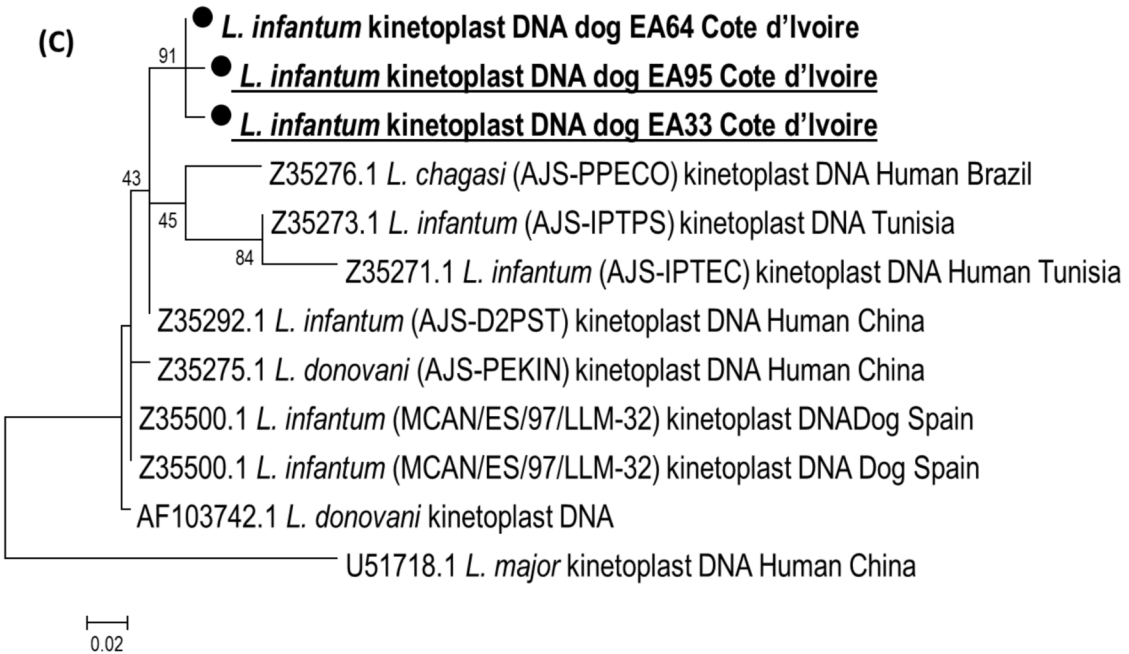
(C)
L. infantum kinetoplast DNA dog EA64 Cote d'Ivoire
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L. infantum kinetoplast DNA dog EA95 Cote d'Ivoire
L. infantum kinetoplast DNA dog EA33 Cote d'Ivoire
43
Z35276.1 L. chagasi (AJS-PPECO) kinetoplast DNA Human Brazil
45
Z35273.1 L. infantum (AJS-IPTPS) kinetoplast DNA Tunisia
84
Z35271.1 L. infantum (AJS-IPTEC) kinetoplast DNA Human Tunisia
Z35292.1 L. infantum (AJS-D2PST) kinetoplast DNA Human China
Z35275.1 L. donovani (AJS-PEKIN) kinetoplast DNA Human China
Z35500.1 L. infantum (MCAN/ES/97/LLM-32) kinetoplast DNADog Spain
Z35500.1 L. infantum (MCAN/ES/97/LLM-32) kinetoplast DNA Dog Spain
AF103742.1 L. donovani kinetoplast DNA
U51718.1 L. major kinetoplast DNA Human China
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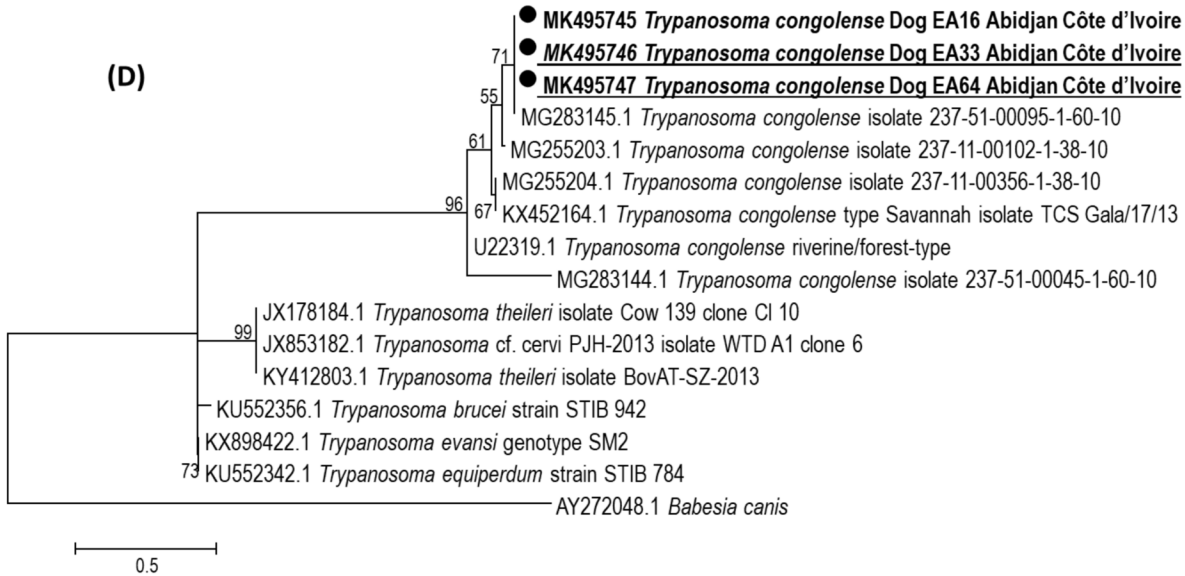
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MK495747 Trypanosoma congolense Dog EA64 Abidjan Côte d'Ivoire
MG283145.1 Trypanosoma congolense isolate 237-51-00095-1-60-10
MG255203.1 Trypanosoma congolense isolate 237-11-00102-1-38-10
MG255204.1 Trypanosoma congolense isolate 237-11-00356-1-38-10
KX452164.1 Trypanosoma congolense type Savannah isolate TCS Gala/17/13
U22319.1 Trypanosoma congolense riverine/forest-type
MG283144.1 Trypanosoma congolense isolate 237-51-00045-1-60-10
JX178184.1 Trypanosoma theileri isolate Cow 139 clone Cl 10
JX853182.1 Trypanosoma cf. cervi PJH-2013 isolate WTD A1 clone 6
KY412803.1 Trypanosoma theileri isolate BovAT-SZ-2013
KU552356.1 Trypanosoma brucei strain STIB 942
KX898422.1 Trypanosoma evansi genotype SM2
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96
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99
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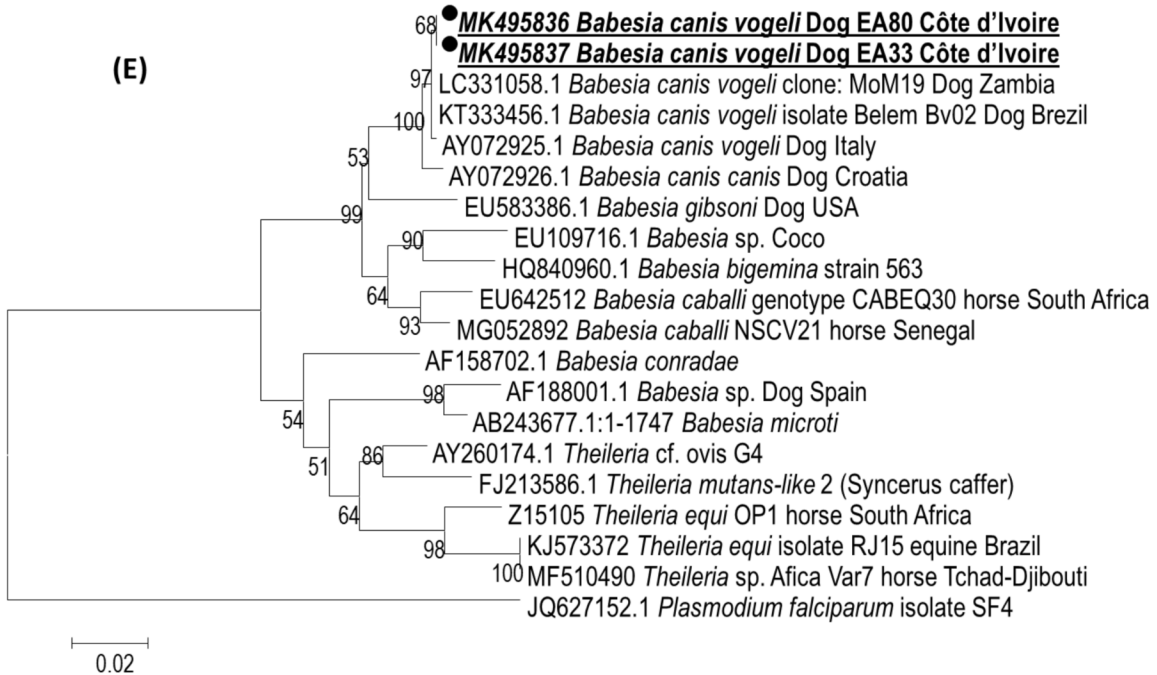
0.5


(E)
68
MK495836 Babesia canis vogeli Dog EA80 Côte d'Ivoire
MK495837 Babesia canis vogeli Dog EA33 Côte d'Ivoire
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LC331058.1 Babesia canis vogeli clone: MoM19 Dog Zambia
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KT333456.1 Babesia canis vogeli isolate Belem Bv02 Dog Brezil
53
AY072925.1 Babesia canis vogeli Dog Italy
AY072926.1 Babesia canis canis Dog Croatia
99
EU583386.1 Babesia gibsoni Dog USA
90
EU109716.1 Babesia sp. Coco
HQ840960.1 Babesia bigemina strain 563
64
EU642512 Babesia caballi genotype CABEQ30 horse South Africa
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MG052892 Babesia caballi NSCV21 horse Senegal
AF158702.1 Babesia conradae
98
AF188001.1 Babesia sp. Dog Spain
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AB243677.1:1-1747 Babesia microti
51
86
AY260174.1 Theileria cf. ovis G4
FJ213586.1 Theileria mutans-like 2 (Syncerus caffer)
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Z15105 Theileria equi OP1 horse South Africa
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KJ573372 Theileria equi isolate RJ15 equine Brazil
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MF510490 Theileria sp. Afica Var7 horse Tchad-Djibouti
JQ627152.1 Plasmodium falciparum isolate SF4
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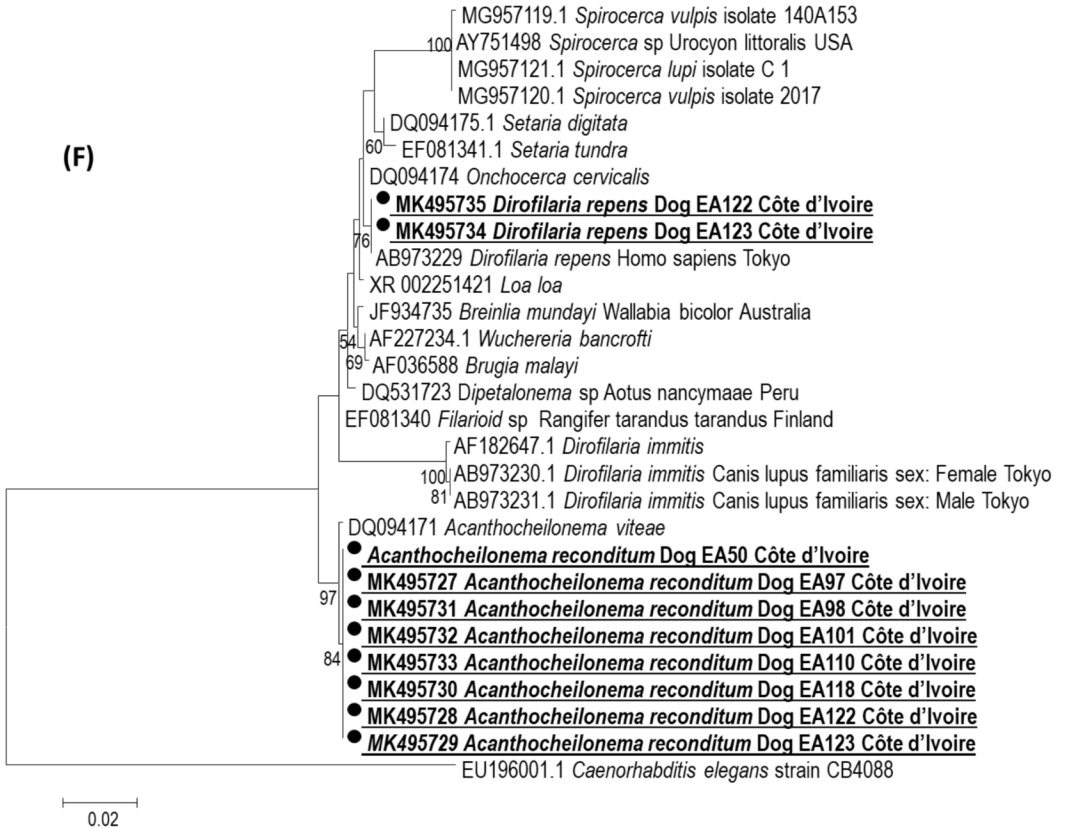
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AY751498 Spirocerca sp Urocyon littoralis USA
MG957121.1 Spirocerca lupi isolate C 1
MG957120.1 Spirocerca vulpis isolate 2017
DQ094175.1 Setaria digitata
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EF081341.1 Setaria tundra
DQ094174 Onchocerca cervicalis
MK495735 Dirofilaria repens Dog EA122 Côte d'Ivoire
MK495734 Dirofilaria repens Dog EA123 Côte d'Ivoire
76
AB973229 Dirofilaria repens Homo sapiens Tokyo
XR 002251421 Loa loa
JF934735 Breinlia mundayi Wallabia bicolor Australia
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AF227234.1 Wuchereria bancrofti
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AF036588 Brugia malayi
DQ531723 Dipetalonema sp Aotus nancymaae Peru
EF081340 Filarioid sp Rangifer tarandus tarandus Finland
AF182647.1 Dirofilaria immitis
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AB973230.1 Dirofilaria immitis Canis lupus familiaris sex: Female Tokyo
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AB973231.1 Dirofilaria immitis Canis lupus familiaris sex: Male Tokyo
DQ094171 Acanthocheilonema viteae
Acanthocheilonema reconditum Dog EA50 Côte d'Ivoire
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MK495727 Acanthocheilonema reconditum Dog EA97 Côte d'Ivoire
MK495731 Acanthocheilonema reconditum Dog EA98 Côte d'Ivoire
MK495732 Acanthocheilonema reconditum Dog EA101 Côte d'Ivoire
84
MK495733 Acanthocheilonema reconditum Dog EA110 Côte d'Ivoire
MK495730 Acanthocheilonema reconditum Dog EA118 Côte d'Ivoire
MK495728 Acanthocheilonema reconditum Dog EA122 Côte d'Ivoire
MK495729 Acanthocheilonema reconditum Dog EA123 Côte d'Ivoire
EU196001.1 Caenorhabditis elegans strain CB4088
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