



HAL
open science

Molecular detection of avian spirochete *Borrelia anserina* in *Argas persicus* ticks in Algeria

Nassim Ouchene, Amira Nebbak, Nadjat Amina Ouchene-Khelifi, Ali Dahmani, Faycal Zeroual, Djamel Khelef, Idir Bitam, Ahmed Benakhla, Philippe Parola

► **To cite this version:**

Nassim Ouchene, Amira Nebbak, Nadjat Amina Ouchene-Khelifi, Ali Dahmani, Faycal Zeroual, et al.. Molecular detection of avian spirochete *Borrelia anserina* in *Argas persicus* ticks in Algeria. *Comparative Immunology, Microbiology and Infectious Diseases*, 2020, 68, pp.101408. 10.1016/j.cimid.2019.101408 . hal-02507197

HAL Id: hal-02507197

<https://hal-amu.archives-ouvertes.fr/hal-02507197>

Submitted on 7 Mar 2022

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

24 chain reaction (qPCR). *Borrelia* spp. screening was performed using primers and probe
25 targeting the 16S rRNA gene. A total of 83 traditional laying hen farms were visited, of which
26 39 (46.98%) were found infested with *A. persicus* tick. Molecular analysis revealed that 2/34
27 (5.88 %) of ticks were infected by *B. anserina*. None of the ticks tested were positive for
28 *Rickettsia* spp., and *Coxiella burnetii*. These results constitute the first report in Algeria of *A.*
29 *persicus* harboring *B. anserina*.

30 **Keywords**

31 *Argas persicus*; laying hens; avian borreliosis; *Borrelia anserina*; Algeria

32

33 **1. Introduction**

34 Ticks are obligate haematophagous ectoparasites of mammals, birds and reptiles. They are
35 distributed worldwide and are vectors of a large variety of human and animal pathogens [1].

36 Two families of ticks are capable of transmitting a broad range of pathogens: Ixodidae (hard
37 ticks) currently comprise over 700 species worldwide, and Argasidae (soft ticks) comprise
38 roughly 200 species [2, 3, 4].

39 Unlike Ixodidae, the duration of bloodmeal of soft ticks is short, particularly for nymphs and
40 adults (15-60 minutes). For larvae, this duration is typically longer (12 hours to several days).

41 This characteristic may be seen as an adaptation to unfavorable external conditions by
42 detaching rapidly from their hosts. After each bloodmeal, these ticks are generally found in
43 the crevices and cracks of the walls of their habitat, in nests and burrows, or on the ground,
44 under the dust [5].

45 In poultry farming, argasid ticks are considered the most important poultry ectoparasites in
46 some countries [6, 7]. Ticks directly affect birds during blood meal, causing anemia, weight
47 loss and reduced egg production [8]. Certain species of argasid ticks may transmit a variety of
48 pathogens of medical and veterinary interest, all of which are capable of causing damage to
49 livestock production and human health [9, 10, 11]. *Carios capensis*, a soft tick of seabirds, is
50 known as reservoir of pathogenic bacteria of medical importance and can be transmitted
51 *Rickettsia* sp. to seabirds [12]. The soft ticks *Argas persicus* infect mostly poultry. It also
52 feeds on turkeys, ducks, geese, pigeons and a variety of wild birds [13]. *A. persicus* is known
53 to carry and transmit several agents, including *Borrelia anserina*, the agent of avian
54 spirochetosis, Kyasanur forest disease virus [14, 15], and the bacterium *Aegyptianella*
55 *pullorum* responsible of aegyptianellosis, an intraerythrocytic tick-borne rickettsial infection
56 of chicken and ducks [13].

57 Avian spirochetosis is a highly fatal septicemic disease of hens, geese, ducks and turkeys in
58 tropical and sub-tropical regions [15, 16, 17]. The disease presents a potential economic
59 problem in places like Africa, where poultry are an important source of protein [17]. It is
60 manifested clinically by hyperthermia, anorexia, greenish diarrhea, paralysis of the legs and
61 wings and sudden death [18, 19].

62 The present study was conducted in Algeria for an inventory of soft ticks in traditional laying
63 hen's farms associated microorganisms.

64 **2. Materials and methods**

65 ***2.1. Study area and collection sites***

66 The study was performed in Ksar El Boukhari (at the south of Medea region) in central-
67 northern of Algeria (35°53'22.33"N 2°44'57.26"E) (Figure 1). This area is mountainous and is
68 630 m above sea level. It has a semi-arid climate characterized by hot summers and cold, wet
69 winters with a rainfall averaging 400 mm per year.

70 The study was conducted in 83 traditional laying hen farms from April 2014 to March 2015.
71 During this period, no anti-parasitic treatments were applied on all farms.

72 All farms were visited only once each season to collect a convenient sample of soft ticks.

73 ***2.2. Tick sampling***

74 The ticks were carefully searched on the hen's body and at the walls crevices of the livestock
75 building. The specimens collected were stored in labeled tubes containing 70° ethanol for
76 morphological identification and DNA extraction.

77 ***2.3. Tick identification***

78 Ticks were identified by morphological criteria using standard taxonomic keys [20]. Each
79 specimen was rinsed twice in distilled water for 10 minutes and then dried on a sterile filter
80 paper. Ticks were individually crushed in sterile Eppendorf tubes. Total DNA was extracted
81 in a final volume of 100 µl from one half of each ectoparasite using the QIAamp Tissue Kit
82 (Qiagen, Hilden, Germany) by Qiagen-BioRobot EZ1 according to the manufacturer's
83 instructions [21]. Genomic DNA was stored at -20°C under sterile conditions.

84 **2.4. Molecular detection of bacteria**

85 To assess the presence of DNA of common microorganisms in ticks, the extracted DNA was
86 screened for the presence of *Borrelia* spp., *Coxiella burnetii* and *Rickettsia* spp. DNA by real-
87 time polymerase chain reaction (qPCR) [22]. Positive results were confirmed by standard
88 PCR.

89 *Borrelia* spp. screening was performed using primers and probe targeting the 16S rRNA gene
90 as previously described [23]. The positive samples in this qPCR were subjected to
91 amplification by standard PCR using *Borrelia*-primers targeting 16S rRNA gene long
92 fragment specific to the 16S rRNA gene of *Borrelia* spp. [22]. For *Rickettsia* identification,
93 DNA samples were screened for all spotted fever group rickettsiae (SFGR) by targeting a
94 partial sequence of the citrate synthase gene (*gltA*) [22].

95 All samples were screened for *Coxiella burnetii* DNA using *IS30A* gene spacers [24].
96 Negative controls (qPCR mix and DNA of uninfected ticks from laboratory colony) and
97 positive controls (*Borrelia crocidurae*, *Rickettsia monacensis*, and *Coxiella burnetii* DNA)
98 were used in each respective qPCR. Ticks analyzed by qPCR were considered positive when
99 the cycle threshold (Ct) value was less than or equal to 36.

100 Conventional PCR amplifications were performed using a Bio-Rad Thermocycler (Bio-Rad
101 Laboratories, Hercules, CA). PCR amplification was verified by migration of products on 2%
102 agarose gel electrophoresis. Products were purified by using a NucleoFast 96 PCR plate

103 (Macherey-Nagel EURL, Hoerdt, France). Purified PCR products were sequenced using the
104 same primers as for a standard PCR using BigDye version 1–1 Cycle Ready Reaction
105 Sequencing Mixture (Applied Biosystems, Foster City, CA, USA) in ABI Prism 3130xl
106 Genetic Analyzer capillary sequencer (ABI PRISM, PE Applied Biosystems, USA). The
107 sequences obtained were analyzed and assembled by ChromasPro version 1.7.7 software
108 (Technelysium Pty. Ltd., Tewantin, Queensland, Australia). Species identity was assessed by
109 performing BLAST ([http:// blast.ncbi.nlm.nih.gov/Blast.cgi](http://blast.ncbi.nlm.nih.gov/Blast.cgi)).

110 **2.5. Statistical analysis**

111 The statistical program used was R i386 3.0.2 for Windows GUI front-end. Chi square test,
112 and multiple range test were used for the statistical analysis. The threshold value of different
113 tests was $P < 0.05$.

114 **3. Results**

115 **3.1. Tick collection**

116 A total of 83 traditional laying hen farms were visited, of which 39 (46.98%) were found
117 infested with soft ticks, 28 (71.80%) during the summer period, significantly higher in
118 comparison to other seasons ($p < 0.001$) (Table 1).

119 A total of 341 soft ticks were collected of which 99 and 242 ticks were collected from the
120 hens' body and wall crevices, respectively. Morphological identification revealed that all these
121 ticks were adult *Argas persicus*.

122 The global percentage of *A. persicus* ticks (87.97%) observed in summer was significantly
123 higher compared to other seasons ($p < 0.001$) (Table 1). The same remark was recorded for
124 hens and farms ($p < 0.001$) (Table 1).

125 The total number of female *A. persicus* collected from hens [n=71, (71.71%)] was
126 significantly higher compared to the total number of males [n=28, (28.28%)] (p<0.001)
127 (Table 1). However, no significant differences were observed for farms (Table 1).

128 **3.2. Molecular analysis**

129 Among the 90 soft ticks that were collected in summer from the hens' body, thirty-four
130 randomly selected specimens (14 males and 20 females) were tested by qPCR after being sent
131 to Marseille, France. Other specimens have been kept for other studies. A total of 2/34 (5.88
132 %) specimens adult females harbored bacteria of the *Borrelia* genus. Sequencing analyses
133 targeting the 16S rRNA fragment lead to the identification of 2 sequences of *B. anserina*
134 (GenBank accession no. CP013704, with 100% similarity). None of the tested ticks was
135 positive for *Rickettsia* spp., and *Coxiella burnetii*.

136 **4. Discussion**

137 In Algeria, studies on soft ticks and associated microorganisms are scarce. They have recently
138 included the detection of *Borrelia crocidurae* in *Ornithodoros sonrai* [25], the detection of an
139 uncultivated *Rickettsia* sp. in *Ornithodoros capensis* s.s., *Ornithodoros erraticus* and
140 *Ornithodoros rupestris* [12], the detection of *Bartonella* spp. and *Anaplasmataceae* bacteria in
141 *A. persicus* and the relapsing fever *Borrelia* spp. in *Carios capensis* and *Ornithodoros*
142 *occidentalis* [26] and the first report of *Ornithodoros capensis* s.s in Algiers [27]. This
143 investigation reports direct evidence of DNA from *B. anserina* bacterium in *A. persicus* ticks
144 collected in the traditional farms of laying hens in Algeria.

145 Quantitative real-time PCR is a powerful method for detecting microorganisms in sample
146 materials from different sources. It has been used to detect *Borrelia* species in clinical

147 samples [28, 29, 30]. Major advantages of qPCR in this context are its simplicity, sensitivity,
148 robustness, and speed [31, 32].

149 The soft tick *A. persicus* has been reported as the most important poultry ectoparasites [7, 33,
150 34]. This poultry tick has previously been described morphologically in the Mediterranean
151 basin [20]. However, in Algeria, knowledge of this tick is still insufficient. It has been
152 identified in chicken and animal shelters in Tamanrasset and Mostaganem respectively by
153 Lafri et al. [26, 35]. In our study, this was the single tick species found in the traditional farms
154 of laying hens. A number of 39/83 (46.98%) laying hen farms were found infested with *A.*
155 *persicus* ticks, of which 28 (71.8%) during the summer period. A total of 341 ticks were
156 collected of which 300 (87.97%) were revealed in summer. The percentage of infested farms
157 and ticks collected in summer were significantly higher than in other seasons. This finding is
158 in agreement with Shahnaz et al. [36] and Phulan et al. [8] in Pakistan. However, lower [37]
159 and higher [38] prevalences have been reported in Ethiopia and Tanzania, respectively. This
160 difference in prevalence of *A. persicus* might be due to the landscape variation in the study
161 area and environmental/climatic factors, including rainfall pattern, relative humidity,
162 atmospheric temperature, seasons, husbandry and or managmental practices [36].

163 *A. persicus* ticks are known as vectors of avian spirochaetosis caused by the bacterium *B.*
164 *anserina* worldwide, an acute septicemic disease of a variety of avian species including geese,
165 ducks, turkeys and chickens [39]. More recently, they have been described as harbouring
166 other potential pathogens ranging from *Mycobacteria*, *Mycoplasma*, *Pasteurella*, *Salmonella*
167 and West Nile virus [14, 15]. The study of Lafri et al. [26] revealed the presence of *Bartonella*
168 spp. and *Anaplasma* spp. in *A. persicus* ticks. Infected *Argas* ticks transmit *B. anserina* both
169 transstadially and transovarially, and the disease causes serious losses of domestic chickens in

170 tropical and warm temperate regions of the world [39]. Outbreak and incidence of fowl
171 spirochaetosis have been reported from poultry in America, Africa and Asia [15, 40, 41].

172 For the first time in Algeria, we highlighted, by sequencing analyses targeting the 16S rRNA
173 gene, the presence of *B. anserina* in *A. persicus* ticks collected in traditional laying hen's
174 farms. In our study, 2/34 (5.88 %) *A. persicus* ticks were found to be infected which is in
175 accordance with Cutler et al. [15] in Ethiopia (7.5%). A higher prevalence of 19.2% was
176 revealed in Pakistan by Aslam et al. [41].

177 **5. Conclusions**

178 In this study, *A. persicus* was the main soft tick in laying hens, and for the first time in
179 Algeria, we identified the presence of *B. anserina* in these ticks. Further studies may help to
180 decipher the economic impact of *B. anserina* on poultry production.

181 **Acknowledgements:**

182 This study received the support of the French government under the Investments of Futures
183 Program managed by the National Agency for Research (reference: Méditerranée Infection
184 10-IAHU-03), France.

185 **References**

- 186 1. RJ. Peter, P. Van den Bossche, BL. Penzhorn, B. Sharp, Tick, fly, and mosquito control-
187 lessons from the past, solutions for the future. *Vet. Parasitol.* 132 (3) (2005) 205-15
- 188 2. A. Estrada-Peña, A.J. Mangold, S. Nava, J.M. Venzal, M. Labruna, A.A. Guglielmone, A
189 review of the systematics of the tick family Argasidae (ixodida). *Acarologia.* 50 (3) (2010)
190 317–333. <https://doi.org/10.1051/acarologia/20101975>

- 191 3. I.G. Horak, J.L. Camicas, J.E. Keirans, The Argasidae, Ixodidae and Nuttalliellidae (Acari:
192 Ixodida): a world list of valid tick names. *Exp. Appl. Acarol.* 28 (2002) 27–54.
- 193 4. A.A. Guglielmo, R.G. Robbins, D.A. Apanaskevich, T.N. Petney, A. Estrada-Peña, I.G.
194 Horak, The hard ticks of the world: (Acari: Ixodida: Ixodidae). London: Springer, 2014, 738p.
195 <http://dx.doi.org/10.1007/978-94-007-7497-1>.
- 196 5. D.E. Sonenshine, Ecology of nidicolous ticks, in: *Biology of ticks*, Vol. 2. Oxford
197 University Press, New York, 1993, 465 p.
- 198 6. F. Jongejans, G. Uilenberg, The global importance of ticks. *Parasitol.* 129 (2004) 3-14.
- 199 7. W.L. Nicholson, D.E. Sonenshine, R.S. Lane, G. Uilenberg, Ticks (Ixodida), in: *Medical
200 and veterinary entomology*. 2nd Edition. Mullen G.R & Durden L.A. (eds), Academic Press,
201 2009, pp. 483-532.
- 202 8. M.S. Phulan, W.M. Bhatti, S.N. Buriro, Incidence of *Argas persicus* in poultry. *Pakistan
203 Vet. J.* 4 (1984) 174–5.
- 204 9. P. Parola, D. Raoult, Ticks and tick-borne bacterial disease in humans: an emerging
205 infection threat. *Clin. Infect. Dis.* 32 (6) (2001) 897-928.
- 206 10. M.J. Turell, C.N. Mores, J.S. Lee, J.J. Paragas, D. Shermuhemedova, T.P. Endy, S.
207 Khodjaev, Experimental transmission of Karshi and Langat (tick-borne encephalitis virus
208 complex) viruses by *Ornithodoros* ticks (Acari: Argasidae). *J. Med. Entomol.* 41 (5) (2004)
209 973-977.
- 210 11. M. Sarwar, Status of Argasid (Soft) Ticks (Acari: Parasitiformes: Argasidae) In Relation
211 To Transmission of Human Pathogens. *Int. J. Vaccines Vaccin.* 4 (4) (2017) 00089.

- 212 12. I. Lafri, H. Leulmi, F. Baziz-Neffah, R. Lalout, C. Mohamed, K. Mohamed, P. Parola, I.
213 Bitam, Detection of a novel *Rickettsia* sp. in soft ticks (Acari: Argasidae) in
214 Algeria. *Microbes Infect.* 17 (2015) 859–861. <https://doi.org/10.1016/j.micinf.2015.09.010>.
- 215 13. A.R. Walker, A. Bouattour, A. Estrada-pena, I.G. Horak, A.A. Latif, R.G. Pergam, P. M.
216 Preston, Ticks of domestic animal in Africa: a guide to Identification of Species. Bioscience
217 Reports, Edinburgh Scotland, U.K., 2003, p. 221.
- 218 14. K. Liu, X.Y. Ding, L.Y. Chen, J.J. Mei, L.J. Zhai, A case report on the diagnosis of
219 gosling spirochetosis. *Jilin Anim. Husb. Vet. Med.* 27 (2006) 42–3.
- 220 15. S. Cutler, A. Abdissa, H. Adamu, T. Tolosa, A. Gashaw, *Borrelia* in Ethiopian ticks.
221 *Ticks Tick borne Dis.* 3 (2012) 14–17. <https://doi.org/10.1016/j.ttbdis.2011.08.004>
- 222 16. A.M. Shommein, A. Khogali, Fowl spirochaetosis haematological and histopathological
223 studies. *Bull. Epiz. Dis. Africa.* 22 (1974) 255–261.
- 224 17. N. Elelu, Tick-borne relapsing fever as a potential veterinary medical problem. *Vet. Med.*
225 *Sci.* 4 (2018) 271-279. <https://doi.org/10.1002/vms3.108>
- 226 18. J. J. Boero, Parasitosis animales. In: *Espiroquetosis aviar*, 4th ed. J. J. Boero, ed. Editorial
227 Universitaria de Buenos Aires S. E. M., Buenos Aires, Argentina. 1976, pp. 90–99.
- 228 19. A.C. Ataliba, J.S. Resende, N. Yoshinari, M.B. Labruna, Isolation and molecular
229 characterization of a Brazilian strain of *Borrelia anserina* , the agent of fowl spirochaetosis.
230 *Res. Vet. Sci.* 83 (2007) 145–149.
- 231 20. A. Estrada-Peña, A. Bouattour, J. Camicas, A.R. Walker, Ticks of Domestic Animals in
232 the Mediterranean Region. A Guide of Identification of Species. Zaragoza: University of
233 Zaragoza Press. 2004, 131 p.

- 234 21. C.E. Wenk, C. Kaufmann, F. Schaffner, A. Mathis, Molecular characterization of Swiss
235 Ceratopogonidae (Diptera) and evaluation of real-time PCR assays for the identification of
236 *Culicoides* biting midges. *Vet. Parasit.* 184 (2012) 258-266.
237 <https://doi.org/10.1016/j.vetpar.2011.08.034>.
- 238 22. A.Z. Diarra, L. Almeras, M. Laroche, J. M. Berenger, A.K. Koné, Z. Bocoum, A. Dabo,
239 O. Doumbo, D. Raoult, Ph. Parola, Molecular and MALDI-TOF identification of ticks and
240 tick-associated bacteria in Mali. *PLoS Negl. Trop. Dis.* 11 (7) (2017) e0005762.
241 <https://doi.org/10.1371/journal.pntd.0005762>
- 242 23. P. Parola, G. Diatta, C. Socolovschi, O. Mediannikov, A. Tall, H. Bassene, J.F. Trape, D.
243 Raoult, Tick-Borne Relapsing Fever Borreliosis, Rural Senegal. *Emerg. Infect. Dis.* 17 (5)
244 (2011) 883–885. <https://doi.org/10.3201/eid1705.100573>
- 245 24. C. Socolovschi, P. Reynaud, T. Kernif, D. Raoult, P. Parola, Rickettsiae of spotted fever
246 group, *Borrelia valaisiana*, and *Coxiella burnetii* in ticks on passerine birds and mammals
247 from the Camargue in the south of France. *Ticks Tick Borne Dis.* 3 (2012) 355–360.
248 <https://doi.org/10.1016/j.ttbdis.2012.10.019>.
- 249 25. J.F. Trape, G. Diatta, C. Arnathau, I. Bitam, M. Sarih, D. Belghyti, A. Bouattour, E.
250 Elguero, L. Vial, Y. Mane, C. Balde, F. Prugnolle, G. Chauvancy, G. Mahe, L. Granjon, J.M.
251 Duplantier, P. Durand, F. Renaud, The epidemiology and geographic distribution of relapsing
252 fever borreliosis in West and North Africa, with a review of the *Ornithodoros erraticus*
253 complex (Acari: Ixodida). *PLoS One* 4 (8) (2013) e78473.
254 <https://doi.org/10.1371/journal.pone.0078473>.
- 255 26. I. Lafri, B. El Hamzaoui, I. Bitam, H. Leulmi, R. Lalout, O. Mediannikov, M. Chergui, M.
256 Karakellah, D. Raoult, P. Parola, Detection of relapsing fever *Borrelia* spp., *Bartonella* spp.

257 and Anaplasmataceae bacteria in argasid ticks in Algeria. PLoS Negl. Trop. Dis. 11 (11)
258 (2017) e0006064. <https://doi.org/10.1371/journal.pntd.0006064>

259 27. F. Baaziz Neffah, T. Kernif, A. Beneldjouzi, A. Boutellis, A. Morsli, Z. Harrat, S.
260 Doumandji, I. Bitam, *Carios capensis* (ACARI: ARGASIDAE) in the nests of the yellow
261 legged Gull (*Larus michahellis*) in the Agueli island of Reghaia, Algeria. Int. J. Bot.
262 Research, 4 (3) (2014) 23–30.

263 28. G. Johnson, M. Ayers, S.C. McClure, S.E. Richardson, R. Tellier, Detection and
264 identification of *Bartonella* species pathogenic for humans by PCR amplification targeting the
265 riboflavin synthase gene (ribC). J. Clin. Microb. 41 (3) (2003) 1069-72.

266 29. L. Ivacic, K.D. Reed, P.D. Mitchell, N. Ghebranious, A LightCycler TaqMan assay for
267 detection of *Borrelia burgdorferi* sensu lato in clinical samples. Diagn. Micr. Infec. Dis. 57
268 (2007) 137-43.

269 30. N.E. Babady, L.M. Sloan, E.A. Vetter, R. Patel, M.J. Binnicker, Percent positive rate of
270 Lyme real-time polymerase chain reaction in blood, cerebrospinal fluid, synovial fluid, and
271 tissue. Diagn. Micr. Infec. Dis. 62 (2008) 464-
272 466. <https://doi.org/10.1016/j.diagmicrobio.2008.08.016>.

273 31. A. Ciervo, L. Ciceroni, Rapid detection and differentiation of *Bartonella* spp. by a single-
274 run real-time PCR. Mol. Cell. Probe. 18 (5) (2004) 307-12.

275 32. P. Wilhelmsson, L. Fryland, S. Börjesson, J. Nordgren, S. Bergström, J. Ernerudh, P.
276 Forsberg, P.E. Lindgren, Prevalence and Diversity of *Borrelia* Species in Ticks That Have
277 Bitten Humans in Sweden. J. Clin. Microbiol. 48 (11) (2010) 4169-4176.
278 <https://doi.org/10.1128/JCM.01061-10>.

- 279 33. E. Colebrook, R. Wall, Ectoparasites of livestock in Europe and the Mediterranean region.
280 Vet. Parasitolo. 120 (2004) 251-274.
- 281 34. R.A. Pantaleoni, M. Baratti, L. Barraco, C. Contini, C.S. Cossu, M.T. Filippelli, L. Loru,
282 M. Romano, *Argas (Persicargas) persicus* (Oken, 1818) (Ixodida: argasidae) In sicily with
283 considerations about its Italian and west-Mediterranean distribution. Parasite 17 (2010) 349-
284 355.
- 285 35. I. Lafri, W. Benredjem, F. Neffah-Baaziz, R. Lalout, K. Abdelouahed, B. Gassen,
286 S. Bakhouch, M. Chergui, M. Karakellah, H. Adjmi-Hamoudi, I. Bitam, Inventory and update
287 on argasid ticks and associated pathogens in Algeria. New Microbe New Infect. 23 (2018)
288 110–114. <https://doi.org/10.1016/j.nmni.2018.02.009>
- 289 36. Z. Shahnaz, F.R. Chaudry, A. Shamim, M.A. Zafar, M. Hasan, M.F. Iqbal, A. Riaz,
290 Research soft tick (*Argas persicus*) Infestation at government layer farms of Pothwar region
291 of Punjab, Pakistan. J. Entomol. Zool. Stud. 4 (4) (2016) 664-667.
- 292 37. A. Mulugeta, C. Mersha, B. Basaznew, Major constraints of village poultry production in
293 Demba Gofa District of Southern Region, Ethiopia. Brit. J. Poultry Sci. 2 (1) (2013) 01-06.
- 294 38. E. Swai, S.M. Kessy, P. Sanka, S. Banga, J.E. Kaaya, A survey on Ectoparasites and
295 haemoparasites of free-range indigenous chickens of Northern Tanzania. Livest. Res. Rural
296 Devel. 22 (2010) (9).
- 297 39. R.S. Lisbôa, R.C. Teixeira, C.P. Rangel, H.A. Santos, C.L. Massard, A.H. Fonseca, Avian
298 spirochetosis in chickens following experimental transmission of *Borrelia anserina* by *Argas*
299 (*Persicargas*) *miniatus*. Avian Dis. 53 (2) (2009) 166–168. <https://doi.org/10.1637/8377->
300 061508-Reg.1

- 301 40. G.L. Cooper, A.A. Bickford, Spirochetosis in California game chickens. *Avian Dis.* 37 (4)
302 (1993) 1167–1171.
- 303 41. B. Aslam, I.Hussain, M.A. Zahoor, M.S. Mahmood, M.H. Rasool, Prevalence of *Borrelia*
304 *anserina* in Argas ticks. *Pakistan J. Zool.* 47 (4) (2015) 1125-1131.

Figure legends

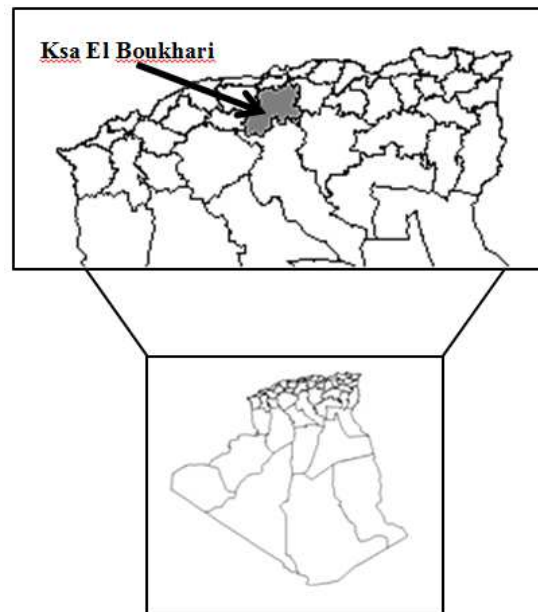


Figure 1. Geographical presentation of the study area: Ksar El Boukhari

Table 1

Number of farms infected by *A. persicus* soft ticks, and number and source of ticks according to the season

Seasons	N° positive farms (%)	Number and source of ticks (%)		
		Total	Hens	Farm
Summer	28 (71.80%)*	300 (87.97%)*:	90 (26.39%)*:	210 (61.58%)*:
		133 M	25 M	108 M
		167 F	65 F	102 F
Autumn	7 (17.94%)	26 (7.62%):	8 (2.34%):	18 (5.27%):
		12 M	3 M	9 M
		14 F	5 F	9 F
Winter	1 (2.56%)	3 M (0.88%):	/	3 M (0.88%):
Spring	3 (7.69%)	12 (3.52%):	1 F (0.29%)	11 (3.22%):
		4 M		4 M
		8 F		7 F
Total	39 (100%)	341 (100%):	99 (29.03%):	242 (70.97%):
		152 M	28 M	124 M
		189 F	71 F	118 F

* These values are significantly higher than the other values in the same column at $p < 0.05$.

M: Males. F: Females.

Graphical abstract

