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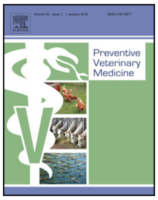

Fox baiting against *Echinococcus multilocularis*: Contrasted achievements among two medium size cities

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ABSTRACT

In Europe, most cities are currently colonized by red foxes (*Vulpes vulpes*), which are considered to be the main definitive host of the zoonotic cestode *Echinococcus multilocularis*. The risk of transmission to humans is of particular concern where high fox populations overlap with high human populations. The distribution of baits containing praziquantel has successfully reduced the infection pressure in rural areas and in small plots within large cities. The purpose of this study was to assess its efficiency in two medium size cities (less than 100,000 inhabitants) in areas of high human alveolar echinococcosis incidence. From August 2006 to March 2009, 14 baiting campaigns of praziquantel treatment were run in Annemasse and Pontarlier (Eastern France), each of which encompassed 33 km^2, with a density of 40 baits/km^2. The bait consumption appeared to be lower in strictly urban context compared to suburban areas (78.9% vs. 93.4%) and lower in Annemasse than in Pontarlier (82.2% vs. 89.5%).

During our study, the prevalence of *E. multilocularis*, as assessed by EM-ELISA on fox faeces collected in the field in Annemasse, was lower within the treated area than in the rural control area. A “before/during” treatment comparison revealed a significant decrease of spring prevalence from 13.3% to 2.2%. No significant change in prevalence was detected in Pontarlier (stable prevalence: 9.1%) where the contamination of the treated area followed the temporal trend observed in the control area. There, a greater resilience of the parasite’s life cycle, probably due to a strong pressure of recontamination from outside the treated area, may have counteracted the prophylaxis treatment.

These contrasted outcomes suggest that the frequency of fox anthelmintic treatment should be adapted to the local situation.

1. Introduction

Alveolar echinococcosis (AE) is a zoonosis caused by the small tapeworm *Echinococcus multilocularis*, whose life cycle depends on small mammals (mainly *Arvicola terrestris* and *Microtus arvalis* in Europe; Eckert and Deplazes, 2004) as intermediate hosts and primarily foxes as definitive

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Table 1
Multicriteria comparison of the two study areas.

	Annemasse	Pontarlier	Data source/comment
Location	6°14′01.9″E, 46°11′35.4″N	6° 21′ 14.6″ E 46° 54′ 14.7″ N	http://www.geoportail.gouv.fr
Population	65,656 inh	23,877 inh	National Institute of Statistics and Economic Studies (INSEE), 2009
Built-up areas	2769 ha (46%)	1053 ha (18%)	1 ha precision over 60 km^2 (Fig. 2)
Population density	23.7 inh/ha	22.7 inh/ha	Built-up areas only
	10.9 inh/ha	4.0 inh/ha	Over the 60 km^2 (Fig. 2)
Grasslands	2%	18%	CORINE Land Cover 2006, French ministry for sustainable development (Meeddat)
EM-prevalence in foxes[a]	49% (CI: 38–61%)	52% (CI: 42–61%)	Combes et al. (2012)
Annual human AE incidence[a]	2.329/10^6 inh	7.619/10^6 inh	Period: 1982–2009; mean national incidence: 0.256/10^6 inh (Grenouillet et al., 2010)

[a] Data given for the whole *département* (French administrative division).

hosts. Epidemiological and genetic studies indicate that the parasite has been spreading throughout Europe since the mid-1980s and is now present in areas where it was not previously known to exist, such as the Netherlands, northern Germany, Poland, the Czech Republic, Slovakia, northern Italy and Romania (Jenkins et al., 2005; Romig et al., 2006; Takumi et al., 2008; Sikó et al., 2011; Davidson et al., 2012).

Between the years 1984–1987 and 2006–2012, the parasite prevalence in fox populations dramatically increased in the historical endemic area of eastern France (Combes et al., 2012), a trend which was similarly observed in Germany and Austria (Berke et al., 2008). Studies in France and Switzerland reported a gradient from low *E. multilocularis* prevalence in foxes in urban areas to high prevalence in the surrounding rural areas (Fischer et al., 2005; Hegglin et al., 2007; Robardet et al., 2008).

Following the end of the rabies epidemic that swept across the continent in the 1970–1980s, fox populations have been steadily increasing in Europe, at least up until the end of the 1990s (Breitenmoser et al., 2000; Chautan et al., 2000). With more and more foxes found in urban areas (Gloor et al., 2001), the establishment of an *E. multilocularis* urban wildlife cycle has led to higher environmental contamination and, therefore, a greater risk of human exposure to the parasite (Deplazes et al., 2004). Such effects have already been highlighted in Switzerland where the overall growth of fox populations was followed by an increase in human cases of AE with a latency of 10–15 years; this delay was most likely due to the long asymptomatic period of incubation of the human disease (Schweiger et al., 2007). These findings advocate the development of prophylaxis methods against *E. multilocularis*, especially in urban environments, where the human population density is high, and contact between humans and the parasite is more likely (Deplazes et al., 2004; Reperant et al., 2007).

In the absence of strong supporting scientific evidence and considering the ethical and logistical issues, the direct control of *E. multilocularis* in any host population by culling seems unwise. For several years, research efforts have focused instead on fox treatment based on the distribution of anthelmintic baits containing praziquantel. Promising results have been obtained in Germany following large-scale baiting programmes in rural areas (Schelling et al., 1997; Tackmann et al., 2001). Hegglin et al. (2003) and Hegglin and Deplazes (2008) showed that monthly bait distribution was effective in reducing the prevalence in foxes in small urban plots (1 km^2 and 6 km^2) within the city of Zurich (Switzerland) while Inoue et al. (2007) achieved a decrease of *E. multilocularis* prevalence in foxes on the periphery of a metropolitan area of 140,000 inhabitants (Otaru City, in the western part of Hokkaido, Japan). In Germany, additional baiting programmes in urban areas (small villages and towns) as part of a large-scale anthelmintic treatment proved to be very effective (Konig et al., 2008). Nonetheless, to our knowledge, no study has been undertaken to date testing the effects of deworming campaigns on foxes within medium size cities (less than 100,000 inhabitants) without intervention in the surrounding rural area. At this scale, the interaction between rural and urban host populations is stronger than in larger cities and may increase the risk of recontamination, thus hampering the efficiency of the treatment. In addition, no studies have been carried out on the financial cost of such a control protocol, although a cost/benefit analysis would be a crucial element for decision-makers.

This study took place in an endemic area of eastern France in order to: (1) determine the efficiency of a hand distribution of anthelmintic baits (praziquantel) on *E. multilocularis* contamination in fox faeces over two medium size cities; and (2) evaluate the cost of such treatments.

2. Materials and methods

2.1. Study areas

The selection of the two medium size cities was based on three criteria, the quantitative limits of the first two being arbitrarily chosen in absence of any official definition of a medium size city: population (20,000–100,000 inhabitants), built-up area of the city (less than 60 km^2), and epidemiological background of the *département*, i.e. the French administrative division in which the cities are located (high human AE incidence: more than 2 cases per 1000,000 inhabitants per year; and high *E. multilocularis* prevalence in foxes). The first city matching our criteria (Table 1) was the city of Annemasse, situated in Haute-Savoie *département* (Fig. 1), which is home to the first described human case of AE in France (late nineteenth century). The second city, Pontarlier, lies in another historical

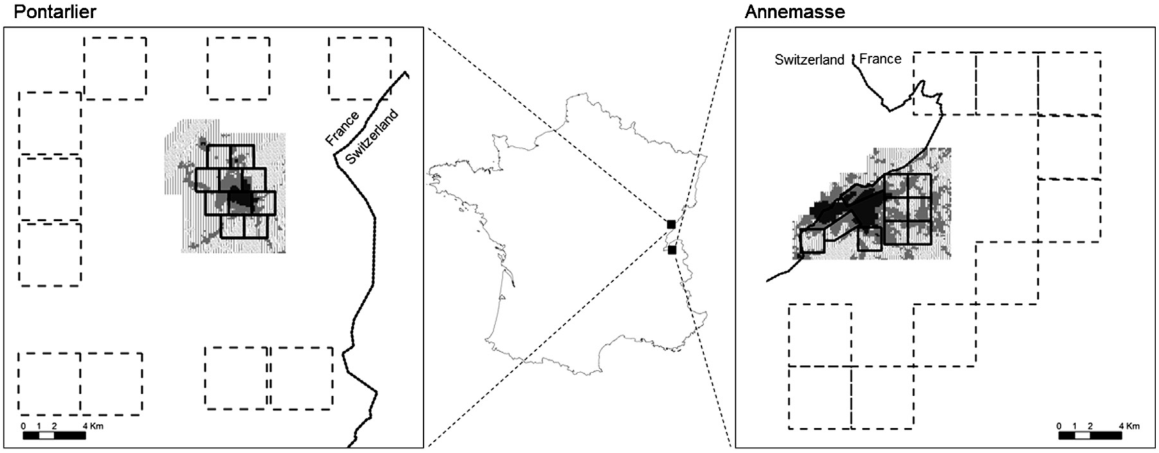

Fig. 1. Fox faeces sampling plots in Annemasse and Pontarlier. Solid lines: 10 plots of 1.5 km sides over the treated areas. Dashed lines: 10 control plots of 4 km sides dispatched around the treated areas. Twice a year (spring and autumn), five faecal samples were collected from each plot.

endemic area, the Doubs *département*, where human cases of AE reach their highest level (Grenouillet et al., 2010). One should consider that human AE incidences and *E. multilocularis* prevalence in foxes reported in Table 1 concern the whole administrative unit and are therefore not relevant to compare epidemiological figures at the city scale.

Though both study areas presented a core of high-density housing blocks surrounded by lower density residential and commercial neighbourhoods (Fig. 2), developed areas covered 46% of the area in Annemasse, compared to only 18% in Pontarlier. Grasslands, the optimal biotopes for the intermediate hosts, were poorly represented around Annemasse, consisting mainly of small patches scattered throughout the area. Per contra, the city of Pontarlier was surrounded by large and continuous meadows and pastures expanding far beyond the treated area.

2.2. Hosts populations

The only available data regarding the fox populations around both cities were from hunting bags recorded by the two local hunting associations: from two districts around Annemasse (total area of 443 km^2), and three districts around Pontarlier (covering 1375 km^2). Hunting activity was assessed as the total number of foxes shot annually divided by the areas considered.

Prior to the treatment campaigns, intermediate host populations were estimated by an index method (Giraudoux et al., 1995; Quere et al., 2000) commonly employed for large-range estimations of small mammal relative densities (Delattre et al., 1999). The method is based on line transect consisting of a succession of 10-pace intervals (~10 m) walked exclusively in grassland habitats. In 2004, 1514 intervals were surveyed in Annemasse

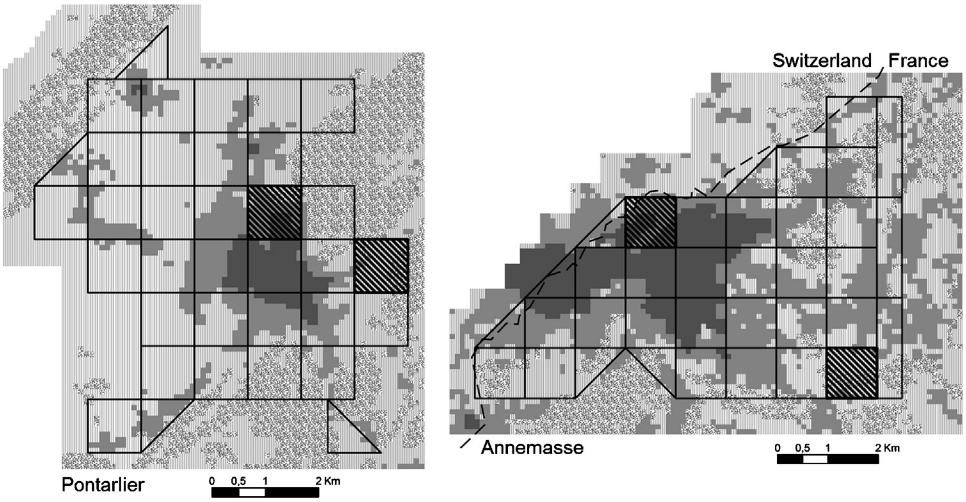

Fig. 2. Predominant land cover in Annemasse and Pontarlier: high-building density (dark grey), low-building density (light grey), open ground (vertical lines) and forest (spotted). The black grids represent the 33 km^2 on which baits were manually distributed with a density of 40 pcs/plot. The two test-plots where the bait consumption was monitored are diagonally striped.

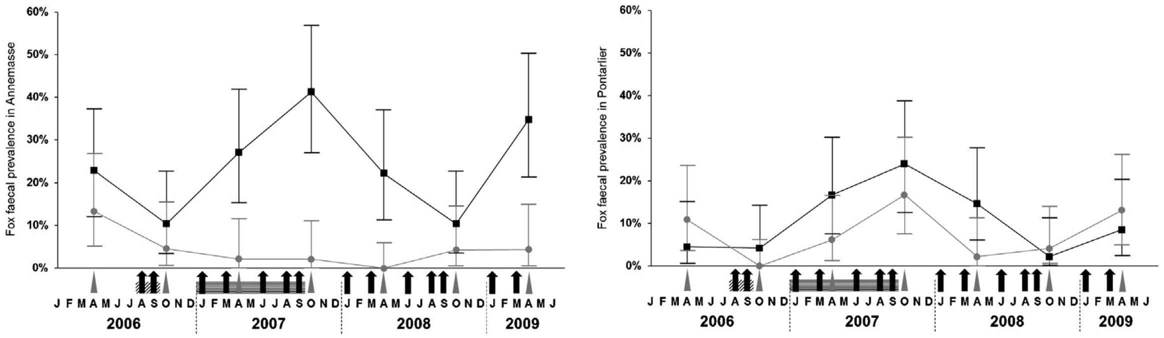

Fig. 3. *E. multilocularis* prevalence in fox faeces in Annemasse and Pontarlier. Chart: treated areas (grey circles) and control areas (black squares) with respective standard error bars. Table: bait distributions (black arrows), faeces sampling (grey triangles), camera trap survey (diagonally strip) and consumption monitoring (horizontally striped).

(76 transects; mean number of intervals per transect: 19.9) and 2324 in Pontarlier (50 transects; mean: 46.5). The survey was repeated in 2005 with 1599 intervals in Annemasse (66 transects; mean: 24.2) and 2473 in Pontarlier (53 transects; mean: 46.7). Each time, a 2-m band was screened on both sides of the transect in order to detect specific indices of presence of *Arvicola terrestris* (patchy distribution of fresh flat earth tumuli; Giraudoux et al., 1995) and of *Microtus* sp. (runways in vegetation and burrow entries, in association with fresh faeces and/or evidence of recent vegetation consumption; Quere et al., 2000). *Microtus arvalis* was sympatric with *M. agrestis* in our study area, the latter at a much lower density. Since both species can share the same habitat and were not distinguishable by their presence indices, they were referred to as *Microtus* sp. Results are expressed as the ratio of positive intervals (presence of intermediate host indices) to the total number of intervals walked.

2.3. Bait distribution and monitoring of bait consumption

From August 2006 to March 2009 (32 months), 14 campaigns of baiting treatment were conducted following a schedule based on hosts and parasite ecology. Each year, two campaigns were performed in August and September (Fig. 3) before foxes start to disperse (and, thus, before they begin dispersing the parasite as well); two campaigns were held in January and March to limit the infection pressure in the spring, when rodent populations start to grow, and a fifth campaign was conducted in June when fox cubs effectively prey on rodents by themselves.

Treatment areas in each city (Fig. 2) consisted of 33 continuous plots of 1 km^2 centred on the urban core, avoiding large forests areas as much as possible. The size of the treated area and the number of plots resulted from a compromise between the built-up areas and our logistical constraints. In Annemasse, the treatment area was limited on its north-western front by the Swiss border. For each campaign, 40 baits per plot were distributed (by foot or car) in the most homogeneous way possible from Monday to Thursday in the two cities successively (over a two-week span). Baits were prepared by IDT Biologik GmbH (Rosslau-Dessau, Germany) with a dose of 50 mg praziquantel (Droncit®, Bayer Vital GmbH, Leverkusen-Bayerwerk, Germany) incorporated in the protein matrix. They were stored at −20 °C until use.

The monitoring of bait consumption was carried out using two test-plots in each city (Fig. 2). To evaluate the impact of urbanization on bait' acceptance, one was located in an “urban” context (~25% high-density building and >50% low-density building) and the second in a “suburban” context (<10% built-up areas and >66% open ground). Two different protocols were implemented: (I) in order to identify the species consuming the baits, three camera traps (CAMTRAKKER, film camera) were installed for 4 consecutive nights on each test-plot near baiting locations during the treatment campaigns in August and September of 2006. When baits were absent in the morning, the likely consumer was defined as the animal photographed during the night; and (II) the consumption of the baits was recorded during the five campaigns of 2007 (Fig. 3) as the presence/absence of the 40 baits of each test-plot 14 days after distribution; this corresponds to the efficiency period of the praziquantel assessed by the supplier. Results are expressed as the ratio of baits consumed to the total number of baits distributed.

2.4. Monitoring of *E. multilocularis* in environmental fox faecal samples

Environmental contamination was monitored by collecting and analysing faecal samples of foxes in the field in spring and autumn from April 2006 (before the beginning of the treatment) until April 2009, for a total of 7 sampling campaigns (Fig. 3). Ten plots of 1.5 km by 1.5 km were defined in both treatment areas in which five faecal samples were collected for each campaign. A second batch of five faecal samples per plot was collected in ten control plots of 4 km by 4 km located around the treatment area, in a rural environment predominantly composed of open habitats and forests (Fig. 1). For each campaign, the 100 faecal samples per city were analysed at the Institute of Parasitology of the University of Zurich by EM-ELISA (Deplazes et al., 1999). Coproantigen detection in fox faeces collected in the field had been successfully used to reveal gradients of environmental contamination on regional (Raoul et al., 2001; Pleydell et al., 2004) and local scales (Stieger et al., 2002), and to assess the effectiveness of the praziquantel-based treatment on foxes in urban environments (Hegglin and Deplazes, 2008). Moreover, this detection method was preferable in that it did not alter the fox populations within the areas studied and estimated the actual contamination of the environment.

2.5. Cost evaluation

With the aim of evaluating bait distribution as a “routine process”, one that is available for sanitary authorities, only the costs of field work and laboratory analyses were considered. The expenses related to salaries were based upon the average wage index of the “Entente de Lutte Interdépartementale contre les Zoonoses (ELIZ)”, which was 16.72€/hour. Travel expenses for staff and vehicles were contractual, accounting for 47€ for one night, 15€ for a meal and 0.36€ per kilometre travelled. The individual cost per bait was 0.73€. For each treatment campaign or faecal sample harvest, four to six people with three vehicles were mobilized during 3–4 days per site.

2.6. Statistical analysis

Comparisons of the intermediate hosts’ transect data from the two study areas were made using the Chi-square tests, with *p*-value estimated by permutation tests (i.e. the distribution of the Chi-square statistic under the null hypothesis was obtained by calculating Chi-square values under 2000 random rearrangements of the observed data points).

The consumption of baits (response variable: bait consumed/not consumed) was modelled by linear logistic regression (GLM), with the following explanatory variables: site (Annemasse vs. Pontarlier), month of distribution (January, March, June, August and September) and urbanization (urban vs. suburban). In the absence of ecological-based criteria to select the order of variables, a series of models was constructed, representing all possible combinations of association and order of appearance of these three variables. Candidate models were compared with Burnham and Anderson (2002) approach to information theory. The model with the lowest AICc was chosen as the one that best represented the variability of bait consumption. When the difference in AICc between two candidate models was less than 2, the principle of parsimony was applied (i.e. the simplest model was selected).

The contamination of faeces (response variable: faeces positive/negative) was modelled at each site with a linear logistic regression (GLM) using the following explanatory variables: season (spring vs. autumn), treatment (treated vs. control) and sampling session (seven consecutive sessions). Model selection was performed as described above.

The pre-treatment faecal prevalence (spring 2006) in both cities was compared to the prevalence observed during the treatment campaigns by combining data from the entire period of treatment (springs 2007–2009). Only the spring data were considered for this final analysis to avoid bias caused by potential seasonal effects (faeces sampled both in spring and autumn during treatment *versus* only in spring before treatment). Comparisons were made using the Chi-square test, with *p*-value estimated by permutation test (2000 permutations). Computing was performed using R version 2.10.1 (R Development Core Team, 2009).

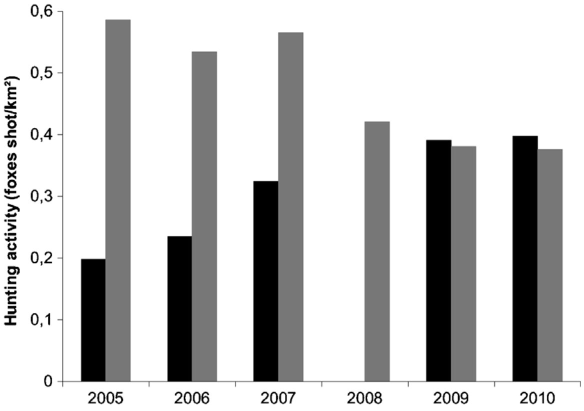

Fig. 4. Hunting activity (number of foxes shot annually/km^2) given by the local hunting associations around Annemasse (grey) and Pontarlier (black): two districts were surveyed around the city of Annemasse (with a total area of 443 km^2), and three districts in Pontarlier (covering 1375 km^2). No data were available in Pontarlier in 2008.

3. Results

3.1. Hosts populations

From 2005 to 2010 (Fig. 4), the hunting activity around Annemasse decreased from 0.59 foxes/km^2 to 0.38 foxes/km^2 while increasing around Pontarlier from 0.20 foxes/km^2 to 0.40 foxes/km^2. The absence of data in 2008 in Pontarlier is due to the test of a different protocol by the hunting association which was deceiving and, consequently, discarded.

Table 2 shows that the relative density of *A. terrestris* was significantly higher in Pontarlier in 2004 (Chi-square = 4.05, *p*-value = 0.044), this difference increased in 2005 (Chi-square = 110.11, *p*-value = 2.20×10^{-16}). Though the relative density of *Microtus* sp. was significantly higher in Annemasse in 2004 (Chi-square = 41.82, *p*-value = 1.00×10^{-10}), this difference was no longer significant in 2005 (Chi-square = 1.60, *p*-value = 0.206).

3.2. Bait distribution and monitoring of bait consumption

Out of the 114 pictures taken by the camera traps, 75 (65.8%) were triggered either by humans, vegetation (stirred by the wind) or malfunctions while 39 (34.2%) showed the presence of animals near the baits. Seven species were detected: foxes (4 consumptions; 6 passing-by), stone martens (4; 1), dogs (1; 6), hedgehogs (1; 1), cats (0; 7), badgers (0; 6) and roe deer (0; 2). Foxes were thus the most photographed species (25.6% of the pictures) and the main consumer, along with the stone

Table 2
Comparison of the relative densities of *A. terrestris* and *Microtus* sp. over the two study areas, in 2004 and 2005, using the Chi-square test.

		Annemasse[a]		Pontarlier[a]		*p*-Value
2004	*A. terrestris*	36.92%	(559/1514)	41.87%	(973/2324)	0.0443[*]
	Microtus sp.	10.24%	(155/1514)	4.48%	(104/2324)	1.00×10^{-10}[***]
2005	*A. terrestris*	22.45%	(359/1599)	45.73%	(1131/2473)	2.20×10^{-16}[***]
	Microtus sp.	5.32%	(85/1599)	4.41%	(109/2473)	0.2056

[a] Number of positive intervals/total number of intervals. All transects were walked in grasslands.
[*] $p < 0.05$.
[***] $p < 0.001$.

martens, each responsible for 40% of bait consumptions. In addition, several observations were made of invertebrates (Hymenoptera, Coleoptera and Gastropoda). Only Gastropoda, represented by slugs and snails, were responsible for relatively large losses of mass on 13 baits during the June and August campaigns (7/160 and 6/160, respectively).

The best model to explain the consumption of the 720 baits monitored in 2007 (320 in Annemasse and 400 in Pontarlier) included the variables “site” and “urbanization” (ΔAICc = 5.124 for comparison with the closest model including the variables “urbanization” and “month of distribution”; odds ratios (OR): site – Pontarlier vs Annemasse: 1.904, 95% CI: 1.229–2.970; urbanization – urban vs. suburban: 0.251, 95% CI: 0.150–0.406). The proportion of baits consumed was thus higher in suburban plots (93.6%, CI: 86.4–100.0%) than in urban plots (78.9%, CI: 68.6–89.2%), and higher in Pontarlier (89.5%, CI: 77.2–100.0%) than in Annemasse (82.2%, CI: 72.7–91.7%). No evidence of monthly difference could be provided.

3.3. Monitoring of *E. multilocularis* in environmental fox faecal samples

Compared with the control area, the parasite faecal prevalence in the treated area in Annemasse decreased after the beginning of treatment and remained below 5% throughout the study (Fig. 3). The best model to explain the faecal prevalence, selected according to the principle of parsimony, retained only the variable “treatment” (ΔAICc = 1.228 for comparison with the model including the variables “treatment” and “season”; odds ratio (OR): plot type – treated vs. control: 0.142, CI: 0.076–0.250) indicating a higher contamination of the control area (24.0%) compared to the treated area (4.3%). In Pontarlier, the temporal change of the parasite faecal prevalence in the treated area was similar to the control area although at a lower level. There, the principle of parsimony indicates that the best model was the null model (ΔAICc = 0.815 for comparison with the closest model including the variable “treatment”) with an overall prevalence of 9.1%.

A “before/during” treatment comparison of the spring faecal prevalence in Annemasse suggests a decrease of the spring contamination from 13.3% in 2006 to 2.2% over the period 2007–2009 (Chi-square = 9.307, *p*-value = 0.005). In Pontarlier, however, we failed to detect any significant difference (10.9% in 2006 and 7.1% over 2007–2009; Chi-square = 0.855, *p*-value = 0.356).

3.4. Cost evaluation

Since the beginning of the experiment and during the three years thereafter, the total cost of the experiment amounted to 235,791€. As operations were performed identically in Annemasse and Pontarlier, this represented a cost per site of 117,895€. If one focuses solely on the prophylaxis treatment (no faeces sampling, no analyses), the cost is reduced to 52,754€ for each city. This means a 1 km^2 treatment plot represented an investment of 114€ per campaign.

4. Discussion

4.1. Bait distribution

Our results revealed that the species consuming the baits were similar to those of Hegglin et al. (2004) in Zurich. The foxes – the main target of the treatment – were the animals most attracted to the baits in both studies (though at similar level than stone martens in our study), with 48% of baits ingested in Zurich and 40% in our study. Hedgehogs and dogs were the main competitors for foxes in Zurich, yet no consumption by stone martens was observed there. By contrast, in our study, stone martens consumed as many bait as foxes did. However, due to the brevity of the camera traps survey (two campaigns and only ten pictures of consumption) and in absence of additional data on stone marten densities over the two study areas, we are presently unable to evaluate to what extent mustelids actually hindered the bait availability for foxes.

The lower consumption of baits in urban areas in comparison to suburban areas could be explained by the greater availability of anthropogenic food in cities (Contesse et al., 2004), resulting in a lower palatability of the baits. Furthermore, the even distribution of baits over treatment plots (some could have been placed in areas that were less accessible and, therefore, less frequented by foxes) may have led to lower consumption. Therefore, the higher bait uptake in Pontarlier may be seen as a consequence of the city’s low proportion of built-up areas. Nonetheless, in both study areas, a final bait consumption of 80% or more, along with foxes responsible for 40% of bait consumption, suggests that a density of 40 baits/km^2, homogeneously distributed, might be adapted in medium size cities, regardless of the month of treatment.

4.2. Contrasted achievement of fox baiting

The comparison between treated and control areas was not sufficient in itself to evaluate the efficiency of treatment since the difference between the two areas may be due to external factors (e.g. host density, egg survival conditions etc.). It was thus necessary to compare faeces contamination before and during the treatment within each city to strengthen our conclusions about the impact of baiting.

Different studies in rural as well as in urban situations have illustrated the role of both fox and intermediate host densities on parasite prevalence in foxes. In a high endemic rural area in Doubs, *E. multilocularis* prevalence in foxes fell after a sharp reduction in the fox population (Raoul et al., 2003), and was positively asymptotically related to the relative densities of both *Arvicola terrestris* and *Microtus* sp. (Raoul et al., 2010). In the city of Zurich, Hegglin et al. (2007) have shown that a decrease in prevalence in foxes from the suburban to the urban area paralleled a decrease in *A. terrestris* density and a decrease in *Arvicolid* occurrence in fox stomach content.

Hunting statistics on predators are subject to many biases and should not be used directly to compare fox densities between different areas. However, when carefully handled, their trend may be used as a population trend indicator (Bögel et al., 1974; Gloor et al., 2001; Cattadori et al., 2003), as long as no significant change occurs in hunters' habits during the time of the survey. We assume this to be the case in both areas over the study period (6 years). Therefore, the declining hunting bags around Annemasse suggest a negative trend in fox populations. At the end of the 1990s, the fox populations of the Geneva basin, especially the south-eastern part where the city of Annemasse is situated, suffered a serious outbreak of sarcoptic mange (Fischer et al., 2003) which decreased the fox density. The disease may still have been circulating among foxes during our study and undermined the population.

Arvicola terrestris and *Microtus arvalis* are prone to multiannual population cycles with outbreaks given a high proportion of grasslands in the landscape (Delattre et al., 1992; Giraudoux et al., 1997). Such is the case in Doubs *département* (including Pontarlier). Small mammal population monitoring was undertaken in 2004 and 2005 and ceased afterwards for logistical reasons. Nevertheless, it suggests a larger *A. terrestris* population density in Pontarlier than in Annemasse and shows no clear pattern for *Microtus* sp. In the absence of long term monitoring of foxes and intermediate hosts in both cities, coupled with prevalence follow-up, it is difficult to assess the actual and detailed impact of hosts' population density variations on the efficiency of baiting campaigns.

However, the positive trend in fox hunting bags and the strong presence of intermediate hosts around Pontarlier are supposed favourable conditions for *E. multilocularis* life cycle (Raoul et al., 2003, 2010). The parasite's resilience may have been strong enough to challenge the efficiency of the baiting campaigns that we had designed, resulting in the absence of significant decrease in prevalence. The large continuous grasslands that expand far beyond the treatment limits and the smaller size of the city of Pontarlier may also have facilitated untreated foxes from outside to come foraging within the treated area, thus maintaining a contamination of the ecosystem. Data on fox home range and/or on genetic distance as described between urban and rural foxes by Wandeler et al. (2003) in Zurich could provide information on population exchange fluxes, thereby challenging our hypothesis.

Retrospectively, given the high variability of prevalence in both control areas, assessing the success of bait distribution in Annemasse with only one sampling period of faeces before the treatment is worrisome. However, the significant decrease in spring *E. multilocularis* faecal prevalence in the treated area after 2006 and the absence of a seasonal peak during 2007 and 2009, as compared with control area, support the effectiveness of baiting prophylaxis. As the residual faecal prevalence reached the level of cross-reactivity of the EM-ELISA, as described by Deplazes et al. (2003), we are uncertain whether the parasite was still present or not in the fox population after treatment. The EM-ELISA is indeed not as sensitive and specific as the Sedimentation and Counting Technique (SCT), which is considered as the "gold standard" method (Eckert and Deplazes, 2004).

In another study, in rural areas of Germany, a sharp decrease of *E. multilocularis* prevalence in foxes from 64% (CI: 59–69%) to 15% (CI: 10–21%) was observed, following an 18-month trial with monthly distribution of baits (Romig et al., 2007). This study highlighted that a decrease in the frequency of bait distribution, from six weeks to three months, maintained low prevalence without further reduction, whereas a frequency of six months resulted in the prevalence rising again to 55% in just 36 months. A baiting protocol implemented by Konig et al. (2008) showed promising results with a decrease of *E. multilocularis* prevalence from 35% (CI: 22–50%) to 1% (CI: 0–4%) within four months (treatment every four weeks with 50 baits/km^2). In an experimental study in an urban context, Hegglin and Deplazes (2008) also assessed that baiting at 3-months intervals is much less effective than monthly baiting campaigns, which better match the parasite's prepatency period (Thompson and Eckert, 1982). Consequently, in our experiment, assuming that bait distribution was well-adapted to target the fox populations, the frequency of treatment and/or the area treated must have been underestimated to actually reduce the faecal prevalence in foxes in Pontarlier. Further trials should be conducted to optimize the bait distribution as a routine protocol to control *E. multilocularis* in this city.

4.3. Cost evaluation

Authorities actively willing to control the parasite must first consider the cost for fox-baiting campaigns. Monitoring of the efficiency of such a control program should always be conducted as well. With regard to our results, frequency and area of treatment should be adapted to each situation. If an increase in frequency of bait distribution up to once per month was to be required, as suggested for instance by Hegglin and Deplazes (2008) in the city of Zurich, the costs for the same area would increase to 45,144€ per year. That said, since the distribution of baits is rather easy to implement, the involvement of local technical staff may reduce these costs by nearly 40% (accommodation, meals, transport). The median treatment cost per patient with AE in Switzerland was evaluated by Torgerson et al. (2008) as 108,762€ (CI: 48,302€–178,568€). The final aim of baiting protocols being to reduce human contamination by the parasite, one may consider the cost-benefit balance for the society if such protocols lead to reduce the number of human AE cases by just one unit per year in the area of interest.

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