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Cystic echinococcosis in three locations in the Middle Atlas, Morocco

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1 **Cystic echinococcosis in three locations in the Middle Atlas, Morocco: estimation of the infection rate in the dog**
2 **reservoir**

3

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18

19 **Abstract**

20 A longitudinal study was carried out in Middle atlas, Morocco (locality of Had Oued Ifrane) in a population of 255 dogs
21 from three localities including two categories of dogs (owned and stray dogs). The dogs were investigated three times
22 over a period ranging from four to eight months between December and August. At each investigation, dogs were treated
23 with arecoline inducing defecation and allowing feces collection. Dogs were further treated with praziquantel to clear
24 them from *E.granulosus*. Microscopic examination of feces ~~were-was~~ performed ~~to assess the infection status of dogs at~~
25 ~~each investigation and eopro-PCR-positive on fecal samples underwent copro-PCR to assess the infection status of dogs~~
26 ~~at each investigation and to determine the circulating strain of *E.granulosus*. A high prevalence of infestation ranging~~
27 ~~from 23.5% to 38.8% and from 51.3% to 68.5% was respectively found in owned and in stray dogs.~~ The PCR results
28 revealed the presence of G1 strain in all positive samples ~~and a high prevalence of infestation, ranged before treatment~~
29 ~~between 23.5% and 38.8% in owned and from 51.3% to 68.5% in stray dogs. The A~~ logistic regression model ~~was~~ used
30 to determine incidence ~~of infestation and~~ showed that stray dogs underwent a significantly higher risk of infection (OR
31 = 14; 95% CI: 6-30; P<0.001) compared to owned dogs. ~~Only anthelmintic treatment intervals of 2 months efficiently~~
32 ~~prevented egg shedding in owned and stray dogs.~~ The seasonal effect was also significant with the highest risk of re-
33 infestation in ~~December (winter)~~ and the lowest risk in ~~August (summer)~~. This study confirms that stray dogs undergo
34 an increased risk of infestation by *E. granulosus* and indicate that infective pressure is influenced by season.

35 **Key words:** *Echinococcus granulosus*, dog, incidence, Morocco.

36

37 **1. Introduction**

38 *Echinococcus granulosus* is a cestode belonging to the family Taenidae. This tapeworm is an intestinal parasite
39 that usually infects canines, especially dogs as definitive host. Eggs of this parasite are eliminated with the feces and
40 transmitted to a wide range of intermediate hosts, including sheep and humans, causing hydatid cysts (larval stage of the
41 parasite). Dogs acquire infection by ingesting infected organs of intermediate hosts (Thompson and McManus 2001).

42 Cystic echinococcosis (CE) is a highly endemic zoonosis in Morocco. The abundance of stray dogs, and slaughter
43 practices allowing dogs to have access to condemned offal especially in rural areas, contributes to its persistence. This
44 disease represents a serious public health problem and has a substantial socio-economic impact. In 2015, 1627 human
45 surgical cases (5.2 cases per 100,000 inhabitants) were recorded for the whole country (Chebli 2017). Surgeries need to
46 be repeated in 3% of cases, and a mortality of 3% was found-reported (ministry Ministry of health, 2012). The
47 treatment costs of treatment was were estimated to be approximately US\$ 1700 and US\$ 3200 for simple and repeat cases
48 respectively and present an important financial burden to the health sector (Andersen et al. 1997). Indirect costs due to
49 recurrence and re-examination, reduced quality of life following surgery, morbidity due to undiagnosed CE and loss of
50 income in fatal cases (ministry of health, 2012) were not considered in these burden calculations and would further
51 increase this estimate (Ministry of Health, 2012).

52 In Morocco, current evidence demonstrates indicates that the transmission cycle of *E.granulosus* relies primarily
53 on a domestic cycle involving dogs and livestock species (sheep, cattle, camels, goats, and equines) (Azlaf and Dakkak
54 2006). Stray dogs in urban areas and free or roaming dogs in rural areas are the main definitive host and are pivotal in
55 transmission in this context (Azlaf and Dakkak 2006; Azlaf et al. 2007; El Berbri et al. 2015a). A study conducted by
56 Azlaf and Dakkak (2006) in several regions of Morocco revealed prevalence rates of 10.58% in sheep, 1.88% in goats,
57 22.98% in cattle, 12.03 % in camels and 17.8% in equines. The study conducted by El Berbri et al. (2015b) in the region
58 of Sidi Kacem revealed a prevalence of 42.9% in cattle, 11% in sheep and 1.5% in goats. In the slaughterhouses, organ
59 refusal due to hydatidosis generates losses estimated at US \$ 100 000 per year at the national level (Azlaf and Kadiri
60 2012). In dogs, the tapeworm prevalence varies between 22% and 68.2% across regions (Ouhelli et al. 1997).
61 Consequently, this high prevalence leads to a very high contamination of the environment with eggs (Gemmell et al.
62 2001), and hence the risk of transmission to farm animals and humans is expected to be very high. For these reasons, and
63 in line with WHO/OIE (2001), detection of infection in dogs is an essential component of epidemiological studies and
64 implementation of CE control programs (Dakkak et al. 2016).

65 In rural regions of Morocco, owned dogs and free roaming or stray dogs are the definitive hosts of *E. granulosus*.
66 Owned dogs are kept as house and livestock guards and are in tight contact with their owners, thereby increasing the risk
67 of contamination of humans, especially women and children (Kachani 1997). On the other hand, their role as shepherd
68 strongly increases the risk of infestation of pastures and thereby leads to infestation of cattle, sheep, goats etc. Infected
69 organs such as liver and lungs from home-slaughtered animals appear as source of infection of owned dogs. Stray dogs
70 are likely to be infested when roaming freely around slaughterhouses and weekly markets (souks) where animals are
71 killed without any access restriction and no appropriate destruction of infected organs (Kachani 1997).

72 Among the pharmacological options aiming at the reduction of the infective pressure for intermediate hosts and
73 humans figure the vaccination of domestic herbivores against *E. granulosus* (Gauci 2005) as well as the regular
74 deworming of dogs (Larrieu 2012). Indeed, a vaccine against G1 strain of *E. granulosus* tested in Argentina prevented
75 cyst development in sheep (Larrieu 2013). Vaccination is therefore considered as a promising option if satisfying parasite
76 control in dogs cannot be achieved. Indeed, effective chimioprevention in dogs can only be achieved if owned and stray
77 dogs undergo deworming at regular intervals (Cabrera 1996). Given the logistic difficulties of deworming campaigns in
78 rural zones, the risk of infection in function of dog type (owned *versus* stray dog) and in function of the parasite egg
79 survival in the environment (winter *versus* summer) appear as important points for the set-up of an efficient deworming
80 strategy. Accordingly, this study aimed at identifying the circulating strain of *E. granulosus* in owned and stray dogs in
81 Middle Atlas of Morocco and at assessing infection risk over time in both dog categories.

82 **2. Material and methods**

83 **2.1. Description of the study area**

84 The study was implemented in Had Oued Ifrane, located in the Middle Atlas which extends from the southwest
85 to the north-east for about 450 km and covers a total area of 27,550 km², corresponding to 15% of Morocco's mountain
86 area (Figure 1). It is an agro-pastoral zone where agriculture and livestock are the main sources of income for the entire
87 rural population. It is a mountainous area where altitude ranges from 800 to 3500 meters. The climate of the region is the
88 mountainous continental Mediterranean type of mountain: cold, rainy and snowy in winter, hot and dry in summer. Had
89 Oued Ifrane was chosen as a case study site, due to the presence of a large canine population, many rural slaughterhouses
90 and a weekly ephemeral fairground market (souk). It is a region known for breeding, particularly sheep farming with a
91 predominance of the Timahdit breed. Livestock production is the main activity for farmers in this region. A study on the
92 prevalence of hydatid cyst in abattoirs in the same region, showed that 30% of cattle, 13% of sheep and 2% of goats carry

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93 one or more hydatid cysts in the liver and lungs suggesting a strong *E. granulosus* infestation in dogs (Fez.Amarir,
94 personal communication 2017).

95 **2.2. Study design**

96 Two populations of dogs were targeted: owned dogs and stray dogs (~~unowned dogs~~). At Had Oued Ifrane, three
97 douars (villages) distant about 20-30 km from each other were selected. They were located near a weekly souk and a
98 slaughterhouse. Their inhabitants were sheep and cattle breeders of similar herd size and with an average of 2 to 3 dogs
99 per household.

100 Each douar was assigned to a dog treatment group (Group A: Douar Assaka, with a two-months treatment
101 interval, Group B: Douar Sanoual with a three-months treatment interval, Group C: Douar Sidi Bel Khir with a four-
102 months treatment interval) and was composed by similar proportions of owned (60-75%) and stray dogs (25-40%).
103 Praziquantel (5mg/kg) was administrated at three occasions (T0, T1, T2) to owned and stray dogs older than one year at
104 intervals of 2, 3 or 4 months to groups A, B and C, respectively (Table 1, Figure 2). The choice to assess the risk of
105 infestation in dogs at different treatment intervals was initially based on site accessibility and lack of knowledge of
106 incidence. Long exposure time is indeed required to compare ~~dog types owned and stray dogs~~ if incidence is low whereas
107 shorter exposure time is indicated to compare higher incidences. All groups were tested for the first time in December
108 2016. Dogs missing a sampling session were no more investigated. Owned dogs were identified and recognized with help
109 of their owner, whereas stray dogs were identified and recognized on the basis of pictures.

110 **2.3. Fecal sample collection and analysis**

111 In order to induce defecation and expulsion of eggs and worms, dogs received meat balls containing arecoline
112 hydrobromide (approximate dose of 4 mg/kg BW). In case of defecation failure, a second dose of 2mg/kg BW was
113 administrated (Cabrera et al. 1995). After sample collection, remaining feces and defecation area were disinfected with
114 alcohol for at least 5 min and burned (Dakkak 2016). To collect feces from fearful stray dogs, levomepromazine (25 mg
115 orally) was used for sedation prior to arecoline administration (according to the protocol described by OIE 2012).

116 The coprological flotation technique described by Riche and Jorgensen (1971) was applied on fecal samples for
117 microscopic examination. Worms and eggs were identified according to Soulsby (1982).

118 Only samples positive at coprology were washed with PBS and DNA extraction was performed according to
119 Mathis et al. (2006) and Abbassi et al. (2003). The DNA was extracted using the Bioline Kit (Bart and al. 2006). The
120 DNA extracted from worms and eggs underwent a PCR amplification by use of the mitochondrial primers

121 EgCOI/EgCOI2 and EgNDI1/EgNDI2 according to the protocol described by Bart and al. (2006). Copro-PCR was
122 reported to be highly sensitive to detect eggs and worms per animal and to identify species of the family Taeniidae (Mathis
123 et al. 2006). The PCR program was made of 35 cycles with, for each cycle, a denaturation step (15 s at 95°C), a
124 hybridization step (15 s at 50°C for EgCOI 1/2 and 52°C for EgNDI 1/2), and an elongation step (10 s at 72°C).

125 Other species of taeniid cestodes were observed during microscopic analyses of fecal samples, such as *Taenia*
126 *hydatigena* and *Dipylidium caninum*. The presence of these parasites was not taken into account in this study, where the
127 focus was specifically given to *E. granulosus*, the only agent responsible for hydatid cysts in farm animals and humans.

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128 2.4. Statistical analysis

129 Logistic regressions were used to analyse the infection status of dogs before and after treatment. At T0, dog type,
130 the location and the interaction between them were used as discrete explanatory variables. T1 and T2 data were analysed
131 using dog type (discrete), location (discrete) and mean calendar time of the exposure period (continuous from January to
132 June; (Figure 2) as explanatory variables. First level interactions were tested and ignored if $P > 0.05$. Linear estimates and
133 the corresponding probabilities (i) were calculated with their 95% confidence intervals. Since exposure periods (e) ranged
134 from 2 to 4 months, estimates were further transformed in monthly risks (r) assuming a prepatent period (p) of one month.

135
$$- r = 1 - (1 - i)^{1/(e-p)}$$

136 3. Results

137 3.1. Strain identification by coproPCR

138 The analysis of the PCR product of 104 positive fecal samples revealed the presence of a single genotype of
139 *E.granulosus*, the G1 which belongs to the sheep strain, characterized by the presence of a single band of molecular
140 weight of 366 bp for COI and 471 bp for NDI (Figure 3 a and b). These specific primers make it possible to avoid
141 confusion of determination of the other species of parasites and to detect only the presence in the samples of the
142 *E.granulosus* species.

143 3.2. Prevalence of dog infections

144 Positive fecal samples at coproscopic analysis were tested with coproPCR technique (Table 1). Numbers Results
145 regarding of *E. granulosus* infestation of dogs are shown in function of site (A, B, C), dog type (ed dogs at different
146 sampling times in each site (A, B, C) and for each dog types (owned versus stray dogs) and time (are shown in Table 1).

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147 At the start begin of the study, the prevalence was significantly higher in stray dogs than in owned dogs (OR = 14; 95%

148 CI: 6 - 30; $P < 0.001$). Dogs of group A were less infected than dogs of group B ($P = 0.03$) and group C ($P = 0.04$).
149 Interactions between sites and dog types were not significant (likelihood ratio test: $P = 0.48$) (Figure 4).

150 **3.3. Risk of dog infections**

151 Modeling the monthly incidence of infection in owned and stray dogs revealed that stray dogs had a significantly
152 higher risk of infection than owned dogs (OR = 14; 95% CI: 6 - 30; $P < 0.001$). The site effect (including effects caused
153 by different locations and exposure times) was also significant. In addition, in the multivariable model, calendar time also
154 had a significant effect ($P = 0.001$) indicating that the risk of infection was significantly higher in winter than in spring
155 and summer (Figure 5). The interaction between site and dog type could not be evaluated since no owned dog was found
156 positive in site A at T1 and T2. Interactions between time and dog type and between time and site were not significant (P
157 = 0.9 in likelihood ratio test between models with and without first level interactions), indicating that the effect of time
158 was the same in all sites and categories.

159 To allow the comparison of incidence between the sites, the model predictions and confidence intervals were
160 transformed in monthly risks. Assuming a constant risk during each exposure period and a prepatent period of 30 days,
161 the monthly risk was lower in site A compared to B and C. The monthly incidence in site C appeared much higher than
162 in sites A and B (Figure 5).

163 **4. Discussion**

164 This is the first report on *E.granulosus* in dogs in Had Oued Ifrane region of the Middle Atlas, Morocco. This
165 Our study revealed the presence of the G1 strain in both, owned and stray dogs, which is in line with. This is in accordance
166 with several previous studies (Azlaf, Dakkak, Chentoufi, & El Berrahmani, 2007; El Berbri et al., 2015a) and which
167 confirms, suggesting the major involvement of dogs in this strain's transmission by infecting animals and humans.
168 Moreover, this study is to our knowledge the first to our knowledge to determine the infection prevalence and the
169 incidence of infection with *E. granulosus* in function of the dog type (stray and versus owned dogs) and in function of
170 exposure time. Thus, this has been conducted in the region of Had Oued Ifrane for the main reason that few studies have
171 attempted to observe such indicators of infestation rate. The focus was also given to the category of dogs (stray and
172 owned) and the frequency by which they have been observed in the region. As reported by Dakkak et al. (2016),
173 determining such indices is thought to be the best indicator regarding the transmission risk of *E.granulosus*
174 transmission in a region (Dakkak et al., 2016).

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175 Our three work sites were carefully selected and were characterized with similarities similar concerning
176 regarding climatic and socio-environmental conditions. The prevalence of dog infestation by *E. granulosus* was expected
177 to be similar between site A, B and C. As a first drawback of this field study, a significantly lower prevalence in dogs of
178 site A (Figure 4) has to be mentioned. Secondly, the different anthelmintic treatment intervals of 2, 3 and 4 months leading
179 to different exposure times should ideally have been applied in all sites. However, given that treatment of stray dogs was
180 complex, the investigators preferred a setting where all dogs of a same site underwent a same treatment schedule.
181 Moreover, treatment of owned dogs strongly depended on owner compliance, which could have been reduced if treatment
182 schedules differed between dogs of the same owner or of a neighbor. Consequently, differences of the baseline (T0) values
183 somewhat weaken the comparisons of absolute prevalence and incidence values in owned and stray dogs.

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184 A last weakness of the design of this field study is the variable length of investigation: indeed, the number of
185 investigations per site was identical, but the total time span varied from 4 to 8 months, thereby introducing a
186 supplementary variable, ie climatic conditions that might influence infectious pressure by increasing or decreasing hydatid
187 cyst survival in the environment. Ideally, the number of investigations should have been adapted in order to achieve an
188 identical length of observation in all sites. On the other hand, contrarily to studies where the effect of long term
189 anthelmintic treatment at different intervals aimed at reducing the development of hydatid cysts in the intermediate host
190 (Cabrera et al, 2002; Zhang et al, 2009), our study assessed the impact of the time of exposure to infective material of the
191 definite host in order to estimate the risk of re-infection after dogs' treatment. Considering that egg excretion by dogs is
192 the source of infection of human, especially in the case of owned dogs living nearby to their owners, valuable information
193 regarding anthelmintic treatment intervals is crucial.

194 Regarding the sample analyses, an underestimation of infestation by *E. granulosus* can not be excluded because
195 only coproscopically positive samples underwent PCR analysis. Indeed, Lahmar and al (2007), who have compared the
196 efficacy of the two tests with arecoline purgation, revealed that microscopic coproscopic control was highly specific but
197 with a less sensitivity of only (~64%) than while the sensitivity of the PCR (almost 100%). It might nevertheless be
198 mentioned that all coproscopically positive sampled were confirmed by PCR reached the 100%. Therefore, even though
199 sampling took place in three different sites to avoid any mixture between groups, the prevalence of infestation has been
200 determined in similar environmental conditions.

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202 Samples were carefully controlled during microscopic analyses. Our experimental design might be defective in the case
203 where eggs or worms escape microscopic control so that the prevalence will be underestimated. Nevertheless, this was
204 not the case in our study because all samples were positive in microscopic test and were confirmed by PCR, which may
205 reflect the endemicity state in this region. In fact, according to Lahmar and al (2007), who have compared the efficacy
206 of the two tests with arecoline purgation, revealed that microscopic control was highly specific but with a sensitivity of
207 only 64% while the sensitivity of the PCR reached the 100%. The fact which may confirms the possibility of getting a
208 high much between the microscopic and PCR results.

209 Before any treatment, the prevalence was high in the both stray and owned dogs (successively surpassing ranging
210 from 23.5% and to 51.3%; Figure 4), and the this difference between the two categories was highly significant present in
211 each of the three groups all sites, revealing matches between them though they all have been shown to be different. I
212 Previously, in a study carried out in Lybia, Buishi et al. (2005) reported slightly lower prevalences, of 21.6% and 25.8%
213 in stray and owned dogs, respectively. Moreover in Tunis, Lahmar et al. (2001) reported a prevalence of found it to be
214 21.0% in stray dogs. The common point between these previous studies and ours is that they all show similarities
215 concerning the prevalence, but not identical since our results revealed it to be higher in stray dogs. The high prevalence
216 in In fact, such result is expected in our case because, in the rural regions of Morocco, stray dogs in our study can be
217 explained by an increased infectious pressure due to free access to harvested and infested organs around slaughterhouses
218 and weekly souks.

219 As shown in Figure 5, the calculated incidence of re-infection varied in function of dog type and site.
220 Independently of study site, risk of e-infection was significantly higher in stray dogs than in owned dogs, which can be
221 related to an increased access to infected organs by stray dogs. Interestingly, the time span of 2 or 3 months between two
222 anthelmintic treatments in owned dogs poorly changed the risk of re-infection of owned dogs and a decrease of re-
223 infection was observed after the 2nd treatment (also see Table 2). These results indicate that a treatment interval of 2 or 3
224 months efficiently controls *E. granulosus* egg shedding in owned dogs. Provided the investigation of owned dogs was
225 strongly dependent on owner compliance, it might be hypothesized that owner awareness for this zoonosis increased over
226 time and thereby changed the feeding strategy of their dogs. Indeed, Marcotty et al (2012) have shown that the population's
227 perception and knowledge of the disease appears as determining factor for the success of control measures may constantly
228 be exposed to risk since they are always free to feed in infested offals, more than owned dogs.

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229 Regarding the risk of reinfection of stray dogs, even the short interval treatment did not completely prevent
230 reinfection, but considerably reduced its risk (Table 1, Figure 5). These results underline to which extent the exposure to

231 infected organs appears to be high in stray dogs. Strikingly, a reduction of reinfection risk was observed in stray dogs
232 between the 2nd and 3rd investigation, despite the absence of a changed feeding strategy as supposed for owned dogs. We
233 hypothesize that this significant effect of time (Table 2) is due to climatic conditions. Indeed, the four-months interval
234 investigation started in December and ended in August, which means that the 2nd exposure period took place under dry
235 and warm conditions. Accordingly, hydatid cysts survival in the environment might have been reduced during this period.
236 If the impact of temperature and humidity is well known on *E. granulosus* egg survival, knowledge is reduced regarding
237 cyst survival in the environment (Atkinson et al. 2012). It might furthermore be assumed that the environmental load is
238 highest in late autumn and during winter because the proportion of slaughtered and potentially infested animals increases
239 at this time point (Thevenet et al. 2003).

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240 After 2 to 4 months of exposure, at post treatment time, the calculated infestation prevalence revealed that in site
241 A, which underwent two consecutive treatments every two months, no owned dogs were infected with a modelled monthly
242 incidence ranging from 0.003 to 0.01 in owned dogs and 0.04 to 0.09 in stray dogs (95% CI in Figure 5). Also, monthly
243 calculated incidences were higher in site B and even more in site C. This is in the line with the study conducted by Cabrera
244 and al., 2002 on the slaughter of sheep for an investigation of the presence of hydatid cysts after a regular dog treatment
245 program. The result of this study, indicate that treatments every 2 months almost totally decrease the risk of infection in
246 owned dogs but probably not sufficiently in stray dogs, in which monthly treatments might be recommended. All this
247 could explain the differences in monthly risk estimates between the 3 study sites, and also this may mainly be related to
248 the large difference in initial prevalence recorded for each site before treatment.

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249 However, since the incidence was calculated at a given time of the year and this time differ from one group to
250 another depending on the period of exposure. The incidence calculated during this study was obtained at different seasons.
251 Indeed, the observed decrease of infestation risk with time indicates that both owned and stray dogs lessly become infected
252 in spring than in winter. In fact, weather changes may have an effect on the survival of the eggs in the environment.
253 Accordingly, Atkinson et al. (2012), examined *E.granulosus* eggs survival under both, field conditions and controlled
254 laboratory condition, which revealed poor eggs survival when exposed either to temperatures >25 ° or extreme cold
255 conditions (-83 °C or below). Nonetheless, temperatures of 18 to 4 °C were optimal with eggs survival for a time ranging
256 from 240 to 478 days. This is why, in our case, we may think that climate conditions in this area of the Middle Atlas may
257 be optimal to conserve ideally the parasite eggs in the environment, except in summer where the temperature exceeds 25
258 °. In this later case, we might say that the number of living eggs could be reduced, even though we could not prove this
259 since the duration of this study did not complete the year. However, in winter this problem does not arise because in

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260 winter there is no temperature below -10° maximum, which has no impact on the survival of the eggs. But seasonal climate
261 variations could have another effect on the quantity of offal infested by the larval stage of the parasite in the environment,
262 knowing that this quantity differs according to the dog status, but it even influences the infectious load in the environment
263 (Thevenet et al., 2003) and this could be attributed to the fact that in late summer and early winter sheep slaughter activity
264 is very high which could lead to an increased hydatid cyst availability. Also, the conservation of hydatid cysts after
265 slaughter might be improved by cold and humid conditions in winter. Then, it might be hypothesized that stray dogs are
266 increasingly roaming around slaughterhouses and weekly souks during winter because alternative food resources are
267 reduced.

268 Interestingly, our results may present an introduction for promoting a program to control echinococcosis despite
269 the short duration of the study. In fact, in a long-term study of 4 years has been conducted by Zhang et al. (2009) in China,
270 as a part of a control program focusing on monthly treatment of dogs with PZQ in two different highly endemic regions.
271 The prevalence of infestation after 4 years of treatment decreased to 0%, a fact that has been attributed to a significant
272 decrease in sheep infestation. Similar results were found in a study conducted by Cabrera et al., 2002 in Uruguay.
273 According to different dog treatment protocols, dogs have been treated, then the infestation rate in sheep has been analysed
274 after slaughter—at which time point after onset of dog treatment? The prevalence of infestation was inversely proportional to
275 treatments frequency: 18.6% for every 4 months, 4.3% for every 3 months and 0% for every 2 months. Considering the
276 short duration of our study, we unfortunately cannot find the same results. Nonetheless the 0% infestation could not be
277 reached in only few months of treatment while dogs were still exposed to infested sheep offal, the reason why the duration
278 of this study was not sufficient to reduce the infectious pressure. From a global point of view, there has been a significant
279 decrease in dog's infestation in our treated regions. This result may be related to the contribution of the Man (the dogs
280 owner) to protect his dogs, because during our deworming actions, the owners were very involved and were implicitly
281 made aware of the importance of the treatment. But, we cannot really be sure of this human effect on the decrease of the
282 infestation since the samples were taken at different times of the year. But according to a study by Marcotty et al., 2012,
283 it has been suggested that a better understanding of the population's perception and knowledge of the disease could
284 improve the situation.

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285 In conclusion, this study confirmed by molecular typing the presence of the G1 *E.granulosus* strain of
286 *E.granulosus* strain in owned and stray dogs in the Middle Atlas of Morocco—the presence of the G1 sheep strain.
287 *E.granulosus* prevalence and incidence of *E. granulosus* was significantly higher in stray dogs, and also
288 incidence was much higher in stray dogs than in owned dogs. As expected, dogs' re-infestation rate increased when
289 treatment intervals increased. Only interval treatments of two months appeared to efficiently control egg shedding in

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290 owned and stray dogs. However, a significantly higher calculated incidence of infestation was found in owned and stray
291 dogs during winter periods than during spring/summer periods, suggesting a seasonal change of infective pressure.
292 Therefore, accordingly, for an effective control strategy in this endemic region of Morocco, several factors must be
293 taken into account. Specifically, the treatment interval must be short and spread over at least 3 to 4 years in order to
294 determine this seasonal effect on the infectious pressure in the environment to be able to visualize this reduction in the
295 intermediate host because a decrease in infestation in sheep will implicitly reduce re-infestation in dogs; also, deworming
296 of dogs must be moderate depending on the status of the dog; stray dogs must be dewormed much more than owned dogs
297 because stray dogs have more chance of reinfection but not always in the same way during the year; and finally, it is
298 also necessary to take into account the importance of raising awareness among the population which will be able to
299 contribute mainly to the control of this zoonosis.

300 **Notes**

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307 Veterinary Institute Hassan II, Rabat, Morocco, for his precious assistance in the field and in the laboratory.

308 **Conflict of Interest Statement**

309 The authors declare that they have no conflict of interest.

310 **Statement of animal rights**

311 This work has been authorized by the animal welfare and ethics committee in Rabat, Morocco, in 2015. The
312 protocol was applied according to the international standards cited in many scientific references and in the 2012 OIE
313 Manual entitled "Manual of diagnostic tests and vaccines for terrestrial animals".

314

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411 **Tables**

412 **Table 1: Proportion of infected owned and stray dogs at different sampling times in the 3 groups of study**

		December	February	March	April	June	August
Group A	Owned dogs	0.24 (15/62)	0 (0/57)	-	0 (0/45)	-	-
	Stray dogs	0.5 (16/32)	0.11 (3/28)	-	0.05 (1/22)	-	-
Group B	Owned dogs	0.40 (30/76)	-	0.06 (4/69)	-	0 (0/65)	-
	Stray dogs	0.63 (15/24)	-	0.30 (6/20)	-	0.18 (3/17)	-
Group C	Owned dogs	0.35 (15/43)	-	-	0.23 (9/39)	-	0.05 (2/39)
	Stray dogs	0.76 (13/17)	-	-	0.76 (13/17)	-	0.50 (7/14)

413 Missing dogs were excluded for the rest of the study.

414

415 **Table 2: Multivariable logistic regression of infection risk; group A and owned dogs are the bases of the site and**
416 **dog type discrete explanatory variables, respectively; time is expressed in months from January (1) to June (6)**

Variables	Odds Ratio	OR [95 % CI]	P-value
Group B / A	6.79	2.01	22.94
Group C / A	61.07	16.82	< 0.001
Stray / owned	13.94	6.27	30.96
dogs			< 0.001
Time	0.68	0.54	0.85

417 OR, Odds Ratio; CI, Confidence Interval

418 **Figure captions**

419 **Fig. 1:** Geographical localization of study site Had Oued Ifrane. Ref:

420 <https://www.google.com/maps/place/Ouad+Ifrane>

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422 **Fig. 2:** Study design

423

424 **Fig. 3:** PCR electrophoresis (pair of NadI primer in Fig 3a and COI primer in Fig 3b) on 1% agarose gel for 9

425 feces samples positive for *E.granulosus* G1 strain represented by band 1 to band 9 with band 10 represents the

426 positive control of G1.

427

428 **Fig. 4:** Initial prevalence of *E.granulosus* infection in owned and stray dogs in 3 study sites (A, B, C) and

429 confidence intervals are shown. Prevalence in stray dogs is significantly higher than in owned dog, P<0.01.

430

431 **Fig. 5:** Modeling of the monthly incidence of infestation with confidence interval (95%)

432 Monthly incidence and confidence interval (95%) within each group and each exposure period are shown.

433 Incidence is significantly higher in stray dogs than in owned dogs, P<0.05.

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438 Figures

439 Fig. 1:



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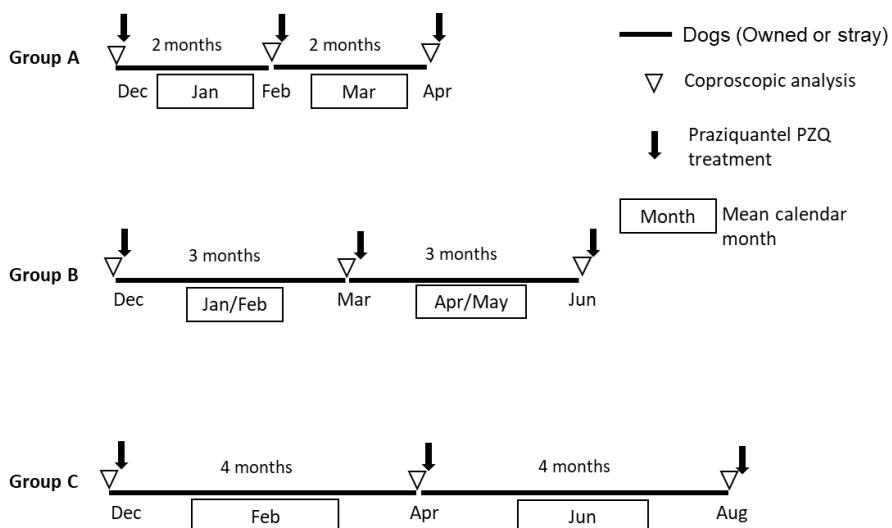
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454 **Fig. 2**



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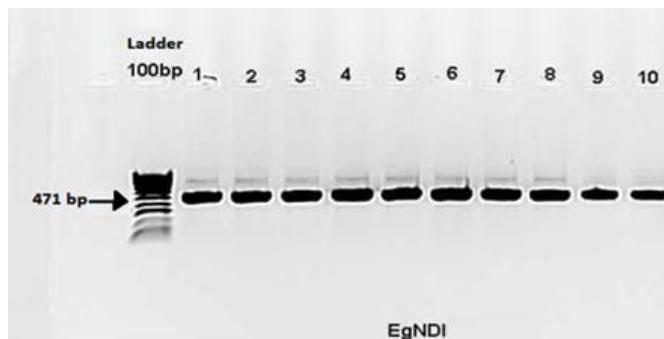
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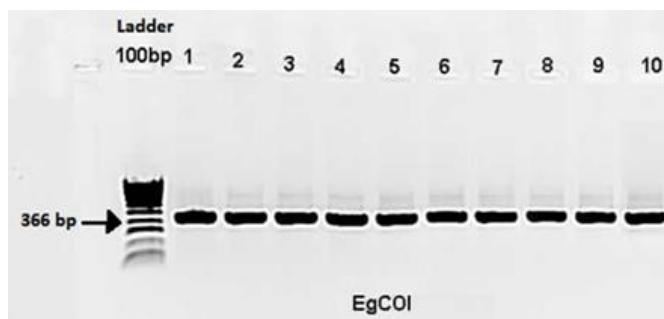
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462 Fig. 3a



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464 Fig. 3b



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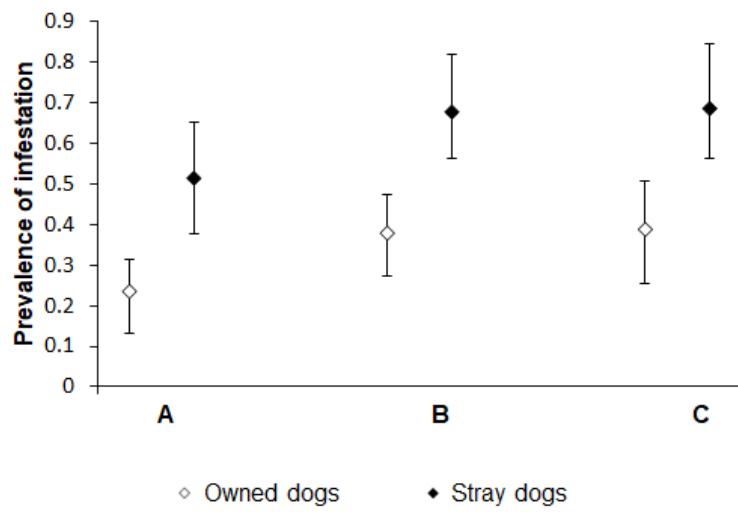
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476 **Fig. 4**



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491 **Fig. 5**

