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Abstract

Toxoplasma gondii is the parasite responsible for toxoplasmosis, a highly prevalent zoonosis that affects humans and warm-blooded animals. Faeces of infected cats can contain millions of *T. gondii* oocysts, which remain infectious in the environment for months. Sites repeatedly used by cats for defecation ('latrines') are recognised as hotspots of *T. gondii* soil contamination, but this contamination varies from one latrine to another. To understand this spatial heterogeneity, camera traps were deployed in 39 cat latrines on three dairy farms with high-density cat populations and programmed to record visits during sixteen 10-day sessions, rotating between three farms over a period of a year. Generalized Linear Mixed Models were used to test the effects of cat sexual maturity, latrine location and season on the number of cat faeces deposited and on the number of cats defecating per latrine, as determined from the analysis of 41,282 video recordings. Sexually immature cats defecated 6.60-fold (95% CI = [2.87–15.25]) more often in latrines located close to a feeding site than in other latrines. This pattern was also observed for mature males (odds ratio [OR] = 9.42, 95% CI = [3.29–26.91]), especially during winter, but not for mature females (OR = 1.77, 95% CI = [0.80–3.94]). The number of defecating cats was also 2.67-fold (95% CI = [1.66 – 4.30], $P < 0.001$) higher in latrines located close to a feeding point than in those located far from it, regardless of cat category and season. Visits by intermediate *T. gondii* hosts (micromammals, birds and others) were also recorded. Out of the 39 latrines, 30 (76.92%) were visited by at least one intermediate host during the study period, and some latrines were highly frequented (up to 8.74 visits/day on average). These results provide evidence that the location of food resources in dairy farms influences the latrine use pattern by cats. Highly frequented latrines can be of high risk of *T. gondii* infection for definitive and intermediate hosts.

Keywords: *Felis silvestris catus*; video trap; cat faeces; toxoplasmosis; intermediate hosts.

1. Introduction

Wild and domestic carnivores are often infected by gastrointestinal zoonotic parasites (Polley 2005; Di Cerbo et al., 2008; Baneth et al., 2016), so their faeces are an important source of environmental contamination with parasitic eggs or (oo)cysts (Robertson and Thompson, 2002). The distribution of infected carnivore faeces largely determines the probability of contact between parasites and their hosts (Traversa et al. 2014). It may also be an important determinant of the zoonotic disease risk for humans especially when faeces are concentrated in human-use environment (Knapp et al., 2018, Bastien et al., 2018). As a consequence, identifying the spatiotemporal pattern of faeces deposition by carnivores constitutes a major issue in understanding the transmission dynamics of zoonotic parasites (Afonso et al., 2008; Milkovic et al., 2009; Raoul et al., 2015).

The faeces deposition pattern by carnivores is usually a non-random process. Several behavioural constraints could exert concurrently, such as the use of faeces for chemical communication (e.g. Rodgers et al., 2015), space use pattern and movements, themselves depending on ecological factors such as microhabitat and/or prey distribution (e.g. Guislain et al., 2007), selection of specific soil substrate to defecate (e.g. Soler et al., 2009), and/or repeated use of shared defecation sites by one or more individuals (e.g. Jordan et al., 2007). Repeated defecation at the same locations, called “latrines”, is observed in several wild carnivore species (see Macdonald, 1985; Rodgers et al., 2015; Buesching et al., 2016) as well as in the domestic cat (*Felis silvestris catus*) when living in social groups (Bradshaw et al., 2012). This particular pattern of faeces deposition may result in the accumulation of free parasite stages in spatially restricted areas, as demonstrated for the high occurrence of *Baylisascaris procyonis* eggs in raccoons (*Procyon lotor*) latrines (Page et al., 1998, Page et al. 2009) or *Toxoplasma gondii* oocysts in domestic cat latrines (Afonso et al., 2008; Simon et al., 2017).

Toxoplasma gondii is a protozoan parasite that can cause serious health issues for humans (Hill and Dubey, 2002) and other warm-blooded intermediate hosts like livestock (Tenter et al., 2000)

and wildlife (Conrad et al., 2005). Felids are the only definitive hosts. They can shed millions of oocysts after ingestion of any of the three infective stages of *T. gondii.* Their transmission pathway is through tachyzoites and bradyzoites contained in preys mainly, through sporozoites to a lesser extent (Dubey, 2006, 2010). After sporulation, oocysts can survive and remain infective for months in soil (Lélu et al. 2012) and water (Lindsay and Dubey, 2009). They represent a potential source of *T. gondii* infection in animals (Van Wormer et al., 2013), as well as in humans through the consumption of contaminated food (fruits and vegetables) and water, or through contact with contaminated soil (Muñoz-Zanzi et al., 2010; Boyer et al., 2011). Among definitive hosts, the domestic cat plays a key-role in the epidemiology of the parasite (Tenter et al., 2000; Dabritz and Conrad, 2010). This is especially true in locations where food resources are deliberately or accidentally provided from human activities. In this case, cats live at high local densities around spatially aggregated resources, such as in livestock farms, around rubbish dumps, industrial and hospital sites, or in certain public areas (Bradshaw et al., 2012). Dairy farms were especially identify as an important source of contamination in rural areas (Lehmann et al., 2003; Richomme et al., 2010; Afonso et al., 2013; Gotteland et al., 2014a). Farm owners use to leave milk available for cats to retain them on the site. This contributes to maintain farm cat populations in densities up to 50 cats/km^2 (Liberg et al., 2000) with high *T. gondii* seroprevalence and infection rates (Simon et al., 2018).

Two studies conducted on free-ranging cats living in high-density groups reported high levels of soil contamination by the parasite in cat latrines compared to other sites: from 9% to 13% and from 39% to 69% of latrine soil sample contained *T. gondii* DNA in an urban area (Afonso et al., 2008) and in dairy farms (Simon et al., 2017) respectively. However, these studies showed that not all latrines were systematically contaminated, suggesting heterogeneity in the spatial deposition of contaminated faeces. The determinants of such heterogeneity are important to understand, especially if latrines are located in areas where animals or humans can be in contact with the soil. On dairy farms, latrines close to a cat feeding area were more often contaminated

than those distant from a feeding area, suggesting their differential use by cats (Simon et al., 2017). However, authors did not find a significant effect of the number of detected faeces per latrine on the occurrence of *T. gondii* DNA in soil samples. They rather suggested a preferential use of latrines close to feeding areas by juvenile cats, known to be the main contributors to oocyst excretion in the environment (Dubey et al., 1995; Weigel et al., 1995; Gauss et al., 2003).

Juvenile cats have small home range centred on the main food resources (Bradshaw et al., 2012; Turner and Bateson, 2013) and are therefore more likely to use defecation sites close to a feeding area. On the contrary, sexually mature males and females living on farms have a larger home range, averaging 2 ha to 430 ha (Germain et al., 2008; Ferreira et al., 2011), which can vary seasonally in response to behavioural and environmental factors. Sexually mature males are known to extend their home range during the mating season (from January to June; Izawa et al., 1982) whereas time spent on farm by sexually mature females may depend on nursing kittens. In addition, adult cats spend more time on farm and move at shorter distances from buildings when precipitations are high and temperatures are low (Goszczynski et al., 2009; Horn et al., 2011). All these factors may influence the space use pattern by cats and their probability of using latrines, but their effect has never been tested.

In this study, based on a camera traps survey and a generalized linear mixed model analysis, we tested the effect of feeding area proximity, season and cat sexual maturity on cat latrine use, in order to ultimately understand why latrine location alter the *T. gondii* contamination of soil. We hypothesized that sexually immature individuals preferentially use latrines located near feeding sites, and that sexually mature cats mainly defecate in farm latrines during cold and wet months, with potential differences between males and females. Camera traps were also used to record latrines visits by *T. gondii* intermediate hosts (mammals and birds) to complete the identification of circumstances leading to hotspots of *T. gondii* transmission in dairy farms.

2. Material and methods

2.1. Study sites

127 The study was conducted on three dairy farms (A, B and C) in a rural area of north-eastern France
128 (49°27'3.49"N, 4°47'0.7"E to 49°27'41.26"N, 5°3'3.35"E; Figure 1). In these farms, cats live in
129 medium to large groups (seven to 24 individuals per group; Table 1). Incidence of *Toxoplasma*
130 *gondii* in the three cat populations ranged from 0.88 to 0.96 seroconversion/cat/year (Simon et al.,
131 2018) and 50% to 66% of the soil samples previously collected were contaminated with oocysts
132 (Simon et al., 2017). The region has a temperate continental climate, with cold wet winters
133 (average monthly temperature ranges from -1°C to 11°C and average monthly precipitation from
134 54 to 71 mm between November and March) and mild summers (average monthly temperature
135 ranges from 4.4°C to 23.5°C between April and October; https://fr.climate-data.org). Cattle were
136 permanently at pasture from April to October/November and remained inside farm buildings
137 during winter. The three studied farms were situated at a distance of 9.67 km to 19.81 km apart
138 and were located on the periphery of a village surrounded by fields of crops and pastures. As
139 previously described by Simon et al. (2017), the study sites corresponded to the farm buildings
140 plus a 20-m surrounding buffer zone, encompassing the core activity area of the cat population
141 living there (Barrat, 1997; Gotteland et al., 2014b). The resulting delimited study area was 0.88 ha
142 in farm A, 1.14 ha in farm B, and 1.58 ha in farm C (Figure 1).

143 **2.2. Cat population monitoring**

144 The cat population on each farm was intensively monitored during one year. Information on cat
145 abundance, cat births and mortality was recorded monthly by direct observation and from
146 information collected from farm owners. As a part of a capture-mark-recapture procedure (Simon
147 et al., 2018), cats were sexed and photographed to create a portfolio used for individual
148 identification based on coat colour pattern. Cats from the same farm with a similar coat colour
149 were also fitted with plastic collars of different colours to be differentiated, as previously made in
150 another study based on camera-traps (Elizondo and Loss, 2016). The age of cats was also
151 estimated based on direct observations at very young age, information collected from farmers and
152 teeth development (see Courchamp et al., 2000).

153 **2.3. Latrine identification**

154 The three studied farms were systematically searched for cat latrines during visits performed
155 before each session of camera trap deployment (see below). Latrine identification was performed
156 by the same person at each visit to avoid inter-individual detection bias. Following Simon et al.
157 (2017), a latrine was defined as a site with at least two faeces of different ages (indicating reuse
158 over time) within one metre of each other. Latrines may also show conspicuous signs of recent cat
159 activity such as presence of footprints and scarification/scratches and/or ploughed substratum.
160 Two latrine sites were considered as different entities when they were separated by at least five
161 metres.

162 **2.4. Camera trap survey**

163 The latrines were surveyed by 10 to 13 infrared motion-triggered camera traps (an average of 12
164 per farm) deployed from August 2014 to July 2015 during sessions of 10 consecutive days per
165 farm, rotating from one farm to another. Ten Bushnell Trophy Cam HD-119437 cameras and
166 three Bushnell Trophy Cam HD-119537 cameras were used. Both models feature an 8-megapixel
167 digital camera and an infrared flash that is considered less likely to disturb animals than visible
168 white-light flashes (Glen et al., 2013; Trolliet et al., 2014, but see Meek et al., 2016). The
169 movement detection range was 15 m for the ten Bushnell-119437cameras and 24 m for the three
170 Bushnell-119537cameras. The trigger speed was set to 1s and the minimal trigger interval to 5s.
171 Depending on the size of a latrine, one to four cameras were deployed to survey the entire surface
172 area used by cats to defecate. The cameras were positioned so as to optimise the detection of cats
173 using the latrine (height and orientation of cameras; Rovero et al., 2010; Glen et al., 2013).
174 However, camera placement was constrained by the availability of appropriate supports on a farm
175 for mounting the camera trap, as this required a stable, solid support unlikely to be removed
176 during the survey session and that did not interfere with the farmer's activities.
177 All the cameras were programmed to record 40s of motion-triggered video footage continuously
178 24 hours a day. The date and time were displayed for each video capture. The duration of the

after-trigger recording time was determined based on a preliminary study performed in June and July 2014 on farm C. Forty seconds of duration was chosen as a good compromise between the time required to record a complete cat defecation sequence (positioning, elimination of faecal matter and, possibly, covering of faeces) and the time required to watch the video footage in order to analyse it. A total of sixteen 10-day recording sessions (five to six sessions per farm) were performed during the one-year study period, theoretically corresponding to a total of 160 recording days. However, due to organizational and unforeseen material issues (loss of battery power, camera failure, change in the field of view or camera disturbance by cats and farmers, *etc.*), some sessions were shorter or longer than 10 days (see Table 1).

2.5. Recorded information

The recorded video footage was watched and analysed by three readers, each analysing one farm. Doubtful events were discussed among readers to avoid divergent interpretations. The information collected from a farm's video footage was entered in a Microsoft Excel database and included the camera site, date, time, session, farm and information regarding the cat and defecation event (see below). The data in the three databases was then verified and homogenised by one person.

2.5.1. Identification of cat and defecation events

Cat defecation events observed at latrines were recorded in the database. An event was entered as 'defecation' when a cat was observed in a typical defecating position (rounded back with the anus close to the ground, with or without a visible elimination of faecal matter). A defecating cat observed in video footage was individually identified based on the colour pattern of its coat and/or coloured collar. Its individual characteristics (sex and age class) at the time of the video footage were also noted in the database. When cat identification was not possible, due to partial observation or to poor readability of the video, the individual was recorded as 'NI' (Not Identified). In this case, a sex and/or an age-class were assigned insofar as possible (see section 2.6).

2.5.2. Latrine visits by intermediate hosts

Intermediate hosts observed in the video footage were categorised in three groups: micromammals (small rodents and shrews), birds and others. A visit from an intermediate host was defined as an observation of one individual of a given species on successive footage within a single 15-min time interval (Kukielka et al., 2013). If several individuals were recorded in the same footage, one visit per individual was recorded. All observations of individuals in the defined latrine area or in its proximity (approximately 5 m around it) were recorded in the database. Visit rates, corresponding to the number of visits per day/latrine/session, were estimated separately for each group of intermediate hosts.

2.6. Definition of variables

For the analysis of latrine use by cats, the explanatory variables were the cat's category (*Cat*) according to its sex and sexual maturity, the season (*Season*) and the location of the latrine in relation to the nearest feeding site (*Distance*). Three cat categories were distinguished: 'sexually immature' (male or female), 'sexually mature male' and 'sexually mature female'. The sexual maturity of females was determined according to age and season: females born in spring and summer (from March to August) were considered mature from the beginning of the breeding period the following year (February), provided they had reached 6 months of age, whereas females born in autumn (after August) were considered mature from 6 months of age (Turner and Bateson, 2013). Based on average values given in published literature, males were considered mature when they reached 10 months of age (Say et al., 1999; Turner and Bateson, 2013).

A latrine was considered near a feeding point when it was at a distance of less than 18.5 m, which corresponds to the estimated average maximum radius of cat activity areas around feeding points in the three studied farms during the study period (Simon et al., 2017). A feeding point corresponds to a site where farm owners feed cats daily (generally with milk, food remains or commercially available cat food).

To determine seasonal variation in latrine use, two ecological seasons were determined on the basis of changes in average monthly precipitation and temperature (data obtained from www.meteofrance.com): 1st April to 30th September ('summer') and 1st October to 31st March ('winter'). This season determination is also relevant from an epidemiological point of view, as cats on the three farms had a higher infection risk from October to March than from April to September (Simon et al., 2018).

2.7. Statistical analyses

The intensity of the latrine use by cats was assessed by two indicators: the number of defecations made by cats of a given category per latrine per session, and the total number of cats of a given category that defecated at least one time in a given latrine per session. Both were analysed in relation to the three fixed factors: *Cat*, *Season* and *Distance*. Generalized Linear Mixed Models (GLMMs) with a Poisson distribution and a logarithm link were used as is usual in analysing count data (Zuur et al., 2009). To take into account the duration of each session and the number of available cats in each farm, both the number of surveillance hours per latrine and per session and the total number of individuals per category and per farm (varying among sessions due to births and mortality) were considered as offsets (Table 1). The *Farm* and *Latrine-Session* (*i.e.* the 10-day survey session of a specific latrine) variables were considered as random effects to take into account the likely dependence of latrines within a given farm and the dependence of cat defecation events within a given latrine. The *Latrine* variable was also considered as a random effect to account for temporal autocorrelations that can result from the repeated survey of a latrine among sessions. These three nested effects were tested using the likelihood ratio test (LRT) on the complete model including all fixed effects and their 2 by 2 interactions.

After selecting random effects, all models were ranked based on Akaike's information criterion for a small sample size corrected for overdispersion (QAICc). The model with the lowest QAICc was considered the best fit to explain the data. A difference of > 2 in the QAICc between two models was used to consider them not equivalent (Burnham and Anderson, 2002). If the

255 difference in QAICc between two models was < 2, only the most parsimonious model (*i.e.* the
256 model with the smallest number of parameters) was retained. From the best model retained,
257 Likelihood Ratio Tests (LRT) were performed to assess the significance of variables. Adjusted
258 odds ratios were estimated with 95% confidence intervals for each comparison of classes, and
259 Wald tests were used to determine the significance of differences between classes of each
260 variable. As variance values were often higher than means, we checked whether over-dispersion
261 was still present in the residuals of the selected models by calculating "sum of squared Pearson
262 residuals/degree of freedom" ratios (Zuur et al., 2009). Residuals were visually examined for
263 normality, homogeneity and independence.
264 All the analyses were performed with R 3.1.3 statistical software (R Core Team, 2014), with a
265 threshold P-value of 0.05. Models were adjusted using the 'lme4' package.

266 **3. Results**

267 **3.1. Pattern of latrine use by cats**

268 ***3.1.1. Collected data***

269 The number of cats per farm varied from six to 14 adults and zero to 13 juveniles, depending on
270 farm and session (Table 1). It increased from spring to early autumn, with the succession of litters,
271 but the high mortality of kittens during their first weeks rapidly reduced the population size.
272 A total of 39 latrines were surveyed during the study (three to nine per session per farm; Table 1).
273 A total of 895 camera-days were recorded on latrines, resulting in 41,282 motion-triggered video
274 recordings. However, of 28% of them were excluded from the database due to camera
275 malfunction (*e.g.* loss of battery power, camera failure) or modifications to the field of view (often
276 due to camera disturbance linked to farming activities). In total, 29,698 videos were analysed,
277 leading to the characterization of 486 defecation events: 99 on farm A, 254 on farm B, and 133 on
278 farm C. The individual identification of cats failed for 20 identification events, but 10 of these cats
279 could be classified in one of the three categories. Defecation occurred in 66 out of the 93 recorded
280 "latrine-sessions" (70.97%), but the frequency of defecation events was highly heterogeneous

among farms, survey sessions and latrines. Depending on the farm, cats were not observed defecating in five to 13 'latrine-sessions'. In contrast, 16 latrines (4 on farm A, 8 on farm B, and 4 on farm C) received 10–38 defecations during a given survey session.

3.1.2. Number of defecation events in latrines

During a 10-days survey session, the average number of defecations per latrine per session was 3.96 (standard deviation (sd) = 6.30) on farm A, 10.58 (sd = 13.33) on farm B, and 3.04 (sd = 3.79) on farm C. Only the nested effects of *Latrine* and *Latrine-Session* had significant random effects (Likelihood ratio test: $\chi^2 = 23.16$, df = 1, $P < 0.001$ and $\chi^2 = 349.20$, df = 1, $P < 0.001$ respectively). The most relevant explanatory model of the number of cat defecation events in latrines included the fixed effects of *Cat*, *Season* and *Distance* (Table 2 and Table 3). Interactions between *Cat* and *Season* and between *Cat* and *Distance* were also retained whereas the interaction between *Season* and *Distance* was not significant (Likelihood ratio test: $\chi^2 = 0.30$, df = 1, P = 0.58).

As expected, a higher number of defecation events from immature cats was observed in latrines located close to a feeding site than in latrines far from a feeding site (OR = 6.60, 95% CI = [2.87 – 15.25], $P < 0.001$; Figure 2). Sexually mature males also defecated more often in latrines close to a feeding site than in latrines far from a feeding site (OR = 9.42, 95% CI=[3.29 – 26.91], $P < 0.001$). However, seasonal variations were observed in mature males behaviour as the number of their faeces deposited in latrines, whatever the location, was higher in winter than in summer (OR = 2.19, 95% CI = [1.14 – 4.18], P = 0.018). Concerning sexually mature females, no difference was observed between their use of latrines close to a feeding site or far from a feeding site in either season (OR = 1.77, 95% CI = [0.80 – 3.94], P = 0.16 and OR = 1.04, 95% CI = [0.54 – 2.03], P = 0.90 respectively).

In summer, the number of faeces deposited in latrines located close to a feeding site was significantly higher from sexually immature individuals than from mature females (OR = 1.64, 95% CI = [1.20 – 2.25], P=0.002) and from mature males (OR = 2.51, 95% CI = [1.81 – 3.48], P

307 < 0.001; Figure 2). Mature females also defecated more often in these latrines than mature males
308 (OR = 1.53, 95% CI = [1.07 – 2.18], P = 0.019). In latrines located far from a feeding site, mature
309 females defecated more often than mature males and immature cats (OR = 7.92, 95% CI = [3.11 –
310 20.21], P < 0.001 and OR = 2.26, 95% CI = [1.16 – 4.41], P = 0.016 respectively) and immature
311 cats defecated more often than mature males (OR = 3.52, 95% CI = [1.33 – 9.30], P = 0.011).
312 In winter, no difference in the number of faeces deposited in latrines close to a feeding point was
313 observed between immature cats and mature males or females (OR = 0.88, 95% CI = [0.63 –
314 1.24], P = 0.48 and OR = 1.32, 95% CI = [0.87 – 1.99], P = 0.18 respectively) neither between
315 mature males and mature females (OR = 1.49, 95% CI = [1.00 – 2.23], P = 0.051; Figure 2). In
316 latrines located far from a feeding site, mature females defecated more often than mature males
317 and immature cats (OR = 3.57, 95% CI = [1.36 – 9.40], P = 0.01 and OR = 2.83, 95% CI = [1.34–
318 5.97], P = 0.006 respectively) but no difference was observed between immature cats and mature
319 males (OR = 1.26, 95% CI = [0.46–3.43], P = 0.65).

320 ***3.3.3. Number of defecating cats***

321 During a video trap session, one to eight cats used the same latrine to defecate. The average
322 number of cats using a latrine was 2.47. The nested effects of *Latrine* and *Latrine-Session* had
323 significant random effects (Likelihood ratio test: $\chi^2 = 8.17$, df = 1, P = 0.004 and $\chi^2 = 7.15$, df = 1,
324 P = 0.007 respectively) whereas the effect of *Farm* was not retained in the model (Likelihood
325 ratio test: $\chi^2 = 0.08$, df = 1, p = 0.77). The most parsimonious model included only the fixed
326 variable *Distance*. The estimated parameters of this model (Table 3) indicated that the latrines
327 located close to a feeding point were used by 2.67-fold more individuals than those located far
328 from a feeding point, regardless of cat category and season (95% CI = [1.66 – 4.30], P < 0.001;
329 Figure 3). The other variables considered in other models for which QAICc < 2 had no significant
330 effects on the number of defecating cats (see Table 2).

331 **3.2. Latrine visits by intermediate hosts**

In total, the video footage for all farms revealed 74 visits to latrines from micromammals (17 rats *Rattus sp.*, 9 mice *Muridae*, four shrews *Soricidae*, one vole *Cricetidae*, and 43 unidentified), 1068 from birds (pigeons, corvids and other passerines) and 94 from other species groups (54 red foxes *Vulpes vulpes*, 38 European hedgehogs *Erinaceus europaeus*, one marten *Martes sp.*, one European hare *Lepus europaeus*). Visits from these intermediate hosts occurred in 30 of the 39 detected latrines corresponding to 76.92% of all the latrines: 64.28% on farm A, 84.61% on farm B and 83.33% on farm C. The visit rate of latrines was heterogeneous between species, farms and latrines (Figure 4). Visit rates were higher for birds (0.02 – 874 visits/day per latrine, with an average visit rate of 1.83 ± 2.28) than for micromammals (0.04 – 1.35 visits/day per latrine, average = 0.38 ± 0.44) and other species groups (0.05 – 1.17 visits/day, average = 0.27 ± 0.28).

4. Discussion

Considering that a cat defecates once a day (Liberg, 1982), and assuming that they defecate only in farm latrines, the expected number of defecation events during the survey period according to the number of cats per farm would be 579 on farm A, 1184 on farm B and 965 on farm C. Compared to these predictions, only 17.10% of defecation events on farm A, 21.45% on farm B, and 13.78% on farm C were observed. Several reasons may explain this result: i) some events may not have been captured due to failure of the cameras before the end of a session or have been mistakenly noted as urination rather than defecation as it was sometime difficult to discern the two behaviours on video; ii) some latrines located in areas inaccessible to humans may have been missed despite our efforts to examine all possible locations during the prospection sessions; iii) cats may also defecate outside latrines in their core activity area, as the presence of isolated faeces has been previously reported on farms (Kitts-Morgan et al., 2015; Simon et al., 2017), or outside the core activity area at a distance from the farm. Ishida and Shimizu (1998) and Forin-Wiart et al. (2014) reported in this regard that only 23.5% and 13.5% of faeces excreted by adult cats after ingestion of marked bait were found in the core activity area, suggesting that a significant proportion of the faeces were deposited at a distance away.

In this study, it was observed that latrines located close to a feeding point were used by almost three-fold more individuals than those located far from a feeding point and are used more intensively by immature cats and mature males than mature females. Both the use of those latrines by several cats and the multiple faecal deposits by same individuals could explain the high occurrence of *T. gondii* DNA previously found in soil samples (Simon et al., 2017). Considering that the probability of a cat becoming infected before age of six months may reach 73% on some farms (Simon et al., 2018) and that the *T. gondii* oocysts shedding period lasts up to 20 days (Dubey, 2010), the intense and repeated use of latrines by juveniles certainly contributes to a large part of the soil contamination in latrines located around food resources in dairy farms. The contamination of *T. gondii* in the most used latrines could also be accentuated by the high survival of oocysts in the environment (Lélu et al., 2012).

In contrast to juveniles, the high number of faeces from sexually mature males observed in the latrines close to a feeding site was less expected. In our study, we considered males to reach sexual maturity at 10 months but it has been shown that some males may delay reproduction until around the age of three years (Say et al., 1999) and that non-reproductive males spend more time around the resource centre than reproductive ones (Macdonald et al., 2000). Delay in access to reproduction may contribute to the contamination of latrines close to feeding areas by some males, especially from October to March when their use of latrines increases and when both cat seroconversion rates and oocyst shedding are high (Hermann et al., 2010, Schares et al., 2016; Simon et al., 2018).

The lower number of defecations from mature males in summer than in winter may result from two behavioural factors that decrease the time spent by cats on farms in summer: larger home ranges and movements during dry and warm months than during other months on one hand (Groszynski *et al*., 2009; Horn *et al*., 2011) and temporary dispersal of reproductive males from January to June on the other (Bradshaw *et al*., 2012). On the opposite, in winter, cats spend most time in and around farm buildings (Horn et al., 2011). Furthermore, in this season, they may be

384 more dependent on human-provided food because of the low availability of prey (rodents and
385 birds; Krauze-Gryz et al., 2017). This may explain the absence of significant difference in the use
386 of latrines located close to a feeding site between the three cat categories in winter, whereas
387 juveniles use significantly more often these latrines than mature males and females in summer.
388 Despite mature females can also increase their movements outside farms during summer, their
389 smaller home ranges (ratio of 3.5:1 male:female; Liberg et al., 2000) and the presence of kittens
390 on farms may constrain them to spend time near farm buildings in summer. That may explain why
391 no seasonal effect was found on the use of latrines by mature females.
392 During the study, farm cats were also observed spending time at latrines for other activities than
393 defecating, such as playing, hunting, feeding and resting. It suggests latrines could be a risk for cat
394 infection by accidental ingestion of *T. gondii* oocysts. This may especially be the case for kittens,
395 which spend a lot of time scratching the ground and so can ingest soil or faeces (the ingestion of
396 cat faeces by a kitten was observed once in our video footage). Latrines may also pose a risk of
397 exposure of intermediate domestic and wild hosts (including cat preys) to *T. gondii* oocysts.
398 Intermediate wild hosts were seen on and around 77 % of the latrines during the study period.
399 Visit rates were highly heterogeneous between micromammals, birds and other species.
400 Compared to other species groups, a high number of bird visits was recorded on the three farms.
401 Most of them were small passerines characterised by a gregarious behaviour (del Hoyo et al.,
402 1992), resulting in a high number of individuals (up to 100) visiting a site at the same time. The
403 cameras position, optimized to detect cats movements rather than smaller animal movements, as
404 well as the characteristics of latrine sites, making them more attractive to certain intermediate
405 hosts than to others (for example exposed cereal storage areas that are used as latrines by cats are
406 also an attractive source of food for birds and rodents) may also explain this heterogeneity in the
407 visit rate of between species. The visits of wild intermediate hosts on latrines confirm that farms
408 may be areas where *T. gondii* circulates between wild and domestic environments (Gilot-Fromont
409 et al., 2012).

This study provides evidence that the location of food supplies provided by humans impacts the pattern of latrine use by cats on farms. Latrines close to a feeding area were : i) used by the highest number of individuals, ii) mainly used by juvenile cats, and iii) intensively used by mature males during autumn and winter that are periods of maximal risk of shedding *T. gondii* oocysts. These latrines could thus be high-risk sites for domestic animal and human *T. gondii* infection on farms especially because cat feeding sites are often located near the milking parlour (personal observation) which is daily frequented by livestock and farmers. In addition, cats usually defecate in areas with loose substratum, such as turn soil, gravel, sand or hay (Bradshaw et al., 2012) resulting in latrines location in areas where frequent contacts with animals and humans can occur (e.g. livestock cubicles with loose bedding material like straw, livestock food storage areas, sand pits, wood storage areas with wood chips, etc.). Previous studies showed that the infection of cattle is common in the region under study, and that the prevalence in cattle herd is associated to the presence of cats (Gilot-Fromont et al. 2009). Although cattle infection is not a major animal health issue, the risk of people to be infected from cattle meat may not be neglected (Tenter et al. 2000). Moreover, oocysts are a major source of direct human infection (VanWormer et al. 2013), thus the risk for people working or visiting farms is at concern. Feeding cats at distance from areas frequented by livestock and humans should modulate the *T. gondii* transmission dynamics in farm and reduce the risk of livestock and human infection. Future investigations are needed to better determine how the positioning of feeding points, as well as the management of areas potentially used as latrines, may influence the risk of infection.

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630 **Figure 1:** Maps of the three studied farms (A, B and C) showing the demarcation of the study
631 area on each farm, the farm layout (buildings and land cover) and the locations of latrines (black
632 boxes) and feeding sites. Sites marked 's' indicate the location of a feeding site in summer; 'w'
633 indicates the location of a feeding site in winter. Absence of 's' or 'w' indicates that the location
634 of the feeding site remained the same throughout the year.

635 **Figure 2:** Number of cat defecation events per latrine and per session predicted by the best model.
636 The number of cat defecations varied according to cat category, latrine location relatively to a
637 feeding point (close: ≤18.5 m or far: >18.5 m) and season. MF: sexually mature female, MM:
638 sexually mature male, I: sexually immature cat. Summer: April–September, Winter: October–
639 March.

640 **Figure 3:** Number of defecating cats per latrine predicted by the best model. The number of
641 cats varied according to latrine location relative to a feeding point (close: ≤18.5 m or far:
642 >18.5 m).

643 **Figure 4:** Average latrine visit rates (number of visits/day/latrine) of micromammals (top),
644 birds (middle) and other species (bottom) by latrine and by farm during the study period. The
645 bars represent standard errors when latrines were visited during several sessions.

646

Table 1: Experimental design of the camera trap survey, composition of the cat population by session and number of recorded defecation events of individuals classified in one of the three categories: sexually immature cat, sexually mature male, sexually mature female.

Farm	Session	Season	No. days	No. cameras	No. detected latrines	No. mature ♂	No. mature ♀	No. immatures	No. defecations from mature ♂	No. defecations from mature ♀	No. defecations from immatures
A	S04	S	10	10	5	6	3	6	4	2	5
	S08	W	12	12	3	5	3	1	14	3	1
	S11	W	11	12	5	5	3	0	11	5	0
	S15	S	15	13	6	4	3	0	15	17	0
	S20	S	13	13	6	3	3	5	0	3	19
B	S3	S	10	13	4	6	3	13	1	12	22
	S7	W	12	12	5	6	4	12	8	8	29
	S12	W	10	12	4	6	6	12	25	15	25
	S16	S	10	13	4	6	6	11	11	13	30
	S19	S	10	12	7	7	7	9	7	8	34
C	S01	S	9	12	7	5	3	7	1	3	9
	S05	S	12	13	7	5	3	8	3	7	18
	S09	W	10	13	7	5	3	8	4	0	11
	S13	W	13	13	9	5	4	4	12	8	12
	S17	S	14	12	8	8	3	2	15	7	3
	S21	S	10	12	6	7	2	4	5	4	7

S: Summer (April–September), W: Winter (October–March), ♀: female, ♂: male.

Table 2: Model selection for the number of cat defecation events per latrine (a) and for the number of cats defecating in latrines (b). Only the first models showing the lowest Akaike's information criterion value corrected for a small sample size and overdispersion (QAICc) are presented. Values in bold correspond to the most parsimonious model. Δi: difference between the QAICc and the lowest QAICc value. Cat: sexually immature cat, sexually mature male or sexually mature female, Distance: location of latrine relative to the nearest feeding point ('close to' or 'far from'), Season: summer (April–September) or winter (October–March).

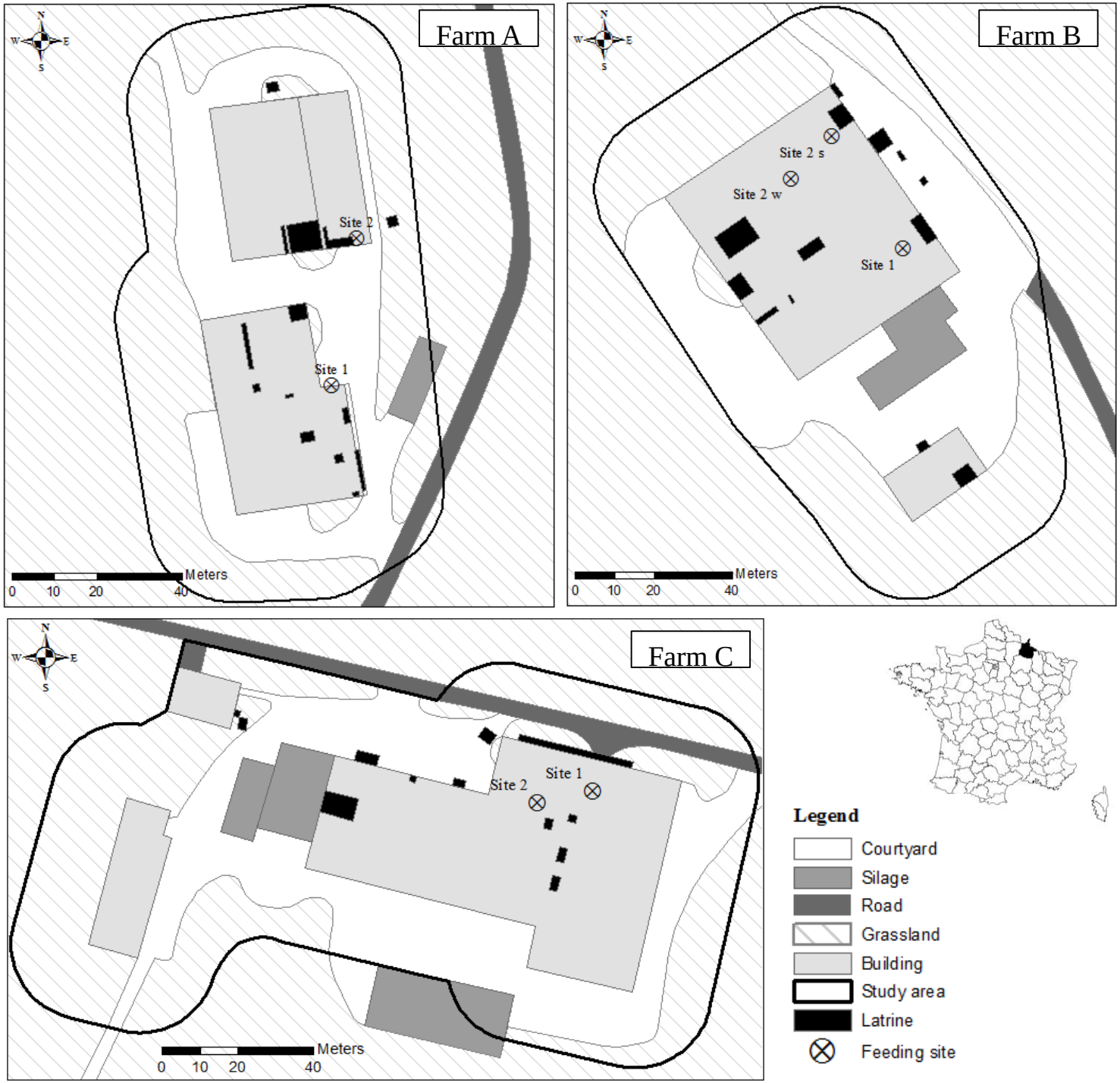
Model	Residual deviance	No. estimated parameters	QAICc	Δ_i	Model weight
(a) Number of defecations					
Cat+Distance+Season+Cat*Distance+Cat*Season	**943.8**	**11**	**440.9**	**0.00**	**0.49**
*Cat+Distance+Season+Cat*Distance+Cat*Season +Distance*Season*	943.5	12	442.9	2.00	0.17
*Cat+Distance+Cat*Distance*	964.5	8	443.5	2.65	0.13
*Cat+Distance+Season+Cat*Season*	962.4	9	444.7	3.86	0.07
*Cat+Distance+Season+Cat*Distance*	964.3	9	445.6	4.72	0.05
(b) Number of defecating cats					
Distance	**549.4**	**4**	**299.4**	**0.00**	**0.20**
*Distance+Season+Distance*Season*	542.3	6	299.8	0.46	0.16
Distance+Season	546.4	5	299.9	0.54	0.15
Cat+Distance	543.7	6	300.5	1.19	0.11
*Cat+Distance+Season+Distance*Season*	536.8	8	301.2	1.80	0.08
Cat+Distance+Season	540.9	7	301.2	1.84	0.08
*Cat+Distance+Cat*Distance*	537.9	8	301.7	2.38	3.06

Table 3: Parameters of the final models retained to explain the number of faeces deposited per latrine (a) and the number of cats defecating in latrines (b). For each variable modality the table indicates the parameter estimate and its standard error (SE). Values in bold correspond to P-values ≤ 0.05. The modalities of each variable were: for Cat: sexually mature males, sexually mature females or sexually immature cats, for Distance: location of latrine relative to the nearest feeding point ('close to' or 'far from'), for Season: summer (April–September) or winter (October–March). The effects of Cat in the upper part of the table stands for the effect of Cat for the reference category, *i.e.*, individuals far from a feeding site and during summer. Because two interactions were selected (Cat*Distance and Cat*Season), the effect of Cat was not similar according to the distance and to the season. The Cat*Distance parameters give the effect of Cat in individuals living close to a feeding site, and the Cat*Season parameters stand for the effect of Cat in winter.

Variable	Description	Parameter estimate (SE)	P-value of the Wald test	Odds ratio	95% confidence interval
(a) Number of defecations					
Cat	Mature male (reference)				
	Mature female	2.07 (0.48)	**<0.001**	7.93	3.11–20.24
	Immature	1.26 (0.50)	**0.011**	3.52	1.33–9.31
Season	Summer (reference)				
	Winter	0.78 (0.34)	**0.019**	2.17	1.14–4.16
Distance	Far from a feeding site (reference)				
	Close to a feeding site	2.24 (0.54)	**<0.001**	9.42	3.29–26.93
*Cat*Distance*	Mature male*Close from a feeding site (reference)				
	Mature female*Close to a feeding site	-1.65 (0.48)	**<0.001**	0.19	0.07–0.49
	Immature*Close to a feeding site	-0.34 (0.50)	0.489	0.71	0.26–1.89
*Cat*Season*	Mature male*Winter (reference)				
	Mature female*Winter	-0.81 (0.27)	**0.002**	0.44	0.26–0.75
	Immature*Winter	-1.03 (0.24)	**<0.001**	0.36	0.22–0.57

(b) Number of defecating cats

Distance	Far from a feeding point (reference)				
	Close to a feeding point	0.98 (0.24)	**<0.001**	2.67	1.66–4.30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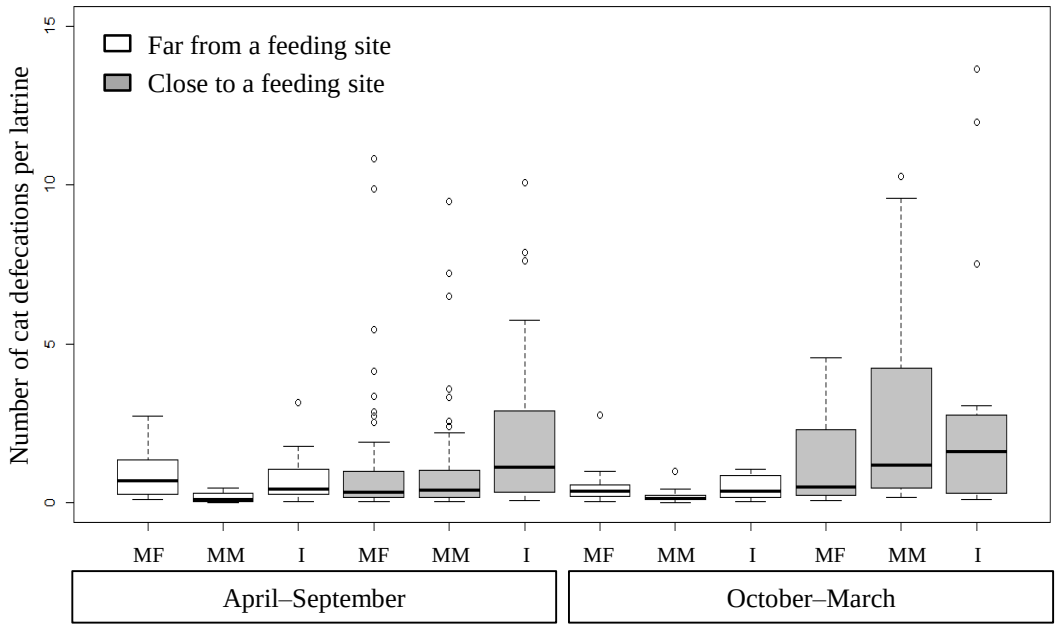
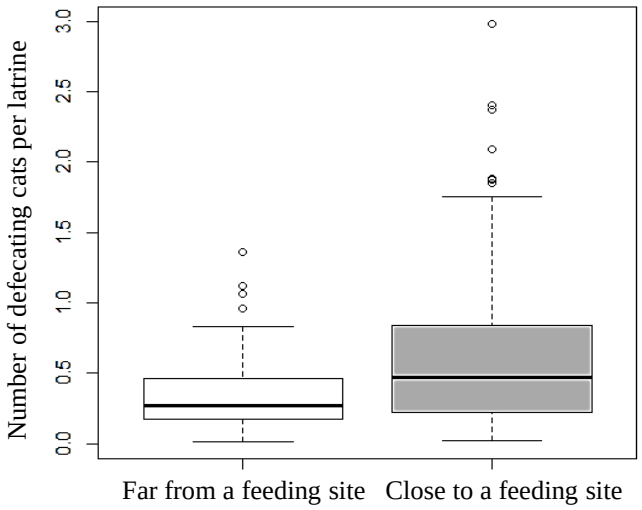
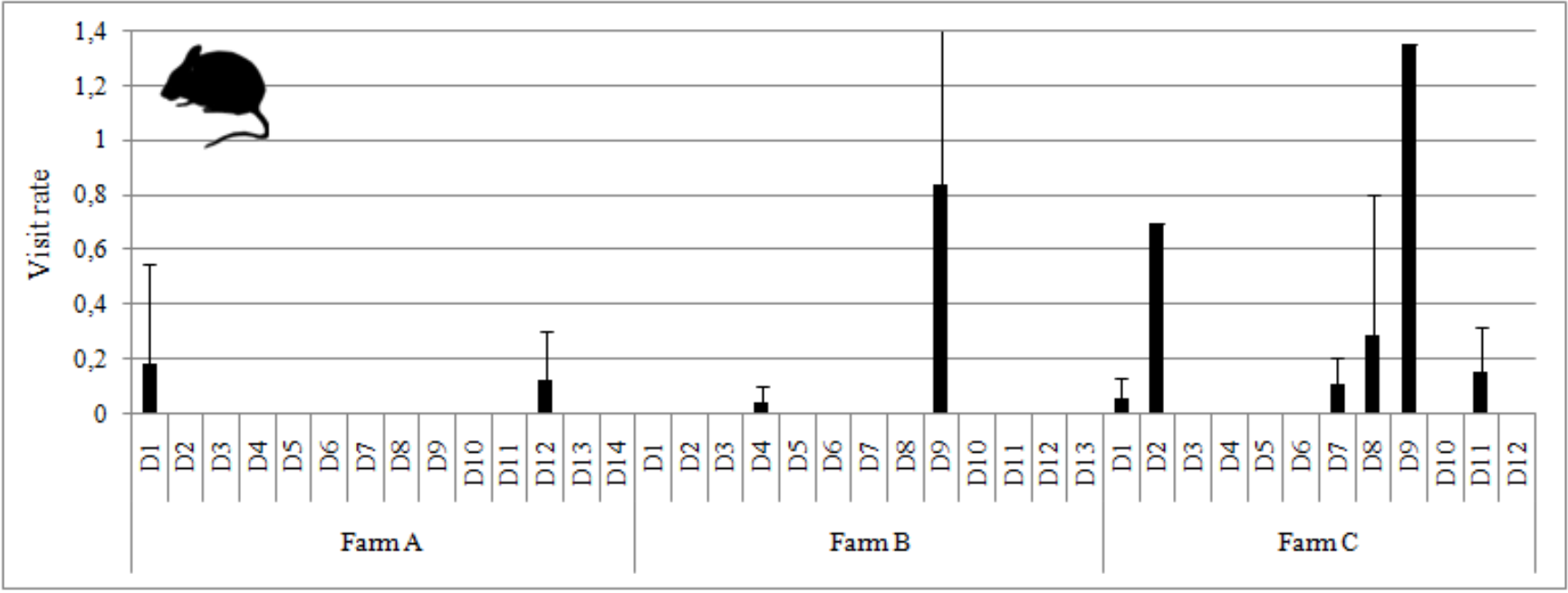
Site 2
Site 1
Meters
0 10 20 40
Farm B
Site 2 s
Site 2 w
Site 1
Meters
0 10 20 40
Farm C
Site 1
Site 2
Meters
0 10 20 40
Legend
Courtyard
Silage
Road
Grassland
Building
Study area
Latrine
Feeding site


Far from a feeding site
Close to a feeding site
Number of cat defecations per latrine
0
5
10
15
MF
MM
I
MF
MM
I
MF
MM
I
MF
MM
I
April–September
October–March


Number of defecating cats per latrine
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Far from a feeding site
Close to a feeding site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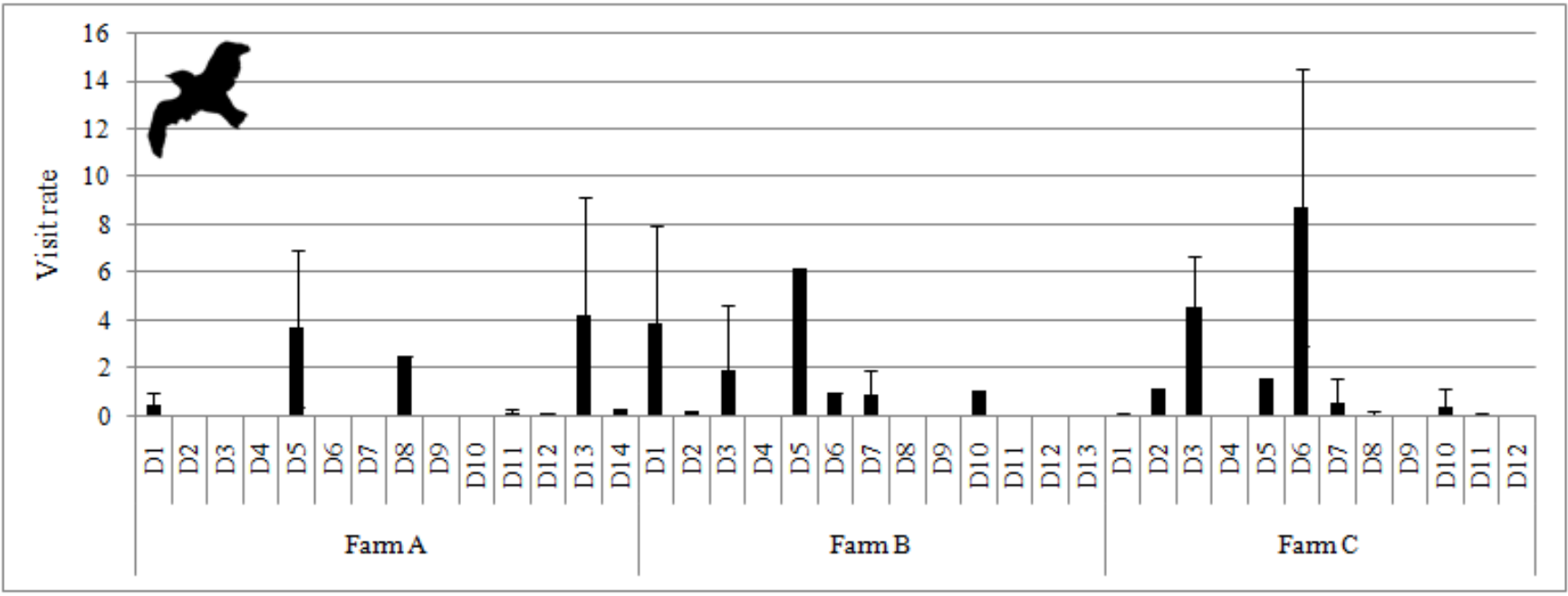
0,6
0,4
0,2
0
D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12 D13 D14
D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12 D13
D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12
Farm A
Farm B
Farm C


Visit rate
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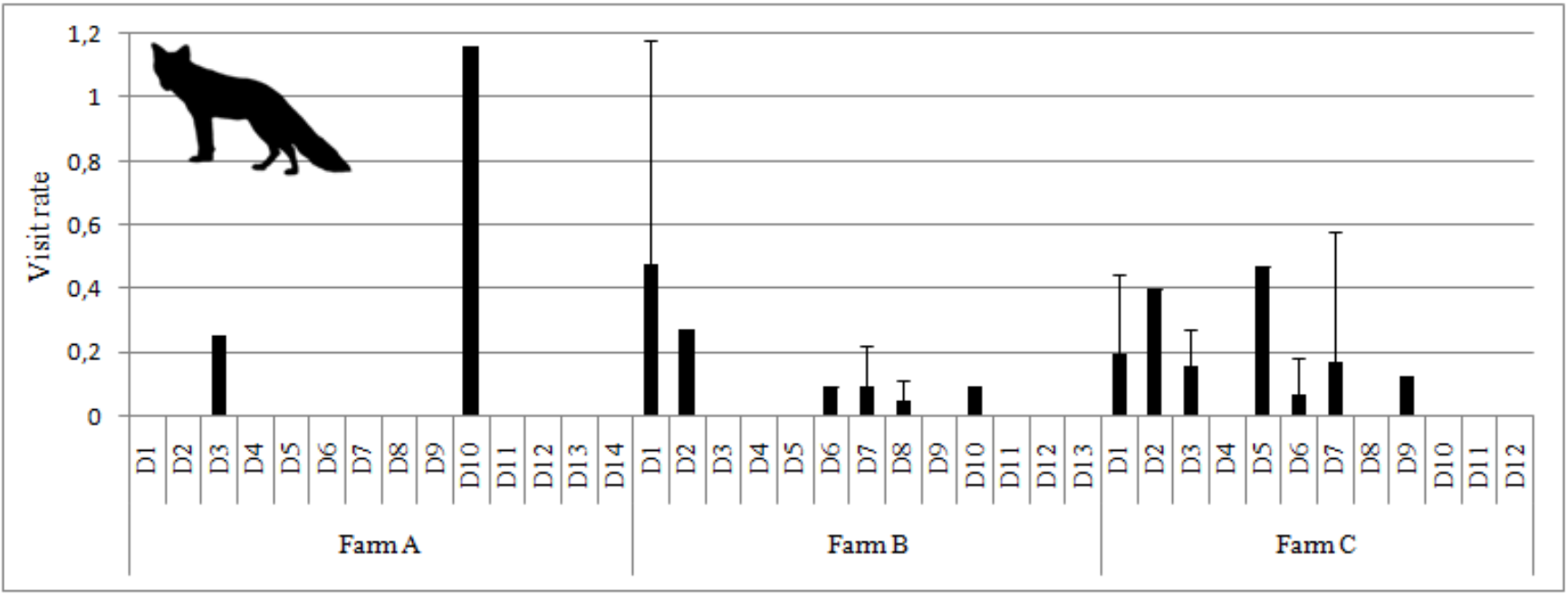
D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12 D13 D14
D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12 D13
D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12
Farm A
Farm B
Farm C


Visit rate
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0,8
0,6
0,4
0,2
0
D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12 D13 D14
D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12 D13
D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12
Farm A
Farm B
Farm C