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1 **Ticks and Tick-borne Diseases**

2 **Research Article**

3

4 **Molecular identification and evaluation of *Coxiella*-like endosymbionts genetic**
5 **diversity carried by cattle ticks in Algeria**

6

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22

23 **Running title:** Diversity of *Coxiella*-like endosymbionts carried by cattle ticks from
24 Algeria

25 **Number of words: 3219**

26 **Abstract**

27 *Coxiella*-like bacteria are a large group of yet-to-isolate and characterize bacteria
28 phylogenetically close to the agent of Q fever, *Coxiella burnetii*, and often associated with
29 ixodid ticks worldwide.

30 This study was designed to assess the presence of *Coxiella*-like endosymbionts (CLE) in
31 ticks and to describe their genetic diversity in different tick species infesting cattle in Algeria.
32 A total of 765 ticks were collected from three locations. The screening of 20 % of sampled
33 ticks (147/765) exhibited the presence of *Coxiella*-like in 51.7 % (76/147). The sequencing of
34 partial 16S rRNA and the *GroEl* genes showed an identity higher than 98 % with different
35 *Coxiella*-like endosymbionts. The phylogenetic analysis based on the 16S rRNA gene showed
36 the positions of identified *Coxiella* bacteria. Eleven of the 13 sequences from *Rhipicephalus*,
37 *Dermacentor* and *Hyalomma* ticks were grouped in a distinct clade, the other two each
38 represent an independent clade.

39 This study reported that CLE are prevalent in cattle ticks. Most of the identified *Coxiella*-
40 like bacteria, from different species of ticks found on cattle, were identical. This may mean
41 that, unlike the currently accepted paradigm, *Coxiella*-like bacteria are not only tick host-
42 associated, but rather can be transmitted from one tick species to another via the vertebrate
43 host.

44

45 **Key words:** *Coxiella*-like, Ticks, Cattle, qPCR, sequencing, Algeria.

46

47 **1. Introduction**

48 Ticks and mosquitoes are considered the most important vectors of pathogenic
49 microorganisms for both humans and terrestrial vertebrate animals in the world (Dehghani et
50 al., 2019). A high number of infectious agents, such as viruses, bacteria and protozoans, are
51 transmitted by ticks (Brinkmann et al., 2019).

52 All tick-borne diseases (TBDs) might be considered zoonotic diseases that represent one of
53 the most rapidly expanding arthropod-borne diseases (Pfäffle et al., 2013), since several tick-
54 borne infections with a significant impact on human and animal's health have been observed in
55 recent decades (Brinkmann et al., 2019).

56 To date, about 900 tick species have been identified and classified worldwide, while in
57 Africa, 223 tick species have been described, 180 of which are classified as hard and 43 as soft
58 ticks (Diarra et al., 2017). Besides, the pathogens carried by these ticks, diverse commensal and
59 symbiotic microorganisms are also present, such as *Coxiella*-like endosymbionts (CLEs)
60 (Špitalská et al., 2018; Zhong, 2012).

61 *Coxiella burnetii*, the Q fever agent distributed worldwide, has already been reported many
62 times in Algeria (Angelakis et al., 2014; Khaled et al., 2016). Multispacer type 20 was
63 determined to be the circulating genotype in cattle in the northern Algeria (Rahal et al., 2018).

64 According to Duron et al. (2015), all strains of *C. burnetii* are the descendants of a
65 *Coxiella*-like progenitor. Consequently, they originated from this group hosted by ticks. No
66 strains of *Coxiella*-like are yet cultured in the environment of vertebrate cells, suggesting that
67 their metabolic needs are different from those of *C. burnetii*. In addition, the recent evolvement
68 of *C. burnetii* from an inherited symbiont of ticks allowed it to infect vertebrates after
69 acquisitions of novel virulence factors (Duron et al., 2015).

70 According to the recent sequencing of *Coxiella*-like bacterium found in *Amblyomma*
71 *americanum* (Smith et al., 2015), recognizable virulence genes have not been found and the
72 main biosynthesis pathways of vitamins and cofactors are encoded by its genome. Thus, it may
73 be a vitamin-provisioning endosymbiont (Duron et al., 2015). Such bacterial functions might be
74 involved in a mutualistic interaction with the tick host by compensating nutritional deficiencies.
75 In fact Guizzo et al. (2017) and Gottlieb et al. (2015) reported that the biosynthetic pathways
76 for the vitamins Biotin (B7), Riboflavin (B2), Pyridoxine (B6), Folic acid (B9) and
77 Pantothenate (B5) are found in all CLE. In addition, the coding genes for the cofactors Flavin

78 adenine dinucleotide (FAD) and Coenzyme A (CoA) are found in all CLEs A hypothesis is
79 supported with the observed decreases in ticks fecundity and life-history traits successive to the
80 symbiont elimination (René-Martellet et al., 2017). The study conducted by Guizzo et al.
81 (2017) and (Zhong et al., 2007) showed that tetracycline treatment of ticks led to the reduction
82 of levels of bacteria in progeny in 74 % for eggs and 90 % for larvae, and hampered the tick to
83 reach the adult life stage, as no tick was able to progress beyond the metanymph stage. While
84 Lalzar et al. (2012), suggested vertical transmission with a high fidelity of CLE since they
85 identified it in eggs and larvae of *Rhipicephalus sanguineus* sensu lato-associated (*R.*
86 *sanguineus* SL-A). This vertical transmission showed 98 % of the 16S rRNA sequence identity
87 in both eggs and larvae (Guizzo et al., 2017), which may explain the high frequency of
88 *Coxiella*-like in the tick populations.

89 However, *Coxiella*-like was confirmed to be a new agent of human infections. These
90 bacteria can possibly lead to an atypical scalp eschar and neck lymphadenopathy syndrome
91 with delayed evolution to crust eschar in the area of the tick-bite (Guimard et al., 2017).

92 A genetic variant of *R. sanguineus* SL-A *Coxiella*-like bacterium was detected in skin
93 biopsy samples and ticks collected from patients with eschar (Angelakis et al., 2016). This is
94 evidence that at least this strain might be responsible for scalp eschar and neck
95 lymphadenopathy after a tick bite (Guimard et al., 2017). Furthermore, a multiorgan infection
96 with a *Coxiella*-like organism was determined to be the cause of death of a female eclectus
97 parrot *Eclectus roratus* (Vapniarsky et al., 2012).

98 Although *Coxiella*-like bacteria and *C. burnetii* share a certain degree of identity, it is not
99 high enough to be clustered in the same species (Angelakis et al., 2016). All *Coxiella*-like
100 bacteria undoubtedly belong to the genus *Coxiella* in the gamma-subgroup of the
101 *Proteobacteria*. This taxonomy was based on the 16S rRNA nucleotide sequences. The
102 sequence identity of the 16S rRNA gene among the *Coxiella* -like bacteria varies from species
103 to species. The assessment of 16S rRNA nucleotide sequencing between *Coxiella*-like strains
104 showed that the maximal identity between them reached 98 %, while the minimal declined to
105 91 % (Zhong, 2012).

106 The aim of this study was to assess the occurrence and genetic diversity of *Coxiella*-like
107 ticks in cattle in Algeria, where no studies on *Coxiella*-like have been conducted to date.

108 **2. Material and Methods**

109 **2.1. Period and area of the study:**

110 Seven hundred sixty-five (765) ticks were collected from dairy cattle between 2013 and
111 2015, mainly in spring and summer. All ticks (engorged and non-engorged) were collected
112 manually with forceps, as described before (Diarra et al., 2017). The ticks were counted, pooled
113 in tubes and kept frozen at -20 C until morphological, molecular and MALDI-TOF MS
114 analyses. Samples were transported from Algeria to the IHU-Méditerranée Infection, Marseille,
115 France to perform analysis.

116 The samples collection was carried out in three provinces in northern Algeria, two of which
117 were in the central northern region named Blida (36° 33' 3.258" N, 2° 54' 31.113" E) and
118 Medea (36° 15' 43.54" N, 2° 45' 18.947" E), and the third location is Batna (35° 39'
119 34.671" N, 6° 19' 42.839" E) located in northeastern of Algeria (Fig. 2).

120 In Blida, 469 ticks were collected, 238 of which were collected from cattle living in the
121 wild in the mountains and brought to the farm only once a month by the farmer. The climate in
122 Blida province is Mediterranean. In winter, precipitation is more abundant than in summer,
123 with about 791 millimeters of precipitation per year. The average annual temperature in Blida
124 is 17.9 °C. However, in Medea (130 ticks), the climate is distinguished by its position above the
125 Tellian Atlas mountains, its altitude of 1,240 meters and its exposed to winds and waves of
126 westerly currents, Blida and Medea are characterized by Mediterranean climate with dry and
127 hot summers (Kottek et al., 2006). While Batna is located in the Aurès Mountains (part of the
128 Atlas Mountains), at 1,048 meters above sea level. Batna (166 ticks) has a cold steppe climate
129 (Kottek et al., 2006), with Mediterranean influences and average annual precipitation of 326
130 mm. Summers are moderately hot (by Saharan standards) and dry. Winters are chilly and
131 wetter, with the possibility of snowfall, more details about the long-term mean temperature of
132 the coldest and the warmest month are shown in the Fig. 1.

133

134 **2.2. Morphological identification**

135 This task was performed down to species level if possible, while engorged ticks were
136 identified at the genus level only. The tick identification was performed twice by two expert
137 tick entomologists and checked using previously established taxonomic identification keys

138 (Walker and Bouattour, 2003). This task was performed under a microscope at a magnification
139 of $\times 56$ (Zeiss Axio Zoom.V16, Zeiss, Marly le Roi, France).

140

141 **2.3. DNA extraction**

142 To complete the molecular identification of ticks and the screening for the presence of
143 *Coxiella*-like bacteria, 147 of the 735 ticks were selected according to their previous
144 morphological identification. The selection was accomplished randomly conferring to their
145 diversities. Thus the selection concerned all species identified morphologically (15 ticks
146 maximum for each specie, in case we had low number of species type e.g. *Ixodes ricinus* 5/735
147 all of them were chosen). Moreover the selected ticks belonged to both sexes (male and
148 female), two life stages (nymph, adult), and different engorgement states (engorged or unfed).

149 DNA extraction was carried out according to the manufacturer's instructions. Briefly,
150 each half tick without legs was incubated at 56°C overnight with 180 μ L of G2 lysis buffer
151 (Qiagen, Hilden, Germany) and 20 μ L proteinase K (Hilden, Germany). Successively, the
152 extraction using an EZ1 DNA Tissue Kit (Hilden, Germany) and EZ1 BioRobot extraction
153 device was realized. Finally, 200 μ L total genomic DNA was eluted in Tris-EDTA (TE) buffer
154 (Hilden, Germany) and kept at -20°C until use.

155

156 **2.4. *Coxiella*-like qPCR screening**

157 All selected ticks (20 %; 147/735) were screened for the presence of *Coxiella*-like
158 endosymbionts, using a qPCR assay targeting the rrs gene (Angelakis et al., 2016). Reaction
159 mix for qPCR assay contained 5 μ l of the DNA template, 10 μ l of Eurogentec Master Mix
160 Roche, 0.5 μ l UDG, 0.5 μ l of each primer (at 20 μ M concentration), and 0.5 μ l of the FAM-
161 labeled probe (at 5 μ M concentration), (Table 1) and 3 μ l of distilled water DNase and RNase
162 free, for a final volume of 20 μ l. The qPCR amplification was performed in a CFX96 Real-
163 Time system (Bio-rad Laboratories, Foster City, CA, USA). with the following thermal profile:
164 the first step consisted in an incubation at 50°C for two min and an initial denaturation step at
165 95°C for five min, followed by 40 cycles of denaturation at 95°C for 5 s and annealing-
166 extension at 60°C for 30 s. We included master mixture in two wells as negative controls for

167 each test, while DNA from *Rhipicephalus bursa* reared in the laboratory, was used as positive
168 control. Samples were considered positive when the cycle threshold (Ct) was lower than 35 Ct.

169

170 **2.5. Molecular identification of ticks by 16S rRNA gene sequencing**

171 The morphological identification of ticks showed positive results during the screening (76
172 ticks) was confirmed by 16S rRNA gene sequencing for 58/76 of them. All selected samples
173 were subjected to standard PCR in an automated DNA thermal cycler. Details about the
174 systems used in the standard PCR are summarized in Table 1.

175 The standard PCR assays consisted of a volume of 25 µl, including 12.5 µl of Amplitaq
176 Gold Master Mix, 0.75 µl of each primer (at 20 µM concentration), 5 µl of DNA model and
177 water. The thermal cycling conditions were: incubation step at 95°C for 15 min, 40 cycles of 1
178 min at 95°C, 30 s annealing at hybridization temperature and one min at 72°C followed by a
179 final extension for five min at 72°C (Table 1). PCR amplification was performed in a Peltier
180 PTC-200 model thermal cycler (MJ Research Inc., Watertown, MA, USA). The results of
181 amplification were visualized by electrophoresis on 2 % agarose gel. The purification of PCR
182 products was performed using Nucleo Fast 96 PCR plates (Macherey Nagel EURL, Hoerd, t,
183 France) according to the manufacturer's instructions. The amplicons were sequenced using the
184 Big Dye Terminator Cycle Sequencing Kit (Perkin Elmer Applied Biosystems, Foster City,
185 CA, USA) with an ABI automated sequencer (Applied Biosystems). The sequences obtained
186 were assembled and analyzed using the ChromasPro software (version 1.34) (Technelysium
187 Pty. Ltd., Tewantin, Australia). Finally, assembled sequences were compared with those
188 available in GenBank using the BLAST server from the National Centre for Biotechnology
189 (<http://www.ncbi.nlm.nih.gov/blast/>).

190

191 **2.6. *Coxiella*-like typing**

192 Following the precise molecular identification of ticks, two strains samples belonging to
193 the same species were selected for the typing by PCR / sequencing of the *Coxiella*-like 16S
194 rRNA and *GroEL* partial genes, since *Coxiella*-like bacteria are hypothetically specific
195 endosymbionts of ticks according to the species (Duron et al., 2015).

196 The experimental protocol for the conventional PCRs, purification of PCR products,
197 sequencing, assembly and sequences analysis were performed as described above. These tasks
198 were repeated four times for each system (*GroEL* and 16S) to support the finding and avoid all
199 possibility of contamination between samples.

200

201 **2.7. Phylogenetic analyses**

202 To carry out the construction of the phylogenetic tree, the *Coxiella*-like obtained sequences
203 in the present study were aligned with reference sequences available in the GenBank database
204 using ClustalW multiple sequence alignment algorithm. A maximum-likelihood method
205 (Kimura 2-parameter model) was used to infer the phylogenetic analyses and tree
206 reconstruction was performed using MEGA6.06 software (Tamura et al., 2013). Bootstrap
207 analyses were conducted using 500 replicates.

208

209 **2.8. Statistical analysis**

210 After set of databases Microsoft Excel® program (Microsoft Corp. Redmont, USA), a
211 descriptive study of ticks' population and of the CLE carriage was performed. The statistical
212 analysis was conducted using XLSTAT Addinsoft version 2018.7 (Data Analysis and
213 Statistical Solution for Microsoft Excel, Paris, France). The comparison of CLE infection's
214 prevalence according to the regions was accomplished using Khi-2 test, for that statistical
215 signification was considered at P-value < 0.05.

216

217 **3. Results**

218 **3.1. Tick identification**

219 In this study, 735 hard ticks were collected from cattle. Thirteen species belonging to five
220 genera were identified as cattle ticks in the three northern provinces of Algeria. Almost all of
221 ticks in north-central Algeria (Blida and Medea) belonged to the genus *Rhipicephalus*, while in
222 Batna, in the northeast, species belonging to genus *Hyalomma* were the most often identified
223 ticks. More details are provided in Fig. 2.

224

225 **3.2. Prevalence of *Coxiella*-like in ticks collected in northern of Algeria**

226 In this study, we performed an extensive screening of *Coxiella*-like on 147 selected ticks
227 (20 % of all ticks collected were selected for DNA extraction). Using qPCR, the prevalence
228 revealed in this study was 51.7 % (76/147) among all the selected ticks for the screening. The
229 prevalence of *Coxiella*-like in hard ticks in Medea province of 80 % (21/26) was higher than in
230 Blida province, 47 % (47/100) (value P = 0.001); traditional breeding cattle, 55 % (27/49) and
231 wild cattle, 39 % (20/51). Prevalence in Medea was also higher than Batna province, 24 %
232 (8/33) (P-value <0.0001). No differences were observed in *Coxiella*-like carriage in ticks
233 between Blida and Batna (P-value= 1.000). *Coxiella*-like DNA was detected in (2/2) of
234 *Dermacentor marginatus* and *Haemaphysalis sulcata*, in 36 % (8/22) of *Hyalomma excavatum*,
235 in (1/4) of *Hyalomma detritum*, in (1/3) of *Hyalomma lusitanicum*, in 14 % (4/28) of
236 *Hyalomma marginatum* and in 15 % (2/13) of unidentified *Hyalomma* spp.

237 Regarding the genus *Rhipicephalus*; *Coxiella*-like DNA was detected in 81 % (26/32) of *R.*
238 *bursa*, in 61 % (11/18) of *R. sanguineus* SL-A and in 64 % (16/25) of unidentified
239 *Rhipicephalus* spp. Finally, 50 % (3/6) of *I. ricinus* were found to be carriers of *Coxiella*-like.

240

241 **3.3. Phylogenetic analysis**

242 Sequencing of the positive samples using the *Coxiella*-like specific primers 16S rRNA
243 failed in 10 of 23 samples, as a result 13 samples were successfully sequenced, and provided a
244 BLAST identity percentage greater than 98 % for better identification (Table 2).

245 Sequencing using the specific *GroEL* primers also failed in 19/23 sequenced with these
246 primers, so only four could be sequenced. The best BLAST hit oscillated between 98.38 % and
247 99.63 %. Of the 4 sequences, two (samples 111 and 118) exhibited 98.38 and 98.63 % of
248 identity with *C. burnetii* str. Schperling (CP014563). The other samples exhibited 98.49 and 98.92
249 % of identity with Candidatus *Coxiella mudrowiae* isolate CRS-CAT (CP024961) (Table 2).

250 The phylogenetic analysis, based on the 16S rRNA gene, showed that *Coxiella* bacteria
251 sequences from *Rhipicephalus*, *Dermacentor*, *Ixodes* and *Hyalomma* species (11 of 13
252 successfully sequenced) are grouped into an independent and distinct clad. While the sequences
253 from a female *R. sanguineus* SL-A from Blida remained distinct to others and share the same
254 common ancestor with *Coxiella* endosymbiont of *R. sanguineus* SL-A isolate Rhsa 2 from
255 Brazil (KP994844). In addition, the 16S rRNA sequence of *Coxiella*-like of a male *H. sulcata*
256 from Blida in this study clustered with the *Coxiella* endosymbiont of *Rhipicephalus* from Italy
257 (KP994831) (Fig. 3).

258 Phylogenetic analysis based on *GroEL* partial gene of *Coxiella*-like bacteria was carried
259 out. Two sequences gave similarity with *C. burnetii* by BLAST. They are grouped with *C.*
260 *burnetii* Nine Mile strain and other *C. burnetii* strains. However, other sequences of *Coxiella*-
261 like endosymbionts identified in *R. sanguineus* SL-A and *H. excavatum* were grouped with
262 endosymbionts of *R. sanguineus* SL-A isolate Rhsa1 chaperonin (*GroEL*) gene and share the
263 same common ancestor (Fig. 4).

264

265 **4. Discussion**

266 This study is to the best of our knowledge the first report on the occurrence and diversity
267 of *Coxiella*-like in cattle ticks from Algeria. The study highlighted the presence of two genera
268 of ticks that constitute the dominant populations of ticks infesting livestock in northern Algeria.
269 In Blida and Medea, *Rhipicephalus* spp. are dominant, especially, *R. bursa* and *R. sanguineus*
270 SL-A (Fig. 2). *R. bursa* is a tick of the Mediterranean climatic region, which is the
271 characteristic climate of the concerned provinces. In Africa, it is confined to the coastal areas
272 from Morocco to Libya including Algeria (Walker and Bouattour, 2003). *R. sanguineus* SL-A,
273 is specialized to domestic dogs, but it may be found on cattle (Walker and Bouattour, 2003).
274 These details support our findings. In Batna, northeastern Algeria, the dominant tick genus was
275 *Hyalomma* especially *H. excavatum*. This species is adapted to the Mediterranean and steppe
276 climatic regions of North Africa and to steppe climatic regions elsewhere in its wide range
277 (Walker and Bouattour, 2003).

278 For the first time in Algeria, we report the occurrence of *Coxiella*-like in ixodid ticks. The
279 prevalence was 51.7 % (76/147) among all the selected ticks for the screening. *Coxiella*-like
280 DNA was detected in all tick species identified in the present study with different percentages.
281 The highest prevalence was reported in the genus *Rhipicephalus* 70 % (53 out of 75), while it
282 was lower in other genera such as *Hyalomma* with 37 % (26 out of 70). This prevalence in
283 Algeria seems to be lower than those reported worldwide. Actually, Guizzo et al. (2017)
284 reported that the treatment of cattle with tetracycline (frequently used in Algeria), leads to the
285 reduction of bacteria levels in the progeny of ticks. These facts might explain the low
286 prevalence reported in this study.

287 In the study conducted by Duron et al. (2015) *Coxiella*-like was detected in >90 % of
288 tested ticks, which is extremely high. The screening was performed in 916 tick specimens from
289 58 species belonging to the two main tick families, Ixodidae (36 species) and Argasidae (22
290 species). *Coxiella* was found in most of the tested genera of hard ticks (*Rhipicephalus*, *Ixodes*,
291 *Amblyomma*, *Dermacentor*, *Haemaphysalis*) and soft ticks (*Ornithodoros*, *Argas*).

292 Actually, the prevalence of *Coxiella*-like varies widely in different tick species. In Europe
293 and North America, it rates from 6.25 % (3/48) in *R. sanguineus* SL-A to 100 % (50/50) in *A.*
294 *americanum* (Zhong, 2012). Machado-Ferreira et al. (2016) reported that *Coxiella* DNA was
295 detected in 37 % (10/27) of *Rhipicephalus microplus* (5/15 adult females, 4/11 adult males and
296 1/1 nymph), and in 61 % (29/47) of the *R. sanguineus* SL-A (15/24 adult females 13/18 adult

297 males 1/5 nymph). These details support our findings in Algeria where the prevalence ranged
298 from 14 % (4/28) for *H. marginatum* to 81 % (26/32) for *R. bursa*, to 2/2 for *D. marginatus*.

299 In Tunisia, Selmi et al. (2019) reported that *Coxiella*-like DNA was detected in 10 ± 0.04
300 % (16/158) of *Hyalomma impeltatum* and 6% (10/160) of *Hyalomma dromedarii* (partially
301 engorged adult ticks) with an overall prevalence of 8 % (26/327). This is close to our results
302 regarding *Hyalomma* genus with 37.1 %. Khoo et al. (2016) reported a prevalence of 89%
303 (49/55) of tested ticks (*Haemaphysalis hystricis*: 6/6 unfed adult females, 13/13 unfed adult
304 males; *Dermacentor steini*: 0/1 unfed adult females, 2/6 unfed adult
305 males; *Dermacentor compactus*: 1/2 unfed adult males; *Amblyomma* sp.: 5/5 engorged adult
306 females, 1/1 unfed adult male; *Haemaphysalis bispinosa*: 18/18 adult females, among them five
307 engorged, 2/2 unfed adult males and 1/1 nymph). In South Korea, Seo et al. (2016) reported the
308 prevalence of 52.4 % (121/231) of adult *Haemaphysalis longicornis*. These different data
309 suggest that the *Coxiella*-like prevalence in ticks may depend, not only on the tick's species,
310 but also on the bioclimatic area. Our results report a higher prevalence in Blida and Medea,
311 characterized by Mediterranean climate, than in Batna with a cold semi-arid climate. In
312 addition, in Malaysia, which is characterized by an equatorial climate, a higher prevalence (89
313 %) than reported in this study was found by Khoo et al. (2016). According to the study carried
314 out by Selmi et al. (2019) the desertic areas reduce the ticks activity to the minimum, especially
315 during summer, regarding the high average of temperature as well as the scarcity of animal
316 species (Selmi et al., 2019).

317 However, the prevalence of *Coxiella*-like bacteria reported here or in other studies, where
318 ticks were collected on host animals, are much higher than in studies reported in ticks collected
319 by flagging over vegetation. Environmental *H. longicornis* ticks, collected in the Chungbuk
320 province (middle of the Korean), showed a *Coxiella*-like prevalence of 2 % (2 /100) of *H.*
321 *longicornis* (Lee et al., 2004); in Queensland, Australia, the prevalence reached 6 % (3/45) (Seo
322 et al., 2016), 0.9 % (1 of 109) in *Haemaphysalis punctata* ticks from Spain (Barandika et al.,
323 2008). Thus, *Coxiella*-like infection in ticks might be originated from host animals since its
324 prevalence in environmental ticks is much lower than that described in ticks collected on
325 animals.

326 The gender of the ticks does not appear to be a factor of genetic variability of the *Coxiella*-
327 like since sequences belonging to the same clade were found in both male and female ticks.
328 According to the 16S rRNA phylogenetic analysis, three different clades of *Coxiella*-like
329 bacteria occur in ticks from Algerian cattle. The dominant one, regrouping 11 of the 13

330 sequences identified seems to be genetically homogenous with few differences among each
331 other (based on 16S rRNA gene). However, *Coxiella*-like bacteria from this genetic group were
332 found in our study in different tick species (*R. bursa*, *R. sanguineus* SL-A, *H. marginatum*, *H.*
333 *excavatum*, *D. marginatus*, *H. sulcata* and *I. ricinus*) and from different regions. This goes
334 against the previous concept that each genetic variant of *Coxiella*-like bacteria is associated
335 with specific tick species. The second 16S-based genetic group was identified in a female of *R.*
336 *sanguineus* SL-A (it is close to *Coxiella* endosymbiont of *R. sanguineus* SL-A from Brazil
337 (Duron et al., 2015) and *Coxiella*-like endosymbiont of *H. excavatum* from Anatolia, Turkey
338 (Brinkmann et al., 2019). The third genetic clade clustered with *Coxiella* endosymbiont of
339 *Rhipicephalus* from Italy (Duron et al., 2015) (Fig. 3). Actually, the studies proofed the vertical
340 transmission of CLE nevertheless; they do not deny the possible horizontal transmission,
341 between ticks' species via infested cattle (the common point between them). For that, we
342 hypothesized that the genetic diversities of the CLE don't only depend on the tick's species but
343 also on the vertebrates' hosts.

344 Only four sequences were obtained for *GroEL* gene. This could be due to the primer's
345 specificity and / or to the use of non-nested PCR protocol to detect *Coxiella*-like DNA which
346 probably decreased, the PCR sensitivity. However, in our study, CLE DNA were successfully
347 sequenced but some of sequences were impossibility assembled with the ChromasPro software
348 due to their poor quality. We avoided the inclusion of these poor sequences in the analysis.

349 Two sequences from female *R. bursa* ticks were almost identical to *C. burnetii* str.
350 Schperling detected on *R. sanguineus* SL-A female tick from Blida province. Two other
351 sequences from *Hyalomma* ticks showed high similarity (>98 %) with *Candidatus* *Coxiella*
352 *mudrowiae* isolate CRS-CAT detected on *R. sanguineus* SL-A and *H. excavatum* ticks from
353 Blida and Batna successively. Whenever we were able, in 3 ticks, to amplify both genes of
354 *Coxiella*-like bacteria (16S rRNA gene and *GroEL*), we may suggest that *GroEL* sequences
355 from ticks 11 and 118 (Fig. 4) represent the same first dominant 16S clade and the sequences
356 from ticks 110 and 146 are the sequences corresponding to the second 16S clade.
357 Unfortunately, we were not able to amplify the *GroEL* gene from the only *H. sulcata* tick
358 where the third 16S-based clade of *Coxiella*-like bacteria was identified.

359

360 5. Conclusion

361 In our study, we report for the first time in Algeria that *Coxiella*-like 16S rRNA gene
362 sequences from different tick species grouped into three clades. Beside the tick species
363 specificities, the genetic diversity of CLE seems to be related to the vertebrate animal hosts,
364 which support the possibility of horizontal transmission between ticks via the vertebrate
365 animals.

366 **Conflict of interest**

367 The authors declare no conflicts of interest.

368

369 **Ethical considerations**

370 The collection of samples from cows was carried out in accordance with the Algerian
371 legislation. Permission was obtained from the Ministry of Higher Education and Scientific
372 Research of Algeria, in collaboration with the University Yahya Fares of Medea, and the
373 National Veterinary High School, Algiers, Algeria.

374

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383

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387

388 **Supplementary data**

389 No.

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Table 1. PCR systems used in the present study, their conditions and sources.

Target	Target Gene	Orientation	Nucleotide sequence (5'-3')	Fragment length	Tm °C	References
<i>Coxiella-like</i>	rrs	Forward	ACCTACCCTTGACATCCTCGGAA	550 bp	54	(Angelakis et al., 2016)
		Reverse	GCAACTAAGGACGAGGGTTG			
		Probe	6FAM-CAGCTCGTGTCTGTGAGATGT-TAMRA			
<i>Coxiella-like</i>	<i>GroEL</i>	Forward 660f	GGCGCICARATGGTTAARGAA	1066 bp	50	(Mediannikov et al., 2003)
		Reverse 1320r	AACATCGCTTTACGACG			
ticks	16S	Forward 16S-F	TTAAATTGCTGTRGTATT3	454 bp	54	(Diarra et al., 2017)
		Reverse 16S-R	CCGGTCTGAACTCASAWC			

505 **Tm:** Annealing temperature.

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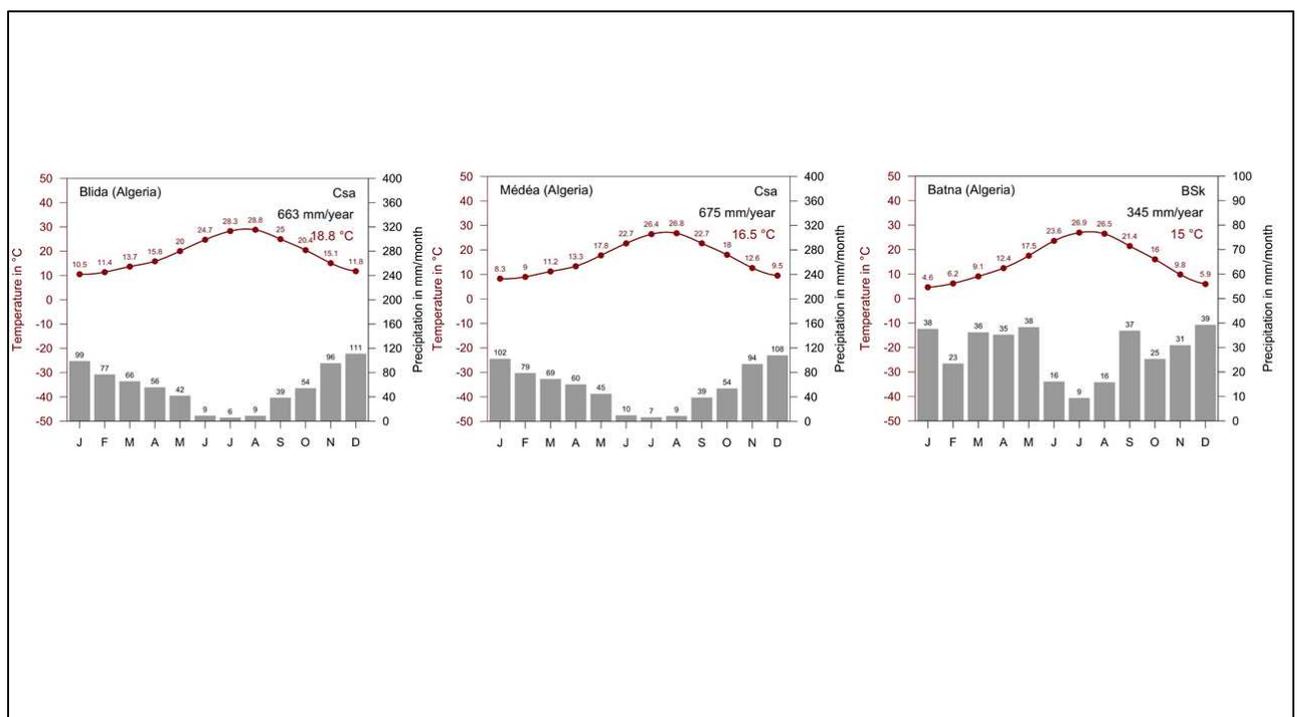
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512 **Table 2.** BLAST analysis of specimens sequenced with 16S rRNA and *GroEL* partial
 513 genes and selected to create the phylogenetic trees.

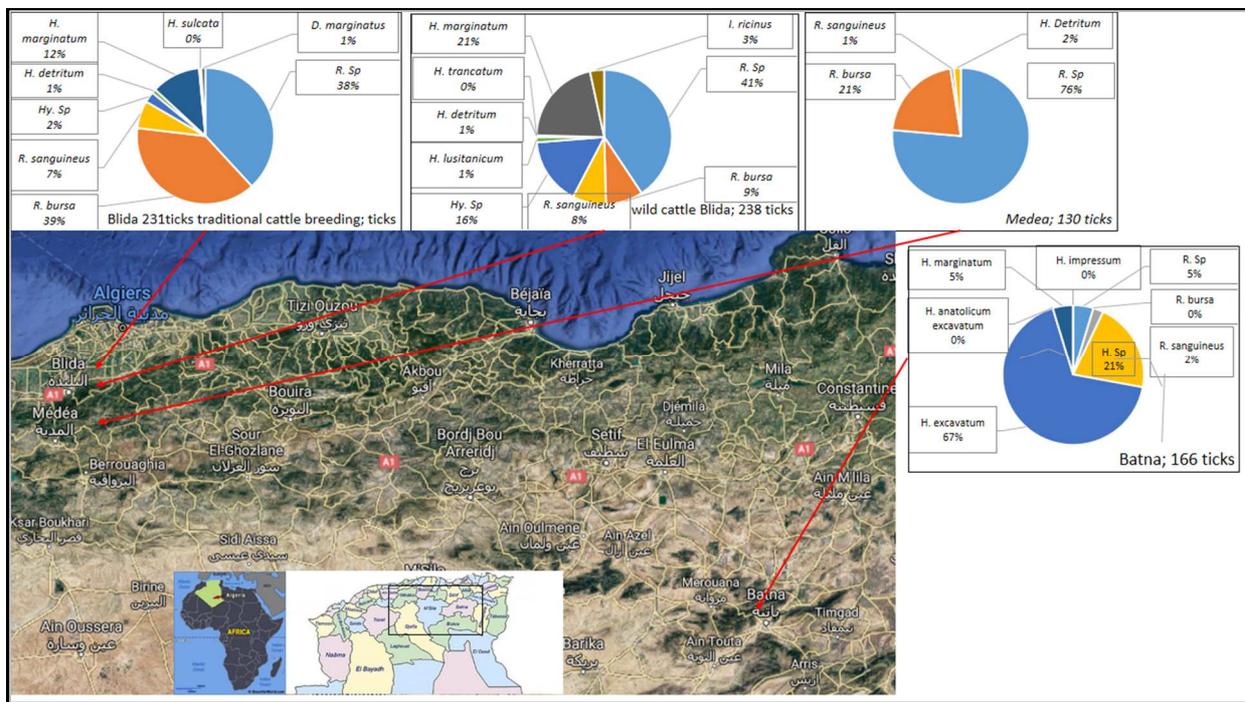
Primers	Localities	No of sequenced / tested	DNA samples ID	Gender tick	of Ticks species	Best BLAST hit	The Percent identity	Accession Numbers	
16S	Medea	1/3	5	Female	<i>R. bursa</i>	Coxiellaceae bacterium CR07	99.39 %	KM079623	
	Blida	12 /16	34	Female	<i>R. bursa</i>	Coxiellaceae bacterium CR07	99.60 %	KM079623	
				69	Male	<i>H. sulcata</i>	Uncultured Coxiella sp. clone 195C	98.64 %	MK164020
				74	Male	<i>R. bursa</i>	Uncultured Coxiella sp. clone 195C	98.64 %	MK164020
				75	Male	<i>D. marginatus</i>	Coxiellaceae bacterium CR07	98.31 %	KM079623
				90	Male	<i>R. bursa</i>	Coxiellaceae bacterium CR07	99.37 %	KM079623
				106	Female	<i>H. marginatum</i>	Coxiellaceae bacterium CR07	99.80 %	KM079623
				110	Male	<i>R. sanguineus</i> SL-A	Coxiellaceae bacterium CR07	100 %	KM079623
				111	Female	<i>R. bursa</i>	Coxiellaceae bacterium AL09	100 %	KM079626
				113	Female	<i>R. bursa</i>	Coxiellaceae bacterium CR07	100 %	KM079623
				118	Male	<i>R. bursa</i>	Coxiellaceae bacterium CR07	100 %	KM079623

			122	Female	<i>I. ricinus</i>	Coxiellaceae CR07	bacterium	100 %	KM0796 23
			148	Male	<i>R. sanguineus</i> SL-A	Coxiellaceae CR07	bacterium	99.80 %	KM0796 23
Batna 0/4									
Medea 0/3									
<i>GroEL</i>	Blida	3/16	110	Female	<i>R. sanguineus</i> SL-A	Candidatus mudrowiae isolate CAT	Coxiella CRS-	98.49 %	CP0249 61
			111	Female	<i>R. bursa</i>	C. burnetii str. Schperling chromosome		99.63 %	CP0145 63
			118	Female	<i>R. bursa</i>	C. burnetii str. Schperling chromosome		98.38 %	CP0145 63
	Batna	1/3	146	Male	<i>H. excavatum</i>	Candidatus mudrowiae isolate CAT	Coxiella CRS-	98.92 %	CP0249 61



515 **Fig. 1.** Climate diagrams for the three Algerian provinces concerned by the tick collection,
 516 showing the long-term mean temperature of the coldest and the warmest month (Rubel, 2020,
 517 pers. communication).

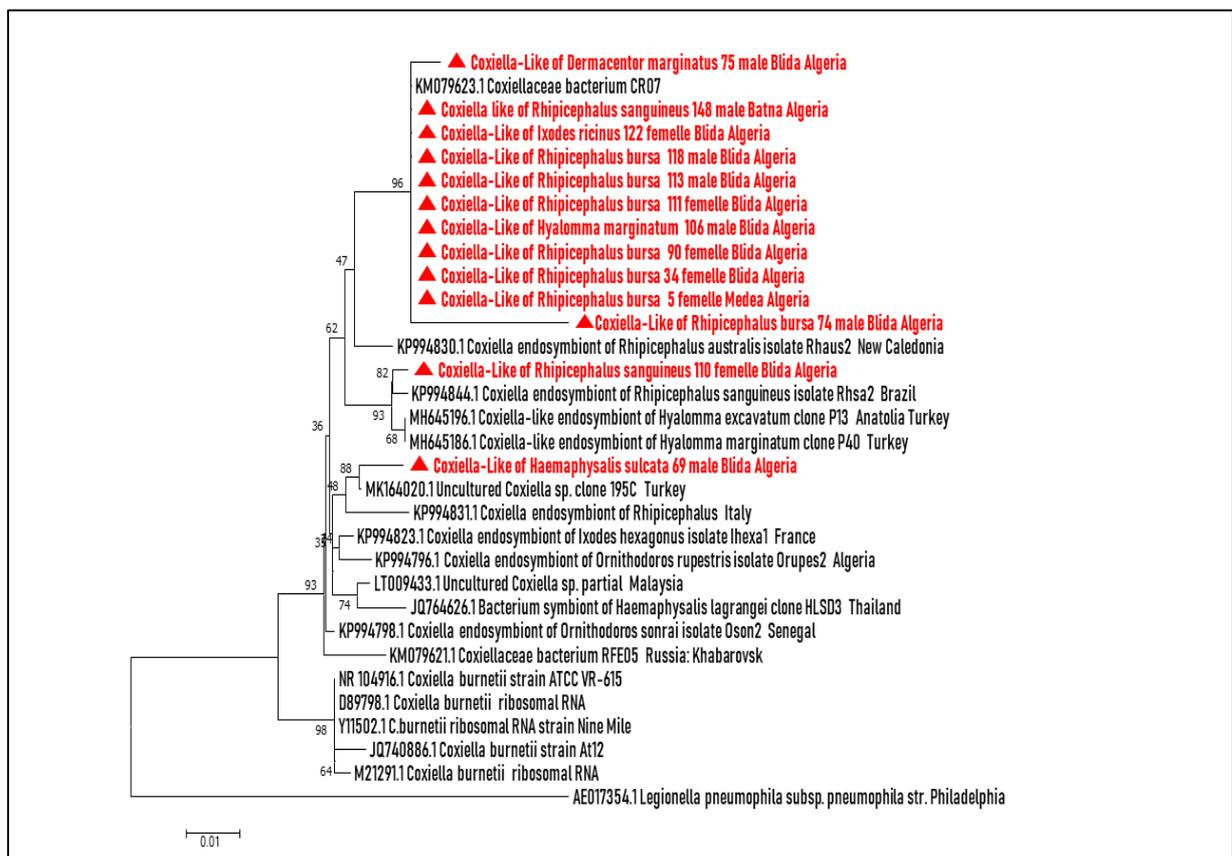
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 528 **Fig. 2.** A map of northern Algeria showing different provinces from which the sample
 529 collections were carried out, with percentage of each tick species found and identified.
 530 (<https://www.google.com/maps/@36.2736941,4.221111,230025m/data=!3m1!1e3?hl=en>).

531 **H:** *Hyalomma / Haemaphysalis*; **R:** *Rhipicephalus*; **D:** *Dermacentor*; **I:** *Ixodes*.

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540 **Fig. 3. Maximum-likelihood phylogenetic analysis of the *Coxiella*-like based on 16S**
 541 **rRNA gene sequences.** Sequences characterized in this study are given in bold and indicated
 542 with the identification (number) of DNA sample, host tick species, its gender and the
 543 geographical origin, respectively. *Legionella pneumophila* subsp. *pneumophila* str. Philadelphia
 544 was included as the outgroup. Sequences were aligned using CLUSTALW, and phylogenetic
 545 inferences was conducted in MEGA6.06 using the maximum likelihood method based on the

546 Tamura-Nei model for nucleotide sequences. The GenBank accession numbers are indicated at
 547 the beginning. Statistical support for the internal branches of the trees was evaluated by
 548 bootstrapping with 5,00 iterations. Bootstrapping under 40 were removed.

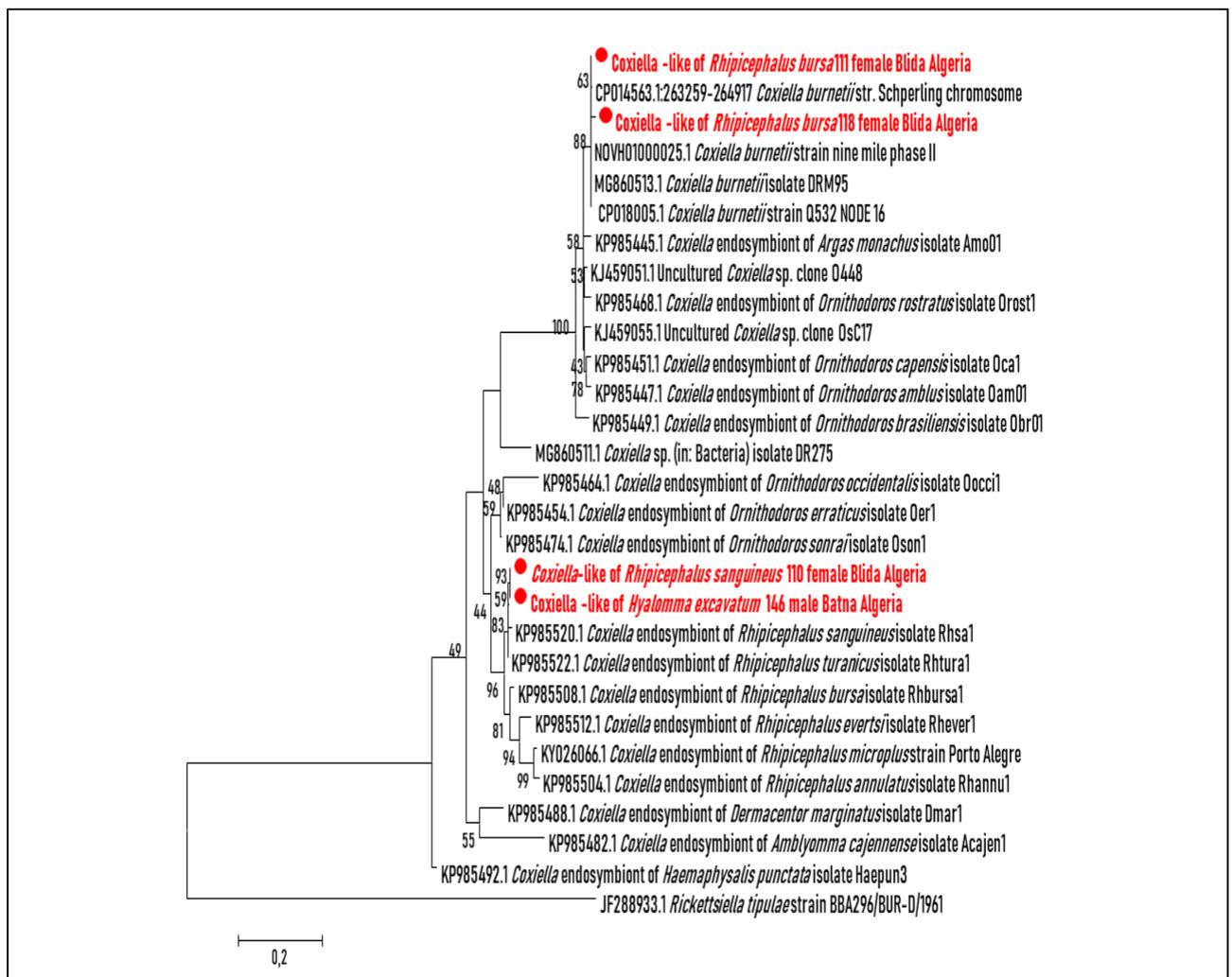
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555 **Fig. 4. Maximum-likelihood phylogenetic tree of the *Coxiella*-like based on *GroEL***
 556 **gene sequences.** Sequences characterized in this study are given bold and indicated with the
 557 identification (number) of DNA sample, host tick species, its gender and the geographical

558 origin, respectively. *Rickettsiella tipulae* strain BBA296/BUR-D/1961 23S ribosomal RNA
559 (rrl) gene was included as the outgroup. Sequences were aligned using CLUSTALW, and
560 phylogenetic inferences was conducted in MEGA6.06 using the maximum likelihood method
561 based on the Tamura-Neimodel for nucleotide sequences. The GenBank accession numbers are
562 indicated at the beginning. Statistical support for the internal branches of the trees was
563 evaluated by bootstrapping with 5,00 iterations. Bootstrapping under 40 were removed.

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