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Echinococcus multilocularis management by fox culling: An inappropriate paradigm

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A R T I C L E   I N F O

A B S T R A C T

With the ongoing spread of *Echinococcus multilocularis* in Europe, sanitary authorities are looking for the most efficient ways of reducing the risk for human populations. Fox culling is one particular tool that has recently shifted from predation control to population health management. Our study aims to assess the effectiveness of this tool in limiting *E. multilocularis* prevalence in fox populations in France.

During four years, a culling protocol by night shooting from cars was implemented around the city of Nancy (eastern France) representing ~1700 h of night work and ~15,000 km driven. The 776 foxes killed represented an overall increase of 35% of the pressure on the fox population over 693 km^2.

Despite this consequent effort of culling, not only did night shooting of foxes fail to decrease the fox population, but it resulted in an increase in *E. multilocularis* prevalence from 40% to 55% while remaining stable in an adjacent control area (585 km^2). Though no significant change in age structure could be described, an increase in immigration and local recruitment is the best hypothesis for population resilience. The increase in prevalence is therefore considered to be linked to a higher rate of juvenile movement within the culled area shedding highly contaminated faeces. We therefore advocate managers to consider alternative methods such as anthelmintic baiting, which has been proven to be efficient elsewhere, to fight against alveolar echinococcosis.

1. Introduction

The spread of alveolar echinococcosis in Europe and the threat it represents to the human population is no longer in question, it's a fact (Romig et al., 2006; Knapp et al., 2009; Osterman Lind et al., 2011; Combes et al., 2012). Also beyond doubt is the obvious link between fox population densities, i.e. *Echinococcus multilocularis'* main definitive host in Europe, and the environmental contamination and direct or indirect human exposure to the parasite (Deplazes et al., 2004; Schweiger et al., 2007; Liccioli et al., 2015). What is at stake today is what should (or can) be done to better prevent further human infections.

The first step to protect human populations has been to develop and optimize medical tools for the diagnosis and the treatment of the disease. Today, the presence of the parasite (asymptomatic for up to 15 years) is, in most cases, discovered soon enough for the patient to receive appropriate medication (Brunetti et al., 2010; Piarroux et al., 2011). Such treatment has reduced the loss of life expectancy from 20 years (1970) to 3 years (2005), but remains toilsome for the patients and onerous for the society (Torgerson et al., 2008).

The eggs being the infective stage for humans and the only free phase of the parasite's life cycle, they may be assumed to be priority targets for the control of *E. multilocularis*. Unfortunately, their microscopic size and their extremely high resistance to humid and cold conditions such as those met in its distribution range prevent any targeted action (Veit et al., 1995). Specific public information campaigns could reduce the contact rate with the eggs by teaching safer behaviours. The real impact of such campaigns is yet very difficult to assess. As for humans, no treatment is currently available to hinder the larval stage within the intermediate hosts, most often small mammals. Moreover, considering the relatively low *E. multilocularis* prevalence in rodent populations and the overdispersion of parasite hotspots (Giraudoux et al., 2002), control options are virtually impossible and predictably ineffective in this compartment. The more promising

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strategies are then focusing on the adult stage of the parasite in red foxes.

Initially developed for the treatment of *E. granulosus* in dogs, praziquantel-based anthelmintic compounds showed very high efficiency in killing the adult worms of *E. multilocularis* in fox intestines. Relying on the successful management of vaccine distribution against rabies in the late 20th century, anthelmintic bait distribution was tested in different countries (as reviewed in Hegglin and Deplazes (2013)). In most cases, a frequent treatment (monthly, at least for an initial period) over one year or more, strongly decreased the parasite prevalence within fox populations (Tackmann et al., 2001; Hegglin and Deplazes, 2008). Yet no effective eradication of *E. multilocularis* has been described, and infection of the fox populations often recovered to pre-treatment levels within months after the end of bait distributions (Romig et al., 2007).

Raoul et al. (2003) showed that a sudden and strong fox population decrease due to indirect poisoning (as a side effect of small mammal control by anticoagulant rodenticide) led to a drastic decrease of *E. multilocularis* contamination in fox faeces. However, fox culling (gas, poison, trapping and shooting) had adverse effects on rabies epidemics in the 1990s, with culls either ineffective and unsustainable on a large scale (Morters et al., 2013). Virus transmission, believed to be directly density dependent, is apparently less complex than two host parasite transmission. Evidence of the feasibility and the effects of such protocol should thus be provided before proposing large scale fox depopulation to control and prevent alveolar echinococcosis.

In 2006, the presence of infected foxes within the city of Nancy was detected by Robardet et al. (2008), triggering concern amongst local authorities about possible human exposure. Fox culling having been suggested as a control tool, we implemented the culling program alongside a monitoring protocol to provide evidence-based information on the effectiveness of this method. We firstly tested the hypothesis that a large scale community based fox culling protocol is effective at significantly reducing fox abundance around a medium-size city. The second hypothesis tested was that the fox culling protocol would in turn induce a decrease in the presence of *Echinococcus multilocularis* within the targeted fox population.

2. Material and methods

2.1. Study area

The city of Nancy is the centre of a large conurbation of 430,000 inhabitants located in north-eastern France (48° 41′ 37″ North; 6° 11′ 05″ East). This region is a long known foci of alveolar echinococcosis (Aubert et al., 1987) with current *E. multilocularis* prevalence in fox population reaching 51.4% (Combes et al., 2012). Ranging from 188 m to 353 m of altitude, the conurbation ($\sim 15\ km^2$) is surrounded by a large forested area (to the west) and an agricultural mosaic of meadows/pastures and crop fields (mainly wheat, corn and colza).

The study area was a circle of 20 km radius centred on the city of Nancy (Fig. 1). It was longitudinally divided by two landscape structures, the highway A31 to the west and the river channel Marne-Rhine to the east. The northern half was dedicated to the fox culling whereas the second half (South) was kept as a control area with no change in hunting and trapping activities. A sampling grid of 3 × 3 km was set on the whole area with 77 grid cells in the North (693 km²) and 65 grid cells in the South (585 km²).

2.2. Fox culling

In France, fox hunting is regionally administered. In our study area, hunters were allowed to shoot foxes from June to February with no quotas. The main practices were stand hunting at dusk, scouting with or without dogs and driving/flushing the animals. In addition, foxes were classified as a "pest" allowing trapping all year round without quotas (restricted to certified trappers). Therefore, the first step of the culling

protocol consisted in contacting the hunters and trappers of the culling area, asking for an increase in fox harvest. Concomitantly, as a sanitary management tool, an administrative authorization was delivered to certified persons to shoot foxes at night by driving with side spotlights. A similar authorisation was given over the control area in order to monitor this population, restricting the sampling effort to one fox per grid cell per year between October and April.

All foxes killed (hunting, trapping and night shooting) were to be brought back to the National Reference Laboratory for *Echinococcus* spp. (located within the study area), along with mention of the date and the grid cell of collection. There, each animal was weighed and sexed. As described by Ruette and Albaret (2010), we considered two age classes: juvenile (prior to first mating period) and adult as per their status on the 1st of February. The reproductive dynamics of the fox population was evaluated by two complementary variables. We firstly considered mating success as the proportion of adult females that did reproduce (presence/absence of placental scars). We also considered the number of placental scars for each active female as an indication of the annual reproductive fitness.

No restriction nor recommendation on the sex or the age were given for the culling operations and the sampling of the control area. Therefore, we consider the foxes killed each year as an opportunistic sample with a similar representativeness of each population. We can thus assume that any observed change in demographics would reflect a change of similar direction in the population.

2.3. Fox relative abundance

The classical method of monitoring fox abundance involves using spotlights from a car driving along a predefined circuit. According to Ruette et al. (2003), simple encounter rates are as effective as distance sampling protocols to monitor the relative abundance of foxes in Europe. Therefore, we measured the fox relative abundance as the total number of foxes seen along two continuous transects of 95 km in the culled area and 80 km in the control area (Fig. 1). During the four winters from 2008 to 2009 to 2011–2012, both transects were simultaneously surveyed in October, November, December and February during two consecutive nights. We kept the highest number of foxes seen between the two repetitions as the closest estimation of the number of foxes actually present along the circuit for a given survey.

Following Frey and Conover (2007), we used the killing success (number of animals killed per hour) during the night shooting operations in the culled area as an alternative/complementary way to assess the impact of the culling on the fox population. If the culling effort to remove one individual increases, this would reflect a decrease in the population (Harding et al., 2001). The night shooting operations in the control area could not be included in this dataset because they were restricted to one fox per grid cell per year.

2.4. E. multilocularis monitoring

As the prevalence of *E. multilocularis* in fox populations may vary during the year, we focused the monitoring during the winter months. Each year, from October to April, the first fox killed on each grid cell from the culled area was screened for *E. multilocularis* adult worms. This sample is then comparable with the sampling of the control area described before. The presence of *E. multilocularis* was assessed using the Segmental Sedimentation and Counting Technique (SSCT) as described in Umhang et al. (2011) and already used in large epidemiological surveys in France and Sweden (Combes et al., 2012; Wahlstrom et al., 2012). *E. multilocularis* prevalence in foxes was calculated annually in both study areas. In addition, the SSCT allowed us to evaluate the worm burden for each animal.
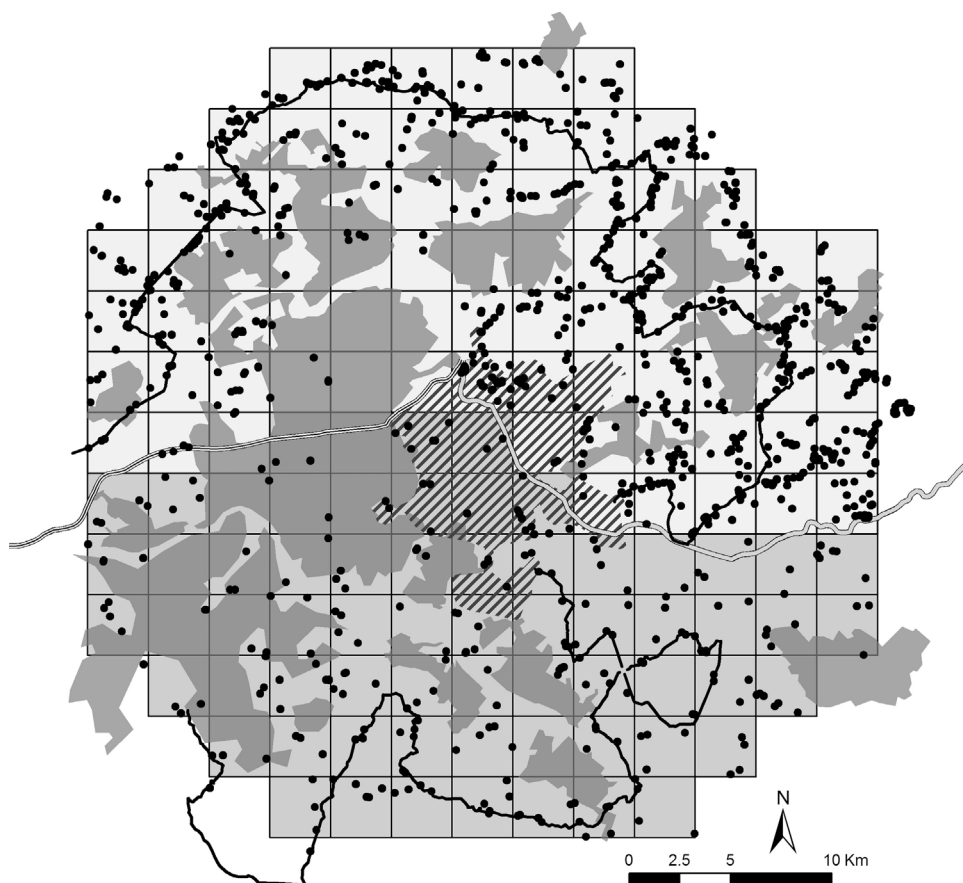
Fig. 1. Study area, spotlight survey circuits and fox culling.

Visual representation of the study area centred on the city of Nancy (dashed area). The area is longitudinally divided in its centre by the A31 highway (striped line to the west) and the river channel Marne-Rhine (grey line to the east). The light grey grid cells represent the culled area (693 km²) and the dark grey grid cells the control area (585 km²). Each black dot represents the location of one fox culled or sampled during the whole study. The two black lines show the spotlight surveys: 95 km in the culled area and 80 km in the control area. The dark grey shapes represent significant forests patches.

2.5. Data analysis

We firstly compared the evolution of demographics parameters in both populations (culled and control) by testing the effect of the year (2008–2012) on the sex ratio (male vs female) and the age (juvenile vs adult) using single predictor generalized linear models (GLM) based on binomial distribution. Similarly, changes in reproductive fitness were evaluated by testing the effect of the year (2008–2012) and the epidemiological status (presence/absence of *E. multilocularis*) on the mating success of adult females (presence/absence of placental scars) and the number of placental scars (active females only). We used GLMs based on binomial and Poisson distributions respectively.

The evolution of the relative abundance of foxes in both areas was assessed by testing the effect of the year (2008–2012), the month (Oct, Nov, Dec, Feb) and the zone (culled vs control) on the number of foxes seen. Considering the structure of the data (counting over a distance), we used GLMs based on a Poisson distribution, with an offset accounting for the sampling effort (log(length of the spotlight transect [km])). In addition, changes in shooting success during the night shooting operations in the culled area were assessed by testing the effect of the year (2008–2012), the distance driven [km] and the number of foxes seen on the number of foxes killed, using a GLM based on a Poisson distribution with an offset accounting for the sampling effort (log(duration of the shooting operation [h])).

We compared the initial *E. multilocularis* prevalence (year one) in the two areas using a Fisher exact test with 2 × 2 contingency tables. The evolution of the prevalence in each area was then tested with a 4 × 2 contingency table using the annual prevalence. A post-hoc pairwise test was then run to refine the results. All tests were performed in R 3.3.3. Individual presence/absence [0,1] of parasites within intestines of foxes was then modelled using a GLM based on a binomial distribution with the following explanatory variables: zone (culled vs

control), year (2008–2012), sex (male vs female) and age (juvenile vs adult).

Finally, the distribution of the worm burden being usually highly over-dispersed, it was modelled using a GLM based on a negative binomial distribution with zone (culled vs control), year (2008–2012), sex (male vs female) and age (juvenile vs adult) as explanatory variables.

In absence of biological precepts for all of the above GLM analyses, all combinations of the variables were fitted and compared in R 3.3.3. The selection of the best model was based on the lowest Akaike index with correction for finite sample sizes, AICc (Burnham and Anderson, 2002). All models with a difference in AICc lower than 2 were considered equal. The principle of parsimony was then applied to select the simpler model including all significant effects. GLM analyses in this study being used to describe the effect of an a priori set of available variables on the observed responses, only the final models are presented following Anderson et al. (2001). The full model selection tables are available as Supplementary data.

3. Results

3.1. Fox culling

From November 2008 to April 2012, 872 foxes were collected from the culled area, mainly killed by night shooting (89.3%). The low proportion of foxes hunted and trapped (8.7%) in the sample reflects a lack of interest in participation to the program rather than a low hunting activity within the area. In fact, the annual records (hunting + trapping) of the hunting association over the administrative area (5246 km² of which 25% consist of the two study areas 1278 km²) were stable throughout the study (Table 1). Consequently, in the following analyses, only the 776 foxes collected during night shooting operations

Table 1
Culling effort and official hunting and trapping records [foxes/km^2].

	2008–2009	2009–2010	2010–2011	2011–2012
Culling (North)[#]	0.15 (103)	0.3 (206)	0.35 (241)	0.33 (226)
Sampling (South)[^]	0.11 (65)	0.11 (62)	0.10 (61)	0.10 (57)
Hunting[^]	0.71 (3736)	0.47 (2459)	0.43 (2258)	0.48 (2517)
Trapping[^]	0.21 (1103)	0.36 (1908)	0.41 (2171)	0.32 (1672)
Hunting + trapping[^]	0.92 (4839)	0.83 (4367)	0.84 (4429)	0.80 (4189)

[#] Foxes killed by spotlight shooting during the culling operations (area = 693 km^2).

[*] Foxes killed by spotlight shooting in the control area (area = 585 km^2).

[^] Hunting and trapping statistics (annual number of foxes killed) over the whole administrative unit (area = 5246 km^2) during the same years.

are considered as the additional culling effort. A total of 194 night shooting operations were performed with two persons present in the car resulting in ~1700 h of night work and ~15,000 km driven, or, in other words, an average of 50 min and 15 km per fox killed. As opposed as for the control site, the culling operations were not restricted in time. Yet, night shooting being easier in winter when the vegetation is low, 75% of the culling operations took place at the same time as the sampling in the control area (October to April).

As presented in Table 1, the annual culling effort increased from the first year to the second year and then stabilized just over 0.3 foxes killed per square kilometre. Considering the average number of foxes hunted and trapped over the whole administrative unit (0.85 foxes/km^2, range: $0.80 < > 0.92$), this represents an increase of nearly 35% of the annual pressure on the fox population over 693 km^2. The sampling in the control area represents 0.1 foxes/km^2 (12%), a third of the actual removal effort in the culled area.

As shown on Table 2a, in the culled area, the odds of killing a male fox increased from year one to year three (1.64) but return to the initial level in the last year. The odds of killing juvenile foxes did not change significantly during our study. Both sex ratio and age structure remained unchanged in the control area. Similarly, we did not detect any effect of the year on the reproductive fitness of the control population (Table 2b). In the culled area, the mating success during the second year was significantly higher than any other year, while active females showed less placental scars after the first year of the culling.

Interestingly, in both areas, the presence of the parasite was linked with a lower mating success and fewer placental scars, the latter being not significant in the culled area.

3.2. Fox relative abundance

The best model explaining the fox relative abundance in our study (Table 3) showed that foxes were globally more abundant in the control area with more foxes seen during the months of October and November independently of the year. Looking at the raw data, these two spotlight counts in year 3 were much higher than any other survey. Each time, the same team of observers was involved with a known tendency to adapt the protocol. A slower driving speed and more time spent trying to get closer to unidentified sightings would certainly increase the population abundance and induce a bias in the results. In fact, when removing these outliers from the data, only the effect of the month remains with October and November being higher than December and February. It may then be safer to consider that fox relative abundance was similar in both areas and that the culling operations had no significant impact on the fox abundance after four years.

In parallel, the best model explaining the killing success during the night shooting operations in the culled area included the number of foxes seen and the distance driven (Table 3) independently of the year. This stability during the whole study support the absence of significant change in fox abundance in the culled area.

3.3. E. multilocularis monitoring

Each year, an average of 84% of the grid cells could be sampled (58–69 in the North and 53–57 in the South) with a total of 478 foxes killed. The first year, considered as the initial level before the culling operations, necropsy analyses showed similar levels of fox contamination (p = 0.705) between the culled area (40%, CI: 27%–53%) and the control area (45%, CI: 31%–59%). During the four years of the study, *E. multilocularis* prevalence in foxes remained stable in the latter (p = 0.931) with an average of 42% (CI: 36%–49%). Table 4 shows that the presence of the parasite in the culled area decreased in year two and then significantly increased between years two and four (p = 0.017,

Table 2a
Reproductive fitness of the fox populations in the culled and control areas.

Model	estimate	std. error	t value	Pr(> |t|)	odd ratio	lower limit CI95%	upper limit CI95%
Culled area							
1. sex ~ year							
(Intercept)	−0.25	0.20	−1.28				
year2	0.28	0.25	1.11	0.27	1.32	0.81	2.15
year3	0.49	0.24	2.07	0.04*	1.64	1.03	2.61
year4	0.35	0.24	1.47	0.14	1.42	0.89	2.27
2. age ~ year							
(Intercept)	−0.81	0.22	−3.78				
year2	0.04	0.27	0.16	0.87	1.05	0.62	1.77
year3	0.29	0.25	1.14	0.25	1.34	0.81	2.20
year4	0.33	0.26	1.29	0.20	1.39	0.84	2.30
Control area							
3. sex ~ year							
(Intercept)	0.16	0.25	0.63				
year2	0.01	0.36	0.02	0.99	1.01	0.50	2.04
year3	0.11	0.36	0.30	0.76	1.12	0.55	2.27
year4	−0.20	0.37	−0.53	0.60	0.82	0.40	1.70
4. age ~ year							
(Intercept)	−0.97	0.28	−3.42				
year2	0.16	0.40	0.40	0.69	1.17	0.54	2.54
year3	−0.02	0.41	−0.04	0.97	0.98	0.44	2.19
year4	0.54	0.39	1.36	0.17	1.71	0.79	3.71

Models 1–4 are based on the foxes killed by spotlight shooting (776 in the culled area and 245 in the control area). We tested the variables sex (male = 1) and age (juvenile = 1) with the explanatory variable year (2008–2012).
We considered a significant effect for p < 0.05* and highly significant for p < 0.01**.

Table 2b
Best models for the reproductive fitness of the fox populations in the culled and control areas.

Model	estimate	std. error	t value	Pr(> |t|)	odd ratio	lower limit CI95%	upper limit CI95%
Culled area							
5. reproduction ~ year + Em analyse							
(Intercept)	1.50	0.77	1.96				
year2	2.66	1.28	2.08	0.04*	14.29	1.17	175.08
year3	0.41	0.87	0.47	0.64	1.51	0.28	8.22
year4	1.72	1.06	1.62	0.10	5.60	0.70	44.84
analysepos	−1.55	0.69	−2.23	0.03*	0.21	0.05	0.83
6. placental scars ~ year + Em analyse							
(Intercept)	2.08	0.25	8.32				
year2	−0.63	0.28	−2.29	0.02*	0.53	0.31	0.91
year3	−0.51	0.27	−1.86	0.06	0.60	0.35	1.03
year4	−0.62	0.29	−2.14	0.03*	0.54	0.30	0.95
analysepos	−0.20	0.15	−1.28	0.20	0.82	0.61	1.11
Control area							
7. reproduction ~ Em analyse							
(Intercept)	1.05	0.30	3.51				
analysepos	−0.60	0.45	−1.32	0.19	0.55	0.22	1.34
8. placental scars ~ Em analyse							
(Intercept)	1.18	0.07	16.24				
analysepos	−0.42	0.14	−3.11	< 0.01**	0.66	0.50	0.86

Presentation of the best models selected on the AICc. All models with a difference in AICc lower than 2 were considered equal. The principle of parsimony was then applied to select the simpler model including all significant effects. The full model selection are available as supplementary data.

Models 5 and 7 are based on the adult females killed by spotlight shooting (culled area: 239; control area: 75). We tested the variable reproduction (presence of placental scar = 1) with the explanatory variables year (2008–2012) and Em analyse (presence of the parasite = 1).

Models 6 and 8 are based on the active adult females (presence of placental scars) killed by spotlight shooting (culled area: 164; control area: 58). We tested the variable placental scars (nb of scars) with the explanatory variables year (2008–2012) and Em analyse (presence of the parasite = 1).

We considered a significant effect for p < 0.05* and highly significant for p < 0.01**.

Table 3
Best models explaining the fox relative abundance.

Model	estimate	std. error	t value	Pr(> |t|)	odd ratio	lower limit CI95%	upper limit CI95%
9. foxes seen ~ offset(log(km)) + month + zone							
(Intercept)	−0.83	0.06	−13.57				
zoneControl	0.11	0.05	2.07	0.04*	1.11	1.01	1.23
month(feb)	−0.11	0.08	−1.34	0.18	0.90	0.77	1.05
month(nov)	0.32	0.07	4.30	< 0.01**	1.37	1.19	1.58
month(oct)	0.35	0.07	4.82	< 0.01**	1.42	1.23	1.64
10. foxes seen ~ offset(log(km)) + month							
(Intercept)	−0.78	0.06	−13.97				
month(feb)	−0.11	0.08	−1.34	0.18	0.90	0.77	1.05
month(nov)	0.26	0.08	3.38	< 0.01**	1.29	1.11	1.50
month(oct)	0.28	0.08	3.64	< 0.01**	1.32	1.14	1.53
11. foxes shot ~ offset(log(duration)) + distance + foxes seen							
(Intercept)	−0.69	0.11	−6.40				
km	0.00	0.00	3.34	< 0.01**	1.00	1.00	1.01
seen	0.02	0.00	7.77	< 0.01**	1.02	1.02	1.03

Presentation of the best models selected on the AICc. All models with a difference in AICc lower than 2 were considered equal. The principle of parsimony was then applied to select the simpler model including all significant effects. The full model selection are available as supplementary data.

Model 9 is based on the 32 spotlight counting operations in both study areas. We tested the variable foxes seen (total number of foxes seen) with an offset to account for the specific length of each circuit (95 km in the culled area and 80 km in the control area) and the explanatory variables zone (culled = 1), year (2008–2012) and month (October, November, December, February).

Model 10 is model 9 run without the two outlier spotlight counting operations of October and November during year 3 in the control area (N = 30).

Model 11 is based on the 194 night shooting operations in the culled area. We tested the variable foxes shot (total number of foxes shot) with an offset to account for the variability of the duration [h] and the explanatory variables distance (total distance driven [km]) and foxes seen (total number of foxes seen).

We considered a significant effect for p < 0.05* and highly significant for p < 0.01**.

post-hoc pairwise Fisher's exact test) reaching 55% (CI: 43%–68%) at the end of the study. The comparison of the models explaining the individual fox contamination show eight models with AICc differences lower than 2. Applying the principle of parsimony, we kept the model with the variable age only (juvenile vs adult) as the best model, showing that juvenile prevalence was 2.34 time higher than in adults (Table 5).

As expected, the number of worms present in the contaminated foxes was highly heterogeneous (1–123,300 worms) with three foxes accounting for nearly half of the total worm burden (47.6%). The best three models of our analysis showed a difference in AICc lower than 2. The best model included the variables year + sex + age to explain the worm burden in our study (Table 5). As a result, worm burdens were much higher during the second year of the study but with similar levels in the three other years. Male foxes had a lower worm burden than females (odds ratio = 0.56) while juvenile foxes harboured on average 2.59 time more worms than adult foxes.

Table 4
Annual number of foxes analysed with subsequent *E. multilocularis* prevalence.

	Culled Area				Control Area			
	pos	neg	prev	IC95%	pos	neg	prev	IC95%
2008–2009	23	35	40%	27–53	25	31	45%	31–59
2009–2010	20	49	29%[*]	19–41	25	32	44%	31–58
2010–2011	22	43	35%	23–47	22	33	40%	27–54
2011–2012	3[*]	29	55%[*]	43–68	21	32	40%	27–54

[*] Significant increase in prevalence between year two and year four of the study (p = 0.017, post-hoc pairwise Fisher's exact test).

4. Discussion

Despite the involvement of the hunting and trapping communities and all the legal tools available to reduce fox populations in France, no significant decrease of relative abundance of foxes could be detected in the study area after four years. Yet, strong variations in *E. multilocularis* prevalence were detected in the culled population resulting in a higher prevalence in the last year, an epidemiological response absent from the adjacent control area. This raises concern for public health considering that foxes are the main spreaders of infectious parasite eggs in the environment (Deplazes et al., 2004; Hegglin and Deplazes, 2013; Liccioli et al., 2015).

Predator control in general and red fox management in particular are strongly debated topics (see for example the discussion between Baker et al. (2002), Leader-Williams et al. (2002) and Aebischer et al. (2003) concerning a hunting ban on foxes in the United Kingdom). Yet, it seems generally accepted that the efficiency of any control management depends on the method used and the spatio-temporal design of the program. Heydon and Reynolds (2000) showed that a given hunting practice had contrasting results in three regions of United Kingdom. In addition, concentrating the shooting effort on juvenile foxes (McLeod and Saunders, 2001) during the winter months (Rushton et al., 2006; Lieury et al., 2015) increases the efficiency of culling operations. In any scenario, the reduction of a population would only be achieved when a large proportion of the population is removed each year. In our study, the human pressure on the fox population (0.3 foxes/km^2, and 1.2 foxes/km^2 when considering the hunting and trapping) was opportunistic and non-selective. Even if most of the night shooting operations were concentrated between October and April, this protocol was likely too weak to significantly reduce the fox abundance in the study area. Recently in France, Lieury et al. (2015) showed in fact that an annual removal pressure of 1.95 foxes/km^2 over ~250 km^2 during 5 years did not induce any significant change in fox abundance.

The design of our study with one long transect in each area (similar to Heydon et al. (2000) comparing fox abundance between different regions of the United Kingdom) may have reduced the sensitivity of the measure. A distance sampling protocol based on multiple shorter transects could have detected smaller local variations (Ruette et al., 2003). The public roads used for the spotlight counts were also used for the culling, never during the same night though. This could still have induced a change in behaviour with foxes avoiding these areas. The apparent stability of the fox abundance would then be negatively biased. Here, the killing success during the night shooting provide a second measure supporting the stability of the fox populations.

This apparent stability of fox abundance is probably the result of a rapid compensatory response. Foxes in Europe have indeed a strong spatial structure with one active pair defending its territory (Macdonald and Bacon, 1982; Poulle et al., 1994), eventually supported by subordinate non breeding females (Baker et al., 1998). If an alpha female is killed, a resident subordinate non-breeding female would rapidly replace her or move from an adjacent social group (Iossa et al., 2009), sustaining the local recruitment. The increase of mating success described in the culled area supports this compensatory reproduction. This could in turn explain the decrease in the average of placental scars in the same population as female foxes produce smaller litters in their first mating season (Ruette and Albaret, 2010).

As for most mammal predators, fox dispersal mainly occurs at the juvenile stage with males moving further away from the parental territory (Allen and Sargeant, 1993). The drift of the sex ratio towards males observed in the culled area may be as a consequence of compensatory immigration. This demographic response has indeed been documented as reducing the success of fox control management (Rushton et al., 2006; Lieury et al., 2015) with mainly young males settling in the freed territories.

Although our data do not show a significant increase in juveniles killed, we brought new insight on their importance for the epidemiological cycle of *E. multilocularis*. Both compensatory mechanisms hypothesized imply that juveniles play an important role in population resilience. Considering their high worm burden, fox juveniles undoubtedly produce highly contaminated faeces. A higher prevalence on top of a roaming and dispersing behaviour will then assure that these contaminated faeces are spread over multiple territories. This certainly contributed to the overall increase in *E. multilocularis* prevalence within the culled population.

Our results suggest a surprising lower worm burden for male foxes. Only a few studies previously compared the parasite load between the sexes with a consistent absence of effect. Two of them compared the average worm burden ((Hofer et al., 2000; Yimam et al., 2002) with a Mann–Whitney *U* test that doesn't consider the highly aggregated distribution of the worms. Only one study from Guislain et al. (2008) tested the effect of sex and age with a GLM showing no significant

Table 5
Best models explaining the *E. multilocularis* contamination and the worm burden of the foxes sampled.

Model	estimate	std. error	t value	Pr(> |t|)	odd ratio	lower limit CI95%	upper limit CI95%
12. Em analyse ~ age							
(Intercept)	−0.67	0.12	−5.63				
age(juv)	0.85	0.20	4.24	< 0.01**	2.34	1.58	3.47
13. worm burden ~ year + sex + age							
(Intercept)	6.50	0.31	20.95				
year2	2.28	0.38	6.04	< 0.01**	9.81	4.68	20.60
year3	0.72	0.38	1.88	0.06	2.06	0.97	4.37
year4	0.67	0.38	1.75	0.08	1.94	0.92	4.10
sex(m)	−0.59	0.29	−2.02	0.04*	0.56	0.31	0.98
age(juv)	0.95	0.27	3.57	< 0.01**	2.59	1.53	4.36

Presentation of the best models selected on the AICc. All models with a difference in AICc lower than 2 were considered equal. The principle of parsimony was then applied to select the simpler model including all significant effects. The full model selection are available as Supplementary data.
Models 12 and 13 are based on the 478 foxes screened for *E. multilocularis*. We tested two variables Em analyse (presence of the parasite = 1) and worm burden (estimated number of worms). For each model, the explanatory variables were zone (culled = 1), age (juvenile = 1), sex (male = 1) and year (year 2008–2012).
We considered a significant effect for p < 0.05* and highly significant for p < 0.01**.

effect. Our study being not specifically designed to test this hypothesis and considering our *p*-value just below the significant threshold, we recommend further trials to better describe the effect of the sex on the worm burden.

This study should be considered as a first descriptive approach of the effects of fox culling on the presence of the parasite. Spatial replicates with varying culling efforts are now needed to test additional variables in our models. Only then a global predictive model could be proposed to better understand the relation between fox culling and *E. multilocularis* contamination. Similarly, our data does not provide information to predict the long term stability of this increase in prevalence nor on its potential effect on human contamination. Considering the very low incidence of alveolar echinococcosis in humans, and its long prepatent period, there is a huge statistical challenge involved in demonstrating a link between an increase in parasite prevalence within a fox population and an increase in human cases on a local scale (but see Schweiger et al., 2007 on a country scale). Here, recent improvements in *E. multilocularis* DNA detection within fox faeces (Knapp et al., 2014) presents a new opportunity to directly monitor the presence of the parasite in the environment, in order to better evaluate the risk of transmission to humans.

Having failed to significantly reduce fox abundance during the four years of our study, we are unable to assess the effect of a reduction of the fox population on the presence of the parasite *E. multilocularis*. Evidence of definitive host density related responses from the parasite are scarce. Complete elimination of the parasite was achieved on a small island in Japan by intensively culling foxes and stray dogs (Kamiya et al., 2007). The geographic context precludes any comparison with open fox populations in continental areas. (Raoul et al., 2003) demonstrate a direct link between a decrease in fox abundance and a decrease in *E. multilocularis* prevalence in France. The reduction of fox abundance in this study was the result of a secondary poisoning by bromadiolone, an anticoagulant used to control vole outbreaks. In Australia, where poison is a legal tool to control the introduced red fox, intense baiting programs show contrasting results (Greentree et al., 2000; Dexter and Murray, 2009) and raise concern for the mortality of non-target species (Dexter and Meek, 1998). Even if technically achievable, direct control of fox populations strong enough to actually reduce the presence of the parasite would necessitate a very large number of foxes killed. This should be put in perspective with the elevated costs, the ecological consequences and the ethical concern of such management (Littin and Mellor, 2005).

Alternative methods should, therefore, be considered to protect human populations. The use of praziquantel-based anthelmintic baits have shown to be an efficient way to control *E. multilocularis* transmission in Europe (Tackmann et al., 2001; Romig et al., 2007; Hegglin and Deplazes, 2008; Konig et al., 2008; Comte et al., 2013) and Japan (Takahashi et al., 2013) when applied on small target areas considered at higher risk for human infection and should be the preferred method for *E. multilocularis* control in wildlife canids.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.prevetmed.2017.09.010.

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