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Spatial distribution of soil contaminated with *Toxoplasma gondii* oocysts in relation to the distribution and use of domestic cat defecation sites on dairy farms

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ABSTRACT

Little information is available on the relationship between the spatial distribution of zoonotic parasites in soil and the pattern of hosts' faeces deposition at a local scale. In this study, the spatial distribution of soil contaminated by the parasite *Toxoplasma gondii* was investigated in relation to the distribution and use of the defecation sites of its definitive host, the domestic cat (*Felis catus*). The study was conducted on six dairy farms with a high number of cats (seven to 30 cats). During regular visits to the farms over a 10 month period, the cat population and cat defecation sites (latrines and sites of scattered faeces) on each farm were systematically surveyed. During the last visit, 561 soil samples were collected from defecation sites and random points, and these samples were searched for *T. gondii* DNA using real-time quantitative PCR. Depending on the farm, *T. gondii* DNA was detected in 37.7–66.3% of the soil samples. The proportion of contaminated samples at a farm was positively correlated with the rate of new cat latrines replacing former cat latrines, suggesting that inconstancy in use of a latrine by cats affects the distribution of *T. gondii* in soil. On the farms, known cat defecation sites were significantly more often contaminated than random points, but 25–62.5% of the latter were also found to be contaminated. Lastly, the proportion of positive *T. gondii* samples in latrines was related to the proximity of the cats' main feeding and resting sites on the farms. This study demonstrates that *T. gondii* can be widely distributed in farm soil despite the heterogeneous distribution of cat faeces. This supports the hypothesis that farms are hotspot areas for the risk of *T. gondii* oocyst-induced infection in rural environments.

1. Introduction

Soil contaminated with helminth eggs or protozoan (oo)cysts is an important source of infection for animals and humans (Torgerson and Macpherson, 2011). The large number of these free-living stage parasites excreted by hosts in the environment and their ability to survive for prolonged periods under a variety of conditions pose a significant threat to veterinary and medical health (Slifko et al., 2000; Alum et al., 2010). Eggs or (oo)cysts are deposited in the environment with definitive host faeces. However, the pattern of faeces deposition by hosts is often a non-random process that generally depends on both behavioural and environmental factors. This is particularly true for species of carnivores (MacDonald, 1980; Buesching and Jordan, in press), which can be definitive hosts of parasites transmissible through the environment to their intermediate or accidental hosts. As a consequence, the deposition pattern of carnivore faeces often leads to a heterogeneous distribution of parasites at the local scale and is notably considered a key parameter in the transmission dynamics of many parasites such as *Toxocara* sp. (Milkovic et al., 2009), *Echinococcus multilocularis* (Raoul et al., 2015) or *Toxoplasma gondii* (Afonso et al., 2008).

Toxoplasma gondii is a protozoan parasite responsible for toxoplasmosis, a prevalent zoonosis in humans and other warm-blooded animals (Tenter et al., 2000). In healthy humans, toxoplasmosis is generally asymptomatic but it can cause serious

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illness in immunocompromised individuals or when acquired congenitally (Robert-Gangneux et al., 2015). Human and animal infection can result from the ingestion of any of the three infective stages of *T. gondii*: tachyzoites and bradyzoites contained in infected hosts and sporozoites contained in sporulated oocysts. Oocysts spread into the environment in faeces of felids, mainly those of the domestic cat (*Felis silvestris catus*), which is the main definitive host of the parasite (Dubey, 2010). When infected for the first time, cats can shed millions of oocysts in their faeces over a period of 7–20 days. Oocysts become infective in the environment within 1–5 days (after sporulation) and can survive for months in soil and water (Yilmaz and Hopkins, 1972; Frenkel et al., 1975; Lélu et al., 2012). Oocysts can be a source of direct infection for humans through the consumption of contaminated water (Villena et al., 2004), shellfish (Chiang et al., 2014), vegetables and fruit (Lass et al., 2012) or through the accidental ingestion of oocysts after contact with contaminated soil (Cook et al., 2000; Tenter et al., 2000). They can also be a source of indirect infection for humans through the consumption of undercooked or raw infected meat from livestock animals exposed to oocyst-contaminated soil (Cook et al., 2000; Tenter et al., 2000; Wilking et al., 2016). Reducing the risk of human and livestock ingestion of *T. gondii* oocysts has been identified as a key issue in toxoplasmosis prevention (Schlüter et al., 2014). This challenge requires identification of the factors that drive the spatial distribution of oocysts in the environment.

The dispersion or potential accumulation of *T. gondii* oocyts in environmental matrices is partly determined by the pattern of cat defecation. Domestic cats living in social groups usually defecate in communal defecation sites called latrines, although some faeces can also be deposited outside these sites (Corbett, L.K. 1979. Feeding ecology and social organization of wildcats (*Felis silvetris*) and domestic cats (*Felis catus*) in Scotland. PhD thesis, University of Aberdeen, Scotland; Bradshaw et al., 2012). This particular pattern of faeces deposition leads to high densities of infective oocysts concentrated in micro-foci where infection risks for animals and humans can be high. In urban areas, several studies have demonstrated that places thought to be favoured by cats for defecation (e.g. sandpits, playgrounds, parks and gardens, areas around rubbish dumps) were often contaminated with *T. gondii* (Frenkel et al., 1995; Lass et al., 2009; Du et al., 2012; Ajmal et al., 2013). In the city of Lyon in France, Afonso et al. (2008) searched for *T. gondii* contamination in soil samples from both cat latrines and random sites and found parasite DNA only in the former. In contrast, in rural areas *T. gondii* distribution in soil has been shown to be largely dispersed within and around villages (Gotteland et al., 2014b,c).

In rural areas, livestock farms have been shown to act as sources of *T. gondii* contamination. This results from several (non-exclusive) factors that characterize these farms: (i) a high density of free-ranging cats (Liberg, 1980; Ferreira et al., 2011; Kitts-Morgan et al., 2015), (ii) a large spatial overlap between definitive and intermediate hosts in a small area, favouring *T. gondii* transmission (Afonso et al., 2013), (iii) a location in an open landscape where contact between domestic and wild animals is frequent (Afonso et al., 2013), and (iv) parasite dissemination from farms to nearby vicinities via soil disruption by livestock (Lehmann et al., 2003; Vasileiou et al., 2015). In animals, the role of farms in *T. gondii* transmission has been demonstrated in both definitive hosts (Afonso et al., 2013) and intermediate hosts (Lehmann et al., 2003; Richomme et al., 2010; Gotteland et al., 2014a), for which the density and proximity of farms influence the proportion of infected individuals. In humans, people living on farms were found to be more frequently exposed to *T. gondii* than others (Sroka et al., 2010; Muñoz-Zanzi et al., 2013). Furthermore, high *T. gondii* prevalence levels have been detected or predicted in soil and water samples collected near farms (Ajmal et al., 2013; Gotteland et al., 2014c). Although the spatial distribution of *T. gondii* oocysts on farms is of special concern in order to prevent the infection of animals and humans living there (Schlüter et al., 2014), as well as to avoid contamination of the surrounding environment (Gilot-Fromont et al., 2012), its determinants are still poorly known.

In this study, to our knowledge for the first time, the spatial distribution of soil contaminated by *T. gondii* oocysts on dairy farms was investigated in relation to the distribution and use of cat defecation sites. Dairy farms were chosen because the milk distributed by farm owners to cats (used for rodent population control; Elton, 1953 in Kitts-Morgan et al., 2015) maintains farm cat populations in densities ranging to 5–50 cats/km^2 with individuals having overlapped home ranges centred on farm buildings (Liberg et al., 2000). These locations were expected to provide reliable information on the influence of cat faeces distribution on soil contamination. Variations in soil contamination were explored both on farms and between farms in relation to the spatial distribution of cat defecation sites and to the size and composition of the cat population. Soil contamination on dairy farms was expected to be higher in cat defecation sites than elsewhere, as observed in urban areas, but was also expected to occur outside latrines due to the dissemination of oocysts, as observed and predicted by Gotteland et al. (2014b,c). In addition, variability in the contamination level between latrines was investigated in relation to both the intensity of latrine use by cats and the locations of latrines in respect to the cat activity pattern on farms.

2. Materials and methods

2.1. Study sites

The study was conducted from March to December 2014 on six dairy farms located in a 137.9 km^2 rural area in the Ardennes region of northeastern France (49°27′3.49″N – 4°47′0.7″E to 49°27′41.26″N – 5°3′3.35″E; Fig. 1A). The climate is temperate continental, with long, cold wet winters; the average monthly temperature ranges from −0.7 °C to 14.9 °C between 1 October and 30 March (www.meteofrance.com) and mean annual precipitation is 958 mm per year. The area is wooded, with cultivated fields and pastures and a low human population density (approximately 16 inhabitants per km^2) spread out in small villages (most of those with less than 200 inhabitants). More than 60% of both the domestic cat and wildcat (*Felis silvestris silvestris*) populations have been described as being infected with *T. gondii* in this area (Afonso et al., 2013).

The number of studied farms (n = 6) was constrained by financial, human and logistic limitations (only one person to collect all data in the field), but a power analysis was conducted on simulated data prior to the study to verify the ability of the sample size to detect significant variations if any (Supplementary Data S1, Supplementary Tables S1 and S2). Livestock production on the farms was semi-intensive to intensive (32–74 dairy cows and 0–58 beef cattle per farm; Table 1). Dairy and beef cattle were annually put out to pasture for summer grazing and remained inside farm buildings during winter. Four of the six farms (farms A, B, C and E) were located 0.25 km to 0.92 km from the nearest village and were mostly surrounded by fields of crops and pastures, whereas two farms (D and F) were located inside villages and surrounded by houses. The study area on each farm was demarcated by a 20 m buffer zone around each building, which corresponded to the core area of the cat population living there (Gotteland et al., 2014b). A compilation of the overlapping 20 m buffer zones resulted in a continuous contour line delimiting the entire study area composed of all the farm buildings, the courtyard and a small proportion of sur-

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