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1 **Molecular evidence of bacteria in *Melophagus ovinus* sheep keds and *Hippobosca equina***  
2 **forest flies collected from sheep and horses in northeastern Algeria.**

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17 **Abstract**

18 The sheep ked, *Melophagus ovinus*, and the forest fly, *Hippobosca equina*, are parasitic  
19 dipteran insects of veterinary importance. As hematophagous insects, they might be  
20 considered as potential vectors of diseases which may be transmissible to humans and  
21 animals. The purpose of this study was to present initial primary data about these two species  
22 in Algeria. To do so, we conducted a molecular survey to detect the presence of bacterial  
23 DNA in flies collected in Algeria. A total of 712 flies including, 683 *Melophagus ovinus* and  
24 29 *Hippobosca equina* were collected from two regions in northeastern Algeria. Monitoring  
25 the monthly kinetics of *M. ovinus* infestations showed something resembling annual activity,  
26 with a high prevalence in January (21.67%) and May (20.94%).

27 Real-time quantitative PCR assays showed that for 311 tested flies, 126 were positive for the  
28 *Bartonella* spp. rRNA intergenic spacer gene and 77 were positive for *Anaplasmataceae*. A  
29 random selection of positive samples was submitted for sequencing. The DNA of *Bartonella*  
30 *chomelii* and *Bartonella melophagi* were amplified in, respectively, five and four *H. equina*.  
31 25 *M. ovinus* positive samples were infected by *Bartonella melophagi*. Amplification and  
32 sequencing of the *Anaplasma* spp. 23S rRNA gene revealed that both species were infected  
33 by *Wolbachia* sp. which had previously been detected in *Cimex lectularius* bed bugs.

34 Overall, this study expanded knowledge about bacteria present in parasitic flies of domestic  
35 animals in Algeria.

36 **Keywords:** Sheep, *Melophagus ovinus*, Horses, *Hippobosca equina*, vector-borne diseases,  
37 Algeria.

## 38 **1. Introduction**

39 Hippoboscidae flies, usually known as keds or louse flies, are obligate hematophagous  
40 Diptera, which bite birds and some mammals [1, 2]. They are organised into more than 19  
41 genera and 150 cosmopolitan species [3-6]. Members of this family, particularly those  
42 relating to sheep (*Melophagus ovinus*), horses (*Hippobosca equina*) and dogs (*Hippobosca*  
43 *longipennis*) bite their hosts and people who take care of these animals [1, 3, 7]. Human  
44 reactions to these bites vary widely, ranging from simple redness followed by pruritic  
45 inflammation to anaphylactic shock requiring emergency treatment [8-11]. In animals, these  
46 ectoparasites are responsible for weight loss, decrease in wool growth and livestock milk  
47 production, and cutaneous myiasis causing, in the long run, significant economic losses [1, 7,  
48 **12-14**].

49 The pathogenic role of Hippoboscidae flies remains insufficiently documented. As they are  
50 often subservient to the hosts they parasitize, this could cast doubt on their ability to transmit  
51 pathogens to other animals or humans [3]. However, recent studies have reported the  
52 molecular detection of several vector-borne pathogens in Hippoboscidae flies collected on  
53 ruminants [15-22], horses [15, 23], dogs [24, 25] and raptors [26]. All these studies support  
54 the hypothesis that Hippoboscidae flies might be vectors of infectious diseases. However,  
55 molecular studies are not sufficient to confirm the vector competence of an arthropod.

56 Although *M. ovinus* is the most studied Hippoboscidae alongside *H. equina* [1, 27, 28], data  
57 on these two species in terms of their biology and involvement in the transmission of  
58 pathogens are entirely lacking in Algeria. The main reason for this is that *M. ovinus* and *H.*  
59 *equina* flies often go unnoticed and are considered by farmers to be harmless pests.

60 It was from this perspective that we investigated Hippoboscidae flies that parasitize sheep and  
61 horses in northeastern Algeria. The aims of the present study were to gather initial data on the  
62 monthly prevalence of *M. ovinus* and high-risk periods, and to use molecular procedures to  
63 investigate the presence of bacteria in *M. ovinus* sheep keds and *H. equina* forest flies.

## 64 **2. Materials and Methods**

### 65 **2.1. Study areas and period of collection**

66 The study was carried out between August 2015 and July 2016 in two regions of the extreme  
67 north east of Algeria: El Tarf (36°51'21.5"N, 8°19'34.5"E) and Mila (36°27'0"N, 6°16'0"E)  
68 (**Fig. 1**). These regions are known for cattle breeding and have fairly similar climates. El Tarf  
69 is made up of two clearly differentiated areas: the northern part is mainly characterised by  
70 alluvial plains and the climate is sub-humid to warm humid; while the southern part has  
71 greater relief and the climate is humid to cool and wet [29]. The Mila region is notable for its  
72 humid climate in the north, sub-humid to semi-arid in the centre, and semi-arid in the south  
73 [29].

### 74 **2.2. Fly collection and identification**

75 *Melophagus ovinus* flies were collected on a monthly basis from three sheep farms located in  
76 the town of Tessala Lematai in the Mila region. These farms culminate at approximately the  
77 same altitude (~ 1,465m) and are each composed of 30 sheep of different sex and age  
78 categories. For every ovine which was examined, its age (young: ≤ 1 year; adult: >1 year) and  
79 sex were recorded. To collect the flies, the fleeces of the parasitized sheep were parted and the  
80 flies were collected directly from the deepest parts of the wool. During the shearing period in  
81 May, the flies were recovered directly and individually from the wool bales placed beside  
82 each sheared sheep. In both cases, sheep keds were carefully removed and immediately placed  
83 in 70% ethanol. The *M. ovinus* pupae were not recovered from infested animals and were left  
84 *in situ*.

85 The *Hippobosca equina* forest flies were collected only once, in June, from two horse barns in  
86 Ain El Kerma, a town in the El Tarf region. The first barn consisted of 11 horses while the  
87 second was composed of nine horses. The flies were caught manually from the inner thigh  
88 and around the perineum of parasitized horses and were directly stored in 70% ethanol.

89 The sampled flies were morphologically identified using a Leica® binocular lens with an  
90 LED light at the IHU Méditerranée Infection, Marseille, France. Identification was essentially  
91 based on the morphotaxonomic criteria reported in Huston and Wall and Shearer dichotomous  
92 keys [30, 31]. Photographs of the dorsal and ventral sides of each species were taken with a  
93 microscope at a magnification of ×56 (Zeiss Axio Zoom.V16, Zeiss, Marly le Roi, France)  
94 (Fig. 2 and 3).

### 95 2.3. DNA extraction

96 DNA extraction was performed on a representative selection of *M. ovinus* flies from each of  
97 the three sheep farms and on all the *H. equina* fly specimens. All experiments and sample  
98 handling was conducted under sterile conditions under a laminar flow biosafety hood. The  
99 sample preparation process was the same for both fly species. The flies were removed from  
100 the ethanol, rinsed for 10 minutes in a sterile distilled water bath and then dried with filter  
101 paper. For each sample, a longitudinal incision was made using a scalpel blade, cutting the fly  
102 into two equal parts. One half was dropped into a sterile tube (Eppendorf; Hamburg,  
103 Germany) while the remaining part was kept at -20°C for further analysis.

104 Each half-fly was then incubated at 56°C overnight with 180µL of G2 lysis buffer (Qiagen,  
105 Hilden, Germany) and 20µL proteinase K (Qiagen, Hilden, Germany). DNA extraction was  
106 processed using an EZ1® DNA Tissue Kit (Qiagen, Hilden, Germany) and EZ1® BioRobot®  
107 extraction device according to the manufacturer's recommendations. Between each batch, all  
108 parts of the device were disinfected and subjected to 20 minutes of ultraviolet light to avoid  
109 any cross contamination. Finally, the DNA from each sample was eluted in 100 µL of Tris-  
110 EDTA (TE) buffer (Qiagen, Hilden, Germany) and stored at -20°C under sterile conditions.

111

112

#### 113 **2.4. Molecular survey and PCR amplification**

114 To investigate the presence of bacterial DNA, all the extracted DNA was subjected to real-  
115 time PCR assay using a CFX Connect™ Real-Time PCR Detection System (Bio-Rad,  
116 Marne la Coquette, France) targeting a fragment of specific genes of five bacteria. The citrate  
117 synthase (*gltA*) “RKND03” gene was used to detect *Rickettsia* spp. [32], the intergenic spacer  
118 (ITS2) gene was used to detect *Bartonella* spp. [33], the 23S ribosomal RNA gene was used  
119 to detect the *Anaplasmataceae* bacteria [34], the ITS4 spacer was used to detect *Borrelia* spp.  
120 [35], and IS30A spacers were used to detect *Coxiella burnetii* [36].

121 15µl of the qPCR reaction mix, without any DNA, and dilutions of DNA extracts of cultured  
122 bacteria strains were used in each test respectively as negative and positive controls, as  
123 previously described [37]. Samples were considered positive when the cycle threshold (Ct)  
124 value given by the Bio-Rad CFX Manager™ v3.1 software (Bio-Rad, Marne la Coquette,  
125 France) was  $\leq 35$ . A random selection of these positive samples was subsequently subject to  
126 conventional PCR prior to sequencing in order to identify pathogens at the species level.

127 Conventional PCR analysis was performed using an automated DNA thermal cycler (Applied  
128 Biosystems, 2720, Foster city, USA), targeting the intergenic transcribed (ITS) gene [38] and  
129 the citrate synthase (*gltA*) gene [39] for qPCR *Bartonella* spp. positive samples. Bacterial  
130 DNA from the *Anaplasmataceae* family was detected using the 23S gene [40]. Amplified  
131 products were then subjected to electrophoresis through a 0.5% agarose gel stained with  
132 SYBR Safe™ and viewed using a ChemiDoc™ MP ultraviolet imager (Bio-Rad, Marnes-la-  
133 Coquette, France).

134 The PCR products were purified using a Macherey-Nagel plate (NucleoFast® 96 PCR,  
135 Düren, Germany), as recommended by the manufacturer. Amplicons sequences were obtained  
136 using the ABI Prism 3130xl (ABI PRISM, PE Applied Biosystems, USA) genetic analyser

137 capillary sequencer and the BigDye® Terminator v1.1, v3.1. 5x Sequencing Buffer (Applied  
138 Biosystems, Warrington, United Kingdom).

## 139 **2.5. Sequence processing**

140 In order to identify bacterial species, the obtained nucleotide sequences were first assembled  
141 and edited using ChromasPro v.1.7.7 software (Technelyium Pty. Ltd., Tewantin,  
142 Queensland, Australia). They were then processed by comparing them with the sequences  
143 available in the GenBank database, using the online Basic Local Alignment Search Tool  
144 (BLAST) ([http:// blast.ncbi.nlm.nih.gov/Blast.cgi](http://blast.ncbi.nlm.nih.gov/Blast.cgi)).

## 145 **2.6. Statistical analysis**

146 Statistical analyses were performed using the SPSS v24.0 HF02 software (IBM SPSS  
147 Statistics for Windows, Version 24.0, Armonk, NY: IBM Corp, 2016), and the Pearson's chi-  
148 squared " $\chi^2$ " test was used to compare the overall prevalence of *M. ovinus* according to the  
149 month of collection and the age and sex of the sheep.

## 150 **2.7. Ethical considerations**

151 Ethical consent for sampling from sheep and horses was granted by the El Tarf University  
152 Animal Ethics Committee. Verbal agreement for the field study was obtained from the  
153 animals' owners and from the local agricultural services office.



## 154 **3. Results**

### 155 **3.1. Fly collection and infestation prevalence**

156 A total of 683 adult *M. ovinus* flies (320 males and 363 females) and 29 *H. equina* flies (14  
157 males and 15 females) were collected respectively from 81/90 (90%) infested sheep belonging  
158 to the three Mila farms and 9/20 (45%) infested horses from the El Tarf barns.

159 The sheep studied here were also co-infested by other ectoparasites such as *Damalinia ovis*  
160 lice, and *Haemaphysalis sulcata* and *Haemaphysalis punctata* ticks, while the horses were  
161 infested by *Damalinia equi* lice and *Boophilus (Rhipicephalus) annulatus* ticks.

162 Monitoring of the monthly kinetics of *M. ovinus* infestation in the three sheep farms showed  
163 that these flies parasitize animals almost all year round, with a high prevalence in January  
164 (21.67%) and May (20.94%) and almost zero prevalence in autumn and summer (**Fig. 4**).

165 These results allow us to define a statistically significant ( $\chi^2$  test,  $\rho = 0.01$ ) period of activity  
166 for this fly species which can be estimated to exist between the months of October and May.

167 In addition, we compared the ovine infestation rate by the age and sex of the sheep. The  
168 results revealed that there was no statistically significant difference in *M. ovinus* infestation  
169 rates between male and female sheep ( $\chi^2$  test,  $\rho = 0.58$ ) and between young and adult sheep  
170 ( $\chi^2$  test,  $\rho = 0.21$ ).

171 The horses' infestation by the *H. equina* forest fly could not be correctly monitored due to the  
172 frequent sales of the horses, which resulted in off-peak periods where no horses were present  
173 in the stables.

### 174 **3.2. Detection of bacteria**

175 From 712 collected flies, 311/712 specimens including, 282 *M. ovinus* (94 specimens from  
176 each of the three farms) and 29 *H. equina* were randomly selected and screened using real-  
177 time PCR for the presence of bacteria. Of the 311 flies tested, 202/311 (64.95%) samples  
178 were positive for at least one of the investigated bacteria, while 58/311 (18.64%) were found

179 to be co-infected by two bacterial genus. The 311 flies tested negative for all spotted fever  
180 group *Rickettsia* species, *Borrelia* spp., and *C. burnetii*. The bacterial identity of a random  
181 selection of the positive samples was later achieved by standard PCR amplification and  
182 sequencing.

183 Real-time assays targeting the intergenic spacer (ITS) gene for *Bartonella* spp. revealed that  
184 126/311 (40.51%) flies, including 104/282 (36.87%) *M. ovinus* and 22/29 (75.86%) *H. equina*  
185 collected respectively from sheep and horses, were *Bartonella*-positive. From 126 *Bartonella*-  
186 positive samples, all 22/29 *H. equina* specimens and 25/104 randomly selected *M. ovinus*  
187 specimens were subject to standard PCR. However, amplification trials using the ITS gene  
188 were not successful. Sequencing analysis using the *gltA* system was subsequently performed  
189 and high quality sequences were obtained for all (25/25) *M. ovinus* specimens and 9/22 of the  
190 *H. equina* flies. The BLAST analysis results revealed 99.48 to 100% identity with the  
191 corresponding 753 base pair fragment of *Bartonella chomelii* (GenBank accession nos.  
192 KM215691.1 and KM215693.1) for 5/9 sequences amplified from *H. equina* and 100%  
193 identity with *Bartonella melophagi* (GenBank accession nos. MG701237.1) for the remaining  
194 sequences (4/9). However, following the BLAST query, all sequences obtained from *M.*  
195 *ovinus* specimens showed 99.50 to 100% identity with the corresponding 323 base pair  
196 fragment of *B. melophagi* (GenBank accession nos. MG701237.1).

197 Of the total 23S qPCR screened flies, 77/311 samples (24.75%) involving 73/282 *M. ovinus*  
198 (25.88%) and 4/29 *H. equina* (13.79%) were infected by bacteria from the *Anaplasmataceae*  
199 family. Within this, 23S rRNA amplification and sequencing was successful for 65/77  
200 samples (61 *M. ovinus* and four *H. equina*). The query of the resulting sequences against the  
201 NCBI GenBank database showed, for both fly species, 99.31 to 100% identity with the  
202 “*Wolbachia endosymbiont of Cimex lectularius*” sequence (GenBank accession nos.  
203 AP013028.1).

#### 204 **4. Discussion**

205 Over last decade, the vast majority of research conducted on parasitic arthropods of animals  
206 in Algeria has essentially focused on ticks, fleas, mosquitoes, and sand-flies. The interest in  
207 these categories of pests largely reflects the fact that they can significantly affect their hosts  
208 by transporting and inoculating several vector-borne diseases [37, 41-45], which can in turn  
209 lead to considerable economic losses.

210 To date, few entomological investigations into haematophagous flies and their associated  
211 microorganisms have been the subject of research in Algeria. The only available records were  
212 inventories on *Nycteribiidae* bat flies, which may be recognized as potential vectors of  
213 *Bartonella tamiiae* [46, 47]. The unpredictable behaviour of the Hippoboscidae fly its voracity  
214 and its spectacular ability to reproduce and quickly increase the number of individuals per  
215 population, as well as and its ability to cover long distances in flight, has significantly  
216 increased the vulnerability of animals to vector-borne pathogens. Consequently, the fly-  
217 bacteria relationship should not be overlooked.

218 In this study, we have observed the prevalence and the period of occurrence of the *M. ovinus*  
219 sheep ked by conducting an annual monitoring of the infestation. An overall prevalence of  
220 90% for *M. ovinus* registered in our study can be explained by the combination of various  
221 crucial factors including the presence of a cold climate and a high altitude region which are  
222 absolutely essential to the fulfilment of *M. ovinus* life cycle [1, 48]; The mismanagement of  
223 the livestock and unhealthy husbandry conditions which could predispose malnourished sheep  
224 to infestation [49]; and finally the highly abusive use of insecticides which could lead to the  
225 emergence of a resistant cohort of keds [12]. In addition, the annual monitoring of *M. ovinus*  
226 population revealed that the number of sheep keds began to increase in late October, peaked  
227 in January and May and observed a seasonal low starting from late May. These findings are in

228 compliance with previous observations where *M. ovinus* populations are reported to be active  
229 in winter and spring and less in summer [50, 51].

230 On the other side, the lack of a statistically significant difference in infestation rates of *M.*  
231 *ovinus* depending on the sex and ages of infested sheep is consistent with previous studies  
232 where the abundance of *M. ovinus* flies in sheep was shown to be related to abiotic  
233 components rather than host dependent factors [1, 49].

234 Molecular tools were used to conduct the bacteriological survey. The reliability of these  
235 techniques in such epidemiological investigations on arthropods and especially on flies has  
236 been demonstrated in previous research [15, 16, 20, 49]. Furthermore, the results reported  
237 here were obtained under sterile conditions using meticulous laboratory procedures and  
238 sophisticated instruments routinely employed in our laboratory. Although a negative and  
239 positive control of each PCR test was used to confirm the accuracy of our results, this cannot  
240 confirm the vector role of the flies studied, as they could have acquired the bacteria after  
241 blood meals on infected animals or crossed other vectors and co-fed with them.

242 This survey demonstrated that 64.95% of tested flies were infected by at least one bacteria. *B.*  
243 *melophagi* was found in *M. ovinus* and *H. equina* while *B. chomelii* was detected in *H.*  
244 *equina*. Phylogenetically, these two bacteria are affiliated, alongside *B. bovis*, *B. capreoli* and  
245 *B. schoenbuchensis*, to *Bartonella* strains which mainly infect ruminants [52]. However, their  
246 pathogenicity and the way that they may be transmitted are poorly understood.

247 Nevertheless, the manifestation of symptoms in patients who are in contact with animals and  
248 who suffered from idiopathic pericarditis, circular red skin lesions, and chronic asthenia have  
249 been associated with the clinical expression of a *B. melophagi* infection which was later  
250 isolated from their blood [11]. Although sheep were reported as the most likely reservoirs of  
251 *B. melophagi* [11, 53, 54] there is no symptomatic description of such infections in animals.

252 However, as certain *Bartonella* species were associated with bovine endocarditis [55, 56]  
253 further investigation of *B. melophagi* are needed.

254 *Bartonella chomelii* has not yet been shown to be pathogenic [57-59]. This *Bartonella* species  
255 was first isolated from domestic cattle blood [60] and was later identified as the most  
256 common *Bartonella* infecting cattle [61].

257 Both species of *Bartonella* adapted to mammal reservoirs and, according to previous studies,  
258 they share biting flies as common potential vectors [54]. This symbiosis is possible when a  
259 variety of adaptation, metabolic interconnections and genomic changes occur between insects  
260 and bacteria.

261 The occurrence of *B. melophagi* within *M. ovinus* sheep keds has already been reported in  
262 previous works [21, 49, 54, 62, 63] and *B. melophagi* DNA was found in the gut of *M. ovinus*  
263 adults and even in the pupal stages, thus suggesting this bacteria is endosymbiotic in nature,  
264 and confirming our findings [15, 21, 49, 62, 64, 65]. However, *B. melophagi* had never been  
265 detected in *H. equina*. To the best of our knowledge, this is the first report of such an  
266 association. The few available reports until now have associated *H. equina* flies with *B.*  
267 *schoenbuchensis* and *B. chomelii* [15, 23].

268 *Bartonella chomelii* has been associated with several arthropods, such as Ixodid ticks and  
269 biting flies [15, 66, 67]. However, its most effective vector remains unknown.

270 Overall, the flies sampled in this survey were infected by two *Bartonella* species and, as  
271 alluded to above, this does not guarantee their role as vectors. Further investigations are  
272 needed to shed light on the role of *H. equina* in the epidemiology of *B. melophagi* and *B.*  
273 *chomelii*.

274 This study also investigated the presence of bacteria from the Anaplasmataceae family. The  
275 ability of hippoboscidae flies to vector bacteria from this family has been recently assessed.  
276 The results confirmed the presence of *Anaplasma ovis* in *M. ovinus* specimens and its vertical

277 transmission among keds [16, 18], thus bringing new evidence regarding the potential  
278 position of these flies as mechanical or biological vectors. In our case, “*Wolbachia*  
279 *endosymbiont of Cimex lectularius*” was detected in 61 *M. ovinus* and four *H. equina*. To the  
280 best of our knowledge, this study provides the first molecular proof of the presence of  
281 *Wolbachia* spp. DNA in *H. equina* adults. However, these bacteria have already been  
282 described as the third most common bacteria genus after *Bartonella* and *Arsenophonus* in the  
283 midgut of *M. ovinus* [63, 64]. In addition, the screening of *M. ovinus* microbiota revealed that  
284 *Wolbachia* was present intracellularly in various tissues such as adipocytes, secretory cells  
285 and intestinal tissue [65]. These multiple locations of *Wolbachia* spp. have previously been  
286 described for other arthropods such as lice and bed bugs [68]. However, no studies have so far  
287 reported the presence of *Wolbachia* spp. or *Anaplasma* spp. in *H. equina* flies.

## 288 **5. Conclusion**

289 In this study, we provided molecular evidence for the presence of bacteria in two fly species  
290 collected from sheep and horses in northeastern Algeria. *B. chomelii* has been detected for the  
291 first time in Algeria and the African continent, while *B. melophagi* is reported for the first  
292 time in Algeria. In view of these facts, more attention should be given to livestock  
293 haematophagous flies, since they can carry zoonotic pathogens. Measures to combat them  
294 could be conducted according to high-risk periods. However, the choice of the most effective  
295 insecticide will have to be made according to two parameters: its long persistence and its  
296 quality/price ratio.

## 297 **Competing interests**

298 The authors declared that they have no competing interests.

299 **Author contributions**

300 **MB** contributed to arthropod collection, performed DNA extractions, PCRs, sequencing and  
301 prepared the first draft of the paper. **NM** helped with fly sampling and contributed to the  
302 manuscript. **AB** contributed to conceiving, designing and coordinating the study. **DR**  
303 contributed reagents/materials/analysis tools. **PP**<sup>\*</sup> coordinated experiments and reviewed the  
304 paper.

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## Figure legends

499 **Fig. 1:** Collection sites of *Melophagus ovinus* and *Hippobosca equina* and their infection rates  
500 by *Bartonella* spp. and *Wolbachia* sp.

501 **Fig. 2:** The “false sheep louse” *Melophagus ovinus* (Diptera: Hippoboscidae): A 4-7 mm fly  
502 with wings which have been reduced to scales and no halteres and a reddish-brown body  
503 covered by dense setae. The head is embedded in the thorax with reduced compound eyes; the  
504 abdomen is heart-shaped (narrow in the male, wider in the female). Three pairs of legs each  
505 ending in large claws. The mouthparts consist of a prominent piercing-sucking proboscis.  
506 Male: Dorsal [a] and ventral view [b]; Posterior end [c]; Pupae are progressively coloured and  
507 tanned from light (right) to dark brown (left) [d]. Female: Dorsal [e] and ventral view [f];  
508 Posterior end [g].

509 **Fig. 3:** The “forest fly” *Hippobosca equina* (Diptera: Hippoboscidae), 7-8 mm, horse, cattle  
510 and dromedary parasite. The wings are large and longer than the body and slightly tinted, with  
511 seven longitudinal veins and two cross-veins [h]. The body is reddish brown with yellow  
512 bands and dense setae, wide and flat thorax and compound big eyes. Three pairs of legs each  
513 ending in strong claws. Moves sideways, like a crab; fast flight. Male: Dorsal [i] and ventral  
514 view [j]; Posterior end [k]. Female: Dorsal [l] and ventral view [m]; Posterior end [n].

515 **Fig. 4:** Overall monthly prevalence of melophagosis in three sheep farms in Mila.

# MEDITERRANEAN SEA

100 km


60 mi



El Tarf

Mila

683



Mila

29



El Tarf

- ★ *Bartonella* spp. (40.51%)
- ◆ *Wolbachia* sp. (25.88%)

- ★ *Bartonella* spp. (75.86%)
- ◆ *Wolbachia* sp. (13.79%)





[a]

[d]



[c]



[b]



[e]



[g]



[f]



[i]



[h]



[k]



[j]



[l]



[n]



[m]

