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Abstract

Argasid ticks are one of the most important poultry ectoparasites. They affect poultry directly through blood meal and indirectly through the transmission of pathogens essentially *Borrelia anserina*, agent of avian borreliosis, one of the most widespread poultry diseases in the world, and is of great economic importance. This study was conducted between April 2014 and March 2015 in the region of Ksar El Boukhari, Algeria, in order to investigate the presence of soft ticks in laying hen farms and to detect *B. anserina* bacteria using molecular tools. DNA was extracted and screened for the presence of *Borrelia* spp. DNA by real-time polymerase

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

Keywords

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

1. Introduction

Ticks are obligate haematophagous ectoparasites of mammals, birds and reptiles. They are distributed worldwide and are vectors of a large variety of human and animal pathogens [1]. Two families of ticks are capable of transmitting a broad range of pathogens: Ixodidae (hard ticks) currently comprise over 700 species worldwide, and Argasidae (soft ticks) comprise roughly 200 species [2, 3, 4].

Unlike Ixodidae, the duration of bloodmeal of soft ticks is short, particularly for nymphs and adults (15-60 minutes). For larvae, this duration is typically longer (12 hours to several days). This characteristic may be seen as an adaptation to unfavorable external conditions by detaching rapidly from their hosts. After each bloodmeal, these ticks are generally found in the crevices and cracks of the walls of their habitat, in nests and burrows, or on the ground, under the dust [5].

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

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

2.5. Statistical analysis

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

3. Results

3.1. Tick collection

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

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

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

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

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

5. Conclusions

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

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Figure legends

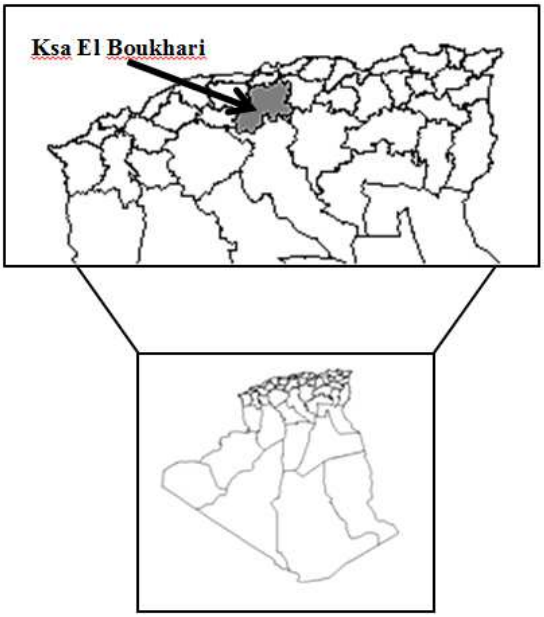

Figure 1. Geographical presentation of the study area: Ksar El Boukhari

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