Role of dog behaviour and environmental fecal contamination in transmission of *Echinococcus multilocularis* in Tibetan communities

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SUMMARY

On the Eastern Tibetan Plateau region (Sichuan province, China) dogs are regarded as important definitive hosts of *Echinococcus multilocularis*. We studied dog spatial behaviour in 4 Tibetan villages in order to determine the role of dogs in environmental contamination and their potential interactions with small mammal intermediate hosts. We identified definitive host species and *Echinococcus* spp. infection status of feces collected in the field by PCR methods and analysed the spatial distribution of canid feces. Nocturnal space utilization of GPS collared dogs in and around villages was also undertaken. *E. multilocularis* DNA was amplified in 23% of dog feces ($n=142$) and in 15% of fox feces ($n=13$) but this difference was not significant. However, dog feces were more frequently observed (78% of collected feces) than fox feces and are therefore assumed to largely contribute to human environment contamination. Feces were mainly distributed around houses of dog owners (0–200 m) where collared dogs spent the majority of their time. Inside villages, the contamination was aggregated in some micro-foci where groups of dogs defecated preferentially. Finally, small mammal densities increased from the dog core areas to grasslands at the periphery of villages occasionally used by dogs; male dogs moving significantly farther than females. This study constitutes a first attempt to quantify in a spatially explicit way the role of dogs in *E. multilocularis* peri-domestic cycles and to identify behavioural parameters required to model *E. multilocularis* transmission in this region.

Key words: transmission ecology, *Echinococcus multilocularis*, domestic dogs, fecal contamination, spatial distribution.

INTRODUCTION

Alveolar echinococcosis (AE) of humans is an often fatal zoonosis, distributed in arctic and temperate regions of the northern hemisphere. It is caused by the ingestion of eggs of the cestode *Echinococcus multilocularis* which are disseminated into the environment by definitive host feces. Definitive hosts are mainly canids and particularly red foxes (*Vulpes vulpes*) in the paleartic Eurasian region (Eckert and Deplazes, 2004). Canids acquire infection by ingestion of small mammal intermediate hosts infected with the metacestode. Once released in the environment, eggs can survive up to 1 year in optimal microclimate conditions, being sensitive to dry and hot conditions (Veit *et al*. 1995). Humans can become infected after accidental ingestion of eggs (e.g. *via* eating contaminated vegetables or after a close contact with infected canids).

The incidence of human AE in endemic areas of Europe can reach 0·74 per 10^5 per year (Vuitton *et al*. 2003). However, some foci of very high prevalences in humans have been recorded in western China, where a prevalence of 14·3% was observed in villages of the eastern Tibetan Plateau (Sichuan province) (Tiaoying *et al*. 2005). The Tibetan Plateau, situated between 3200 and 5000 m above sea level, is inhabited by a large diversity and abundance of host species and is regarded as a méta-stable reservoir for *Echinococcus multilocularis* (Giraudoux *et al*. 2006). The landscape is dominated by grassland and the main resource for

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people is provided by yak breeding (*Bos grunniens*). Livestock, especially grazing pressure, has been linked to the provision of optimal habitats for small mammal intermediate hosts, i.e. pikas *Ochotona curzoniae and O. cansus,* voles *Microtus limnophilus* and *M. leucurus*, and hamster *Cricetulus kamensis*, whose spatial distributions are clustered in colonies (Raoul *et al*. 2006). Four wild canid species (*Vulpes vulpes, Vulpes ferrilata, Vulpes corsac* and *Canis lupus*) as well as the domestic dog (*Canis familiaris*) are potential definitive hosts in this area. High prevalences of 59·1% and 57·1% for *E. multilocularis* have been recorded in Tibetan foxes and red foxes respectively (Qiu *et al*. 1995), though what proportion was actually *E. shiquicus* in Tibetan foxes is not clear (Xiao *et al*. 2005). Lower prevalences (12–15%) have been found in owned dogs following purgation studies in Shiqu's county (Budke *et al*. 2005*a*; Wang *et al*. 2010). However, dogs are numerous in Tibetan villages where they are kept as guard dogs, and stray dogs are protected by the local Buddhism religion. It is a rarity to see a house without dogs and stray dogs are frequently observed roaming in and around villages. In summer, some of the dogs go with the herdsmen in the summer pastures in high elevation grasslands.

Epidemiological studies have identified dog ownership, number of dogs owned and relationships with dogs (i.e. contact) as risk factors for human AE in Tibetan communities in northwest Sichuan (Tiaoying *et al*. 2005; Budke *et al*. 2005*a*), where small mammal population cycles have been documented (Giraudoux *et al*. 2006). Preliminary analysis of dog feces collected in Shiqu's county showed that small mammal remains occurred in 28·8% of the samples (Wang *et al*. 2010). Furthermore, evidence for dog/small mammal interactions have been outlined by a positive correlation found between the proportion of fenced pastures, optimal habitats for small mammals, and both *E. multilocularis* prevalences and worm burden in owned dogs (Wang *et al*. 2004, 2010). In such context, dogs are currently assumed to be the main definitive host in a peri-domestic cycle of *E. multilocularis* (parallel to the wildlife cycle). However, to date there have been no studies of the behavioural ecology of the dog population in Tibetan communities in this highly human AE endemic area.

Generally, studies on the role of behavioural ecology of dogs in parasite transmission cycles are rare despite the fact that dogs are known to be hosts for up to 60 zoonoses (Macpherson *et al*. 2000) and are often considered as the main source of contamination of the human environment (Craig *et al*. 2000; Macpherson, 2005; Thompson *et al*. 2009). Behavioural ecology provides a means to study the role of dogs in transmission by identifying and quantifying defecation and predation behaviours. Most studies of dog populations in an epidemiological context provide measurements of population size, sex and age structure that can be used to guide control programmes (Anvik *et al*. 1974; WHO and WSPA, 1990). The spatial distribution of zoonotic pathogens in human environments can be assessed by analysing soil contamination arising from definitive host feces such as for *Toxoplasma gondii* (Afonso *et al*. 2008), *Toxocara canis* (Habluetzel *et al*. 2003) or *Echinococcus* spp. (Craig *et al*. 1988; Shaikenov *et al*. 2004), or by studying host defecation behaviour (Anvik *et al*. 1974; Robardet *et al*. 2008). Modern non-invasive methods have been developed to facilitate the assessment of definitive host infection status from fecal material *via* DNA analysis, e.g polymerase chain reaction (PCR), or immunological analysis, e.g. copro-antigen enzyme-linked immunosorbent assays (ELISA) (Dinkel *et al*. 1998; Raoul *et al*. 2001; Eckert and Deplazes, 2004). Analysis of movements of the definitive host has proven useful in epidemiological studies: for example, telemetry technology provided relevant data to understand the spatial behaviour of foxes in cities and the risk they induce for the transmission of *Echinococcus multilocularis* (Robardet *et al*. 2008). Concerning dogs, Watson-Jones and Macpherson (1998) have studied free-roaming dog movements by visual observations in Turkanian villages and found that the time dogs spent in the community settlement was correlated with other risk factors of *Echinococcus granulosus* transmission and with the incidence of the hydatid disease in the community.

Our general objective was to quantify and spatialize the role of domestic dogs in an *E. multilocularis* peridomestic transmission cycle within Tibetan villages situated on the eastern Tibetan Plateau. Spatial patterns of dogs and fox's defecation and dog's movements were studied to address the following questions. (i) Which of dog or fox feces constitute the main source of *E. multilocularis* contamination in the human environment? (ii) How are *E. multilocularis* contaminated canid feces distributed within and at the periphery of the human environment (i.e. village areas)? (iii) What are the distributions (extent and frequency) of dog movements within the human and wild intermediate host environment and how and why spatial behaviours vary between individual dogs? (iv) Are there specific areas in the human environment where dogs could potentially be infected due to overlap of their movement range with small mammal colonies?

A new coprodiagnostic test (real-time PCR) combining parasite detection with discrimination of the canid host species from which the fecal sample derived, provided us with a tool to quantify the role of dogs versus foxes in *E. multilocularis* environmental contamination. Spatial distribution of canid feces (characterized by their specific identification and infection status) was investigated in relation to human and wild intermediate host population habitats (in and around village areas), together with the

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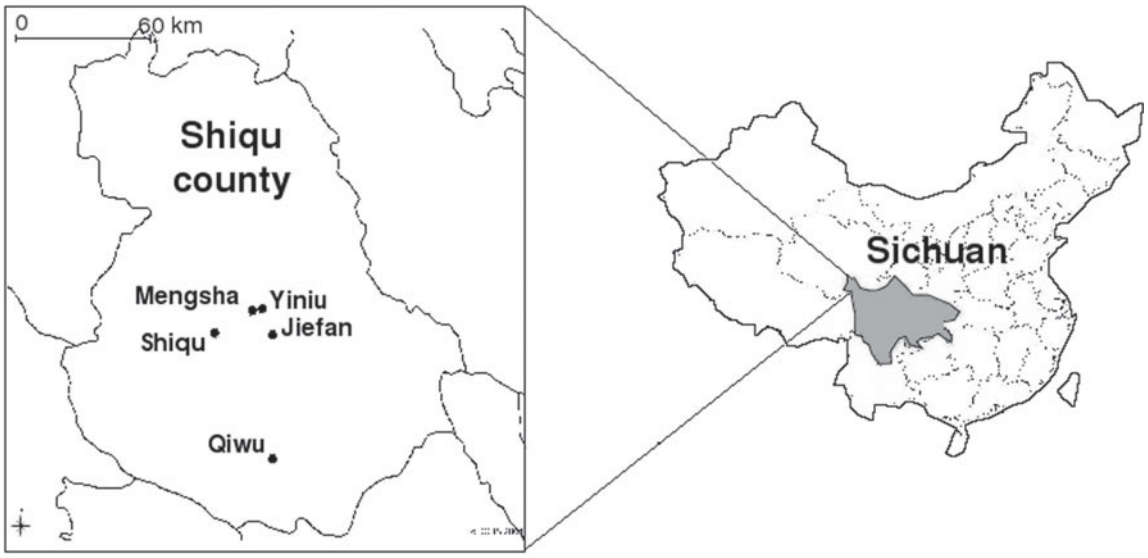

Fig. 1. Localization of the study sites in Shiqu county (China).

distribution of individual dog nocturnal movements in relation to dog owners' houses. The influences of dog individual traits (e.g. sex, relationships with owners) on inter-individual variability of movement were also analysed. Finally, we analysed the spatial overlap between small mammal population distributions and dog acitivity areas.

MATERIALS AND METHODS

Study sites

Shiqu township (Sichuan province, China) is situated between 3900 and 4200 metres above sea level on the Tibetan Plateau. Four villages in the vicinity (20–85 km) of Shiqu township were investigated (Fig. 1). The study started in May 2006 in Yiniu and Qiwu villages which were revisited in May 2007 along with 2 further sites: Mengsha and Jiefan. Villages were characterized by hundreds of scattered houses spread out along rivers and crossed by tracks. Within villages, areas of bare ground were common in contrast to the surrounding landscape which was dominated by grassland (fenced or grazed). Bushes were sparsely distributed on sloped grassland whilst wet grassland with micro-topography could be found near rivers.

The 4 villages were selected according to their epidemiological situation regarding the human AE prevalence (Tiaoying *et al*. 2005). Qiwu and Mengsha were identified as relatively low AE prevalence areas (2 ± 0·9% and 3 ± 5·8%, respectively) whilst AE prevalences of 11 ± 13·3% and 12 ± 8·8% were observed in Jiefan and Yiniu respectively. An epidemiological survey done twice in 2006 provided an estimate of 31–64 owned dogs per village (Jasmin Moss, unpublished data, Table 2). Dog population size was underestimated in Jiefan since only 70% of the households have been sampled.

Canid feces and environmental contamination

Fecal sampling. Canid feces were sampled in 1 hectare quadrats located inside and outside villages. A total of 33 quadrats have been sampled during 6 sampling surveys: 17 inside and 16 outside villages (Fig. 2). Inside villages, quadrats were in close vicinity to houses, while outside villages, quadrats were situated in grassland surrounding village cores. All the feces found within each quadrat were counted and geo-referred systematically. Fecal densities were estimated as the number of counted feces per quadrat.

Fox and dog feces were discriminated visually (based on their shape, size and smell) by field experts who previously experienced fox feces collection in the field, from captive foxes or from necropsies. The expert accuracy to discriminate dog to fox feces was then tested by comparing the expert identification to the identifications of host species from molecular analysis (see following).

Copro-assays for host species DNA and parasite DNA. In 27 out of the 33 quadrats we collected a minimum of 25 (in 2006) and 15 (in 2007) fecal samples for coprodiagnostic tests in order to identify DNA of *E. multilocularis* and to discriminate canid host species directly from feces.

DNA from fecal samples was extracted as described previously by Dinkel *et al*. (1998). For the specific detection of *E. multilocularis* and canid host species DNA (*Vulpes vulpes,* red fox; *V. ferrilata,* Tibetan fox; *V. corsac,* sand fox; and *Canis lupus/familiaris,* wolf/dog) we used 2 newly-developed hybridization probe-based real-time LightCycler (Roche, Mannheim, Germany) PCR assays (Dinkel *et al*. 2011) as follows. (1) A real-time multiplex nested PCR (rtm-nested PCR) with fluorescence resonance energy transfer (FRET) probes for specific

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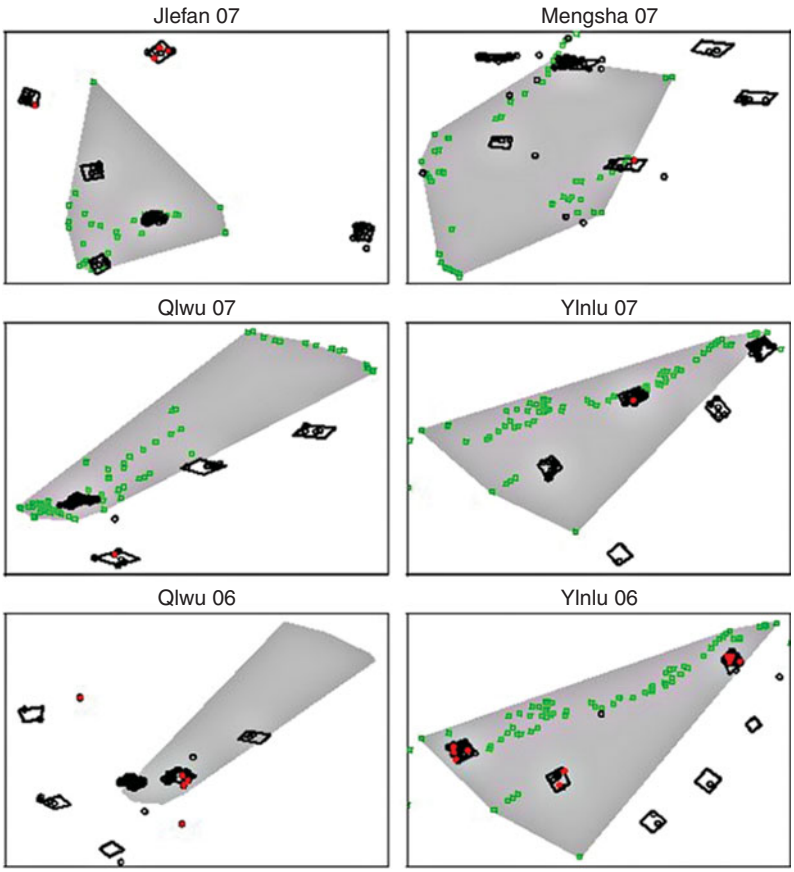

Fig. 2. Feces sampling design per sampling survey. Village areas (90% MCP) and village houses are represented by grey areas and small green squares respectively. Large black squares represent quadrats and small black and red circles represent collected dog and fox feces respectively.

DNA detection and discrimination of *E. multilocularis* and canid host species in a single reaction, enabling specific species identification by analysis of the melting curves. The target sequence for *E. multilocularis* was part of the mitochondrial 12S rRNA gene, whereas a part of the mitochondrial cytochrome b gene was used as target for the canid host species identification. (2) A real-time nested PCR (rt-nested PCR) with FRET probes only for the detection and discrimination of the host species described above by melting curve analysis.

Specificity of the assays was tested with DNA isolated from *Echinococcus granulosus* (G1), *E. ortleppi, E. canadensis* (G6, G7), *Taenia crassiceps, T. hydatigena, T. mustelae, T. pisiformis, T. serialis, T. taeniaeformis, Mesocestoides leptothylacus, Microtus arvalis, Arvicola terrestris, Felis catus* and *Martes foina*. No cross-reactions were detected. The detection sensitivity of the rtm-nested PCR obtained with serial dilutions (100 ng – 1 fg) of genomic *E. multilocularis* DNA added to 10 μl total DNA solution from *E. multilocularis*-negative canid feces was 10 fg (Dinkel *et al*. 2011). Based on a comparison of 47 fecal samples from China, the proportion of positives tested by the real-time mutliplex nested PCR was in the same range as when tested by the previously evaluated nested PCR (38% *vs* 30%) which showed a sensitvity of 70% to 100% depending on the number and gravidity status of worms present in the intestine (Dinkel *et al*. 1998).

Both, the rtm-nested and rt-nested PCR were conducted in 2 steps. For the first pre-amplification PCR, which allows the parallel amplification of DNA from various cestodes (355 bp fragment) and different carnivores (401 bp fragment) the primer mix contained a set of 4 primers (cf. Appendix 1; Dinkel *et al*. 2006, 2011). A total of 10μl of DNA from fecal samples was added to a 90 μl reaction mixture (cf. Appendix 1) and hot-start PCR was done for 40 cycles in a thermal block cycler (2720 Thermal cycler, Applied Biosystems, cf. Appendix 1). In a second step the LightCycler instrument (Version 1.5, Roche, Mannheim, Germany) was used for amplification of the target DNA and to monitor the development of the PCR product after each cycle. The rtm-nested PCR was performed with a final volume of 20μl in sealed LightCycler glass capillaries (cf. Appendix 1; Dinkel *et al*. 2011). Differentiation was obtained by detection of mismatches with FRET probes using subsequent melting curve analysis (cf. Appendix 1).

To increase the sensitivity in samples which gave no result for the identification of canid host species we additionally used the rt-nested PCR. The detection of *E. multilocularis* was confirmed by using a

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published nested PCR assay (Dinkel *et al*. 1998). All PCRs included a negative control to monitor possible contamination and a PCR positive control. The isolated DNA from each fecal sample underwent an inhibition control to exclude false-negative results due to inhibition factors present in fecal samples.

Prevalences and their corresponding 95% confidence intervals were estimated. The confidence intervals were computed on the score-test statistic for binary data (Pearson chi-square test) using the *prop.test* function of the *stats* R package. Then, the difference in *E. multilocularis* fecal prevalence between quadrats inside and outside of village areas was tested using a Chi-squared test.

Feces distribution in the environment. Random versus aggregated distributions of feces were assessed in each sampling survey over all quadrats. The ratio of feces density variance over its mean was used as an index of the aggregation. Null models, Generalized Linear Models (GLM) assuming Gaussian, Poisson (random) or Negative Binomial (aggregated) density distributions, fitted on all quadrat densities, were then compared. Density distribution providing the lowest second-order bias corrected Akaike Information Criterion (AICc) was then used in further modelling.

The effect of the type of quadrat (inside *vs* outside village) was then tested on feces density. Because houses were scattered and village boundaries hard to assess, analysis was completed by investigating how feces density varied according to quadrat distances from houses. This provided additional information concerning the range of feces distribution and potential environmental contamination around houses. Generalized Linear models incorporating the effect of the type of quadrat (inside *vs* outside village) and the effect of the minimum distance between the quadrat and the nearest village house, these effects being considered alone and associated, were evaluated using AICc and corresponding Akaike weights (*wi*) that provide the probability of each model to be the best given the data and the set of candidate models (Burnham and Anderson, 1998). Before running the models, we tested whether it would be beneficial to add random effects on the year and village (sampling survey). Finally, aggregation in feces distributions within each quadrat was tested by estimating the Clark and Evans index (R index) (Clark and Evans, 1954) using the *spatstat* package under R statistical software (R Development Core Team, 2008). The hypothesis that the proportion of quadrats with aggregative distribution differed inside and outside village areas was tested using a Chi-squared test.

Dog movement survey

Dog class and data collection. In Tibetan communities dogs could be free roaming, partially free or always tethered. Two categories of dogs were sampled: dogs restrained all day and released at night (owned tethered = OT dogs) and dogs always free (owned free = OF dogs). Dog owners supplied information regarding the sex of the dog and whether it usually visits higher altitude summer pastures. The latter variable was named 'pastoral activity' in the following analysis. Relocations were recorded by GPS collars (WildTrax, Bluesky Telemetry) that were set up on dogs during the day and removed the following day, 1 location being recorded every 10 min.

Since OT dogs were only free from their tether at night, we focused on studying only nocturnal movements for the whole dog population. The time-period of relocation records was homogenized between dogs: the beginning of the night (8:00 pm) was set as the earliest hour at which owned and tethered dogs were known to be released, while the end of the night was considered as the latest hour at which the same dogs were known to be re-tethered (8:00 am), providing trajectories of 12 h for all dogs. However, the lack of accurate information on each dog's hours of freedom and when owners actually re-tethered them may mean that some OT dogs could have been free for less than 12 h and their trajectories may include an unspecified period when they were tethered.

Analysis of distributions of single-night dog movements. We characterized 3 areas of dog population space use: the core areas, the mean dog population activity areas and areas covered by excursive trajectories (extended dog movements).

First, relocation distributions were analysed in relation to the distances between the dog and its release point (RP, i.e. its owner's house). The core area, classically used in the context of home range estimation (Seaman and Powell, 1990; Hodder *et al*. 1998), was used here as a means to define areas around dog owners' houses where dog relocations were aggregated, i.e. where dogs spent the majority of their time. Minimum Convex Polygon (MCP) areas were estimated for different percentages of included relocations on the basis of their distances to their barycentre, i.e. to the dog RP. Friedman test followed by multiple comparisons were done, using the function *friedmanmc* of the *pgirmess* R package, to identify the percentage of relocations included in MCP estimation that lead to a significant increase in MCP area. The nocturnal core area was estimated per dog as the MCP area that included the relocation below such threshold. Dog trajectories were described by estimating the maximum distance travelled from the dog RP inside and oustide nocturnal core areas. Additionally, the mean distance travelled per hour provided a measure of their nocturnal activity (Ciucci *et al*. 1997).

The only night sampling period used to characterize dog movements prevented estimation of the

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dogs' home ranges. Instead, we defined the 'one night activity ranges' of the dog population of one village – or their home range estimated on a delimited time-period (Okubo and Levin, 2001) – by subsampling each dog trajectory in 1 location/h/dog. Utilization Distribution functions were then estimated by the Kernel method (Worton, 1989). The smoothing parameter *href* estimated by the ad-hoc method available in the function *kernelUD* of the *adehabitat* package (Calenge, 2006; R Development Core Team, 2008) is known to suffer from over-estimation of the area when multiple clustering is observed in the relocation data set (Seaman and Powell, 1996). As dog relocations were aggregated around their RP, we estimated an optimal *h* for each sampling survey as a re-scaling of *href* which provided an area that did not connect clusters between which no movements (successive relocations) have been recorded. The dog population nocturnal activity range was defined as the area in which dogs spent the majority of their time - we defined this on 90% of the relocations.

Finally, the delineation of dog nocturnal activity range allowed us to define excursive trajectories using dog relocations falling outside the dog population activity range. The number of such excursive relocations was assessed per dog and main patterns of excursive trajectories were identified. Then, excursive visiting paths were identified as at least 3 successive excursive relocations. We finally explored the existence of aggregated relocations for each dog, as a potential surrogate for foraging behaviours, by estimating the Clark and Evans index on excursive relocations.

Correlations of inter-individual dog movement parameters. Correlations between movement parameters, i.e the maximum distance from the release point, the speed, the core area and the number of excursive relocations per dog, were assessed by Spearman correlation tests. Then, the influences of dog sex, freedom from tether and pastoral activity on normalized (log transformed) movement parameters were investigated among the dog population *via* linear models, except for the effects on the number of excursive relocations which were assessed by a GLM assuming a Negative Binomial distribution. We compared AICc of the null models with all possible combinations of covariates. For each movement parameter, we carried out a preliminary test for the addition of random effects on the sampling survey and individual dogs.

Spatial interactions between dogs and small mammal populations

In the 4 villages, we walked radial transects along which indices of activity of small mammals (mainly feces and holes of *Ochotona* spp., *Microtus* spp. and *Cricetulus kamensis*) were recorded within every interval of 10 paces (Raoul *et al*. 2006). Transects started from the centre of the village and ended in the surrounding grassland. We first estimated the minimum distance separating positive intervals for small mammal presence to all dog RP's at each village. Differences in frequency distribution of positive intervals were assessed between the dog population activity areas and the excursive areas only. This was due to low or even null numbers of transect intervals being situated within individual dog one night core areas. The presence/absence of small mammals were modelled with a Generalized Linear Mixed Effects (GLME) model assuming a logistic binomial link function, against the different dog areas and including a random effect on the sampling survey. Differences in small mammal presence indices between dog areas were assessed by comparing model AICc with the null models and by estimating their risk ratio (Bailey and Alimadhi, 2008).

All statistical analyses were achieved using the R statistical software (version 2.10) (R Development Core Team, 2008).

RESULTS

Canid feces and environmental contamination

Fecal species identification and prevalences. A total of 980 feces were counted and geo-referred in the 4 villages, among which 284 feces were analysed for host species identification and presence of *E. multilocularis* contamination (Table 1).

PCR inhibition problems were observed for 74 (46·8%) of the 158 feces collected in 2007 and 30 (23·8%) of the 126 feces collected in 2006 (Table 1). These feces could not be used for further analysis of fecal prevalences.

Among the remaining 180 feces collected, based on expert knowledge, 142 were attributed to dog, 13 to fox (*Vulpes ferrilata*) and 25 could not be identified. Actually, PCR analysis showed that 84·6% (11/13) of the feces attributed to fox and that 100% (142/142) of feces attributed to dog were correctly classified. Thus, a very low proportion of foxes feces (2 of 155) were confused with dog feces in the field. Among the 284 feces collected in quadrats and analysed by PCR for *E. multilocularis* DNA only, experts identified a total of 14% of fox feces.

The overall coproPCR prevalence for *E. multilocularis* DNA was 20·5%, with 22·5% and 15·4% respectively for dogs and foxes (Table 1). However, there was no significant difference between prevalences of *E. multilocularis* infection in dog and fox feces samples tested (Pearson's Chi-squared test with Yates' continuity correction, Chi-squared = 0·06, D.F. = 1, *P* value = 0·81).

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Table 1. Fecal samples in all quadrats of each sampling survey: total number, aggregation index, number of PCR analyses, number of non-inhibited PCR analyses and prevalence of *E. multilocularis* DNA for all samples and dog and fox feces separately

	All feces					Dog feces		Fox feces	
Sampling survey	Total number	Aggregation index	Nb Tested[a]	Nb PCR[b]	Prevalence (CI)[c]	Nb PCR	Prevalence (CI)	Nb PCR	Prevalence (CI)
Jiefan 07	92	20·6	48	24	12·5 (3·3–33·5)	14	14·3 (2·5–43·9)	5	20 (1·1–70·1)
Mengsha 07	67	9·4	28	14	14·3 (2·5–43·8)	10	20·0 (3·5–55·8)	1	0 (0–94·5)
Qiwu 07	155	91·7	43	21	28·6 (12·2–52·3)	16	25·0 (8·3–52·6)	1	100 (5·5–100)
Yiniu 07	118	25·7	39	25	20 (7·6–41·3)	23	17·4 (5·7–39·6)	1	0 (0–94·5)
Yiniu 06	171	55·7	103	75	24 (15·2–35·5)	60	28·3 (17·8–41·6)	4	0 (0–60·4)
Qiwu 06	377	324·4	23	21	14·3 (3·8–37·4)	19	15·8 (4·2–40·5)	1	0 (0–94·5)
Total	980	142·5	284	180	20·5 (15·1–27·3)	142	22·5 (16·1–30·5)	13	15·4 (2·7–46·3)

(a) Number of feces analysed by PCR; (b) number of identified samples (non failed PCR); (c) 95th confidence intervals for prevalences (in%).

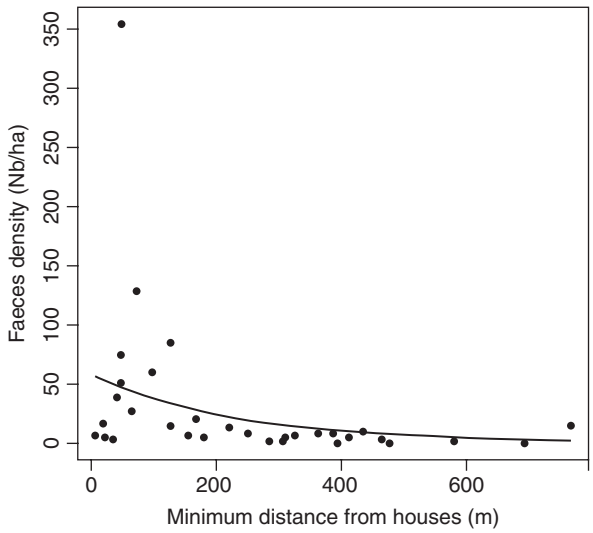

Fig. 3. Distribution of feces densities (number of feces per hectare) according to the minimum distance between quadrats and village houses.

We also failed to detect a significant difference in overall fecal prevalence of *E. multilocularis* between feces samples located inside villages (22·1%, 32/145) versus those located outside villages (14·3%, 5/35, Pearson's Chi-squared test with Yates' continuity correction, Chi-squared = 0·62, D.F. = 1, P value = 0·43).

Feces distributions. Aggregation indices were greater than 1 in each sampling survey as well as for the total (Table 1). Clearly, feces were over-dispersed in villages and fecal density distributions were better captured on the whole data set by a model incorporating a Negative Binomial distribution (AICc = 281·62 versus AICc = 372·32 and AICc = 2343·72 for Gaussian and Poisson distribution respectively). Consequently we used a Negative Binomial fecal distribution for further modelling.

We found no effects of the year and the village on feces densities (GLM, $\Delta_{\mathrm{AICc}} = 0{\cdot}03$, $w_i = 0{\cdot}5$ and $\Delta_{\mathrm{AICc}} = 2{\cdot}49$, $w_i = 0{\cdot}2$, respectively in comparaison to the null model AICc). Those variables were thus not considered as random effects in the further models. We also failed to detect an effect of inside village versus outside village quadrat on feces densities (GLM, AICc = 284·3, $w_i = 0$). However, the model including the minimum RP distance provided the approximate best fit (GLM, AICc = 138·1, $w_i = 1$). Feces density reached a maximum (354 feces/ha) in 1 quadrat situated at a minimum distance of 48 m from village houses while all quadrats situated farther than 200 m from village houses contained less than 14 feces (Fig. 3). Low feces density was observed for 4 quadrats situated at less than 50 m from houses suggesting that the model particularly over-fitted feces densities for those quadrats.

Finally, feces were aggregated in 84% of the quadrats. No significant difference was observed between the proportion of quadrats showing aggregation situated inside (88%, 15/17) and outside (81%, 13/16) villages (Chi-squared = 0·01, D.F. = 1, P value = 0·9).

Dog movement survey

Sampling effort. Seventy-eight dogs were fitted with GPS collars for 1 night, 7 were fitted for 2 nights, 2 for 3 nights and 1 for 4 nights. A total number of 96 one-night trajectories were recorded in the 4 villages (Table 2). Collected GPS collar connections to satellites induced a mean of 19·5% (S.D. = 22·9%) missing values per dog trajectories that were excluded for further analysis. The mean positioning error provided by GPS collars and estimated over all dog relocations varied according to the sampling survey from: 4·7 m (S.D. = 2·9) to 10·5 m (S.D. = 4·6) horizontally and from 6·8 m (S.D. = 5) to 15·20 m (S.D. = 8·3) vertically.

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Table 2. Number of GPS collared dogs (sampling effort) per sampling survey and dog category (freedom level, pastoral activity and sex)

Freedom	Owned tethered									Owned free										
	SP[a]			nSP[b]			u.[d]			SP			nSP			u.				
Pastoral Sex	F	M	u.	F	M	u.	F	M	u.	F	M	u.	F	M	u.	F	M	u.	Total	% pop.[c]
Jiefan 07	1	3	0	2	3	0	0	0	1	1	1	0	2	6	0	0	0	0	**20**	64(31)
Mengsha 07	0	0	0	1	5	0	0	0	2	0	1	0	0	4	0	0	0	2	**15**	29·7(64)
Qiwu 06	1	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	**5**	13·95(43)
Qiwu 07	0	3	0	0	3	0	0	0	0	0	3	0	1	3	0	0	0	0	**13**	30(43)
Yiniu 06	0	2	0	1	5	0	0	0	0	0	1	0	0	3	0	0	0	0	**12**	31·57(57)
Yiniu 07	0	0	1	0	4	0	0	0	1	0	0	0	5	1	0	0	0	1	**13**	35·09(57)

(a) Summer pasture dog; (b) non summer pasture dog; (c) percentage of sampled dogs over the total number of dogs estimated (into brackets); (d) undetermined.

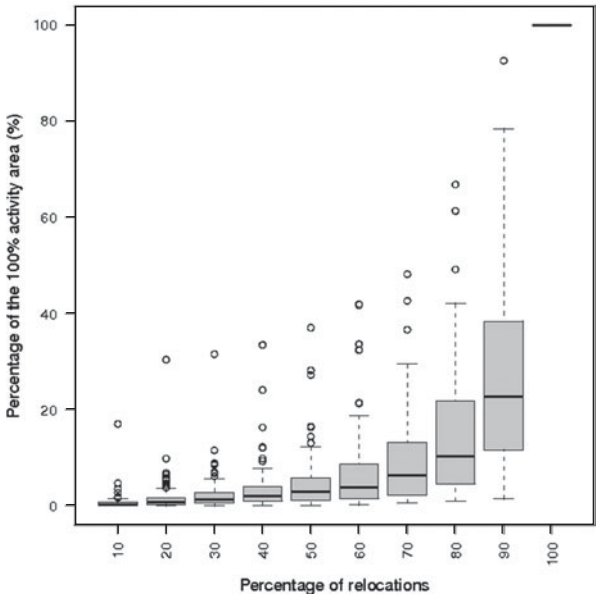

Fig. 4. Boxplots of the proportion of the maximum MCP area according to the percentage of the total number of relocations included in the estimation of MCP area.

Distribution of single-night dog movements. MCP areas differed according to the percentages of relocations included in their estimation (Friedman test, Friedman chi-squared = 837, D.F. = 9, *P* value <2·2e-16) (Fig. 4). Most of the relocations were aggregated in areas smaller than the total activity area which relied on few exceptional relocations. For example, when including 80% of the relocations, we observed that a median value of about 10% and an outlier below 70% of the total activity area could be captured among dogs. A very low proportion of relocations (the last 10%) provided more than 70% of the total area for half of the dogs. Because a significant difference was found between the inclusion of 80 and 100% of relocations (*P* value = 0·05), the one night core areas were estimated for each dog as the MCP area including 80% of its relocations.

Dogs were located at 10 to 1500 m from their release points but were generally under 250 m from this point, moving slowly (162 m/h on average) within a less than 1 ha core area for the large majority of them. Among individual dogs, one night core areas and maximum RP distances showed dispersed (standard deviations larger than the means) distributions aggregated around low values. Fifty percent of the dogs had night core areas smaller than 0·16 ha, stayed within a 36 m range around their RP and travelled a maximum distance of 152 m from their RP. However, 5% of them had night core area larger than 1·19 ha, moved at 115 m maximum from their RP and travelled a maximum distance of 921 m. Optimal *h* (smoothing parameter) estimates for each sampling survey ranged from 35·7 (scaling factor = 0·2) to 133·9 (scaling factor = 0·5) (Table 3). The main activity areas of dogs ranged from 32·5 to 174·5 ha according to the village and sampling survey; 39% of the dogs had at least 1 relocation falling outside the population activity area (Table 3). The mean number of excursive relocations per dog varied from 2·7 to 5·1 depending on the sampling survey. The mean distance of such excursive relocations from the dog RP varied between 309 and 583 m according to the village, with a minimum distance of 58 m.

Among those trajectories, 2 patterns of dog excursive relocations were observed: visiting paths consisting of a minimum of 2 successive relocations (Fig. 5, a–d), and 'away and back' movements relying on isolated excursive relocation from the dog one-night core areas (Fig. 5, e)). Eleven dog trajectories were considered as visiting paths among which 5 of them showed aggregative excursive relocations (mean (R) = 0·85; S.D. (R) = 0·11) such as the trajectory illustrated on Fig. 5, f).

A maximum of 9 successive relocations (or 90 min excursive trajectories) were recorded for a male dog fitted in Qiwu village (2007) and which regularly visit summer pastures.

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Table 3. Parameters of the dog population activity areas and excursive ('exc') relocations for each sampling survey

Sampling survey	Area	Optimal *h*	Exc dogs	Nb exc relocations			Dist exc relocations[a]			Visiting dogs[b]	Nb successive exc. relocations
	ha		Nb[c]	Mean (sd)	Min	Max	Mean (sd)	Min	Max	Nb	Max
Jiefan 07	174·5	133·9	4	3·25 (3·9)	1	9	583 (373)	186	1125	1	5
Mengsha 07	118·2	129·3	7	2·71 (1·7)	1	5	437 (163)	187	683	1	3
Qiwu 07	32·5	80·9	5	4·8 (7·3)	1	18	360 (217)	64	732	1	9
Yiniu 07	67·9	37·5	9	5·11 (4·1)	1	13	403(231)	60	1174	4	6
Yiniu 06	53·9	75·4	10	3 (3)	1	9	309(197)	99	902	2	8
Qiwu 06	15·2	49	4	4·75 (3·2)	2	8	331 (453)	58	1519	2	5
Total			39	3·8 (3·9)	1	18	388 (274)	58	1519	11	9

(a) Distance of excursive relocations from dog RPs; (b) dogs having at least 3 successive excursive relocations (visiting path); (c) number.

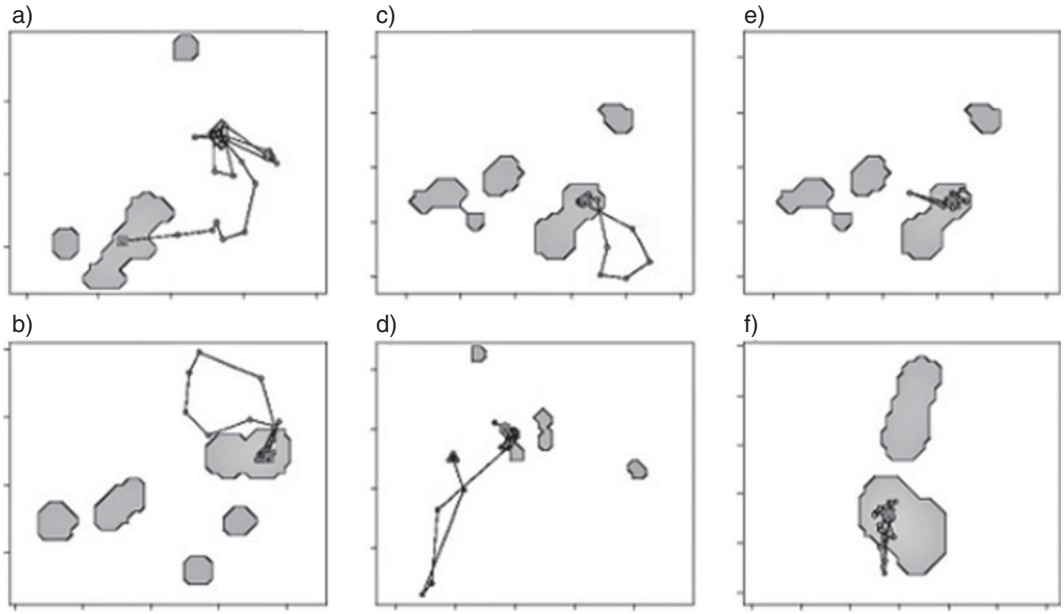

Fig. 5. Example of trajectories (black lines) and excursive relocations (dots) for 6 dogs, from (a) to (f), in reference to the dog population main activity area (grey polygons). Dog trajectories shown from (a) to (d) correspond to visiting paths while dog trajectories shown in (e) and (d) correspond to 'way and back' paths.

Correlations of inter-individual movement parameters. As expected, all movement parameters were significantly correlated to each other (*P* values <0·01). Spearman correlation coefficients ranged from 0·53 between the number of excursions and the core area, to 0·84 between the maximum distance from RP and the speed. No effects of the individual, the year and the village have been identified using AICc comparisons. Therefore those variables were not incorporated as random effects in the models. Dog sex slightly influenced dog maximum RP distances (LME, AICc = 236·4, ΔAICc = 2·4, *wi* = 0·3), median area being larger for males (median = 165) than for females (median = 117). No effects of the other factors on other movement parameters have been detected.

Spatial interactions of dog and small mammal populations. A total of 42 standard radial transects were done. Evidence for small mammal presence was found close to dog owners' houses at a minimum distance varying between 32 and 122 meters according to the sampling survey (Table 4). Positive transect intervals (92%) were identified in 2006 inside dog individual one night core areas in Qiwu village (Table 4). The proportion of transect intervals with the presence of small mammals was higher in the dog excursive area than in the dog population activity area (15·5–56·5% versus 3–37%, GLME, AIC = 5275, ΔAICc = 138; Risk ratio = 2·36 (1·86–2·93)).

DISCUSSION

In western China, including Tibetan regions, domestic dogs appear to play an important role in the risk of human alveolar echinococcosis caused by

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Table 4. Small mammal spatial distributions inside the three type of dog areas of activity. Confidence intervals for the percentages and risk ratios provided in parentheses were estimated on the inverted score test statistic (Pearson chi-square) using the prop.test function of the stats R package

	Nb transects[a]	Minimum distance[b]	Core areas		Population activity area		Excursive area		Risk ratios
			Total[c]	% Sm[d]	Total	% Sm	Total	% Sm	
Jiefan 07	11 (356)	65	9	0 (0–37·1)	214	22·9 (17·6–29·2)	543	56·5 (52·2–60·7)	1·8 (1·5–2·2)
Mengsha 07	8 (133)	32	17	0 (0–22·9)	239	6·7 (4·0–10·8)	254	46·1 (39·8–52·4)	1·4 (1·2–1·7)
Qiwu 07	4 (110)	56	0	0 (NA)	34	2·9 (0·2–17·1)	422	25·8 (21·8–30·3)	1·5 (1·3–1·8)
Yiniu 07	5 (185)	50	0	0 (NA)	87	36·8 (26·9–47·9)	523	29·3 (25·4–33·4)	2·0 (1·7–2·4)
Yiniu 06	7 (163)	122	0	0 (NA)	64	9·4 (3·9–19·9)	1016	15·5 (13·3–17·9)	2·1 (1·8–2·4)
Qiwu 06	7 (423)	38	13	92·3 (62·1–99·6)	33	36·4 (21·0–54·9)	1023	39 (36·0–42·1)	2·0 (1·7–2·3)

(a) Small mammal sampling effort: numbers of transect. Number of transect intervals in parentheses; (b) minimum distance from dog RP; (c) total number of transect intervals; (d) percentage of positive transect intervals.

exposure to eggs of the fox tapeworm *E. multilocularis* (Macpherson and Craig, 2000; Craig *et al*. 2006). High prevalences (>10%) of *E. multilocularis* have been recorded in owned dogs in Tibetan communities of northwest Sichuan province located in the eastern edge of the Tibetan Plateau (Budke *et al*. 2005*a*; Wang *et al*. 2010). However, in contrast to studies of fox definitive hosts in European and Japanese *E. multilocularis* endemic areas, there is virtually no information on dog behavioural ecology and its potential role in the peri-domestic transmission of *E. multilocularis*. To address this issue we used a multi-disciplinary approach that combined PCR analysis (of both host and parasite targets) of spatially distributed fecal samples and dog behavioural ecology study.

In the 4 Tibetan villages situated in the area of highest endemicity for *E. multilocularis* on the Tibetan Plateau, 7% of feces analysed were identified by host DNA amplification as Tibetan fox (*Vulpes ferrilata*) origin. This endemic fox is known to inhabit grassland areas far (>1000 m) from human influences (Gong and Hu, 2003; Wang *et al*. 2007) but our research demonstrates that Tibetan foxes also forage and defecate in and around villages. Considering that 15% of fox feces were positive for *E. multilocularis* DNA then foxes could be considered as a source of human environmental contamination. However, fox feces were found in much smaller proportions than dog feces in and around villages (8% of feces analysed by multiplex PCR). Prevalence of *E. multilocularis* did not differ significantly between dog and fox feces but the larger density and number of dog feces found in human environments suggests an important role of dogs in fecal contamination of the environment in Tibetan villages in this area (Shiqu county, Ganze prefecture, Sichuan).

An important drawback of our parasitological analysis remains the large proportion of inhibited PCRs that constrains the feces sample size and consequently the interpretation of observed prevalences on their own. Since both species exhibit quite similar foraging behaviours in village areas (eating remains of human food or carrion), apart from predation behaviour, no credible argument towards a high sensitivity of fox or dog DNA to inhibitors can be assumed. Feces identification by experts in the field provides additional information on the contamination distribution and can help to compensate the current limitations of the molecular methods used here. This emphasizes the importance of using multiple methods for feces collected from the analysis of environment contamination.

All dogs that were GPS collared shared a common pattern of single-night relocation distributions: i.e. they were aggregated around owners' houses. The area over which owned dogs spent the majority of their time at night ranged from 0·004 to 10 ha,

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and 95% of dogs stayed for 80% of their time at a maximum distance of 115 m from their owners' houses. That area probably corresponds to the small and easily defendable territory where aggressive dog behaviours are mostly expressed (Boitani *et al*. 1995; Macpherson *et al*. 2000). In contrast, 40% of dogs were recorded outside their main population activity area, at distances varying from 58 to 1519 m from their release points and during a maximum time-period of 90 min. Even if recorded for only 12 h and at the dog individual level, the patterns of relocation distributions identified here are in agreement with those observed in feral or free-roaming dogs for which core areas (50% of their home range) corresponded to 5·7% of the total home range area, including dens, resting and retreat sites (Boitani *et al*. 1995). The large dispersion we observed in the dog maximum release point distance, core area and core release point distance emphasized the existence of 2 types of dog behaviour: fast and long distance movements versus a large proportion of relatively slow movements concentrated around the owner's home. The fact that all movement variables were correlated to each others suggests that collared dogs which moved farthest also had a tendency to have larger core areas, speeds and excursive relocations.

Our observations on dog nocturnal movements agree with Meek (1999) who found inter-individual variability in free-roaming dog home ranges and discriminated sedentary (mean home range = 2·6 ha) from wandering dogs (mean home range = 927 ha). We found that only dog sex could partly explain this observed variability (maximum distance travelled by males being more active than females) which confirms previous studies (Boitani *et al*. 1995). The inter-individual variation in nocturnal behaviour might interact and hide the effects of the nature of dog/human relationships (pastoral activity and free roaming) on nocturnal spatial behaviour. It is well known that dog-owner relationships influence social organization of the dog and consequently their territory definition and spatial utilization - owned dogs having smaller home range size than owner-less dogs (Macpherson *et al*. 2000). Repeated trajectory recordings for each individual dog and stratified sampling of the dog population would help to identify those effects. Additionally, one weakness of our movement survey is that dogs were followed during a limited time-span in May. However, dog movements might vary between months and this variation should be investigated to fully assess dog space uses in relation to the transmission risk of the parasite. For example, in winter, individual movements might be even more concentrated in village houses and their close surroundings due to the climatic conditions. Also, at the population level, the number of dogs and their frequentation of the village area should be higher because of the lower mobility of herdsmen.

Dog feces densities were found to be higher in quadrats situated inside villages than outside villages and were over-dispersed regarding their minimum distance from houses. This aggregative distribution of feces matched with dog nocturnal relocation distributions. The distance range of feces contamination (0 to 200 m) and the maximum density of feces observed at 50 m fell within the maximum distance from the owner's house recorded within the core areas (345 m). Therefore, dogs mainly defecated within their core areas. In urban areas, dog feces contamination has been observed in places close to houses, in parks and residential areas or in public playgrounds and were associated with a high rate of soil contamination by eggs of the zoonotic nematode *Toxocara* (Anvik *et al*. 1974; O'Lorcain, 1994). Concerning pastoral areas of the Tibetan Plateau, the close relationship and spatial proximity between dogs and humans may cause a considerable increase in risk of *Echinococcus* spp. transmission. Given the average *E. multilocularis* fecal prevalence found (21%) and the dog spatial defecation behaviour outlined above, we suggest that the village area, and particularly within about 200 meters around dog owners' houses, might be the place for probable higher contact between dog feces and the human population, and thus increased risk of exposure to the parasite. On the other hand, the transmission risk is likely to decrease with distance from a dog owner's house. Since the Tibetan villages we studied did not vary in feces spatial distribution patterns (and prevalences), we could cautiously generalize that a similar pattern probably occurs in other villages of the study region. Furthermore, at a higher resolution, feces were found to be aggregated within quadrats inside the village area. Scent-marking behaviour and social interactions among dogs, arising within the population area of activity, might explain such aggregation (Boitani and Ciucci, 1995). Feces of different dogs are likely to be found within such micro-aggregation zones inside villages where *E. multilocularis* environment contamination might be particularly high.

In a control programme perspective, it would be necessary to relate contamination patterns with dog individual traits, for instance to quantify the relative role of owned *vs* stray dogs in environmental contamination, and to estimate whether village contamination by feces is due to a large or low numbers of dogs. The use of DNA micro-satellites to relate individual dogs to feces sample is promising for such a purpose. Concerning the predicted transmission risk to humans, interpretations of our findings are drawn assuming that risk factors related to human behaviours are constant along a gradient from house to outside village. However, human behaviours are known to influence the transmission risk and such parameters are often missing in transmission risk estimation (Macpherson, 2005). Hygienic conditions and behaviours that induce

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direct contact with the dogs such as feeding it, playing with it and allowing it to stay inside the house, or direct contact with feces have been found to be risk factors (Craig *et al*. 2000; Wang *et al*. 2001; Schantz *et al*. 2003; Tiaoying *et al*. 2005). Investigating the spatial distribution of 'at risk' human behaviours within and around the villages would probably provide a more complete assessment of the transmission risk.

We found that dogs also defecated sporadically during their excursive trajectories, further than 200 m from houses and outside village areas up to a distance of 1519 m and therefore could contribute to the contamination of the wild reservoir (small mammals) mainly inhabiting adjacent grassland areas. Small mammals infected with *E. multilocularis* metacestodes can contain thousands of protoscoleces and dog infection pressure appears high when they predate on small mammals (Budke *et al*. 2005*b*). The same authors ran an epidemiological model to estimate the contact rate between dogs and small mammals and found a value of 0·52 infective insult per year to explain actual dog prevalences in the same study area (i.e. villages surrounding Shiqu township). This contact rate in the case of *E. multilocularis* transmission is known to rely on prey densities and prevalences (Giraudoux *et al*. 2002; Raoul *et al*. 2010) as well as on predator behaviour (Hegglin *et al*. 2007 ; Raoul *et al*. 2010). On the Tibetan Plateau, relatively high prevalences of *E. multilocularis* have been recorded in *Microtus irene* (25%) and *Ochotona curzoniae* (up to 7·7%) (Qiu *et al*. 1999). In parallel, such species are susceptible to undergo multi-annual population fluctuations (Giraudoux *et al*. 2006) with high abundance phases in particular habitats (see Raoul *et al*. (2006) for *Microtus limnophilus* and *Ochotona curzoniae* in the study area).

The current study suggests that small mammal density increases while intensity of spatial use by dogs decreases with the distance from houses. Spatial overlapping of the two host populations is therefore maximal in dog activity areas, excluding core areas, where small mammal densities are intermediate. Similar spatial patterns have been found between foxes and small mammals in peri-urban areas in Switzerland (Deplazes *et al*. 2004). Indices for small mammal species presence were found with largest frequency outside dog core activity areas, i.e. in the areas where dogs would only visit on occasional excursions. Nevertheless, small mammals were also observed, even at lower densities inside dog population areas, and even in dog core areas for one of the studied villages. This suggests that contact between dogs and grassland small mammals could even occur inside the village area and does not necessarily require dog excursions into the surrounding grassland. Thus, the possibilities of a peri-domestic parasite cycle are real, and it could be maintained in close vicinity of the dog owner houses. The villages occupied during winter or by non-transhumant human and dog populations are potential areas of small mammals/dog interactions and therefore dog contamination. This situation is probably similar in summer pastures where dogs are generally attached close to the tents and released for the night (Giraudoux, *personal observation*).

At the population level, our study constitutes a preliminary step in the quantification of the frequency of dog and small mammal interactions. Further data are required to investigate the plasticity of dog predation behaviour in relation to small mammal prey densities by dietary analysis. Since dog predation pressure could shift according to the variation in anthropogenic resources (Macpherson *et al*. 2000), particular effort should be put on studying the effects of dog 'domestication states' as defined by the feralization model developed by Boitani and Ciucci (1995), on predation behaviours. In parallel, small mammal habitats have already been defined in this region of China (Raoul *et al*. 2006; Marston, 2008) but further work is required to build an accurate predictive mapping of small mammal occurrence within the whole area surrounding villages. Ultimately such contact rate could be of particular relevance as a parameter of epidemiological models.

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REFERENCES

Afonso, E., Lemoine, M., Poulle, M.-L., Ravat, M.-C., Romand, S., Thulliez, P., Villena, I., Aubert, D., Riche, B., Rabilloud, M. and Gilot-Fromont, E. (2008). Spatial distribution of soil contamination by *Toxoplasma gondii* in relation to cat defecation behaviour in an urban area. *International Journal for Parasitology* **38**, 1017–1023.

Anvik, J., Hague, A. and Rahaman, A. (1974). A method of estimating urban dog populations and its application to the assessment of canine fecal polution and endoparasitism in Saskatchewan. *Canine Veterinary Journal* **15**, 219–223.

Bailey, D. and Alimadhi, F. (2008). logit. mixed: Mixed effects logistic. In: *Zelig: Everyones Statistical Software* (ed. Kosuke, I., King, G. and Lau, O.). URL http://gking.harvard.edu/zelig

Boitani, L. and Ciucci, P. (1995). Comparative social ecology of feral dogs and wolves. *Ethology Ecology and Evolution* **7**, 49–72.

Boitani, L., Francisci, F., Ciucci, P. and Andreoli, G. (1995). Population biology and ecology of feral dogs in Central Italy. In *The Domestic Dog: its Ecology, Behaviour and Evolution* (ed. Serpell, J. E.), pp. 217–244. Cambridge University Press, Cambridge, UK.

Budke, C. M., Campos-Ponce, M., Qian, W. and Torgerson, P. R. (2005*a*). A canine purgation study and risk factor analysis for echinococcosis in a high endemic region of the Tibetan Plateau. *Veterinary Parasitology* **127**, 43–49.

Budke, C. M., Jiamin, Q., Craig, P. and Torgerson, P. (2005*b*). Modeling the transmission of *Echinococcus granulosus* and *Echinococcus multilocularis* in dogs for a high endemic region of the Tibetan Plateau. *International Journal for Parasitology* **35**, 163–170.

Burnham, K. and Anderson, D. (1998). *Model Selection and Multimodel Inference*, 2nd Edn. Springer-Verlag, New York, USA.

Calenge, C. (2006). The package 'adehabitat' for the r software: A tool for the analysis of space and habitat use by animals. *Ecological Modelling* **197**, 516–519.

Ciucci, P., Boitani, L., Francisci, F. and Andreoli, G. (1997). Home range, activity and movements of a wolf pack in central Italy. *The Zoological Society of London* **243**, 803–819.

Clark, P. and Evans, F. (1954). Distance to nearest neighbour as a measure of spatial relationships in populations ecology. *Ecology* **35**, 445–453.

Craig, P., Giraudoux, P., Shi, D., Bartholomot, B., Barnish, G., Delattre, P., Quéré, J., Harraga, S., Bao, G., Wang, Y., Lu, F., Ito, A. and Vuitton, D. (2000). An epidemiological and ecological study of human alveolar echinococcosis transmission in South Gansu, China. *Acta Tropica* **77**, 167–177.

Craig, P. S., MacPherson, C. N. L., Watson-Jones, D. L. and Nelson, G. S. (1988). Immunodetection of Echinococcus eggs from naturally infected dogs and from environmental contamination sites in settlements in Turkana, Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **82**, 268–274.

Craig, P. and The Echinococcosis Working Group in China (2006). Epidemiology of human alveolar echinococcosis in China. *Parasitology International* **55**, 221–225.

Deplazes, P., Hegglin, D., Gloor, S. and Romig, T. (2004). Wilderness in the city: the urbanization of *Echinococcus multilocularis*. *Trends in Parasitology* **20**, 77–84.

Dinkel, A., Kern, S., Brinker, A., Vaniscotte, A., Giraudoux, P., Mackenstedt, U. and Romig, T. (2011). A real-time multiplex nested PCR system for coporological diagnosis of *Echinococcus multilocularis* and host species. *Parasitological Research*. doi: 10.1007/s00436-011-2272-0. Online First.

Dinkel, A., Romig, T. and Mackenstedt, U. (2006). PCR system for the combined detection of *Echinococcus multilocularis* and host species from faecal samples. *Deutsche Gesellschaft für Parasitologie* **22** Jahrestagung, Wien, 22.–25.02.2006, ISBN 3-200-00559-9:138.

Dinkel, A., Von Nickisch-Rosenegk, M., Bilger, B., Merli, M., Lucius, R. and Romig, T. (1998). Detection of *Echinococcus multilocularis* in the definitive host: coprodiagnosis by PCR as an alternative to necropsy. *Journal of Clinical Microbiology* **36**, 1871–1876.

Eckert, J. and Deplazes, P. (2004). Biological, Epidemiological, and Clinical Aspects of Echinococcosis, a Zoonosis of Increasing Concern. *Clinical Microbiology Review* **17**, 107–135.

Giraudoux, P., Delattre, P., Takahashi, K., Raoul, F., Quéré, J., Craig, P. and Vuitton, D. (2002). Transmission ecology of Echinococcus multilocularis in wildlife: what can be learned from comparative studies and multiscale approaches? In *Cestode Zoonoses*: *Echinococcosis* and *Cysticercosis* (ed. Craig, P. S. and Pawlowski, Z.), pp. 251–266. IOS Press Amsterdam, The Netherlands.

Giraudoux, P., Pleydell, D., Raoul, F., Quéré, J., Wang, Q., Yang, Y., Vuitton, D., Qiu, J., Yang, W. and Craig, P. (2006). Transmission ecology of *Echinococcus multilocularis*: what are the ranges of parasite stability among various host communities in China? *Parasitology International* **55**, 237–246.

Gong, M. H. and Hu, J. (2003). The summer microhabitat selection of Tibetan fox in the northwest plateau of Sichuan. *Acta Theriologica Sinica* **23**, 267–269.

Habluetzel, A., Traldi, G., Ruggieri, S., Attili, A., Scuppa, P., Marchetti, R., Menghini, G. and Esposito, F. (2003). An estimation of *Toxocara canis* prevalence in dogs, environmental egg contamination and risk of human infection in the Marche region of Italy. *Veterinary Parasitology* **113**, 243–252.

Hegglin, D., Bontadina, F., Contesse, P., Gloor, S. and Deplazes, P. (2007). Plasticity of predation behaviour as a putative driving force for parasite life-cycle dynamics: the case of urban foxes and *Echinococcus multilocularis* tapeworm. *Functional Ecology* **21**, 552–560.

Hodder, K., Kenward, R., Walls, S. and Clarke, R. (1998). Estimating core ranges: a comparison of techniques using the Common buzzard (*Buteo buteo*). *Journal of Raptor Research* **32**, 82–89.

Macpherson, C. (2005). Human behaviour and the epidemiology of parasitic zoonoses. *International Journal for Parasitology* **35**, 1319–1331.

Macpherson, C. and Craig, P. S. (2000). Dogs and cestode zoonoses. In *Dogs, Zoonoses and Public Health* (ed. Macpherson, C., Meslin, F. and Wandeler, A.), pp. 177–211. CABI International, Wallingford, UK.

Macpherson, C., Meslin, F. and Wandeler, A. (2000). *Dogs, Zoonoses and Public Health*. CABI International, Wallingford, UK.

Marston, C. (2008). Spatial modelling of small mammal distributions in relation to parasite transmission in western china. Ph.D. thesis, School of Environment and Life Sciences University of Salford, Salford, UK.

Meek, P. (1999). The movement, roaming behaviour and home range of free-roaming domestic dogs, *Canis lupus familiaris*, in coastal New South Wales. *Wildlife Research* **26**, 847–855.

Okubo, A. and Levin, S. (2001). Diffusion and ecological problems: modern perspectives. In *Interdisciplinary Applied Mathematics* 2nd Edn. (ed. Antman, S. S., Marsden, J. E., Sirovich, L. and Wiggins, S.), Springer Verlag, New York, USA.

O'Lorcain, P. (1994). Prevalence of *Toxocara canis* ova in public playgrounds in the Dublin area of Ireland. *Journal of Helminthology* **68**, 237–241.

Qiu, J., Chen, X., Ren, M., Luo, C., Liu, D. and Liu, X. (1995). Epidemiological study on alveolar hydatid disease in qinghai-xizang (Tibetan) plateau. *Journal of Practical Parasitic Diseases* **3**, 106–109.

Qiu, J., Liu, F., Schantz, P., Ito, A., Carol, D. and He, J. (1999). Epidemiological survey of hydatidosis in Tibetan areas of Western Sichuan Province. *Archivos Internacionales de la Hidatidosis, 23–84*.

R Development Core Team (2008). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org

Raoul, F., Deplazes, P., Nonakac, N., Piarroux, R., Vuitton, D. and Giraudoux, P. (2001). Assessment of the epidemiological status of *Echinococcus multilocularis* in foxes in France using elisa coprotests on fox feces collected in the field. *International Journal for Parasitology* **31**, 1579–1588.

Raoul, F., Deplazes, P., Rieffel, D., Lambert, J. C. and Giraudoux, P. (2010). Predator's dietary response to prey density variation and consequences for a cestode transmission. *Oecologia* **164**, 129–139.

Raoul, F., Quéré, J., Rieffel, D., Bernard, N., Takahashi, K., Scheifler, R., Ito, A., Wang, Q., Qiu, J., Yang, W., Craig, P. and Giraudoux, P. (2006). Distribution of small mammals in a pastoral landscape of the Tibetan Plateau (Western Sichuan, China) and relationship with grazing practices. *Mammalia* **70**, 214–225.

Robardet, E., Giraudoux, P., Caillot, C., Boue, F., Cliquet, F., Augot, D. and Barrat, J. (2008). Infection of foxes by *Echinococcus multilocularis* in urban and suburban areas of Nancy, France: infuence of feeding habits and environment. *Parasite* **15**, 77–85.

Schantz, P. M., Wang, H., Qiu, J., Liu, F. J., Saito, E., Emshoff, A., Ito, A., Roberts, J. M. and Delker, C. (2003). Echinococcosis on the Tibetan Plateau: prevalence and risk factors for cystic and alveolar echinococcosis in Tibetan populations in Qinghai Province, China. *Parasitology* **127**, 109–120.

Seaman, D. E. and Powell, R. A. (1990). Identifying patterns and intensity of home range use. Bears: Their Biology and Management. *A Selection of Papers from the Eighth International Conference on Bear Research and Management, Victoria, British Columbia, Canada* **8**, 243–249.

Seaman, D. E. and Powell, R. A. (1996). An evaluation of the accuracy of kernel density estimators for home range analysis. *Ecology* **77**, 2075–2085.

Shaikenov, B. S., Rysmukhambetova, A. T., Massenov, B., Deplazes, P., Mathis, A. and Togerson, P. R. (2004). Short report: the use of a polymerase chain reaction to detect *Echinococcus Granulosus* (G1 Strain) eggs in soil samples. *American Journal of Tropical Medicine and Hygiene* **71**, 441–443.

Thompson, R. A., Kutz, S. J. and Smith, A. (2009). Parasite zoonoses and wildlife: emerging issues. *International Journal of Environmental Health Research* **6**, 678–693.

Tiaoying, L., Qiu, J., Wen, Y., Craig, P. S., Xingwang, C., Xiao, N., Ito, A., Giraudoux, P., Wulamu, M., Wen, Y. and Schantz, P. (2005). Echinococcosis in Tibetan populations, Western Sichuan province, China. *Emerging Infectious Diseases* **11**, 1866–1873.

Veit, P., Bilger, B., Schad, V., Schäfer, J., Frank, W. and Lucius, R. (1995). Influence of environmental factors on the infectivity of *Echinococcus multilocularis* eggs. *Parasitology* **110**, 79–86.

Vuitton, D., Zhou, H., Bresson-hadni, S., Wang, Q., Piarroux, M., Raoul, F. and Giraudoux, P. (2003). Epidemiology of alveolar echinococcosis with particular reference to China and Europe. *Parasitology* **127**, 87–107.

Wang, Q., Raoul, F., Budke, C., Craig, P. S., Xiao, Y., Vuitton, D. A., Campos-Ponce, M., Qiu, D. C., Pleydell, D. and Giraudoux, P. (2010). Grass height and transmission ecology of *Echinococcus multilocularis* in tibetan communities, China. *Chinese Medical Journal* **123**, 61–67.

Wang, Q., Vuitton, D., Qiu, J., Giraudoux, P., Xiao, Y., Schantz, P., Raoul, F., Li, T., Wen, Y. and Craig, P. (2004). Fenced pasture: a possible risk factor for human alveolar echinococcosis in Tibetan pastoralist communities of Sichuan, China. *Acta Tropica* **90**, 285–293.
Wang, Z., Wang, X. and Liu, X. (2007). Selection of land cover by the Tibetan fox *Vulpes ferrilata* on the eastern Tibetan Plateau, western Sichuan Province, China. *Acta Theriologica* **52**, 215–223.
Watson Jones, D. L. and Macpherson, C. N. L. (1988). Hydatid disease in the Turkana district of Kenya V1. Man:dog contact and its role in the transmission and control of hydatidosis amongst the Turkana. *Annals of Tropical Medicine and Parasitology* **82**, 343–356.
WHO and WSPA (1990). *Guidelines for Dog Population Management*. World Health Organisation, Geneva, Switzerland.
Worton, B. J. (1989). Kernel methods for estimating the utilization distribution in home-range studies. *Ecology* **70**, 164–168.
Xiao, N., Qiu, J., Nakao, M., Li, T., Yang, W., Chen, X., Schantz, P. M., Craig, P. S. and Ito, A. (2005). *Echinococcus shiquicus* n. sp., a taeniid cestode from Tibetan fox and plateau pika in China. *International Journal for Parasitology* 35, 693–701.

APPENDIX 1. Technical details of copro-assays

*Pre-amplification PCR (*Dinkel *et al*. 2006*,* 2011*)*

- Primers: P60 short.for (5′-TGG TAC AGG ATT AGA TAC CC-3′)/P375 short.rev (5′-TGA CGG GCG GTG TGT ACC-3′) and CVF for (5′-TTA ATG ACC AAC ATT CGA AA-3′)/CVF rev (5′-AGG/T ACA/G TAG/C CCC ATA/G AAA/T GC-3′)
- PCR reaction mixture: 15 mM Tris–HCl (pH 8·0), 50 mM KCl, 2·5 mM $MgCl_2$, 200 μM of each dNTP, 30 pmol of each cestode primer (P60 short.for/P375 short.rev), 40 pmol of each carnivore primer (CVF for/CVF rev) and 2·5 units AmpliTaq Gold (Applied Biosystems).
- Cycles: denaturation for 30 s at 94 °C, annealing for 1 min at 54 °C and elongation for 40 s at 72 °C.

*Rtm-nested PCR (*Dinkel *et al*. 2011*)*

- LightCycler glass capillaries: 5 pmol of primer Pnest.for (5′-ACA ATA CCA TAT TAC AAC AAT ATT CCT ATC-3′) and Pnest.rev (5′-ATA TTT TGT AAG GTT GTT CTA-3′) (Dinkel *et al*. 1998), 10 pmol of primer CVF light.for and CVF light.rev, 2 μM of each hybridization probe (emulti-fl, emulti-705, CaVuFe1-fl and CaVuFe2-640), 2 μl of LightCyler DNA Master HybProbe (Roche, Mannheim, Germany), 2·5 mM $MgCl_2$ and 1 μl of PCR product of the first PCR. gg
- Cycling conditions: 15 s at 94 °C, followed by 45 cycles of 4 s at 94 °C, 15 s at 50 °C and 25 s at 72 °C.
- Melting curve analysis: 4 s at 94 °C, 15 s at 50 °C and 25 s heating to 90 °C with a ramping rate of 0·2 °C/s for emulti-fl/emulti-705 and 10 s at 95 °C, 10 s at 30 °C and heating to 90 °C (0·2 °C/s) for CaVuFe1-fl and CaVuFe2-640 (melting temperature [Tm] 60·0 °C *E. multilocularis; V. vulpes* 56·0 °C; *V. corsac* 45·4 °C; *V. ferrilata* 44·8 °C; *C. lupus/familiaris* 38·0 °C).

*Rt-nested PCR (*Dinkel *et al*. 2011*)*

- PCR reaction mixture: 10 pmol of each primer (CVF light. for and CVF light.rev), 2 μM of each hybridization probe (CaVuFe1-fl and CaVuFe2-640), 2 μl of LightCyler DNA Master HybProbe (Roche, Mannheim, Germany), 2·5 mM $MgCl_2$ and 1 μl of PCR product of the first PCR.
- Thermal cycling and melting curve analysis was done as described for rtm-nested PCR.

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