



HAL
open science

Molecular characterization of some equine vector-borne diseases and associated arthropods in Egypt

H. Abdulla, Dina Aboelsoued, T. Fang, S. Abdel-Shaft, K. Megeed, Philippe Parola, Didier Raoult, Oleg Mediannikov, Hend H.A.M. Abdullah, Tarek Farag, et al.

► **To cite this version:**

H. Abdulla, Dina Aboelsoued, T. Fang, S. Abdel-Shaft, K. Megeed, et al.. Molecular characterization of some equine vector-borne diseases and associated arthropods in Egypt. *Acta Tropica*, 2022, *Acta Tropica*, 227, pp.106274. 10.1016/j.actatropica.2021.106274 . hal-03823751

HAL Id: hal-03823751

<https://amu.hal.science/hal-03823751>

Submitted on 8 Jan 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

15 **Abstract**

16 Equine vector-borne diseases (EVBDs) are emerging and re-emerging diseases, and most
17 of them are zoonotic. This study aimed to investigate EVBDs in equines and associated
18 arthropods (ticks and flies) from Egypt using molecular analyses, in addition to a preliminary
19 characterization of associated ticks and flies by the matrix-assisted laser desorption/ionization
20 time of flight (MALDI-TOF) and molecular techniques. In this study, 335 blood samples were
21 obtained from equines that appeared to be in good health (320 horses and 15 donkeys) in
22 Cairo and Beni Suef provinces, Egypt. From the same animals, 166 arthropods (105 sucking
23 flies and 61 ticks) were collected. Ticks and flies were preliminary characterized by the
24 MALDI-TOF and molecular tools. Quantitative PCR (qPCR) and standard PCR coupled with
25 sequencing were performed on the DNA of equines, ticks, and flies to screen multiple
26 pathogens. The MALDI-TOF and molecular characterization of arthropods revealed that louse
27 fly (*Hippobosca equina*) and cattle tick (*Rhipicephalus annulatus*) infesting equines.
28 *Anaplasma platys*-like (1.6%), *Anaplasma marginale* (1.6%), *Candidatus Ehrlichia rustica*
29 (6.6%), a new *Ehrlichia* sp. (4.9%), and *Borrelia theileri* (3.3%) were identified in *R.*
30 *annulatus*. *Anaplasma* sp. and *Borrelia* sp. DNAs were only detected in *H. equina* by qPCR.
31 *A. marginale*, *Anaplasma ovis*, and *Theileria ovis* recorded the same low infection rate (0.6%)
32 in donkeys, while horses were found to be infected with *Theileria equi* and a new *Theileria*
33 sp. *Africa* with recorded prevalence rates of 1.2% and 2.7%, respectively. In conclusion,
34 different pathogens were first detected such as *A. platys*-like, *Candidatus E. rustica*, and a
35 new *Ehrlichia* sp. in *R. annulatus*; *A. marginale*, *A. ovis*, and *T. ovis* in donkeys; and a new
36 *Theileria* sp. “Africa” in horses.

37

38

39

40 **1. Introduction**

41 Equine vector-borne diseases (EVBDs) are emerging and re-emerging diseases, and
42 most of them are zoonotic (Onmaz et al., 2013). Among hematophagous arthropods affecting
43 *Equidae*, two major groups are to be mentioned as prominent infectious diseases vectors:
44 ixodid ticks (*Acari*) and hexapod *Diptera* (true flies) (Kaufman et al., 2008). As a result of the
45 vectors' parasitism, *Equidae* suffers from allergy, paralysis, myiasis, and the risk of
46 transmission of various viral, bacterial, spirochetal, and rickettsial diseases (Onmaz et al.,
47 2013). Since there is a distinct correspondence between the epidemiology of EVBDs and the
48 distribution of vectors (Wise et al., 2013), equines and their vectors play a significant role in
49 the maintenance and circulation of EVBDs throughout the world (Štefanidesová et al., 2008
50 Onmaz et al., 2013).

51 The *Anaplasmataceae* family includes intracellular bacteria such as *Anaplasma* and
52 *Ehrlichia*, with a significant medical and veterinary importance (Dumler et al., 2001; Pruneau
53 et al., 2014). *Anaplasma* spp. are distributed worldwide especially in tropical and subtropical
54 Africa and are responsible for granulocytic anaplasmosis in equine as well as in canine
55 populations and ruminants (Laus et al., 2013; Vichova et al., 2014). These diseases are tick-
56 borne and occur during spring and autumn seasons regarding tick activity (Bown et al 2003).
57 Equine anaplasmosis (EA) has been reported in most of European countries, but the
58 prevalence and incidence of EA in African countries, including Egypt, are limited (Saleem et
59 al., 2018).

60 The genus *Borrelia* includes pathogenic spirochetes, which cause relapsing fevers and
61 Lyme borreliosis (Haitham et al., 2013). Lyme disease is transmitted by hard ticks, while
62 relapsing fever borreliosis is transmitted by soft ticks (Vial et al 2006; Trape et al., 2013).
63 Equine borreliosis (EB) is characterized by shifting leg lameness, generalized stiffness,
64 muscular weakness, lethargy, and behavioral abnormalities (Divers et al., 2018). Due to the
65 neuromuscular and musculoskeletal effects of EB, it has been included in differential

66 diagnosis in situations of lameness and poor performance in sport horses (Swinebroad, 2018).
67 Data regarding the incidence and prevalence of EB and their vectors are absent in Egypt.
68 *Borrelia burgdorferi* was detected in ticks (Adham et al., 2010; Hassan et al., 2017), and
69 *Borrelia theileri* was detected in *Rhipicephalus annulatus* (Hassan et al., 2017). The *Borrelia*
70 *flaB* and 16S rRNA genes were used for *Borrelia* detection (McCoy et al., 2014).

71 Equine piroplasmosis (EP) is a tick-borne disease that is endemic in Europe, Asia,
72 Russia, Africa, and the United States (Kouam et al., 2010). EP is caused by one of the
73 hemoprotozoan parasites: *Theileria equi* and *Babesia caballi* (Wise et al., 2013, Mahmoud et
74 al., 2016). EP is characterized by fever, hemoglobinuria, jaundice, ventral edema, pale
75 mucous membranes, anemia, weakness, lethargy, mild colic, abortion in mares, and death
76 (Rothschild and Knowles, 2007; Zobba et al., 2008). The mortality rate for *B. caballi* is 10%,
77 while it reaches 50% for *T. equi* (Tamzali, 2013; Wise et al., 2013). The recovered horses may
78 become asymptomatic carriers in case of *T. equi*, whereas *B. caballi* is usually self-limiting
79 for up to 4 years (Rothschild, 2013). For these reasons, the equid movement across borders
80 may be restricted (Knowles, 1996). Subsequently, EP is considered one of the biggest
81 problems in the international equid trade (Kouam et al., 2010; Scoles and Ueti, 2015).

82 In Egypt, the most prevalent ixodid tick genera infesting equines are *Hyalomma* and
83 *Rhipicephalus*. The genus *Hyalomma* includes *Hyalomma excavatum*, *Hyalomma dromedarii*,
84 and *Hyalomma marginatum* that are the commonest species that infest equines (Abdel-Shafy,
85 1994; Abdel-Shafy, 2000). Moreover, the genus *Rhipicephalus* (formerly *Boophilus*) includes
86 *R. annulatus*, which mainly infests cattle and is rarely found on equines (Abdel-Shafy et al.,
87 2013). The common dipteran infesting equines is the louse fly *Hippobosca equina* (Onmaz et
88 al., 2013). Recently, changes in tick distribution and introduction of exotic ticks with
89 infectious agents into previously unaffected areas have been recorded (Estrada-Pena and
90 Venzal, 2007, Estrada-Pena, 2008; Chitimia-Dobler et al., 2016). Therefore, arthropod
91 identification is essentially significant for the epidemiological mapping of arthropods and

92 arthropod-borne diseases (Dantas-Torres et al., 2013; Lu et al., 2013). Recently, the proteomic
93 approach was applied for arthropod identification, which is called matrix-assisted laser
94 desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) (Dvorak et al.,
95 2014; Kumsa et al., 2016). The MALDI-TOF successfully identified arthropods through the
96 spectra obtained from fresh, frozen, or even alcohol-preserved vector leg specimens, avoiding
97 difficulties of morphological taxonomy for identification due to damaged samples or
98 immature stages (Karger et al., 2012; Yssouf et al., 2013; Kumsa et al., 2016; Diarra et al.,
99 2017). Furthermore, this approach has been characterized by time saving, low cost, and
100 applicability on large-scale studies compared with molecular identification (Araya-Anchetta
101 et al., 2015; Yssouf et al., 2016).

102 The availability and significant increase in DNA sequence data due to the high
103 sensitivity and accuracy of molecular techniques have attracted the interest of evolutionary
104 researchers to identify and characterize previously detected and/or novel species and/or
105 genotypes more accurately. Moreover, studying phylogenetic inferences and epidemiology of
106 vector-borne pathogens contributes to the knowledge needed for disease control and
107 prevention. In Egypt, reports for the epidemiology of EVBDs have been limited, except few
108 reports regarding EP (Mahmoud et al., 2016; Elsayy et al., 2021). Therefore, this study aimed
109 to screen Egyptian *Equidae* and associated arthropods for EVB pathogens such as
110 *Piroplasma*, *Anaplasma*, *Borrelia*, *Rickettsia*, *Coxiella burnetii*, and *Bartonella* using
111 molecular analyses. In addition, equine arthropods were identified using the MALDI-TOF and
112 molecular techniques.

113 **1. Materials and Methods**

114 *2.1. Animals and blood sampling*

115 This study is a cross-sectional study and included 335 apparently healthy *Equidae*
116 (320 horses and 15 donkeys) using a convenience sampling strategy. The study was approved
117 by the Medical and Veterinary Research Ethics Committee at the National Research Centre,

118 Egypt under the number 19059. Animals were clinically examined, body temperature was
119 measured and inspected for the presence of arthropods on different parts of their body. Blood
120 samples were collected from the jugular vein in a sterile EDTA tube from different localities
121 of Capital Cairo and Beni Suef province in Egypt between 2016 and 2017. All blood samples
122 were stored at -20°C for molecular investigations.

123 *2.2. Arthropod collection*

124 A total of 166 ectoparasites were obtained from equines (105 sucking flies and 61
125 ticks) from different localities, as mentioned earlier. All flies and ticks were carefully
126 collected to avoid physical damage. Arthropods from the same animal were counted and
127 gathered in a single tube containing 70% ethanol and transferred to the laboratory for
128 morphological, molecular, and MALDI-TOF MS analyses.

129 *2.3. Arthropod identification*

130 *2.3.1. Morphological identification*

131 Arthropods were identified under the microscope of X56 (Zeiss Axio Zoom.V16,
132 Zeiss, Marly-le-Roi, France) using taxonomic identification keys (Walker, 2003; Estrada-
133 Pena et al., 2004; Mullen and Durden, 2009; Onmaz et al., 2013).

134 *2.3.2. MALDI-TOF MS identification*

135 *2.3.2.1. Sample preparation*

136 Ticks and sucking flies that were preserved in 70% ethanol were washed twice in
137 distilled water for 1 min for each time and then dried using sterile filter paper (Kumsa et al.,
138 2016). The legs of the arthropod were cut using a sterile scalpel, put in a sterile 1.5-mL
139 Eppendorf, and incubated at 37°C overnight to evaporate any alcohol residue (Yssouf et al.,
140 2013; Diarra et al., 2017). The rest of the sample was cut longitudinally in two parts; one-half
141 was dissected into small pieces for molecular purpose, and the remaining half was stored at
142 -20°C as backup for any additional studies.

143 *2.3.2.2. Sample homogenization and loading on MALDI-TOF target plates*

144 On the cutoff legs, a nip of glass powder (Sigma, Lyon, France) was added into 30 μ L
145 of a mixture of 70% (v/v) formic acid and 50% (v/v) acetonitrile (Fluka, Buchs, Switzerland;
146 Kumsa et al., 2016). The legs were crushed and homogenized using a TissueLyser II device
147 (Qiagen, Hilden, Germany) with 30 movements per second for 1 min and repeated three times
148 (Nebbak et al., 2017). After centrifugation of homogenized arthropod legs (at 2,000 g for
149 30 s), 1 μ L of the supernatant of extracted protein was dropped onto a spot of a MALDI-TOF
150 polished steel plate (Bruker Daltonics, Wissembourg, France) in quadruplicate (Nebbak et al.,
151 2017). At room temperature, the plate was left to dry, and then, each spot was covered with
152 1 μ L of cyano-4-hydroxycinnamic acid (CHCA) matrix solution that was composed of
153 saturated α -CHCA (Sigma, Lyon, France), 50% acetonitrile (v/v), 2.5% trifluoroacetic acid
154 (v/v) (Sigma-Aldrich, Dorset, UK), and HPLC grade water (Yssouf et al., 2016). After drying,
155 the plate was loaded into the microflex LT MALDI-TOF MS apparatus (Bruker Daltonics,
156 Germany) for analysis. Matrix solution was loaded in duplicate onto each MALDI-TOF plate
157 with or without a bacterial test standard (Bruker Protein Calibration Standard I) to control
158 loading on the MS target plate, matrix quality, and MALDI-TOF apparatus performance
159 (Nebbak et al., 2017).

160 *2.3.2.3. MALDI-TOF MS parameters*

161 The protein mass profiles of each sample were obtained using a microflex LT
162 spectrometer with the flexControl software (Bruker Daltonics) with special parameters
163 recommended by Kumsa et al. (2016). The spectra obtained were visualized by the
164 flexAnalysis 3.3 software and transferred to ClinProTools version v.2.2 and MALDI Biotyper
165 v.3.0 (Bruker Daltonics, Germany) for analysis (Karger et al., 2012).

166 *2.3.2.4. Spectrum analysis and reference database creation*

167 For the spectrum analysis, the ClinProTools v2.2 and flexAnalysis v3.3 software
168 programs were applied to evaluate the reproducibility of the MS spectra obtained from the
169 same arthropods' species and assess intraspecies specificity (Fotso Fotso et al., 2014; Nebbak

170 et al., 2016). After the morphological and molecular confirmation of arthropod identification,
171 two to five high-quality reproducible profiles with a high peak intensity of each species
172 conserved in 70% ethanol were selected and loaded into the MALDI Biotyper 3.0 to create as
173 reference spectra (Kumsa et al., 2016; Boucheikhchoukh et al., 2018). As for the upgrade
174 arthropod MALDI-TOF database, reference spectra were established by spectra at least two
175 specimens per species of both genders using the algorithm MALDI Biotyper software v3.0
176 (Bruker Daltonics; Diarra et al., 2017; Boucheikhchoukh et al., 2018).

177 *2.3.2.5. Blind test*

178 All specimens were subjected to a blind test, except those used as reference spectra.
179 The log score values (LSVs) were calculated by the MALDI Biotyper software v.3.0 (Bruker
180 Daltonics) to estimate the reliability of species identification. The LSVs ranged from 0 to 3;
181 these corresponded to the degree of similarity between the spectra submitted by the blind test
182 and the MS reference spectra in the database. The identification was considered reliable with
183 an LSV of at least 1.8 (Yssouf et al., 2013; Kumsa et al., 2016). The identification of blind
184 tested samples was assessed by taking the highest LSVs associated with a spectrum quality.

185 *2.4. Molecular techniques*

186 *2.4.1. DNA extraction*

187 Each dissected half of arthropod was cut in pieces, put in a sterile 1.5-mL tube
188 containing 200 μ L of G2 lysis buffer and 25 μ L of proteinase K (Qiagen, Hilden, Germany),
189 and incubated at 56°C overnight. Then, 200 μ L of supernatant was transferred into a new
190 sterile tube after centrifugation of the mixture. Subsequently, 200 μ L of each blood sample
191 was subjected to DNA extraction and arthropods using the EZ1 DNA Tissue Kit (Qiagen),
192 according to the manufacturer's instructions. The extracted DNA was stored at -20°C until
193 use in molecular methods.

194 *2.4.2. Screening of pathogen DNA by quantitative PCR in equines and associated arthropods*

195 Quantitative PCR (qPCR) was performed in all extracted DNA samples (equines and
196 associated arthropods) for multi-pathogen DNA screening using genus-specific primers and
197 probes targeting the 5.8S rRNA gene of *Piroplasmida* (Dahmana et al., 2019), 23S rRNA
198 gene of *Anaplasmataceae* (Dahmani et al., 2017), *gltA* gene *Rickettsia* sp. (Rolain et al.,
199 2002), 16S rRNA gene *Borrelia* sp. (Bottieau et al., 2012), IS1111 intergenic spacer for *C.*
200 *burnetii* (Mediannikov et al., 2010), and 16S-23S intergenic spacer for *Bartonella* sp. (Raoult
201 et al., 2006) (Table 1). The qPCR was applied using the CFX96 Real-Time System (Bio-Rad,
202 Marnes-La-Coquette, France). The mixture of qPCR contained 10 µL of Eurogentec Probe
203 PCR MasterMix (Eurogentec, Liège, Belgium), 0.5 µM of primers and FAM-labeled probe,
204 5 µL of DNA template, and 3.5 µL of sterile distilled water to complete the reaction volume
205 to 20 µL. The negative (without any DNA) and positive controls (corresponding pathogen
206 DNA) were added to each reaction to evaluate the reaction. The samples were considered
207 positive with the cycle threshold (Ct) lower than 35 Ct (Sokhna et al., 2013).

208 2.4.3. Standard PCR for pathogens identification

209 The positive qPCR samples were subjected to standard PCR and sequencing. For the
210 identification of *Piroplasmida* (*Theileria* sp. and *Babesia* sp.), *Anaplasmataceae* (*Anaplasma*
211 sp. and *Ehrlichia* sp.), and *Borrelia* sp., 1,100 bp of the 18S rRNA gene, 520 bp fragment of
212 the 23S rRNA gene, and 1,200 bp of the 16S rRNA gene were used, respectively (Bottieau et
213 al., 2012; Dahmani et al., 2017; Dahmana et al., 2019). The PCR reactions were performed on
214 a thermal cycler (Applied Biosystems, Paris, France) using the AmpliTaq Gold[®] 360 Master
215 Mix (Thermo Fisher Scientific, USA), according to the manufacturer's recommendations. In
216 each reaction, negative and positive controls were used. Moreover, 1.5% agarose gel stained
217 with SYBR[®] Safe (Invitrogen, USA) electrophoresis was applied to confirm the amplification
218 and visualized by Image Lab software (Bio-Rad, Marnes-La-Coquette, France).

219 2.4.4. Molecular identification of arthropods

220 Standard PCR was applied to confirm MALDI-TOF identification. Mitochondrial
221 gene (CO1 and 16S rRNA genes) sequencing was used for the identification of flies and tick
222 species through DNA automated thermal cycler (Applied Biosystems, Paris, France) under the
223 same condition as previously described (Table 1) (Chitimia et al., 2010; Abdullah et al., 2016;
224 Boucheikhchoukh et al., 2018). PCR products were purified and sequenced as mentioned
225 earlier (Boucheikhchoukh et al., 2018).

226 *2.4.5. Sequencing and phylogenetic analyses*

227 The purification of PCR products was applied using NucleoFast 96 PCR plates
228 (Macherey–Nagel, Düren, Germany), according to the manufacturer’s recommendations. The
229 purified PCR products were sequenced using the BigDye Terminator Cycle Sequencing Kit
230 (3130X1 Genetic Analyzer, ABI PRISM). The sequences obtained were assembled and edited
231 by ChromasPro software (ChromasPro 1.7, Technelysium Pty Ltd., Tewantin, Australia), and
232 the corrected sequences were compared with the reference sequences available in GenBank
233 by the BLASTn (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The obtained sequences of
234 Egyptian EVB genotypes and arthropods were recorded in GenBank. These sequences
235 obtained and sequences available in GenBank were aligned using CLUSTAL W in MEGA
236 software X (Kumar et al., 2018). The phylogenetic trees were inferred using the maximum
237 likelihood method and Tamura-Nei model with 1,000 bootstrap replicates in MEGA X
238 software (Tamura and Nei, 1993; Kumar et al., 2018).

239 **3. Results**

240 *3.1. Morphological identification of arthropods*

241 A total of 105 louse flies were collected from 311 horses from Cairo and
242 morphologically identified as *H. equina* (70 males and 35 females, Fig. 1). In addition, we
243 examined 15 donkeys and nine horses from Upper Egypt province (Beni Suef) and found 54
244 *R. annulatus* individuals infesting 15 donkeys and eight horses. Moreover, we found seven
245 *Rhipicephalus* spp. infesting three horses and four donkeys, whose need further identification

246 using the MALDI-TOF technique and molecular biology. The details of collected arthropods
247 are listed in Table 2.

248 3.2. MALDI-TOF MS analyses of arthropods

249 For *H. equina*, the random MALDI-TOF test revealed that the obtained spectra did not
250 exist in our database and all tested flies were identical to each other. However, the random
251 MALDI-TOF test of *Rhipicephalus* spp. revealed that the obtained spectra were not identical
252 to each other and they did not exist in our database. Therefore, the reference database was
253 essential for the accurate identification of arthropod specimens. After molecular confirmation,
254 *H. equina* and *R. annulatus* genotypes from Egypt were introduced as reference MS spectra
255 into the laboratory database. Once the database updated, a blind test was performed on the rest
256 of arthropods. The microflex LT MALDI-TOF MS enabled the identification of 105/105
257 (100%) for *H. equina* ($1.22 \leq \text{LSVs} \leq 2.48$, Fig. 2) and 61/61 (100%) for *R. annulatus*
258 ($1.62 \leq \text{LSVs} \leq 2.72$, Fig. 3).

259 3.3. Molecular identification of arthropods

260 Molecular identification was applied on specimens selected for the creation of
261 reference MS spectra. For *H. equina*, based on the CO1 gene, standard PCR and sequencing
262 were applied and succeeded in identifying sucking flies as *H. equina* (GenBank: MK737646)
263 with 99% (589/590) identity to those of *H. equina* from Portugal (GenBank: MN868873). In
264 addition, DNA markers (CO1 and 16S rRNA genes) were used in standard PCR and
265 sequencing on *Rhipicephalus* spp. specimens selected for the creation of reference MS
266 spectra. The BLAST analysis revealed that *R. annulatus* was the only *Rhipicephalus* sp.
267 detected in this study with different genotypes. By the 16S rRNA gene, *R. annulatus*
268 (GenBank: MK737647 and MK737648) was identified and collected from horses and
269 donkeys in Beni Suef province with 99% (430/434) identity to those of *R. annulatus* derived
270 from cattle from Beheira province, Egypt (GenBank: KY945491). In further identification
271 through the CO1 gene, the BLAST search confirmed that *Rhipicephalus* sp. was *R. annulatus*

272 (GenBank: MZ505561 and MZ505565) with 99% (802/809) similarity to *R. annulatus* from
273 cattle from the United States (GenBank: KX228534). The phylogenetic position of the five
274 obtained *R. annulatus* compared with other reference tick species is illustrated in Fig. 4.

275 3.4. Pathogen detection in arthropods

276 All DNA samples of arthropods were screened for the detection of pathogen DNA of
277 *Piroplasma* sp., *Anaplasma* sp., *Rickettsia* sp., *Borrelia* sp., *C. burnetii*, and *Bartonella* sp.
278 The result revealed that the DNA of *Anaplasma* and *Borrelia* sp. were detected in *R.*
279 *annulatus* collected from donkeys and *H. equina* from horses, while *R. annulatus* collected
280 from horses were free from any pathogen DNA (Table 3). All arthropods were found to be
281 free from *Piroplasma*, *Rickettsia*, *C. burnetii*, and *Bartonella* infections.

282 *Anaplasma* DNA was detected using qPCR based on the 23S rRNA gene in 21 of 166
283 (12.7%) arthropods, including 27.9% among *R. annulatus* (17/61) and 3.8% in *H. equina*
284 (4/105). We were able to amplify and sequence the portion of 23S rRNA gene in nine of the
285 17 samples of *R. annulatus* collected from donkeys. The BLAST research reported the
286 presence of two different genotype groups that were attributed to two genera of
287 *Anaplasmataceae* (*Anaplasma* and *Ehrlichia*) in *R. annulatus*. A new *A. platys* genotype
288 (GenBank: MN614105) was detected in one *R. annulatus* with 100% (415/415) identity with
289 *A. platys*-like genotype detected in cattle and sheep from the same locality of Beni Suef
290 province (GenBank: MN626397 and MN626398) and clustered in the same clade (bootstrap
291 value 99, Fig. 5). In addition, this genotype had 99% (409/415) identity with those detected in
292 the blood of New Caledonian dogs (GenBank: KM021425). Another *Anaplasma* sp. that was
293 detected in *R. annulatus* was *Anaplasma marginale* genotype (GenBank: MN614106) with
294 100% (380/380) identity to *A. marginale* detected in the blood of cattle from Algeria
295 (GenBank: MH321194). Moreover, two *Ehrlichia* spp. with three different genotypes were
296 found in *R. annulatus*; a new genotype close to *Candidatus Ehrlichia rustica* was derived from
297 one *R. annulatus* (GenBank: MN614107) with 99% (413/415) identity to those of *E. rustica*

298 found in *Amblyomma variegatum* from Côte d'Ivoire (GenBank: KT364330), and *Candidatus*
299 *E. rustica* genotype was derived from three *R. annulatus* (GenBank: MN614109) with 100%
300 (415/415) identity to the same reference. Finally, a novel potential *Ehrlichia* sp. was derived
301 from three *R. annulatus* (GenBank: MN614110) with 98% (404/413) identity with *Candidatus*
302 *Ehrlichia urmitei* detected in *Rhipicephalus bursa* in France. In the phylogenetic tree, the
303 sequence of novel potential *Ehrlichia* sp. clustered in a well-supported branch (bootstrap
304 value 55) with *Ehrlichia ruminantium* (Fig. 5). The phylogenetic position of these genotypes
305 is illustrated in Fig. 5.

306 For *Borrelia* sp., the result of qPCR revealed that 5 of 166 (3%) were positive for
307 *Borrelia* sp., including 3.3% in *R. annulatus* (2/61) and 2.9% (3/105) in *H. equina*. Using 16S
308 rRNA gene sequencing, we identified it as *B. theileri* that derived from two *R. annulatus*. The
309 result revealed that a new genotype of *B. theileri* (GenBank: MN619805) was identical to *B.*
310 *theileri* previously detected in sheep and cattle from the same locality of Beni Suef province
311 (GenBank: MN621893 and MN621894). The phylogenetic position of this *B. theileri*
312 genotype is illustrated in Fig. 6.

313 3.5. Pathogen detection in Equidae

314 All blood samples were screened by qPCR to determine the presence of pathogen
315 DNA of piroplasmids, *Anaplasmataceae*, *Rickettsia* spp., *Borrelia* spp., *C. burnetii*, and
316 *Bartonella* spp. DNAs of *Piroplasmida* and *Anaplasma* spp. were detected in horses and
317 donkeys, while equines were free from DNA of other pathogens (Table 3).

318 For *Anaplasma* sp., 33 of 335 (9.8%, 28 horses and five donkeys) were positive by the
319 qPCR system. We obtained good-quality 23S rDNA sequences from only four samples,
320 probably due to the low amount of DNA in the blood samples. Based on the BLAST analyses,
321 we identified two species of *Anaplasma*, and a genotype of *Anaplasma ovis* was derived from
322 two donkeys (GenBank: MN614104) with 100% (454/454) identity to *A. ovis* detected in
323 sheep and cattle blood of the same locality (GenBank: MN626392 and MN625933). Another

324 species identified was *A. marginale*, identified in two donkeys (GenBank: MN614103) with
325 100% (455/455) identity to those detected in the blood of cattle collected from Egypt
326 (GenBank: MN625935). The position of these genotypes was shown in the phylogenetic tree
327 (Fig. 5).

328 Piroplasmida DNA was detected by qPCR in 27 of 335 animals (8.1%, 25 horses and
329 two donkeys). As a result, PCR and sequencing of the 18S rRNA gene portion were
330 performed and allowed the identification of 15 of the 27 qPCR positive samples (4.5%, 13 in
331 horses and two in donkeys). According to the BLAST search, we identified three *Piroplasma*
332 species: *T. equi*, a new potential *Theileria* sp. “Africa,” and *Theileria ovis*. In horses, we
333 identified two *Piroplasma* sp. (*T. equi* and *Theileria* sp. “Africa”). By the BLAST research,
334 we obtained two genotypes of *T. equi*; one genotype was derived from one horse, and another
335 genotype was derived from three horses (GenBank: MN625897 and MN625898) with 100%
336 (922/922) identity to those of *T. equi* detected in horses from Israel (GenBank: MK063843
337 and MK063842, respectively). Moreover, we found four genotypes of *Theileria* sp. “Africa”
338 (GenBank: MN625900, MN625901, MN625902, and MN625903) that clustered in a separate
339 clade with a good bootstrap support with the other *Theileria* sp. “Africa” previously detected
340 in African horses (Fig. 7). In addition, by the BLAST analyses, one genotype from horse was
341 considered to be a new genotype with 99% (917/918) identity to *T. equi* detected in horse
342 blood from Brazil (GenBank: MG052913). Moreover, three genotypes were identified as *T.*
343 *equi* with 100% (923/923) identity to those detected in horse blood from Turkey and Sudan
344 (GenBank: MG569896, MG569893, and AB515309, respectively). In donkeys, *T. ovis* shared
345 100% (919/919) identity with sheep and buffalo previously detected in the same province in
346 Egypt (GenBank: MN625886 and MN625887).

347 **2. Discussion**

348 *Equidae* are used in several beneficial activities for human such as police services,
349 agriculture, and pharmaceutical purposes, in addition to competitive and noncompetitive

350 leisure pursuits (Payne et al., 2017). Generally, *Equidae*, especially donkeys, play a
351 significant role in the transmission of vector-borne diseases by acting as a domestic reservoir
352 and carrying vectors to a broad host range or even to humans (Onmaz et al., 2013). The
353 spectrum of EVBDs increased and drew the attention of veterinarians and clinicians to
354 diseases such as piroplasmosis, anaplasmosis, borreliosis, rickettsiosis, bartonellosis, and Q
355 fever (Dantas-Torres et al., 2013). In addition, advances in molecular biology tools and the
356 availability of DNA sequence data facilitate the detection of new pathogen species and even
357 genotypes (Ehounoud et al., 2016). In this study, epidemiological and entomological data on
358 the prevalence of EVBDs infecting *Equidae* and associated arthropods in two regions of
359 Egypt (Capital Cairo and Beni Suef province) were summarized. Moreover, equine arthropod
360 parasites were identified by the MALDI-TOF and molecular techniques.

361 Ixodid ticks *Hyalomma* and *Rhipicephalus* and dipteran *H. equina* are the most
362 common arthropods infesting equines (Abdel-Shafy, 1994; Abdel-Shafy, 2000; Onmaz et al.,
363 2013). In this study, traditional taxonomic keys of arthropods revealed the presence of
364 *Rhipicephalus* sp. as ixodid ticks collected from horses and donkeys of Beni Suef province. In
365 addition, *H. equina* was morphologically identified that were collected from horses in Cairo.
366 In support of these findings, previous studies found *Rhipicephalus* sp. (especially *R.*
367 *annulatus*) in Egypt as the main ixodid ticks infesting cattle and that may infest equines
368 (Abdel-Shafy et al., 2013) and *H. equina* was louse fly of horses (Hafez et al., 1978). The
369 morphological similarities at both intra- and interspecies levels limit the worth of
370 morphological taxonomic keys such as in *Rhipicephalus* sp. (Walker et al., 2000;
371 Guglielmone et al., 2014; Nava et al., 2015). Therefore, a combination of the MALDI-TOF
372 MS and DNA markers was applied to identify *H. equina* and *Rhipicephalus* sp. Molecular
373 markers (16S rRNA and CO1) revealed one genotype of *H. equina* and five novel genotypes
374 of *R. annulatus*. Thereafter, the MS spectra of *H. equina* and *R. annulatus* were deposited in the
375 database as the reference MS spectra, and then, a blind test was applied on all arthropod

376 samples. Therefore, the combination of molecular identification and MALDI-TOF MS was
377 successful in the identification of arthropods infesting equines. To our knowledge, *H. equina*
378 and *R. annulatus* were first identified using the combination between MALDI-TOF MS and
379 molecular identification in Egypt. Later, Elhelw et al. (2021) identified *R. annulatus* from
380 cattle by a molecular marker in Egypt.

381 The family Anaplasmataceae includes, among others, two significant genera,
382 *Anaplasma* and *Ehrlichia*, which can cause significant infections in a wide range of animal
383 hosts and humans. These infections are mainly transmitted by ticks (Rar and Golovljova,
384 2011). In this study, the overall prevalence of Anaplasmataceae infection in *Rhipicephalus* sp.
385 collected from *Equidae* was 14.7%. This study reports for the first time a novel potential
386 *Anaplasma* sp. in *R. annulatus* in Egypt with 1.6% infection rate. This *Anaplasma* sp. was
387 genetically close to canine *A. platys* with 99% homology; thus, it was commonly named *A.*
388 *platys*-like. The phylogenetic tree revealed that our *A. platys*-like was grouped in the same
389 clade with canine *A. platys* (bootstrap value 89, Fig. 5). Furthermore, it forms a separate clade
390 with *A. platys*-like detected in cattle and sheep from Beni Suef province where *R. annulatus*
391 was collected from donkeys (Abdullah et al., 2021). To our knowledge, the presence of *A.*
392 *platys*-like has never been reported in *R. annulatus*. Recently, *A. platys*-like was reported in
393 large and small ruminants in Beni Suef, camels in Giza and Cairo (Abdullah et al., 2021), and
394 in cattle from Menoufia province of Egypt (Tumwebaze et al., 2020), implying that this novel
395 potential pathogen circulates in different animal hosts and different provinces of Egypt.
396 Therefore, *R. annulatus* may act as a vector of this pathogen in Egypt. Moreover, *A. platys*-
397 like was identified in different animal hosts as cattle in Italy (Zobba et al., 2014), Algeria
398 (Dahmani et al., 2015), and Tunisia (Said et al., 2017), camels in Tunisia (Belkahia et al.,
399 2015; Selmi et al., 2019), and sheep and goat in South Africa (Berggoetz et al., 2014) and
400 Senegal (Dahmani et al., 2019). In addition, *A. marginale* was detected in *R. annulatus* with
401 1.6% infection rate. In Egypt, *A. marginale* was endemic in different animal hosts such as

402 cattle (Fereig et al., 2017; Al-Hosary et al., 2020; Parvizi et al., 2020), buffaloes (Elhariri et
403 al., 2017), and camels (El-Naga and Barghash, 2016). Recently, Al-Hosary et al. (2021)
404 reported *A. marginale* in *R. annulatus*. For the genus *Ehrlichia*, we recorded for the first time
405 two different genotypes of *Candidatus E. rustica* in *R. annulatus* in Egypt with 6.6% infection
406 rate. One novel genotype of *Candidatus E. rustica* was 99% homology with those of *E.*
407 *rustica* found in *Amblyomma variegatum* from Côte d'Ivoire, and another genotype was 100%
408 homology to the same reference (Ehounoud et al., 2016). Moreover, we have identified a new
409 potential *Ehrlichia* sp. in three *R. annulatus* with 4.9% infection rate. The sequence of this
410 potential *Ehrlichia* sp. clustered in a separated clade with *E. ruminantium* (bootstrap value
411 55). As a result, we had a new potential *Ehrlichia* sp. and *Candidatus E. rustica* in *R.*
412 *annulatus* that had never been reported before in Egypt. Interestingly, these potential new
413 species were identified in three different regions in the world (France, Côte d'Ivoire, and
414 Egypt) and from different tick species (*Rhipicephalus*, *Amblyomma*, and *Hyalomma* sp.)
415 (Ehounoud et al., 2016). Thus, *R. annulatus* could be a vector for *Anaplasmataceae* in Egypt,
416 and there is a risk of transmission of other novel potential vector-borne diseases. This should
417 be evaluated in future studies.

418 In Africa, most of *Borrelia* spp. were detected in soft ticks, such as *Ornithodoros* sp.,
419 which is the main vector (Parola and Raoult, 2001). To date, in Africa, *Borrelia* sp. was
420 identified in hard ticks (*Amblyomma* and *Rhipicephalus* sp.) in Ethiopia (Mediannikov et al.,
421 2013; Kumsa et al., 2015), Mali (McCoy et al., 2014), Côte d'Ivoire (Ehounoud et al., 2016),
422 Egypt (Hassan et al., 2017), Madagascar (Hagen et al., 2018), and Ecuador (Gioia et al.,
423 2018). In this study, a new potential *B. theileri* was identified in two *R. annulatus* with 3.3%
424 infection rate. The obtained sequence was 99% identical to *B. theileri* found in *Rhipicephalus*
425 *geigy* in Mali (McCoy et al., 2014). Likewise, the phylogenetic analysis revealed that a new
426 genotype of *B. theileri* was clustered in the same clade with *B. theileri* detected in sheep and

427 cattle from the same locality of Beni Suef province (Abdullah et al., 2021). In Egypt, *B.*
428 *theileri* had previously been detected in *R. annulatus* (Hassan et al., 2017).

429 Regarding *H. equina*, *Anaplasma*, and *Borrelia* sp., DNAs were detected by qPCR.
430 However, we were unable to amplify and sequence these samples, which could be attributed
431 to the high sensitivity of qPCR compared with that of standard PCR or to the low
432 concentration of pathogenic DNA in fly tissues. Kowal et al. (2016) reported the role of
433 Hippoboscids in the transmission of bacterial pathogens such as *Anaplasma* and *Bartonella*.
434 Moreover, Boucheikhchoukh et al. (2019) detected *Bartonella* and *Wolbachia* sp. in *H.*
435 *equina*.

436 In *Equidae* blood, we reported *Anaplasmataceae* DNA in donkeys and *Piroplasmida*
437 DNA in horses. For *Anaplasmataceae*, the overall prevalence of anaplasmoses in donkeys
438 was 26.6%, while horses were found free from *Anaplasma* sp. This result was in accordance
439 with Ehounoud et al. (2016) who did not find any *Anaplasma* in horses. *A. ovis* and *A.*
440 *marginale* were the common *Anaplasma* pathogens of sheep and cattle, respectively
441 (Dahmani et al., 2019). However, in our study, we found *A. ovis* and *A. marginale* in donkeys.
442 *A. ovis* shared 100% identity to those of *A. ovis* in sheep and cattle blood from the same
443 locality of Beni Suef province (Abdullah et al., 2021). Another *Anaplasma* sp. was *A.*
444 *marginale* that shared 100% similarity with those of *A. marginale* detected in the blood of
445 cattle collected from the same locality of Beni Suef province (Abdullah et al., 2021). *A. ovis*
446 and *A. marginale* have never been reported yet in donkeys in Egypt and even Africa. Later, *A.*
447 *marginale* has been reported in donkeys in Pakistan (Asif et al., 2019).
448 Therefore, donkeys should be involved in the epidemiology of tick-borne diseases.

449 EP is a protozoan disease caused by *T. equi* and *B. caballi* (Wise et al., 2013;
450 Mahmoud et al., 2016). Our study reported an overall prevalence of EP at 4.5% (1.2% for *T.*
451 *equi*, 2.7% for *Theileria* sp. “Africa,” and 0.6% for *T. ovis*), but we did not detect *B. caballi*.
452 This may be attributed to self-limiting of *B. caballi* infection and the lifetime persistence of *T.*

453 *equi* (Friedhoff and Soule, 1996). In this study, two genotypes of *T. equi* were pooled in a
454 separate clade with *T. equi* that has already been reported in horses in America (Hall et al.,
455 2013) and Israel (Margalit et al., 2018). Moreover, novel potential *Theileria* sp. “Africa”
456 genotypes were clustered in a separate clade of a good bootstrap support with the other
457 *Theileria* sp. “Africa” previously detected in African horses in Senegal and Chad (Fig. 7)
458 (Dahmana et al., 2019). To our knowledge, *Theileria* sp. “Africa” has never been reported yet
459 in Egypt. *T. ovis* was detected in donkeys with a prevalence rate of 0.6%, representing its first
460 detection in donkeys in Africa. Recently, *T. ovis* was reported in horses and donkeys in
461 Turkey (Ozubek and Aktas, 2018). In the last decade, several researchers have found different
462 piroplasmid species in horses and donkeys and have reduced the host specificity of
463 piroplasmids (Fritz, 2008; Zanet et al., 2017).

464 **3. Conclusion**

465 This study summarized the epidemiological and entomological data of EVBDs
466 infecting equines and associated arthropods in Cairo and Beni Suef province in Egypt. We
467 applied, for the first time in Egypt, a combination between the MALDI-TOF and molecular
468 markers for the correct identification of arthropods. In addition, we reported the first detection
469 of *A. platys*-like, *Candidatus* *E. rustica*, and a new *Ehrlichia* sp. in *R. annulatus*, *A.*
470 *marginale*, *A. ovis*, and *T. ovis* in donkeys and a new *Theileria* sp. “Africa” in horses in
471 Egypt. Therefore, equines, especially donkeys, should be involved in the epidemiology of
472 tick-borne diseases as they serve as reservoirs for these emerging and re-merging pathogens to
473 other animals.

474 **Ethical Committee approval**

475 This study was approved by the Medical and Veterinary Research Ethics Committee at
476 the National Research Centre, Egypt under the number 19059.

477 **Declaration of Competing Interest**

478 The authors declare no conflict of interest.

479 **Acknowledgment and Funding**

480 This study was supported by the Institut Hospitalo-Universitaire (IHU) Méditerranée
481 Infection, the National Research Agency under the program “Investissements d'avenir”,
482 reference ANR-10-IAHU-03, the Région Provence-Alpes-Côte d'Azur and European funding
483 FEDER PRIMI.

484 The authors acknowledge funding from the Science and Technology Development
485 Fund (STDF) and Institut Francais d’Egypte (IFE) (ID: 30652) for the support of this
486 research.

487 **Authors' contributions**

488 All authors were involved in the design of the study. HHAMA, DA and TKF collected
489 blood and vector samples. HHAMA, DA, TKF, KNA and SA identified fly and ticks
490 morphologically. HHAMA and DA shared in DNA extraction. HHAMA, SA, DR and OM
491 shared in molecular protocols and data analysis. HHAMH, PP and OM shared in MALDI-
492 TOF protocols and data analysis. HHAMH wrote the first draft of the manuscript. All authors
493 revised and approved the last version of the manuscript.

494 **References**

- 495 Abdel-Shafy, S., 1994. Morphological description of ixodid immature stages and research of
496 blood parasites in farm animals in Egypt. MSc Thesis, Fac. Agric. Cairo Univ. 150pp.
- 497 Abdel-Shafy, S., 2000. Microbiological and control studies on ticks infesting farm animals
498 and poultry. PhD. Thesis Fac. Agr. Cairo Univ. 209pp.
- 499 Abdel-Shafy, S., Habeeb, S.M., El Namaky, A.H., Abou-Zeina, H.A.A., 2013. Scanning
500 electron microscopy of nymphal and larval stages of the cattle tick *Rhipicephalus*
501 (*Boophilus*) *annulatus* (say) 1821(Acari: Ixodidae). Global Vet. 10, 1–8.
502 <https://doi.org/10.5829/idosi.gv.2013.10.1.71150>.

503 Abdullah, H.H.A.M., Amanzougaghene, N., Dahmana, H., Meriem, L., Raoult, D.,
504 Mediannikov, O., 2021. Multiple Vector-borne Pathogens of Domestic Animals in Egypt.
505 PLOS Neg. Trop. Dis. 15, e0009767. <https://doi.org/10.1371/journal.pntd.0009767>.

506 Abdullah, H.A.M.H., El-Molla, A., Salib, A.F., Allam, A.T., Ghazy, A.A., Abdel-Shafy, S.,
507 2016. Morphological and molecular identification of the brown dog tick *Rhipicephalus*
508 *sanguineus* and the camel tick *Hyalomma dromedarii* (Acari: Ixodidae) vectors of
509 rickettsioses in Egypt. Vet. World 9, 1087–1101.
510 <https://doi.org/10.14202/vetworld.2016.1087-1101>.

511 Adham, F.K., Emtithal, M., Abd-El-Samie, E.M., Refaat, M., Gabre, R.M., El Hussein, H.,
512 2010. Detection of tick blood parasites in Egypt using PCR assay I- *Borrelia burgdorferi*
513 *sensu lato*. J. Egypt. Soc. Parasitol. 40, 553–564. [https://doi.org/10.1007/s00436-009-](https://doi.org/10.1007/s00436-009-1443-8)
514 1443-8.

515 AL-Hosary, A., Răileanu, C., Tauchmann, O., Fischer, S., Nijhof, M.A., Silaghi, C., 2020.
516 Epidemiology and genotyping of *Anaplasma marginale* and co-infection with piroplasms
517 and other Anaplasmatataceae in cattle and buffaloes from Egypt. Parasit. Vectors 13, 495.
518 <https://doi.org/10.1186/s13071-020-04372-z>.

519 AL-Hosary, A., Răileanu, C., Tauchmann, O., Fischer, S., Nijhof, A. M., Silaghi, C.,
520 2021. Tick species identification and molecular detection of tick-borne pathogens in
521 blood and ticks collected from cattle in Egypt. Ticks and Tick-Borne Dis. 12,
522 101676. <https://doi:10.1016/j.ttbdis.2021.101676>.

523 Araya-Anchetta, A., Busch, J.D., Scoles, G.A., Wagner, D.M., 2015. Thirty years of tick
524 population genetics: a comprehensive review. Infect. Genet. Evol. 29, 164–179.
525 <https://doi.org/10.1016/j.meegid.2014.11.008>.

526 Asif, M., Parveen, A., Ashraf, S., Hussain, M., Aktas, M., Ozubek, S. Shaikh, R.S., Iqbal, F.,
527 2019. First report regarding the simultaneous molecular detection of *Anaplasma*
528 *marginale* and *Theileria annulata* in equine blood samples collected from southern

529 Punjab in Pakistan. *Acta Parasitologica* 65, 259–263. <https://doi.org/10.2478/s11686-019->
530 00141-w.

531 Belkahia, H., Ben Said, M., Sayahi, L., Alberti, A., Messadi, L., 2015. Detection of novel
532 strains genetically related to *Anaplasma platys* in Tunisian one-humped camels (*Camelus*
533 *dromedarius*). *J. Infect. Dev. Ctries.* 9, 1117–1125. <https://doi.org/10.3855/jidc.6950>.

534 Berggoetz, M., Schmid, M., Ston, D., Wyss, V., Chevillon, C., Pretorius, A.M., Gern, L.,
535 2014. Tick-borne pathogens in the blood of wild and domestic ungulates in South Africa:
536 interplay of game and livestock. *Ticks Tick Borne Dis.* 5, 166–175.
537 <https://doi.org/10.1016/j.ttbdis.2013.10.007>.

538 Bottieau, E., Verbruggen, E., Aubry, C., Socolovschi, C., Vlieghe, E., 2012.
539 Meningoencephalitis complicating relapsing fever in traveler returning from Senegal.
540 *Emerg. Infect. Dis.* 18, 697–698. <http://dx.doi.org/10.3201/eid1804.111771>.

541 Boucheikhchoukh, M., Laroche, M., Aouadia, A., Dib, L., Benakhla, A., Raoult, D., Parola,
542 P., 2018. MALDI-TOF MS identification of ticks of domestic and wild animals in
543 Algeria and molecular detection of associated microorganisms. *Comp. Immunol.*
544 *Microbiol. Infect. Dis.* 57, 39–49. <https://doi.org/10.1016/j.cimid.2018.05.002>.

545 Boucheikhchoukh, M., Mechouk, N., Benakhla, A., Raoult, D., Parola, P., 2019. Molecular
546 evidence of bacteria in *Melophagus ovinus* sheep keds and *Hippobosca equina* forest flies
547 collected from sheep and horses in northeastern Algeria. *Comp. Immunol. Microbiol.*
548 *Infect. Dis.* 65, 103–109. <https://doi.org/10.1016/j.cimid.2019.05.010>.

549 Bown, K.J., Begon, M., Bennett, M., Woldehiwet, Z., Ogden, N.H., 2003. Seasonal dynamics
550 of *Anaplasma phagocytophila* in a rodent-tick (*Ixodes trianguliceps*) system, United
551 Kingdom. *Emerg. Infect. Dis.* 9, 63–70. <https://doi.org/10.3201/eid0901.020169>.

552 Chitimia-Dobler, L., Nava, S., Bestehorn, M., Dobler, G., Wolfel, S., 2016. First detection of
553 *Hyalomma rufipes* in Germany. *Ticks Tick Borne Dis.* 7, 1135–1138.
554 <https://doi.org/10.1016/j.ttbdis.2016.08.008>.

555 Chitimia, L., Lin, R., Cosoroaba, I., Wu, X., Song, H., Yuan, Z., Zhu, X., 2010. Genetic
556 characterization of ticks from Southwestern Romania by sequences of mitochondrial
557 *cox1* and *nad5* genes. *Exp. Appl. Acarol.* 52, 305–311. [https://doi.org/10.1007/s10493-](https://doi.org/10.1007/s10493-010-9365-9)
558 [010-9365-9](https://doi.org/10.1007/s10493-010-9365-9)

559 Dahmana, H., Amanzougaghene, N., Davoust, B., Carette, O., Normand, T., Demoncheaux,
560 J.P., Mulo, B., Fabrizio, B., Scandola, P., Chik, M., Fenollar, F., Raoult, D.,
561 Mediannikov, O., 2019. Great diversity of Piroplasmida in *Equidae* in Africa and Europe,
562 including potential new species. *Vet. Parasitol. Regional Studies and Reports* 18,
563 e100332. <https://doi.org/10.1016/j.vprsr.2019.100332>.

564 Dahmani, M., Davoust, B., Benterki, M.S., Fenollar, F., Raoult, D., Mediannikov, O., 2015.
565 Development of a new PCR-based assay to detect Anaplasmatidae and the first report of
566 *Anaplasma phagocytophilum* and *Anaplasma platys* in cattle from Algeria. *Comp.*
567 *Immunol. Microbiol. Infect. Dis.* 4, 39–45. <https://doi.org/10.1016/j.cimid.2015.02.002>.

568 Dahmani, M., Davoust, B., Rousseau, F., Raoult, D., Fenollar, F., Mediannikov, O., 2017.
569 Natural Anaplasmatidae infection in *Rhipicephalus bursa* ticks collected from sheep in
570 the French Basque Country. *Ticks Tick Borne Dis.* 8, 18–24.
571 <https://doi.org/10.1016/j.ttbdis.2016.09.009>.

572 Dahmani, M., Davoust, B., Sambou, M., Bassene, H., Scandola, P., Ameer, T., Raoult D.,
573 Fenollar, F., Mediannikov, O., 2019. Molecular investigation and phylogeny of species of
574 the Anaplasmatidae infecting animals and ticks in Senegal. *Parasit. Vectors* 12, 495.
575 <https://doi.org/10.1186/s13071-019-3742-y>.

576 Dantas-Torres, F., Chomel, B.B., Otranto, D., 2013. Ticks and tick-borne diseases: A one
577 health perspective. *Trends Parasitol.* 28, 437–346.
578 <https://doi.org/10.1016/j.pt.2012.07.003>.

579 Diarra, A.Z., Almeras, L., Laroche, M., Berenger, J.M., KoneÂ, A.K., Bocoum, Z., Dabo, A.,
580 Doumbo, O., Raoult, D., Parola, P., 2017. Molecular and MALDI-TOF identification of

581 ticks and tick-associated bacteria in Mali. PLoS Negl. Trop. Dis. 11, e0005762.
582 <https://doi.org/10.1371/journal.pntd.0005762>.

583 Divers, T.J., Gardner, R.B., Madigan, J.E., Witonsky, S.G., Bertone, J.J., Swinebroad, E.L.,
584 Schutzer, S.E., Johnson, A.L., 2018. *Borrelia burgdorferi* infection and lyme disease in
585 North American horses: a consensus statement. J. Vet. Intern. Med. 32, 617–632.
586 <https://doi.org/10.1111/jvim.15042>.

587 Dumler, J.S., Barbet, A.F., Bekker, C.P., Dasch, G.A., Palmer, G.H., Ray, S.C., Rikihisa, Y.,
588 Rurangirwa, F.R., 2001. Reorganization of genera in the families Rickettsiaceae and
589 Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with
590 *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six
591 new species combinations and designation of *Ehrlichiaequi* and ‘HGE agent’ as
592 subjective synonyms of *Ehrlichia phagocytophila*. Int. J. Syst. Evol. Microbiol. 51,
593 2145–2165. <https://doi.org/10.1099/00207713-51-6-2145>.

594 Dvorak, V., Halada, P., Hlavackova, K., Dokianakis, E., Antoniou, M., Volf, P., 2014.
595 Identification of phlebotomine sand flies (Diptera: Psychodidae) by matrix-assisted laser
596 desorption/ ionization time of flight mass spectrometry. Parasit. Vectors 7, 21.
597 <https://doi.org/10.1186/1756-3305-7-21>.

598 Ehounoud, C.B., Yao, K.P., Dahmani, M., Achi, Y.L., Amanzougaghene, N., KacouN’Douba,
599 A., N’Guessan, J., Raoult, D., Fenollar, F., Mediannikove, O., 2016. Multiple pathogens
600 including potential new species in tick vectors in Côte d’Ivoire. PLoS Negl. Trop. Dis.
601 10, e0004367. <https://doi.org/10.1371/journal.pntd.0004367>.

602 Elhelw, R., Elhariri, M., Hamza, D., Abuowarda, M., Ismael, E., Farag, H., 2021. Evidence of
603 the presence of *Borrelia burgdorferi* in dogs and associated ticks in Egypt. BMC Vet.
604 Res. 17, 49. <https://doi.org/10.1186/s12917-020-02733-5>.

605 Elhariri, M.D., Elhelw, R.A., Hamza, D.A., Soliman, D.E., 2017. Molecular detection of
606 *Anaplasma marginale* in the Egyptian water buffaloes (*Bubalus bubalis*) based on major
607 surface protein 1. J. Egypt. Soc. Parasitol. 47, 247–252.

608 El-Naga, T.R.A., Barghash, S.M., 2016. Blood Parasites in Camels (*Camelus dromedarius*) in
609 Northern West Coast of Egypt. J. Bacteriol. Parasitol. 7, 258.

610 Elsayy, B.S.M., Nassar, A.M., Alzan, H.F., Bhoora, R.V., Ozubek, S., Mahmoud, M.S.,
611 Kandil, O.M., Mahdy, O.A., 2021. Rapid detection of equine piroplasms using Multiplex
612 PCR and first genetic characterization of *Theileria haneyi* in Egypt. Pathogens. 10, 1414.
613 <https://doi.org/10.3390/pathogens10111414>.

614 Estrada-Pena, A., Bouattour, A., Camicas, J.L., Walker, A.R., 2004. Ticks of Domestic
615 Animals in the Mediterranean Region. A guide to Identification of Species. 1st Ed. p131.

616 Estrada-Pena, A., Venzal, J.M., 2007. Climate niches of tick species in the Mediterranean
617 region: modeling of occurrence data, distributional constraints, and impact of climate
618 change. J. Med. Entomol. 44, 1130–1138. <https://doi.org/10.1093/jmedent/44.6.1130>.

619 Estrada-Pena, A., 2008. Climate, niche, ticks, and models: what they are and how we should
620 interpret them. Parasitol. Res. 103, 87–95. <https://doi.org/10.1007/s00436-008-1056-7>.

621 Fereig, R.M., Mohamed, S.G.A., Mahmoud, H., AbouLaila, M.R., Guswanto, A., Nguyen,
622 T.T., Mohamed, A.A., Inoue, N., Igarashi, I., Nishikawa, Y., 2017. Seroprevalence of
623 *Babesia bovis*, *B. bigemina*, *Trypanosoma evansi*, and *Anaplasma marginale* antibodies
624 in cattle in southern Egypt. Ticks Tick Borne Dis. 8, 125–131.
625 <https://doi.org/10.1016/j.ttbdis.2016.10.008>.

626 Fotso Fotso, A., Mediannikov, O., Diatta, G., Almeras, L., Flaudrops, C., Parola, P.,
627 Drancourt, M., 2014. MALDI-TOF mass spectrometry detection of pathogens in vectors:
628 the *Borrelia crociduræ/ Ornithodoros sonrai* paradigm. PLoS Negl. Trop. Dis. 8, e2984.
629 <https://doi.org/10.1371/journal.pntd.0002984>.

630 Friedhoff, K.T., Soule, C., 1996. An account on equine babesioses. Rev. Sci. Tech. 15, 1191–
631 1201. <https://doi.org/10.20506/rst.15.3.972>.

632 Fritz, D., 2010. A PCR study of piroplasms in 166 dogs and 111 horses in France (March
633 2006 to March 2008). Parasitol. Res. 106, 1339–1342. [https://doi.org/10.1007/s00436-](https://doi.org/10.1007/s00436-010-1804-3)
634 010-1804-3.

635 Gioia, G.V., Vinueza, R.L., Marsot, M., Devillers, E., Cruz, M., Petit, E., Boulouis, H.J.,
636 Moutailler, S., Monroy, F., Coello, M.A., Gondard, M., Bournez, L., Haddad, N.,
637 Zanella, G., 2018. Bovine anaplasmosis and tick-borne pathogens in cattle of the
638 Galapagos Islands. Transbound. Emerg. Dis. 65, 1262–1271.
639 <https://doi.org/10.1111/tbed.12866>.

640 Guglielmone, A.A., Robbins, R.G., Apanaskevich, D.A., Petnery, T.N., Estrada-Pena, A.,
641 2014. The Hard Ticks of the World, (Acari: Ixodidae). Springer, Dordrecht. 738.
642 <https://doi.org/10.1007/978-94-007-7497-1>.

643 Hafez, M., Hilali, M., Fouda, M., 1978. Ecological studies on *Hippobosca equina* (Linnaeus,
644 1758) (Diptera: Hippoboscidae) infesting domestic animals in Egypt. Zeitschrift für
645 Angewandte Entomologie. 87, 327–335. [https://doi.org/10.1111/j.1439-](https://doi.org/10.1111/j.1439-0418.1978.tb02458.x)
646 0418.1978.tb02458.x.

647 Hagen, R.M., Frickmann, H., Ehlers, J., Krüger, A., Margos, G., Hizo-Teufel, C., Fingerle,
648 V., Rakotozandrindrainy, R., von Kalckreuth, V., Im, J., Pak, D.G., Jeon, J.H.,
649 Rakotondrainiarivelo, P.J., Heriniaina, N.J., Razafindrabe, T., Konings, F., May, J.,
650 Hogan, B., Ganzhorn, J., Panzner, U., Schwarz, G.N., Dekker, D., Marks, F., Poppert, S.,
651 2018. Presence of *Borrelia* spp. DNA in ticks, but absence of *Borrelia* spp. and of
652 *Leptospira* spp. DNA in blood of fever patients in Madagascar. Acta Trop. 177, 127–134.
653 <https://doi.org/10.1016/j.actatropica.2020.105339>.

654 Haitham, E., Raoult, D., Drancourt, M., 2013. Relapsing fever borreliae in Africa. Am. J.
655 Trop. Med. Hyg. 89, 288–292. <https://doi.org/10.4269/ajtmh.12-0691>.

656 Hall, C.M., Busch, J.D., Scoles, G.A., Palma-Cagle, K.A., Ueti, M.W., Kappmeyer, L.S.,
657 Wagner, D.M., 2013. Genetic characterization of *Theileria equi* infecting horses in North
658 America: evidence for a limited source of U.S. introductions. *Parasit. Vectors* 11, 35.
659 <https://doi.org/10.1186/1756-3305-6-35>.

660 Hassan, M.I., Gabr, H.S.M., Abdel-Shafy, S., Hammad, K.M., Mokhtar, M.M., 2017.
661 Prevalence of tick-vectors of *Theileria annulata* infesting the one-humped camels in Giza,
662 Egypt. *J. Egypt. Soc. Parasitol.* 47, 425–432. <https://doi.org/10.12816/jesp.2017.77797>.

663 Karger, A., Kampen, H., Bettin, B., Dautel, H., Ziller, M., Hoffmann, B., Süß, J., Klaus, C.,
664 2012. Species determination and characterization of developmental stages of ticks by
665 whole-animal matrix-assisted laser desorption/ionization mass spectrometry. *Ticks Tick*
666 *Borne Dis.* 3, 78–89. <https://doi.org/10.1016/j.ttbdis.2011.11.002>.

667 Kaufman, E., Koehler, G., Butler, J.F., 2008. *External Parasites on Horses*. Cooperative
668 Extension Service, University of Florida: US Department of Agriculture.
669 <http://edis.ifas.ufl.edu/IG139>.

670 Knowles, D. Jr., 1996. Equine babesiosis (piroplasmosis): a problem in the international
671 movement of horses. *Br. Vet. J.* 152, 123–126. [https://doi.org/10.1016/S0007-](https://doi.org/10.1016/S0007-1935(96)80066-2)
672 [1935\(96\)80066-2](https://doi.org/10.1016/S0007-1935(96)80066-2).

673 Kouam, M.K., Masuoka, P.M., Kantzoura, V., Theodoropoulos, G., 2010. Geographic
674 distribution modeling and spatial cluster analysis for equine piroplasms in Greece. *Infect.*
675 *Genet. Evol.* 10, 1013–1018. <https://doi.org/10.1016/j.meegid.2010.06.014>.

676 Kowal, J., Nosal, P., Kornaś, S., Wajdzik, M., Matysek, M., Aga, M.B., 2016. Biodiversity
677 and importance of hippoboscids infection in ceroids. *Med. Weter.* 72, 745–749.
678 <https://doi.org/10.21521/mw.5602>.

679 Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular
680 Evolutionary Genetics Analysis across computing platforms. *Mol. Biol. Evol.* 35, 1547–
681 1549. <https://doi.org/10.1093/molbev/msy096>.

682 Kumsa, B., Socolovschi, C., Raoult, D., Parola, P., 2015. New *Borrelia* species detected in
683 ixodid ticks in Oromia, Ethiopia. *Ticks Tick Borne Dis.* 6, 401–407.
684 <https://doi.org/10.1016/j.ttbdis.2015.03.009>.

685 Kumsa, B., Laroche, M., Almeras, L., Mediannikov, O., Raoult, D., Parola, P., 2016.
686 Morphological, molecular and MALDI-TOF mass spectrometry identification of ixodid
687 tick species collected in Oromia, Ethiopia. *Parasitol. Res.* 115, 4199–4210.
688 <https://doi.org/10.1007/s00436-016-5197-9>.

689 Laus, F., Veronesi, F., Passamonti, F., Paggi, E., Cerquetella, M., Hyatt, D., Tesei, B.,
690 Fioretti, P., 2013. Prevalence of tick-borne pathogens in horses from Italy. *J. Vet. Med.*
691 *Sci.* 75, 715–720. <https://doi.org/10.1292/jvms.12-0449>.

692 Lu, X., Lin, X.D., Wang, J.B., Qin, X.C., Tian, J.H., Guo, W.P., Fan, F.N., Shao, R., Xu, J.,
693 Zhang, Y.Z., 2013. Molecular survey of hard ticks in endemic areas of tick-borne
694 diseases in China. *Ticks Tick Borne Dis.* 4, 288–296.
695 <https://doi.org/10.1016/j.ttbdis.2013.01.003>.

696 Mahmoud, M.S., El-Ezz, N.T., Abdel-Shafy, S., Nassar, S.A., El Namaky, A.H., Khalil,
697 W.K., Knowles, D., Kappmeyer, L., Silva, M.G., Suarez, C.E., 2016. Assessment of
698 *Theileria equi* and *Babesia caballi* infections in equine populations in Egypt by
699 molecular, serological and hematological approaches. *Parasit. Vectors* 9, 260.
700 <https://doi.org/10.1186/s13071-016-1539-9>.

701 Margalit Levi, M., Tirosh-Levy, S., Dahan, R., Berlin, D., Steinman, A., Edery, N., Savitski,
702 I., Lebovich, B., Knowles, D., Suarez, C.E., Baneth, G., Mazuz, M.L., 2018. First
703 detection of diffuse and cerebral *Theileria equi* infection in neonatal filly. *J. Equine Vet.*
704 *Sci.* 60, 23–28. <https://doi.org/10.1016/j.jevs.2017.10.016>.

705 McCoy, B.N., Maïga, O., Schwan, T.G., 2014. Detection of *Borrelia theileri* in *Rhipicephalus*
706 *geigy* from Mali. *Ticks Tick Borne Dis.* 5, 401–403.
707 <https://doi.org/10.1016/j.ttbdis.2014.01.007>.

708 Mediannikov, O., Diatta, G., Fenollar, F., Sokhna, C., Trape, J.F., Raoult, D., 2010. Tick-
709 borne rickettsioses, neglected emerging diseases in rural Senegal. PLoS Negl. Trop. Dis.
710 4, e821. <https://doi.org/10.1371/journal.pntd.0000821>.

711 Mediannikov, O., Abdissa, A., Socolovschi, C., Diatta, G., Trape, J.F., Raoult, D., 2013.
712 Detection of a new *Borrelia* species in ticks taken from cattle in Southwest Ethiopia.
713 Vector Borne Zoonotic Dis. 13, 266–269. <https://doi.org/10.1089/vbz.2011.0874>.

714 Mullen, G.R., Durden, L.A. 2009. Medical Veterinary Entomology. Academic Press, Elsevier.

715 Nava, S., Estrada-Pena, A., Petney, T., Beati, L., Labruna, B.M., Szabo, P.J.M., Venzal, M.J.,
716 Mastropaolo, M., Mangold, J.A., Guglielmo, A.A., 2015. The taxonomic status of
717 *Rhipicephalus sanguineus* (Latreille, 1806). Vet. Parasitol. 208, 2–8.
718 <https://doi.org/10.1016/j.vetpar.2014.12.021>.

719 Nebbak, A., Illcox, A.C., Bitam, I., Raoult, D., Parola, P., Almeras, L., 2016. Standardization
720 of sample homogenization for mosquito identification using an innovative proteomic tool
721 based on protein profiling. Proteomics. 16, 3148–3160.
722 <https://doi.org/10.1002/pmic.201600287>.

723 Nebbak, A., El Hamzaoui, B., Berenger, J.M., Bitam, I., Raoult, D., Almeras, L., Parola, P.,
724 2017. Comparative analysis of storage conditions and homogenization methods for tick
725 and flea species for identification by MALDI-TOF MS. Med. Vet. Entomol. 31, 438–348.
726 <https://doi.org/10.1016/j.vetpar.2014.12.021>.

727 Onmaz, A.C., Beutel, R.G., Schneeberg, K., Pavaloiu, A.N., Komarek, A., van den Hoven, R.,
728 2013. Vectors and vector-borne diseases of horses. Vet. Res. Commun. 37, 65–81.
729 <https://doi.org/10.1007/s11259-012-9537-7>.

730 Ozubek, S., Aktas, M., 2018. Genetic diversity and prevalence of piroplasm species in equids
731 from Turkey. Comp. Immunol. Microbiol. Infect. Dis. 59, 47–51.
732 <https://doi.org/10.1016/j.cimid.2018.08.005>.

733 Parola, P., Raoult, D., 2001. Ticks and tick-borne bacterial diseases in humans: an emerging
734 infectious threat. *Clin. Infect. Dis.* 32, 897–928. <https://doi.org/10.1086/319347>.

735 Parvizi, O., El-Adawy, H., Melzer, F., Roesler, U., Neubauer, H., Mertens-Scholz, K., 2020.
736 Seroprevalence and molecular detection of bovine anaplasmosis in Egypt. *Pathogens*. 9,
737 64. <https://doi.org/10.3390/pathogens9010064>.

738 Payne, V.K., Mbafor, F.L., Pone, J.W., Tchoumboué, J., 2017. Preliminary study of
739 ectoparasites of horses in the western highlands of Cameroon. *Vet. Med. Sci.* 3, 63–70.
740 <https://doi.org/10.1002/vms3.56>.

741 Pruneau, L., Moumène, A., Meyer, D.F., Marcelino, I., Lefrançois, T., Vachiéry, N., 2014.
742 Understanding Anaplasmataceae pathogenesis using “Omics” approaches. *Front Cell*
743 *Infect. Microbiol.* 4, 86. <https://doi.org/10.3389/fcimb.2014.00086>.

744 Raoult, D., Roblot, F., Rolain, J.M., Besnier J.M., Loulergue, J., Bastides, F., Choutet, P.,
745 2006. First isolation of *Bartonella alsatica* from a valve of a patient with endocarditis. *J.*
746 *Clin. Microbiol.* 44, 278–279. <https://doi.org/10.1128/JCM.44.1.278-279.2006>.

747 Rar, V., Golovljova, I., 2019. *Anaplasma*, *Ehrlichia*, and “*Candidatus Neoehrlichia*” bacteria:
748 pathogenicity, biodiversity, and molecular genetic characteristics, a review. *Infect. Genet.*
749 *Evol.* 11, 1842–1861. <https://doi.org/10.1016/j.meegid.2011.09.019>.

750 Rolain, J.M., Stuhl, L., Maurin, M., Raoult, D., 2002. Evaluation of antibiotic susceptibilities
751 of three rickettsial species including *Rickettsia felis* by a quantitative PCR DNA assay.
752 *Antimicrob. Agents Chemother.* 46, 2747–2751. [https://doi.org/10.1128/AAC.46.9.2747-](https://doi.org/10.1128/AAC.46.9.2747-2751.2002)
753 [2751.2002](https://doi.org/10.1128/AAC.46.9.2747-2751.2002).

754 Rothschild, C.M., 2013. Equine piroplasmiasis. *J. Equine Vet. Sci.* 33, 497–508.
755 <https://doi.org/10.1016/j.jevs.2013.03.189>.

756 Rothschild, C., Knowles, D., 2007. Equine piroplasmiasis. pp. 465–473. In: *Equine Infectious*
757 *Diseases* (Sellon DC and Long MT eds), Saunders, St. Louis.

758 Said, B.M., Belkahia, H., El Mabrouk, N., Saidani, M., Alberti, A., Zobba, R., Cherif, A.,
759 Mahjoub, T., Bouattour, A., Messadi, L., 2017. *Anaplasma platys*-like strains in
760 ruminants from Tunisia. *Infect. Genet. Evol.* 49, 226–233.
761 <https://doi.org/10.1016/j.meegid.2017.01.023>.

762 Saleem, S., Ijaz, M., Farooqi, S.H., Rashid, M.I., Khan, A., Masud, A., Mehmood, K., Zhang,
763 H., 2018. First molecular evidence of equine granulocytic anaplasmosis in Pakistan. *Acta*
764 *Trop.* 180, 18–25. <https://doi.org/10.1016/j.actatropica.2017.12.032>.

765 Scoles, G., Ueti, M.W., 2015. Vector ecology of equine piroplasmosis. *Annu. Rev. Entomol.*
766 60, 561–580. <https://doi.org/10.1146/annurev-ento-010814-021110>.

767 Selmi, R., Ben Said, M., Dhibi, M., Ben Yahia, H., Messadi, L., 2019. Improving specific
768 detection and updating phylogenetic data related to *Anaplasma platys*-like strains
769 infecting camels (*Camelus dromedarius*) and their ticks. *Ticks Tick-borne Dis.* 10,
770 101260. <https://doi.org/10.1016/j.ttbdis.2019.07.004>

771 Sokhna, C., Mediannikov, O., Fenollar, F., Bassene, H., Diatta, G., Tall, A., Trape, J.F.,
772 Drancourt, M., Raoult, D., 2013. Point-of-care laboratory of pathogen diagnosis in Rural
773 Senegal. *PLoS Negl. Trop. Dis.* 7: e1999. <https://doi.org/10.1371/journal.pntd.0001999>.

774 Štefanidesová, K., Kocianová, E., Boldiš, V., Košťánová, Z., Kanka, P., Nemethová, D.,
775 Špitálska, E., 2008. Evidence of *Anaplasma phagocytophilum* and *Rickettsia helvetica*
776 infection in free-ranging ungulates in central Slovakia. *Eur J Wild Res.* 54, 519–524.
777 <https://doi.org/10.1007/s10344-007-0161-8>.

778 Swinebroad, E.L., 2018. Borreliosis in Sport Horse Practice. *Vet. Clin. North. Am. Equine*
779 *Pract.* 34, 313–343. <https://doi.org/10.1016/j.cveq.2018.04.011>.

780 Tamzali, Y., 2013. Equine piroplasmosis: an updated review. *Equine Vet. Educ.* 25, 590–598.
781 <https://doi.org/10.1111/eve.12070>.

782 Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control
783 region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* 10, 512–526.
784 <https://doi.org/10.1093/oxfordjournals.molbev.a040023>.

785 Trape, J.F., Diatta, G., Arnathau, C., Bitam, I., Sarih, M., 2013. The epidemiology and geo-
786 graphic distribution of relapsing fever borreliosis in West and North Africa, with a review
787 of the *Ornithodoros erraticus* complex (Acari: Ixodida). *PLoS One.* 8, e78473.
788 <https://doi.org/10.1371/journal.pone.0078473>.

789 Tumwebaze, A.M., Lee, S.H., Adjou Moumouni, P.F., Mohammed-Geba, K., Sheir, S.K.,
790 Galal-Khallaf, A., Abd El Latif, M.H., Morsi, S.D., Bishr, M.N., Galon, M.E.,
791 Byamukama, B., Liu, M., Li, J., Li, Y., Ji, S., Ringo, E.A., Abdo Rizk, M., Suzuki, H.,
792 Ibrahim, M.H., Xuan, X., 2020. First detection of *Anaplasma ovis* in sheep and
793 *Anaplasma platys*-like variants from cattle in Menoufia governorate, Egypt. *Parasitol. Int.*
794 78, 102150. <https://doi.org/10.1016/j.parint.2020.102150>.

795 Vial, L., Diatta, G., Tall, A., Ba el, H., Bouganali, H., Durand, P., Sokhna, H., Rogier, C.,
796 Renaud, F., Trap, J.F., 2006. Incidence of tick-borne relapsing fever in West Africa:
797 longitudinal study. *Lancet.* 368, 37–43. [https://doi.org/10.1016/S0140-6736\(06\)68968-X](https://doi.org/10.1016/S0140-6736(06)68968-X).

798 Vichova, B., Majlathova, V., Novakova, M., Stanko, M., Hviscova, I., Pangracova, L.,
799 Chrudimsky, T., Curlik, J., Petko, B., 2014. *Anaplasma* infections in ticks and reservoir
800 host from Slovakia. *Infect. Genet. Evol.* 22, 265–272.
801 <https://doi.org/10.1016/j.meegid.2013.06.003>.

802 Walker, J.B., Keirans, J.E., Horak, I.G., 2000. The genus *Rhipicephalus* (Acari, Ixodidae): A
803 guide to the brown ticks of the world. Cambridge University Press, Cambridge, UK.

804 Walker, A.R., 2003. Ticks of domestic animals in Africa: Guide to identification of species.
805 Edinburgh, Biosciences Reports.

806 Wise, L.N., Kappmeyer, L.S., Mealey, R.H., Knowles, D.P., 2013. Review of equine
807 piroplasmosis. *J. Vet. Intern. Med.* 27, 1334–1346. <https://doi.org/10.1111/jvim.12168>.

808 Yssouf, A., Flaudrops, C., Drali, R., Kernif, T., Socolovschi, C., Berenger, J.M., Raoult, D.,
809 Parola, P., 2013. Matrix-assisted laser desorption ionization-time of flight mass
810 spectrometry or rapid identification of tick vectors. *J. Clin. Microbiol.* 51, 522–528.
811 <https://doi.org/10.1128/JCM.02665-12>.

812 Yssouf, A., Almeras, L., Raoult, D., Parola, P., 2016. Emerging tools for identification of
813 arthropod vectors. *Future Microbiol.* 11, 549–566. <https://doi.org/10.2217/fmb.16.5>.

814 Zanet, S., Bassano, M., Trisciuglio, A., Taricco, I., Ferroglio, E., 2017. Horses infected by
815 Piroplasms different from *Babesia caballi* and *Theileria equi*: species identification and
816 risk factors analysis in Italy. *Vet. Parasitol.* 236, 38–41.
817 <https://doi.org/10.1016/j.vetpar.2017.01.003>.

818 Zobba, R., Ardu, M., Niccolini, S., Chessa, B., Manna, L., Cocco, R., Pinna Parpaglia, M.L.,
819 2008. Clinical and laboratory findings in equine piroplasmosis. *J. Equine. Vet. Sci.* 28,
820 301–308. <https://doi.org/10.1016/j.jevs.2008.03.005>.

821 Zobba, R., Anfossi, A.G., Pinna Parpaglia, M.L., Dore, G.M., Chessa, B., Spezzigu, A.,
822 Rocca, S., Visco, S., Pittau, M., Alberti, A., 2014. Molecular investigation and phylogeny
823 of *Anaplasma* sp. in Mediterranean ruminants reveal the presence of neutrophil-tropic
824 strains closely related to *A. platys*. *Appl. Environ. Microbiol.* 80, 271–280.
825 <https://doi.org/10.1128/AEM.03129-13>.

826

827

828 **Table 1.** Primers and probes used for qPCR, standard PCR, and sequencing in this study

Microorganisms	Targeted Sequence	Primers f, r (5'-3') and Probes p (6FAM-TAMRA)	References
Quantitative PCR primers and probes			
Piroplasmida	5.8S rRNA	f_AYYKTYAGCGRTGGATGTC r_TCGCAGRAGTCTKCAAGTC p_TTYGCTGCGTCCTTCATCGTTGT	Dahmana et al. (2019)
Anaplasmataceae	23S rRNA (TtAna)	f_TGACAGCGTACCTTTTGCAT r_TGGAGGACCGAACCTGTTAC p_GGATTAGACCCGAAACCAAG	Dahmani et al. (2017)
<i>Rickettsia</i> sp.	gltA (RKND03)	f_GTGAATGAAAGATTACACTATTTAT r_GTATCTTAGCAATCATTCTAATAGC p_CTATTATGCTTGCGGCTGTCGGTTC	Rolain et al. (2002)
<i>Borrelia</i> sp.	Internal transcribed spacer 16S RNA (Bor ITS4)	f_GGCTTCGGGTCTACCACATCTA r_CCGGGAGGGGAGTGAAATAG p_TGCAAAAGGCACGCCATCACC	Bottieu et al. (2012)
<i>Coxiella burnetii</i>	Insertion Sequence (IS1111)	f_CAAGAAACGTATCGCTGTGGC r_CACAGAGCCACCGTATGAATC p_CCGAGTTCGAAACAATGAGGGCTG	Mediannikov et al. (2010)
<i>Bartonella</i> sp.	Internal transcribed spacer16S (BartoITS3)	f_GATGCCGGGGAAGGTTTTTC r_GCCTGGGAGGACTTGAACCT p_GCGCGCGCTTGATAAGCGTG	Raoult et al. (2006)
Standard PCR and Sequencing primers			
Piroplasmida	18S rRNA	F1- GCGAATGGCTCATTATAACA F4-CACATCTAAGGAAGGCAGCA F3-GTAGGGTATTGGCCTACCG R3-AGGACTACGACGGTATCTGA	Dahmana et al. (2019)
Anaplasmataceae	23S rRNA	Ana23S-212F-ATAAGCTGCGGGGAATTGTC Ana23S-753R-TGCAAAAGGTACGCTGTCAC	Dahmani et al. (2017)
<i>Borrelia</i> sp.	16S rRNA	16S-F-GCTGGCAGTGCGTCTTAAGC 16S-R-GCTTCGGGTATCCTCAACTC	Bottieu et al. (2012)
Arthropods Identification primers	16S rRNA CO1	16Stic.F-CCGGTCTGAACTCAGATCAAGT 16Stic.R-GCTCAATGATTTTTTAAATTGCTGT CO1F-GGAACAATATATTTAATTTTTGG CO1R-ATCTATCCCTACTGTAAATATATG	Boucheikhchoukh et al. (2018) Chitimia et al. (2010)

829 **Table 2.** Information data from the animals studied and their arthropods

Provinces Animals (Total)	Gender Male/Female	Breed Native/Foreign/Arabic	Tick species (<i>Rhipicephalus</i> sp.) (M/F)	Sucking Flies (<i>Hippobosca equina</i>) (M/F)
Cairo Horses (311)	202/109	271/34/6	--	105 (70/35)
Beni Suef Horses (9)	3/6	9/0/0	0/28	--
Donkeys (15)	0/15	15/0/0	4/29	--
Total	205/130 (335)	295/34/6 (335)	4/57 (61)	105

830

831 **Table 3.** The prevalence of pathogens in equines and associated arthropods

832

833

Animal Hosts /Arthropods	No. of examined Animals/Arthropods	Pathogens amplified	No. of infected Animals/Arthropods (%)
Horse	320	Piroplasmida <i>Theileria equi</i> <i>Theileria sp. Africa</i>	13/320 (4.1%) 4/320 (1.3%) 9/320 (2.8%)
Donkeys	15	Anaplasmataceae <i>A. marginale</i> <i>A. ovis</i> Piroplasmida <i>T. ovis</i>	4/15 (26.6%) 2/15 (13.3%) 2/15 (13.3%) 2/15 (13.3%) 2/15 (13.3%)
<i>R. annulatus</i>	61	Anaplasmataceae <i>A. platys</i> -like <i>A. marginale</i> <i>Candidatus E. rustica</i> <i>Ehrlichia sp.</i> <i>Borrelia sp.</i> <i>B. theileri</i>	9/61 (14.7%) 1/61 (1.6%) 1/61 (1.6%) 4/61 (6.6%) 3/61 (4.9%) 2/61 (3.3%) 2/61 (3.3%)

834

835 **Figure 1.** The arthropods collected from horses and donkeys, the louse fly *Hippobosca equina* (a–
836 c), and ixodid tick *Rhipicephalus* (formerly *Boophilus*) *annulatus* (d–g): (a) dorsal view of *H.*
837 *equina*, (b) ventral view of *H. equina* female, (c) ventral view of *H. equina* male, (d) dorsal view of
838 *R. annulatus* female, (e) ventral view of *R. annulatus* female, (f) dorsal view of *R. annulatus* male,
839 and (g) ventral view of *R. annulatus* male

840 **Figure 2.** Mass spectrometry profiles of *Hippobosca equina* aligned using flexAnalysis v3.3
841 software. [Intens] Intensity; [a.u] arbitrary units; [m/z] mass-to-charge ratio. A random color is
842 used for the spectrum of each specimen

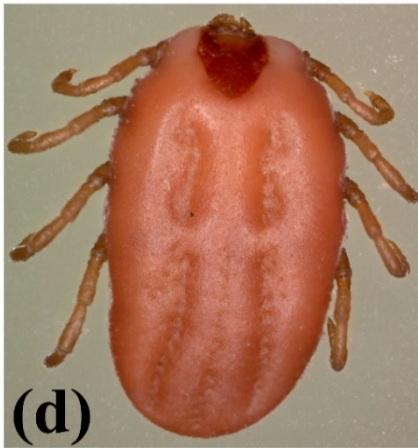
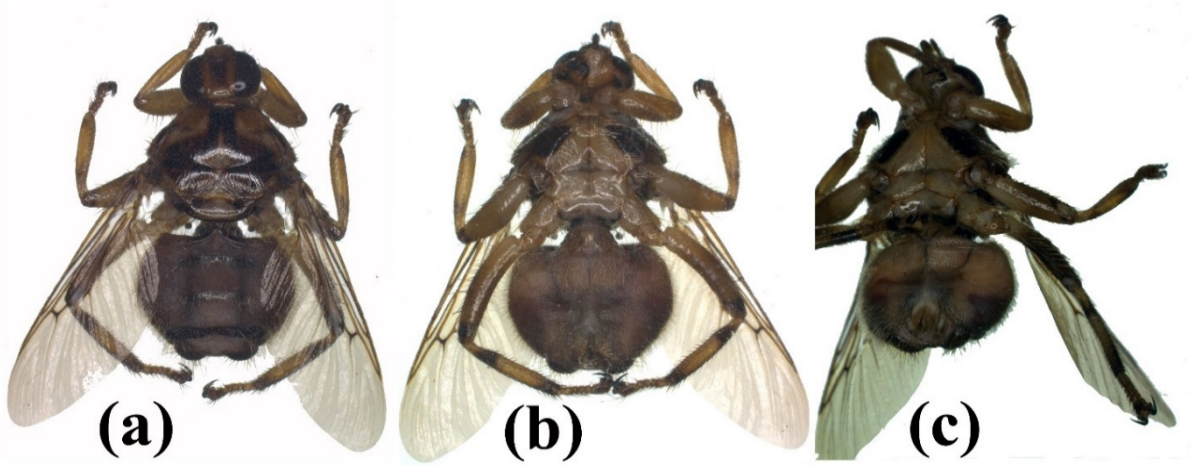
843 **Figure 3.** Mass spectrometry profiles of *Rhipicephalus annulatus* genotypes aligned using
844 flexAnalysis v3.3 software. [Intens] Intensity; [a.u] arbitrary units; [m/z] mass-to-charge ratio. A
845 random color is used for the spectrum of each specimen

846 **Figure 4.** Phylogenetic analysis using the maximum likelihood method based on CO1 for
847 *Rhipicephalus* sp. The new obtained sequences in this study are highlighted (**bold**)

848 **Figure 5.** Phylogenetic analysis using the maximum likelihood method based on 23S rRNA for
849 *Anaplasma* sp. and *Ehrlichia* sp. The new obtained sequences in this study are highlighted (**bold**)

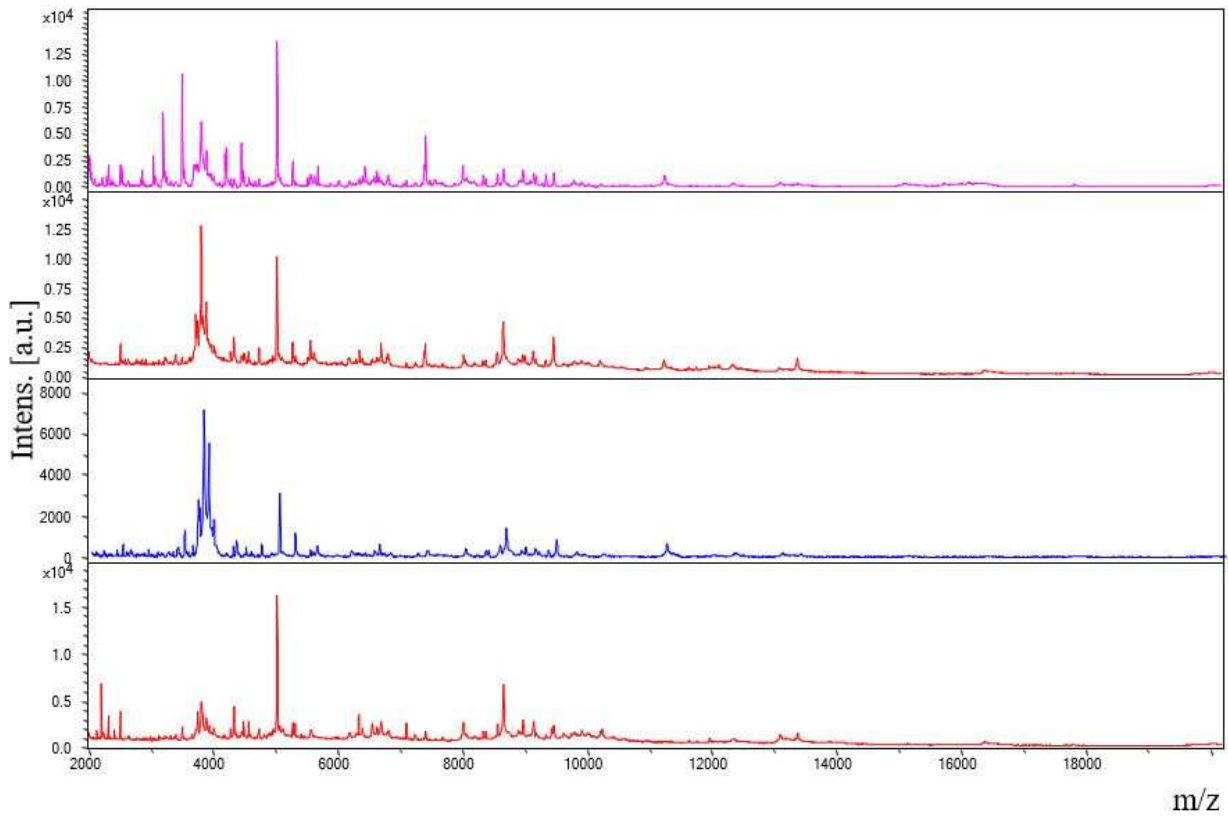
850 **Figure 6.** Phylogenetic analysis using the maximum likelihood method based on 16S rRNA for
851 *Borrelia* sp. The new obtained sequences in this study are highlighted (**bold**)

852 **Figure 7.** Phylogenetic analysis using the maximum likelihood method based on 18S rRNA for
853 *Theileria* sp. The new obtained sequences in this study are highlighted (**bold**)



1
2
3

Figure 1.



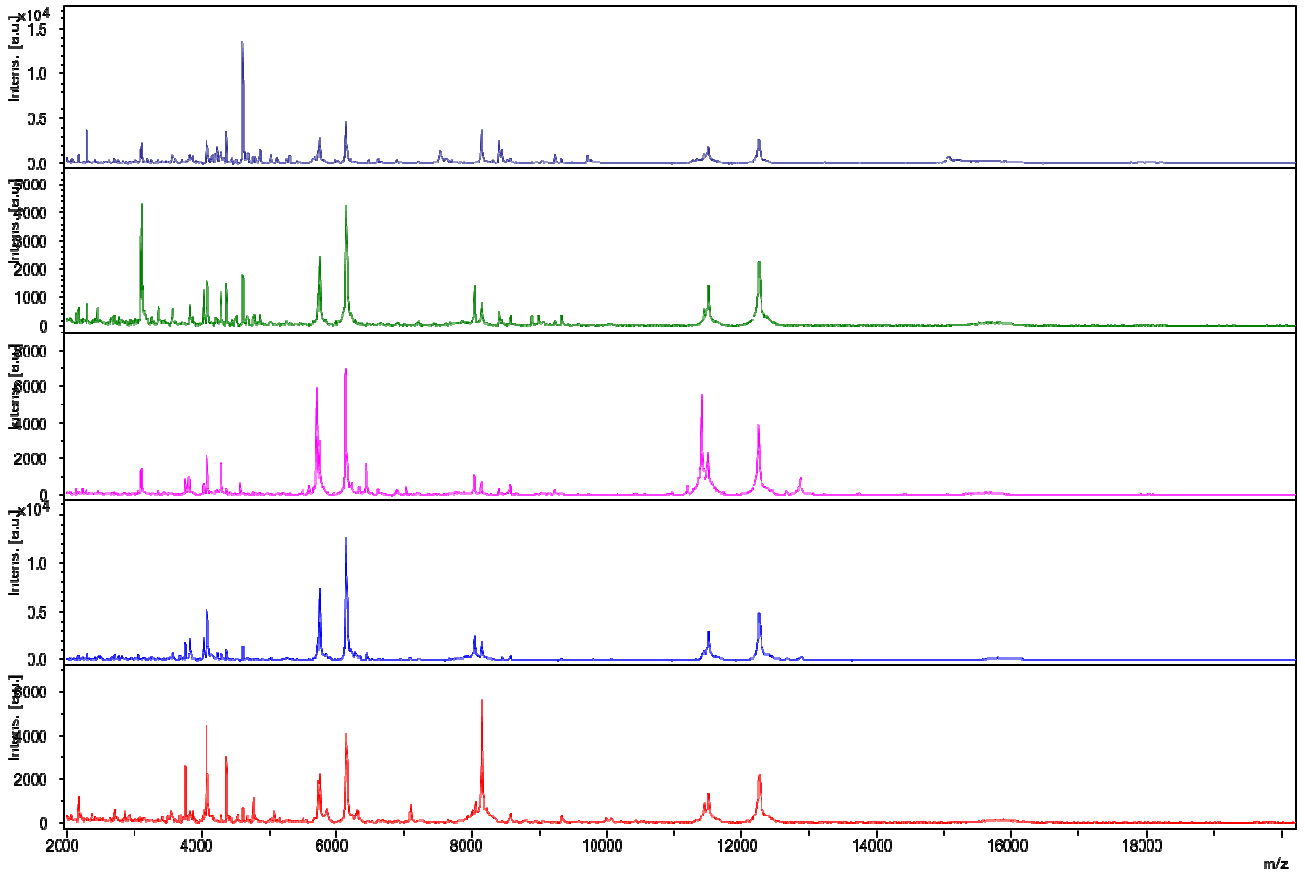
4

5

6

Figure 2.

7



8

Figure 3.

9

10



11

12

13

Figure 4.



0.05

14

15

Figure 5.

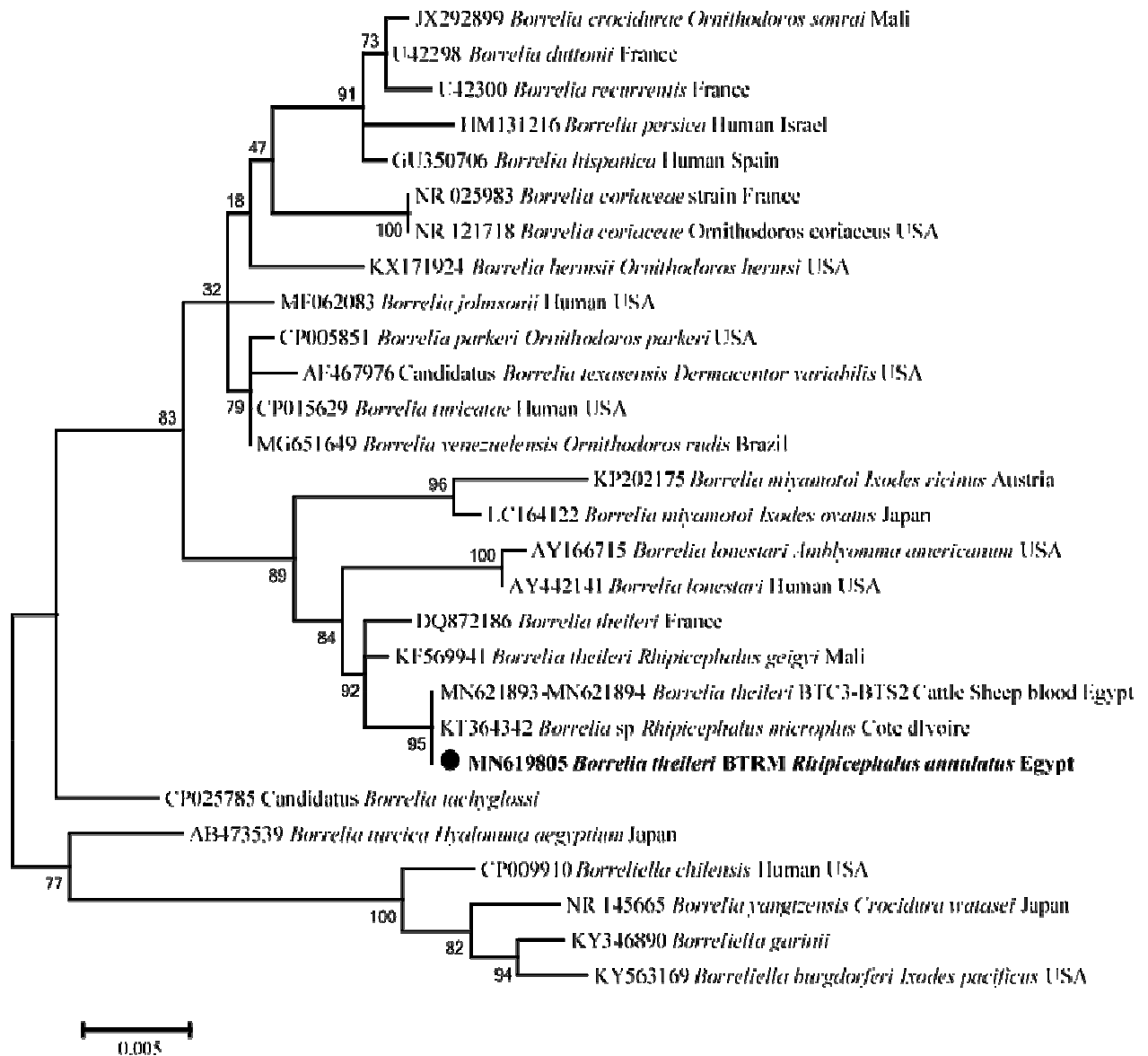
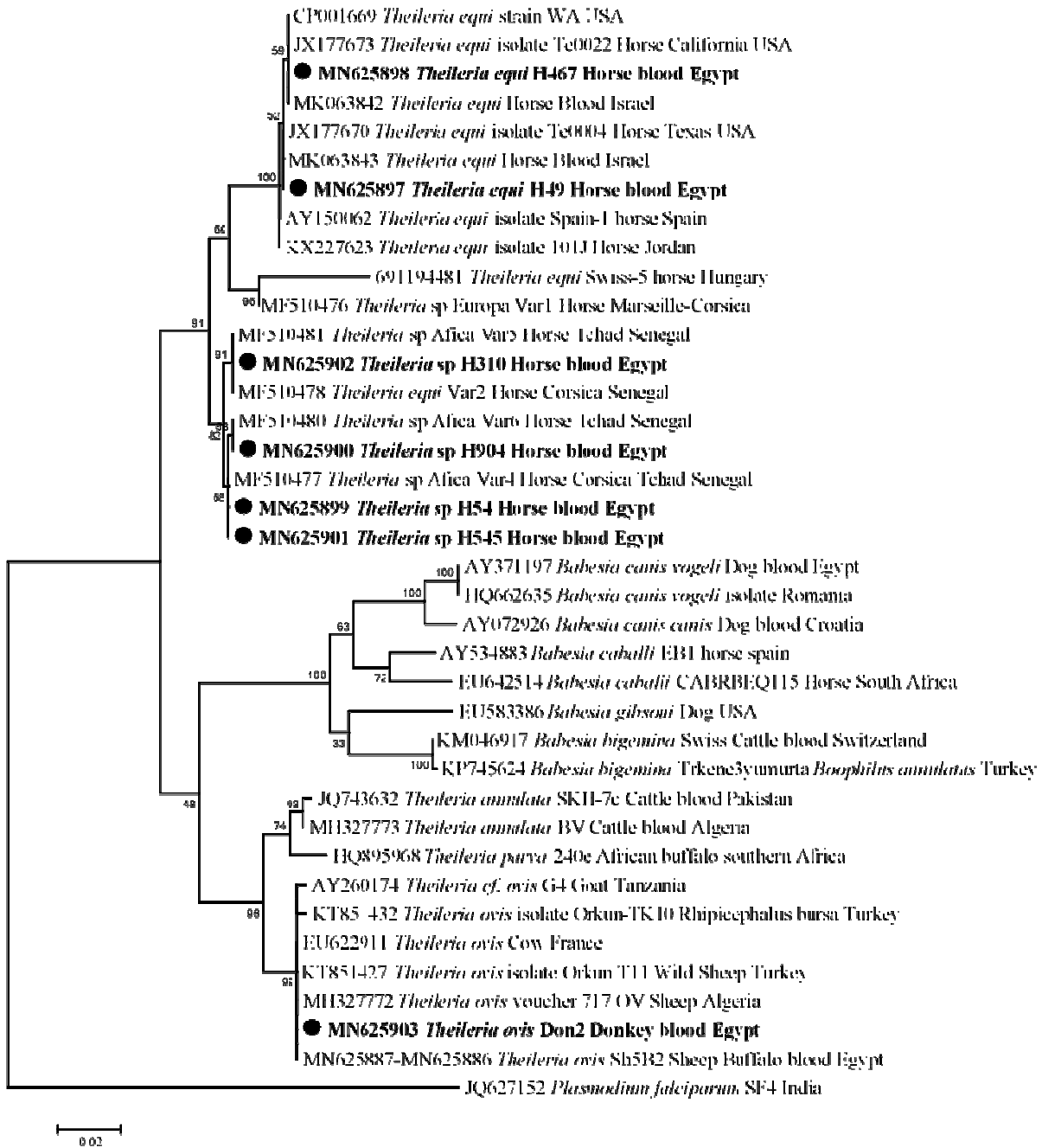


Figure 6.

16

17

18



19

20

Figure 7.