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

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Abstract

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

Key words: *Echinococcosus granulosus*, dog, incidence, Morocco.

1. Introduction

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

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

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

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

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

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2. Material and methods

2.1. Description of the study area

The study was implemented in Had Oued Ifrane, located in the Middle Atlas which extends from the southwest to the north-east for about 450 km and covers a total area of 27,550 km^2, corresponding to 15% of Morocco's mountain area (Figure1). It is an agro-pastoral zone where agriculture and livestock are the main sources of income for the entire rural population. It is a mountainous area where altitude ranges from 800 to 3500 meters. The climate of the region is the mountainous continental Mediterranean type of mountain: cold, rainy and snowy in winter, hot and dry in summer. Had Oued Ifrane was chosen as a case study site, due to the presence of a large canine population, many rural slaughterhouses and a weekly ephemeral fairground market (souk). It is a region known for breeding, particularly sheep farming with a predominance of the Timahdit breed. Livestock production is the main activity for farmers in this region. A study on the prevalence of hydatid cyst in abattoirs in the same region, showed that 30% of cattle, 13% of sheep and 2% of goats carry one or more hydatid cysts in the liver and lungs suggesting a strong *E. granulosus* infestation in dogs (Fez.Amarir, personal communication 2017).

2.2. Study design

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

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

2.3. Fecal sample collection and analysis

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

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

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

EgCOI1/EgCOI2 and EgNDI1/EgNDI2 according to the protocol described by Bart and al. (2006). Copro-PCR was reported to be highly sensitive to detect eggs and worms per animal and to identify species of the family Taeniidae (Mathis et al. 2006). The PCR program was made of 35 cycles with, for each cycle, a denaturation step (15 s at 95°C), a 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).

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

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2.4. Statistical analysis

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

– $r = 1-(1-i)^{1/(e-p)}$

3. Results

3.1. Strain identification by coproPCR

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

3.2. Prevalence of dog infections

Positives fecal samples at coproscopic analysis were tested with coproPCR technique (Table 1). Numbers Results regarding of *E. granulosus* infestation of dogs are shown in function of site (A, B, C), dog type (ed dogs at different sampling times in each site (A, B, C) and for each dog types (owned *versus* stray dogs) and time (are shown in Table 1). 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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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). Interactions between sites and dog types were not significant (likelihood ratio test: P = 0.48) (Figure 4).

3.3. Risk of dog infections

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

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

4. Discussion

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

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

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

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

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

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

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

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

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

~~After 2 to 4 months of exposure, at post-treatment time, the calculated infestation prevalence revealed that in site A, which underwent two consecutive treatments every two months, no owned dogs were infected with a modelled monthly 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 calculated incidences were higher in site B and even more in site C. This is in the line with the study conducted by Cabrera and al., 2002 on the slaughter of sheep for an investigation of the presence of hydatid cysts after a regular dog treatment program. The result of this study, indicate that treatments every 2 months almost totally decrease the risk of infection in owned dogs but probably not sufficiently in stray dogs, in which monthly treatments might be recommended. All this could explain the differences in monthly risk estimates between the 3 study sites, and also this may mainly be related to the large difference in initial prevalence recorded for each site before treatment.~~

~~However, since the incidence was calculated at a given time of the year and this time differ from one group to another depending on the period of exposure. The incidence calculated during this study was obtained at different seasons. Indeed, the observed decrease of infestation risk with time indicates that both owned and stray dogs lessly become infected in spring than in winter. In fact, weather changes may have an effect on the survival of the eggs in the environment. Accordingly, Atkinson et al. (2012), examined *E.granulosus* eggs survival under both, field conditions and controlled laboratory condition, which revealed poor eggs survival when exposed either to temperatures >25 °or extreme cold conditions (-83 °C or below). Nonetheless, temperatures of 18 to 4 °C were optimal with eggs survival for a time ranging 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 be optimal to conserve ideally the parasite eggs in the environment, except in summer where the temperature exceeds 25 °. In this later case, we might say that the number of living eggs could be reduced, even though we could not prove this 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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Mis en forme : Couleur de police : Rouge winter there is no temperature below -10 maximum, which has no impact on the survival of the eggs. But, seasonal climate variations could have another effect on the quantity of offal infested by the larval stage of the parasite in the environment, knowing that this quantity differs according to the dog status, but it even influences the infectious load in the environment (Thevenet et al. 2003) and this could be attributed to the fact that in late summer and early winter sheep slaughter activity is very high which could lead to an increased hydatid cyst availability. Also, the conservation of hydatid cysts after slaughter might be improved by cold and humid conditions in winter. Then, it might be hypothesized that stray dogs are increasingly roaming around slaughterhouses and weekly souks during winter because alternative food resources are reduced.

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

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

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

Notes

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

Statement of animal rights

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

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Tables

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)

Missing dogs were excluded for the rest of the study.

Table 2: Multivariable logistic regression of infection risk; group A and owned dogs are the bases of the site and 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	0.002
Group C / A	61.07	16.82	221.66	< 0.001
Stray / owned dogs	13.94	6.27	30.96	< 0.001
Time	0.68	0.54	0.85	0.001

OR, Odds Ratio; CI, Confidence Interval

Figure captions

Fig. 1: Geographical localization of study site Had Ouad Ifrane. Ref: https://www.google.com/maps/place/Ouad+Ifrane

Fig. 2: Study design

Fig. 3: PCR electrophoresis (pair of NadI primer in Fig 3a and COI primer in Fig 3b) on 1% agarose gel for 9 feces samples positive for *E.granulosus* G1 strain represented by band 1 to band 9 with band 10 represents the positive control of G1.

Fig. 4: Initial prevalence of *E.granulosus* infection in owned and stray dogs in 3 study sites (A, B, C) and confidence intervals are shown. Prevalence in stray dogs is significantly higher than in owned dog, P<0.01.

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

Monthly incidence and confidence interval (95%) within each group and each exposure period are shown. Incidence is significantly higher in stray dogs than in owned dogs, P<0.05.

Figures

Fig. 1:

Fig. 2

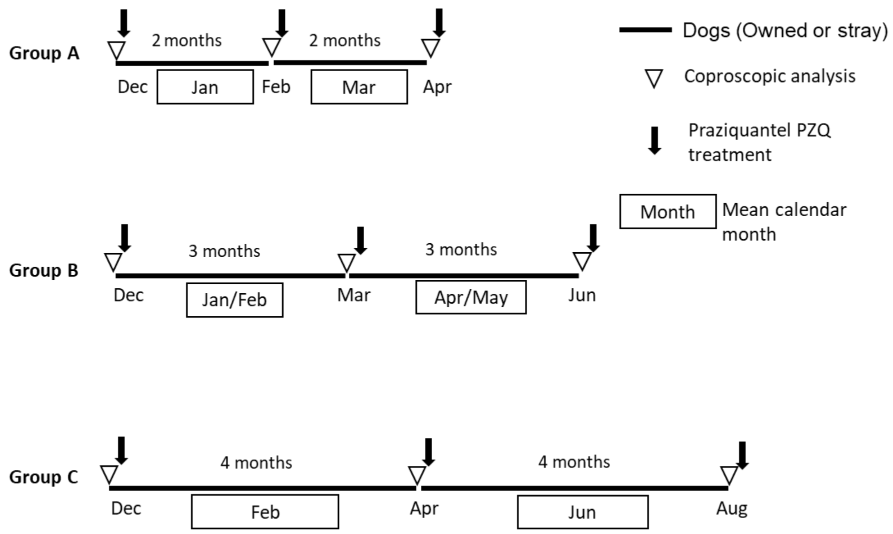

Group A
2 months
2 months
Dec
Jan
Feb
Mar
Apr
Dogs (Owned or stray)
Coproscopic analysis
Praziquantel PZQ treatment
Month
Mean calendar month
Group B
3 months
3 months
Dec
Jan/Feb
Mar
Apr/May
Jun
Group C
4 months
4 months
Dec
Feb
Apr
Jun
Aug

Fig. 3a

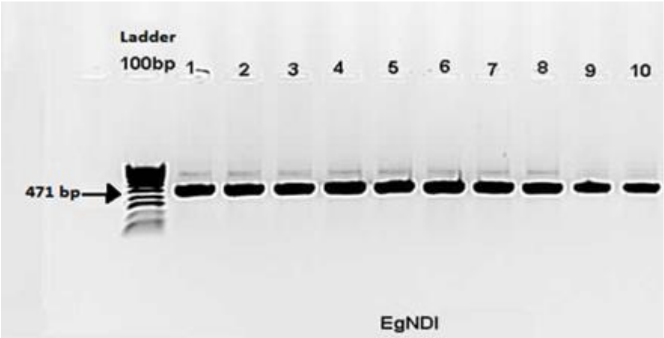

Ladder
100bp 1 2 3 4 5 6 7 8 9 10
471 bp
EgNDI

Fig. 3b

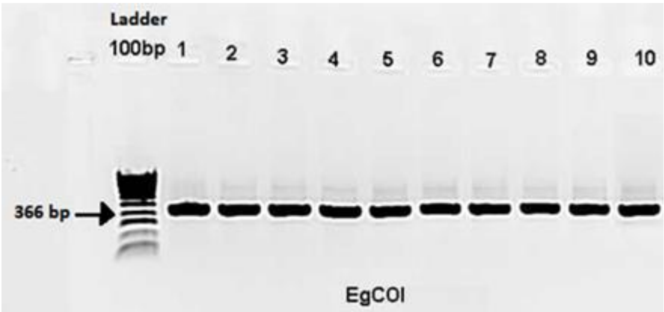

Ladder
100bp 1 2 3 4 5 6 7 8 9 10
366 bp
EgCOI

Fig. 4

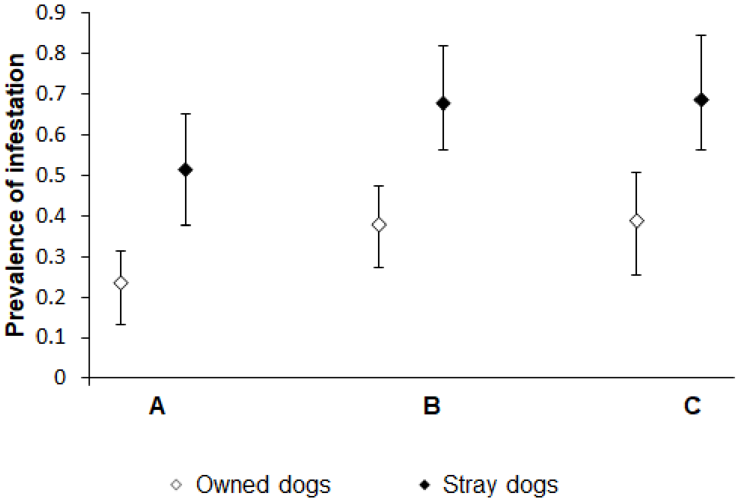
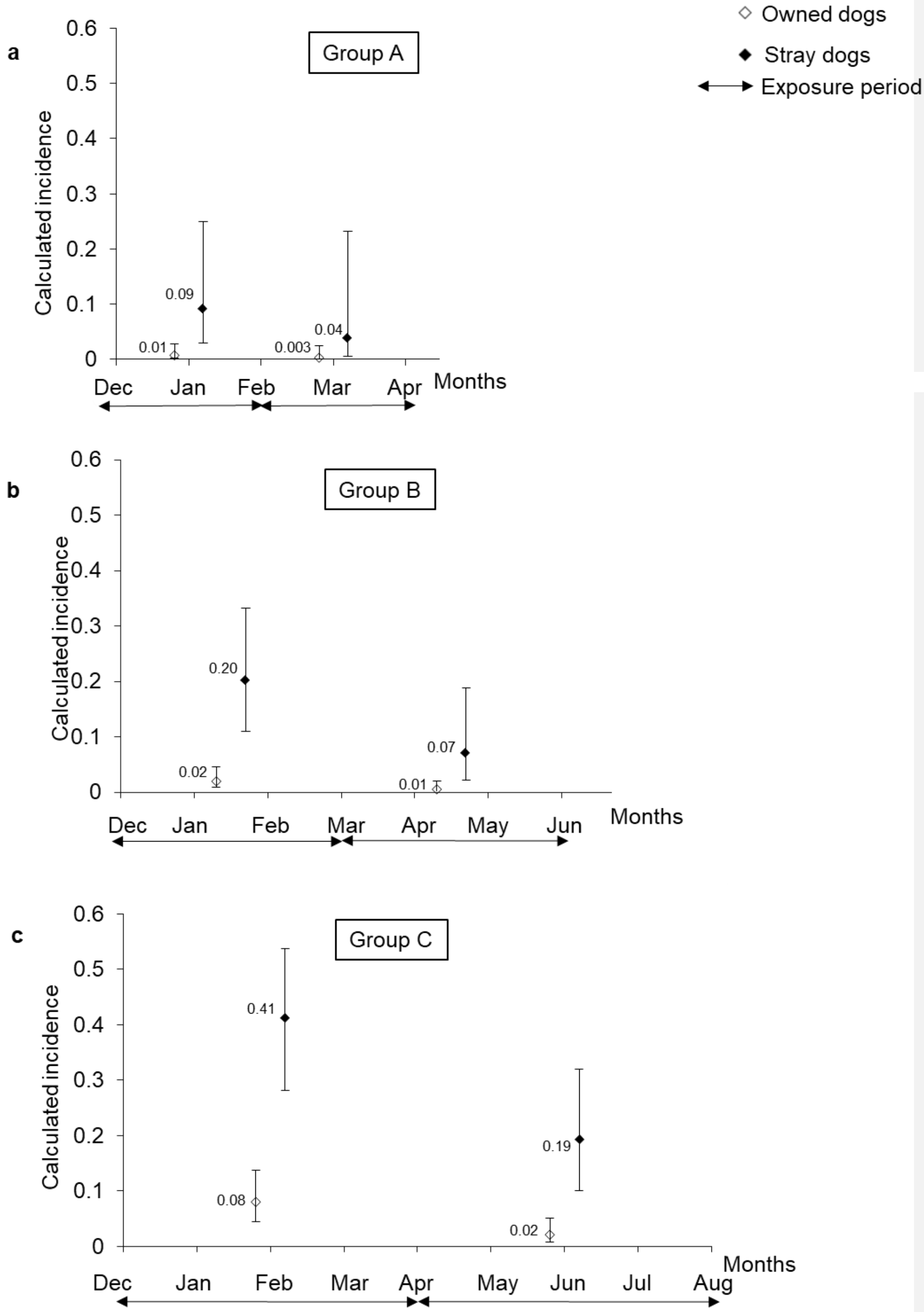

Prevalence of infestation
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
A
B
C
Owned dogs
Stray dogs

Fig. 5

492



Owned dogs
Stray dogs
Exposure period
a
Group A
0.6
0.5
0.4
0.3
0.2
0.1
0
Calculated incidence
0.09
0.01
0.04
0.003
Dec
Jan
Feb
Mar
Apr
Months
b
Group B
0.20
0.02
0.07
0.01
Dec
Jan
Feb
Mar
Apr
May
Jun
Months
c
Group C
0.41
0.08
0.19
0.02
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Months