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### Cystic echinococcosis in three locations in the Middle Atlas, Morocco

Amarir, Fatima Ezzahra; Saadi, Aouatif; Marcotty, Tanguy; Rhalem, Abdelkbir; Oukessou, Mohamed; Sahibi, Hamid; Obtel, Majdouline; Bouslikhane, Mohammed; Sadak, Abderrahim; Kirschvink, Nathalie

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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  
4 Amarir Fatima Ezzahra<sup>a, b, 1</sup>, Saadi Aouatif<sup>a, d</sup>, Marcotty Tanguy<sup>b</sup>, Rhalem Abdelkbir<sup>a</sup>, Oukessou Mohamed<sup>f</sup>, Sahibi  
5 Hamid<sup>a</sup>, Obtel Majdouline<sup>e</sup>, Sadak Abderrahim<sup>e</sup>, and Kirschvink Nathalie<sup>b</sup>.

6 *<sup>a</sup>Department of Pathology and Veterinary Public Health, Laboratory of Parasitology, Hassan II Agronomy and*  
7 *Veterinary Institute (IAV), Rabat, Morocco.*

8 *<sup>b</sup>Integrated Veterinary Research Unit, Department of Veterinary Medicine, Namur Research Institute for Life*  
9 *Sciences (NARILIS), University of Namur, Belgium.*

10 *<sup>c</sup>Laboratory of Zoology and General Biology, Parasitological and Ecological Unit, Faculty of Sciences, Mohammed*  
11 *V University, Rabat, Morocco.*

12 *<sup>d</sup>Fundamental and Applied Research for Animals and Health (FARAH), University of Liège, Belgium.*

13 *<sup>e</sup>Laboratory of Community Health, Preventive Medicine and Hygiene; Laboratory of Epidemiology and Clinical*  
14 *Research, Department of Public Health, Faculty of Medicine and Pharmacy, University Mohammed V, Rabat,*  
15 *Morocco.*

16 *<sup>f</sup>Department of Veterinary Biological and Pharmaceutical Sciences, Unit of Physiology and Therapeutics, Hassan II*  
17 *Agronomic and Veterinary Institute, Rabat, Morocco.*

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 healthHealth, 2012).** The  
47 **treatment costs of treatment waswere** 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 de 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 of 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 km<sup>2</sup>, corresponding to 15% of Morocco's mountain  
86 area (Figure1). 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<sub>s</sub>, 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 EgCOI1/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.

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

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

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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 degree 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 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 1) 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 microscopicoproscopic 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 arceoline 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 (suecessively surpassingranging  
210 from 23.5% and to 51.3%; Figure 4), and the this difference between the two categories was highly signifieantpresent in  
211 each of the three groupsall 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 inIn 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 2<sup>nd</sup> 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.

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

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231 infected organs appears to be high in stray dogs. Strikingly, a reduction of reinfection risk was observed in stray dogs  
232 between the 2<sup>nd</sup> and 3<sup>rd</sup> 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 2<sup>nd</sup> 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).

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.

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 ( < 3 °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 (Thevenot 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 unit ? 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.

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 Prevalence and incidence of *E. granulosus* e was significantly higher in stray dogs, and also  
288 incidence was much higher in stray dogs than in owned dogs. As expected D, dogs' re-infestation rate increased when  
289 treatment intervals increased. Only interval treatments of two months appeared to efficiently control egg shedding in

Mis en forme : Barré

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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 reinfestation 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.

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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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407 <sup>1</sup>Correspondence address: [amarir.f@gmail.com](mailto:amarir.f@gmail.com), phone: + 212 (0) 6 45353149

408 Full postal address: *Hassan II Agronomy and Veterinary Institute (IAV), Department of Pathology and Veterinary*  
409 *Public Health, Laboratory of Parasitology , BP 6202 Madinat Al Irfane – Rabat 1010, Morocco.*

410 ORCID iD: 0000-0003-3367-8435

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)**

<b>Variables</b>	<b>Odds Ratio</b>	<b>OR [95 % CI ]</b>		<b>P-value</b>
<b>Group B / A</b>	6.79	2.01	22.94	0.002
<b>Group C / A</b>	61.07	16.82	221.66	< 0.001
<b>Stray / owned dogs</b>	13.94	6.27	30.96	< 0.001
<b>Time</b>	0.68	0.54	0.85	0.001

417 OR, Odds Ratio; CI, Confidence Interval

418 **Figure captions**

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

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

421

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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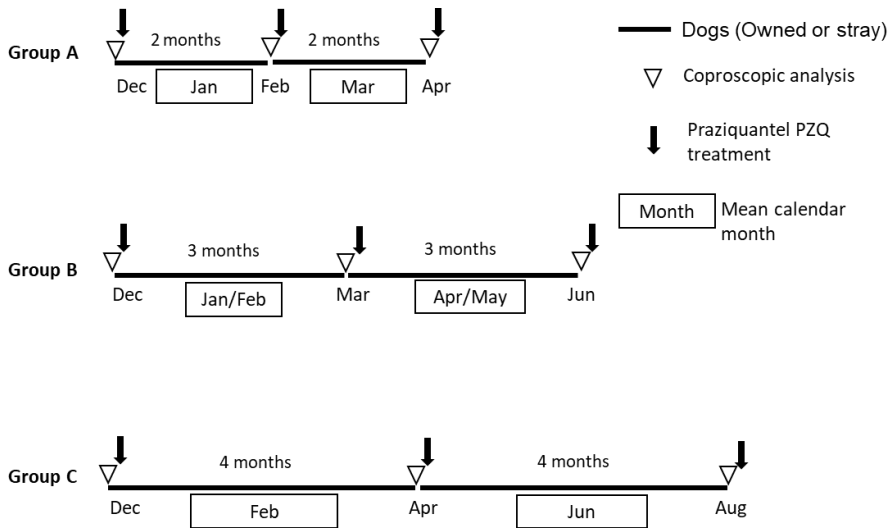
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454 **Fig. 2**



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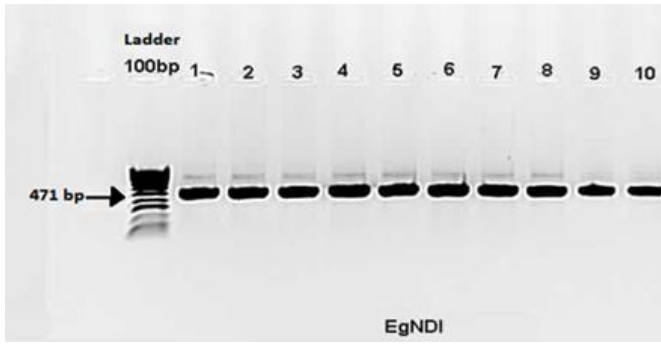
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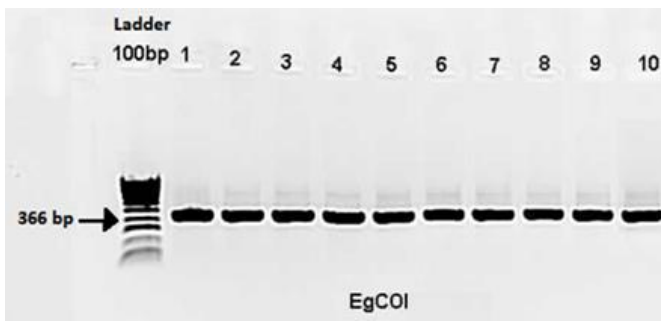
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462 Fig. 3a



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464 Fig. 3b



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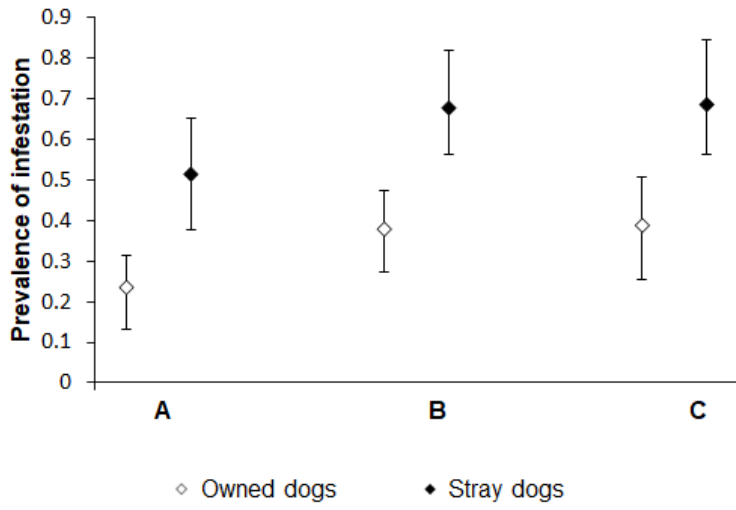
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476 Fig. 4



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491 Fig. 5

