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Wolf scat detection dog improves wolf genetic monitoring in new French colonized areas

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Abstract. A detection dog and handler team were used to recover scats in areas newly colonized by wolves outside the Alpine mountains of France between October 2018 and May 2019. Survey areas were classified as occupied by a resident wolf pack (WP) or dispersers (no-WP). The efficiency of monitoring by a targeted dog-handler team was compared to opportunistic monitoring by trained observers. Use of the detection dog allowed up to 99.6% time savings relative to monitoring by trained observers. Wolf scats found by the dog represented 82.1% of genetically confirmed samples in the 12 sample units (each being 10 × 10 km) monitored by both trained observers and the dog-handler team. Occupancy modelling was used to estimate wolf detection probabilities. Ten kilometres of survey with the dog were required to reach a 98% detection probability in WP territories and 20 km to reach 96% in no-WP areas. By contrast, two years of opportunistic monitoring by trained observers were required to obtain a 90% and 76% probability of detecting wolves in WP and no-WP areas, respectively. The use of the detection dog via dog-team surveys greatly increased the collection of viable samples for genetic analysis and individual genotype identification. Our study offers further confirmation that dog-handler teams can be very effective at locating scats from target carnivores, to supplement or complement human search efforts.

Key words: conservation dogs, genetic analysis, faecal samples, large carnivores, wolves, non-invasive monitoring

Introduction

Wolves (*Canis lupus*) are currently recolonizing parts of their historic range in France, following the

first official confirmation of the species presence in the early 1990s in the French Alps (Peillon & Carbone 1993, Valière et al. 2003, Ciucci et al. 2009, Louvrier et al. 2018b). In France, wolf recovery

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is occurring in areas of intensive sheep breeding activity. In these regions, livestock depredation attributed to wolves can be considerable (> 12,000 head in 2019), causing substantial economic and social impacts (Données DDT(M)-DREAL Auvergne Rhône-Alpes 2019). The grey wolf is strictly protected in France (European commission 2006) and the French government must deal with the challenge of mitigating human-wildlife conflict, decreasing damage to livestock and ensuring wolf conservation (Treves et al. 2009, Chapron & Treves 2016). A lethal control programme was established in 2001 with the aim of ensuring population viability (Chapron et al. 2003). In 2018 and 2019, 47 and 94 wolves respectively were legally culled by shooting, but obtaining reliable estimates of wolf distribution and population trends remains challenging for French authorities. The conflict over wolves is particularly prominent in areas of new wolf colonization as people are unaccustomed to sheep depredation and herds are not often well protected (Chapron et al. 2014). Predation by dogs represents a further threat that sheep owners may be confronted with.

Monitoring large carnivores is exigent because they are nocturnal, elusive, highly mobile and occur in low densities over wide territories (Long et al. 2012, Ausband et al. 2014). In this context, applying a standard random sampling design for the purpose of monitoring wolf populations offers little chance of detecting signs of wolf presence. Therefore, citizen science, using volunteers, could be useful and is considered an effective source of information for assessing changes in the distribution of a species over large areas (Schmeller et al. 2009, van Strien et al. 2013). In France, the “French Wolf Network” and its data collection constitute the basis for the study of geographical wolf distribution and expansion (Duchamp et al. 2012). The French Wolf Network (FWN) has increased in parallel with the wolf population expansion from a few hundred people in 1994, to more than 4,000 trained observers in 2019. Wolf signs collected by this network include prey remnants bearing the characteristic marks of an attack by wolves, complete or partial wolf carcasses, faeces, footprints/tracks, auditory and visual observations of wolves, preferably confirmed by camera trapping. In the French Alps, the combination of both camera trap monitoring and genetic sampling of wolf scats routinely offer greater efficiency (Karanth et al. 2006, Long et al. 2007a, 2011, Mattioli et al. 2018). All suspected signs are subject to a standardized control process

to prevent misidentification, and a wolf coordinator validates the data (Duchamp et al. 2012). When wolf signs have been reported for two consecutive years in an area and DNA analysis or camera trapping confirmed the identity of the species, signalling a wolf territory, it acquires the status of “Permanent Presence Zone” (PPZ). The “Minimum Number of Wolves” (MNW) is estimated on each PPZ by winter tracking and through visual counts.

A second method of determining the number of individuals is to apply a “Capture-Mark-Recapture” (CMR) model to the wolf genetic samples collected. We use the CMR model to estimate population size and survival rates (Duchamp et al. 2012). Wolf scats are thus opportunistically and routinely collected in the French Alps and are the main source of genetic samples (600-700 each year), along with carcasses of culled wolves. The collection of wolf scats occurs mainly during winter since snow cover in the French Alps facilitates the detection of signs of wolf presence. However, in newly colonized areas outside the Alpine mountains, scat collection is more difficult for three main reasons: 1) snow events may be rare, 2) a lack of recruited observers and 3) the probability of detecting a lone and mobile disperser is low (Marescot et al. 2011). As a result, there is a dearth of genetic samples from newly colonized French territories and the first wolf signs are usually unreliable.

To address these challenges, we decided to incorporate the use of a trained scat detection dog and handler, to increase the efficiency of scat collection in newly colonized areas. The use of trained dogs in large carnivore management and conservation has gained increasing attention among researchers during the last decade (Browne et al. 2006). Scat detection dog teams have successfully been used to locate genetic samples of various carnivores (Long et al. 2007b) but the intrinsic difficulty of detecting large carnivores remains and it is still possible to miss animals even where they are present. To account for “imperfect” detection, we built a single-season occupancy model with capacity to account for false negatives (MacKenzie 2006, Hines et al. 2010, Thorn et al. 2011, Sunarto et al. 2012).

In this study, we aimed to investigate whether using a detection dog and handler team facilitated fast and efficient collection of wolf scat in newly colonized areas. Additionally, we aimed to compare the team’s efficiency and reliability to that of trained observers.

Material and Methods

Dog training

The dog team comprised one dog and the handler (first author), the handler was previously trained as an FWN observer (see below). The dog (female Belgian shepherd selected by a professional dog-breeder) had been living with its handler from the age of two months and was trained to recognize wolf scats. The training protocol, based on positive reinforcement, was adapted from previous conservation detection work (Smith et al. 2003, Wasser et al. 2004, Sentilles et al. 2016; Appendix S1) and remains fundamentally the same. The dog progressed to an interim training stage similar to that described in Statham et al. (2020), graduating from controlled settings with artificial scat provision into known, occupied wolf territories in order to encounter naturally occurring wolf scats. The handler trained the dog with samples from 60 genetically confirmed wolf scats originating in the French Alps. The selection of scats for training included genetically confirmed samples from target species (scats from male and female wolf haplotype group w22 (sensu Pilot et al. 2010), i.e. characteristic haplotype from grey wolves originating in the Apennine Peninsula and French Alps). Scats known to be from non-target species (red fox *Vulpes vulpes* and domestic dogs fed with wild ungulate meat) were also provided during training (as per DeMatteo et al. 2019). The training protocol consisted of searching off leash, with the dog indicating having found wolf scat by freezing, lying down, and barking. For each find the dog was rewarded by playing with a ball. Once the dog showed consistent recognition and detection of naturally occurring wolf scat, the handler deemed it ready to deploy on surveys.

French Wolf Network observer training

Wolf range expansion was monitored at the country level without standardized experimental design being implemented by the French Office of Biodiversity. Instead, opportunistic sampling was conducted by a network of field observers, the FWN. These field observers come from governmental (French Office of Biodiversity) or non-governmental organizations (e.g. game and wildlife services, hunting associations, forestry offices, naturalists, farmers). All observers completed a 3-day training course, covering species identification and surveying methods (Duchamp et al. 2012). After completing the training, the observers were asked to search for

naturally occurring wolf scat samples on road trails and crossroads (detailed below).

Newly colonized areas and choice of sampled unit

The dog-handler team was deployed in 12 newly colonized areas declared as Permanent Presence Zones (PPZ), i.e. areas where wolf signs have been reported for two consecutive years and the identity of the species has been confirmed by DNA analysis or camera trapping. These sample units were surveyed by both the dog team and FWN observers. Each sample unit (i) were defined as 10 × 10 km cells (European Commission 2006). We used the same grid used by Louvrier et al. (2018b), available online at: <http://carmen.carmencarto.fr/38/Loup.map>. We assumed that one grid cell of 100 km² corresponded to the minimal home range size for grey wolves at these latitudes (Okarma et al. 1998, Mech & Boitani 2010, Mancinelli et al. 2018). One sample unit was in the Maritime Alps (a new peri-urban colonized area), the others were outside the French Alps. In the case of the Maritime Alps, FWN observers suspected a new wolf pack, but only one genetic sample had been obtained. In one sample unit (in Camargue, an agricultural lowland, where one rogue wolf was suspected in sheep depredation) no genetic samples were available despite intense search effort by local FWN observers. For all these PPZ, we lacked genetic samples to implement the CMR model (i.e. no or few genetic samples collected each year). A designated wolf referee coordinated the network activity, controlling the technical reliability of each presence sign that was reported. We decided to deploy the dog team in each sample unit where FWN observers reported difficulties in finding wolf signs. Based on FWN coordinator expertise, we identified seven PPZ as held by a wolf pack (WP), and five zones with no wolf pack (no-WP) but where at least one solitary wolf had been reported for two consecutive years. We examined photographs and considered the quantity of prey remnants showing characteristic wolf attack marks, and the MNW on each PPZ (if available). During 2018-2019 FWN observers were asked to continue trying to collect wolf scats in the areas surveyed by the dog-handler team. All samples collected by FWN observers were genetically analysed. Dog team field surveys were conducted from October 2018 to May 2019. Surveys occurred mainly in mountainous areas (altitudes ranging from 600 to 1,460 m), but one sample unit occurred on agricultural lowland (in Camargue).

Dog-handler team sampling

The team surveyed predetermined transects that were preferably circular for logistical reasons (return to the vehicle), in some cases a member of the FWN transported the dog and handler, thus allowing a line-transect along forest roads. In each sample unit two to four transects were chosen, the aim being to cover more or less the same distance in each sample unit. The dog team surveyed trails previously covered by local FWN observers (including those where no scat had been detected but local knowledge considered wolf presence to be possible) or where camera trapping captured at least one wolf. The dog handler had no previous knowledge of the survey areas. We equipped both the dog and the handler with GPS devices (Dogtra pathfinder) to record survey tracks and distance covered, and to enable mapping of all scats indicated by the dog. All collected scats were genetically analysed, as described below. The dog team spent nine non-consecutive days surveying in 2018 and 10 non-consecutive days in 2019 (see Table 1 for details).

French Wolf Network opportunistic sampling

Both dog-team and FWN observers surveyed the same group of twelve sample units. The number of FWN observers per sample unit ranged from ten to > 55; the ratio of professionals/non-professionals ranged from 0.2 to 0.5 (Appendix S3b). In new areas of wolf colonisation, state rangers from the

French Office of Biodiversity carried out baseline monitoring in all districts where wolf signs were still considered as unreliable. The rangers belong to the FWN and were already deployed across France for environmental policy and wildlife monitoring; they might opportunistically find scats during this time. Non-professional FWN observers usually go into the field specifically to search for wolf signs including scats. However, we did not require the FWN observers to record details of their time spent in the field.

All scats collected by the FWN observers were genetically analysed (see below). According to Barja et al. (2004), wolves tend to travel along forest roads and trails and to mark them with tracks and scat, especially at forest crossroads. As such, we asked FWN observers to pay attention to forest crossroads and to collect all scat found there. The proportion (number of professionals over the total number of FWN observers) was 0.2 to 0.5. This proportion also depended on the unit (see also Louvrier et al. (2018b) for detailed analysis of sampling effort by FWN observers based on the number of observers per grid cell). However, no information on total time spent surveying opportunistically by FWN observers was available.

DNA extraction and genetic analysis

Allscatswerestoredindividually,withoutdesiccant, in a sealed plastic bag. Samples were then frozen. Scat samples were analysed using a mitochondrial DNA (mtDNA) species identification test, as well as a Polymerase Chain Reaction (PCR) multitube approach based on 20 microsatellite markers and one sex-specific marker for individual and gender identification. Extraction methods, PCR protocols, protocols for individual identification, molecular sexing and for screening wolf samples from other animal species samples are described in detail in Valière et al. (2003) and Duchamp et al. (2012). We used these genetic analyses to test the reliability of the scat-detection dog's ability to discriminate wolf scats from other (nontarget) species scats. They were also essential to evaluate the efficiency of the scat collected by the dog-handler team *vs.* humans-only teams, to provide genotypes or detect different individuals. Genetic analyses were performed at the ANTAGENE company (<https://www.antagene.com/en>).

Data modelling and analysis

To model the probability of detection by the dog team, we used a single season site-occupancy model

Table 1. Time and spatial parameters of the wolf detection dog surveys.

Parameter	Result
total number of search days	19
total time in field, including logistics	31
total resident wolf pack (WP) segments sampled	143
total dispersers (no-WP) segments sampled	140
mean WP segments per sample unit	20.4 (range 6-27)
mean no-WP segments per sample unit	28 (range 20-32)
daily segments sampled	14.8
total search time (h:min)	97:13
daily search time (h:min)	05:07
mean time per sampled segment (min)	21

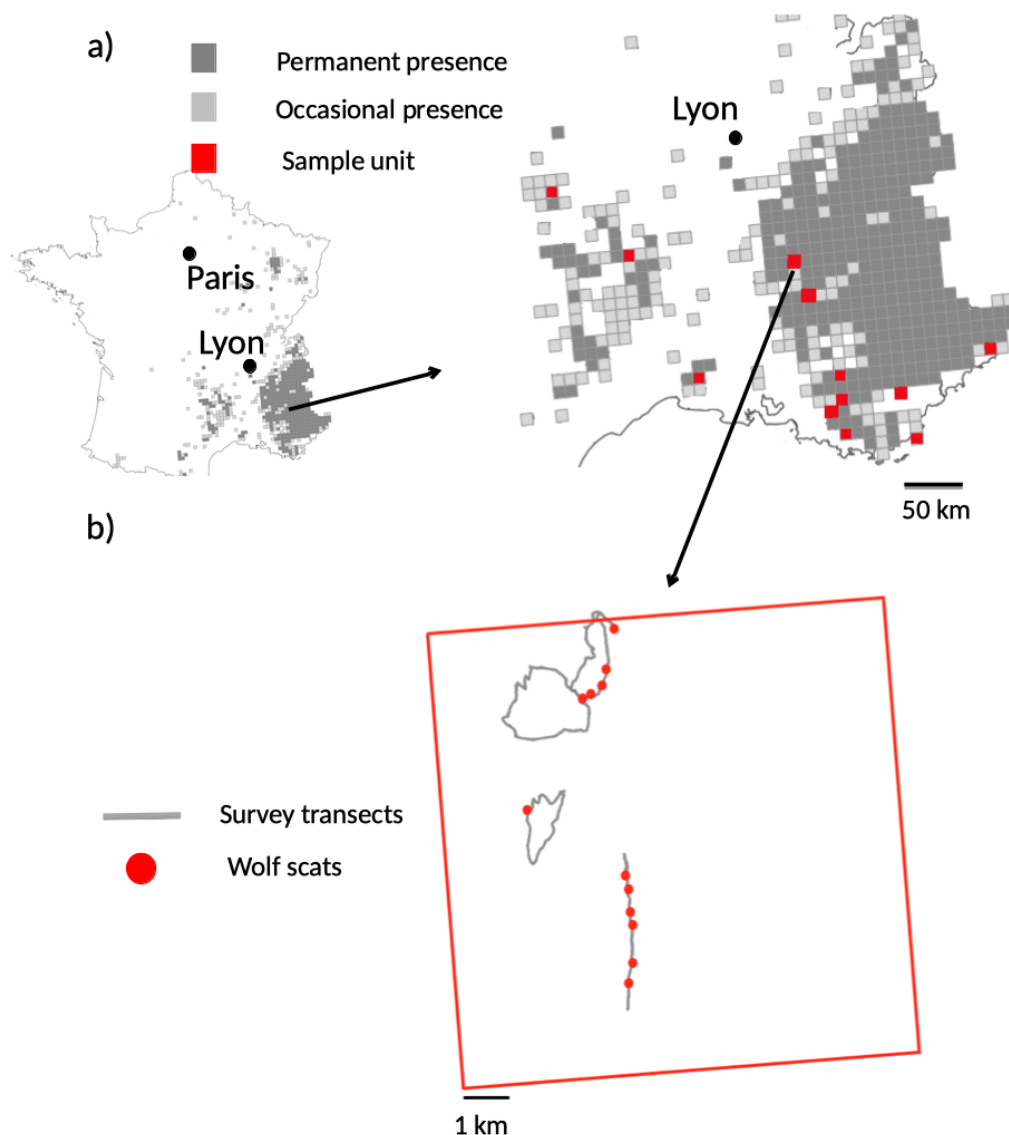


Fig. 1. a) Wolf distribution in France (right) and localization of sample units (left), b) example of surveys in one sample unit. Permanent Presence Zone refers to areas where wolf signs have been reported for two consecutive years (PPZ), occasional presence refers to areas where wolf signs have been reported in less than two consecutive years.

to account of for what we termed “imperfect” detection (i.e. missing a scat when present) and to estimate the power of the dog survey method (MacKenzie 2006, MacKenzie et al. 2009). The survey was carried out over 12 separated grid cells (Fig. 1), and it lasted less than three days in each sample unit “i”. Each sample unit was in one PPZ. Within each sample unit “i” the protocol ensured that the dog team passed through a point where wolf presence signs were previously detected.

The model (adapted from Hines et al. 2010; Appendix S2) was implemented in WinBugs (Kéry 2010) with 30,000 iterations and three chains. The

distance covered was subdivided into segments “j” of equal length (i.e. 1 km) that were treated as spatial replicates in the occupancy analysis. The data was subdivided into spatial replicates (1 km transect segments) for the survey in each sample unit “i”. The number of 1-km replicate segments surveyed per sample unit ranged from six to 32. We checked convergence visually by inspecting the chains and by checking that the Rhat statistic was below 1.1 (Kéry 2010, Brooks & Gelman 2012; Appendixes S2, S3a).

We used a t-test to compare mean number of scats per kilometre of survey in areas where only dispersers were identified *vs.* wolf pack territories.

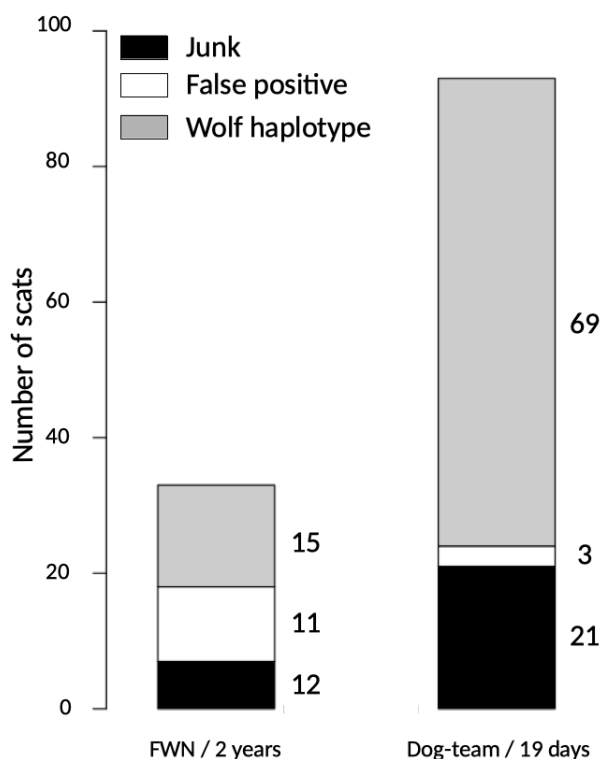


Fig. 2. Total number of scats collected by French Wolf Network (FWN) observers and the dog-handler team in twelve sample units. Grey bars – wolf haplotypes. White bars – false positive (i.e. nontargets). Black bars – scats not suitable for analysis (failed to amplify, see also Table 2).

All statistical analyses (other than occupancy with WinBugs) were performed using R-software.

To model the probability of detection by FWN observers, we used a single season site occupancy model. One approach to modelling detection/non detection from multiple seasons is to effectively apply a single season model to the data collected in each of the seasons (MacKenzie 2006). Under this approach, occupancy in one season is considered to be a random process in the sense that the

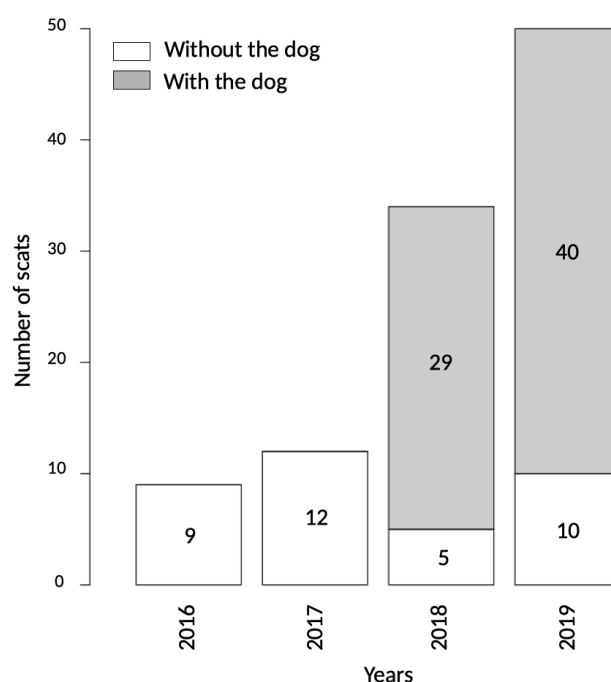


Fig. 3. Number of scats collected annually between 2016 and 2019 in twelve sample units. White bars – scats collected by trained observers from the FWN. Grey bars – scats collected via the dog-handler team. The year 2018 corresponds to the first year of using the dog team.

occupancy status of a unit in the previous season has no effect on the probability of occupancy in the same unit in the current season. Regardless of the underlying processes of change in occupancy, only the resulting pattern of level of occupancy each season is modelled.

The model was implemented in WinBugs (Kéry 2010) with 30,000 iterations, three chains, a burn-in of 4,000 and a thinning of five. The time covered was subdivided into time surveys “j” of equal duration (i.e. one month) that were treated as temporal replicates in the occupancy analysis.

Table 2. Species identification of wolf scat samples located by dog-handler team and FWN observers in France. ^aThe percentage of the total number of DNA-amplified samples confirmed as wolf.

Species	Dog-team	FWN observers	Dog-team + FWN observers
Wolf	69	15	84
Red fox	3	5	8
Dog	0	6	6
Number amplified	72	26	98
Number failed to amplify	21	7	28
Total	93	33	126
% amplified	74.8	78.7	77.7
% wolf ^a	95.8	57.7	66.7

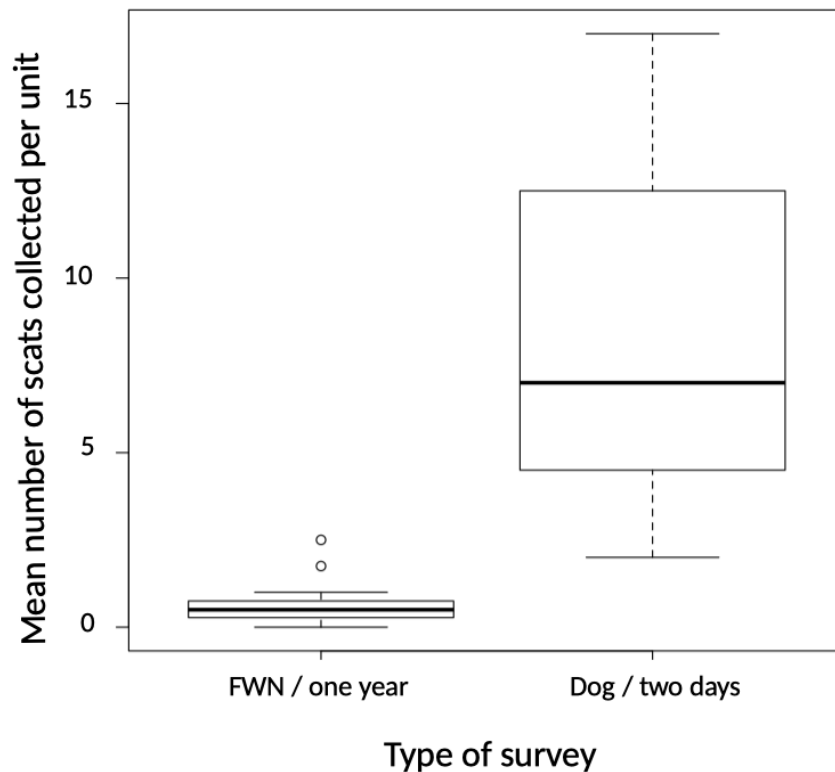


Fig. 4. Comparison of the mean number of scats collected in each sample unit between 2018-2019 by FWN observers and by the dog-handler team. Opportunistic scat collection by FWN observers spanned one year, whereas the dog team spent two days of targeted monitoring to collect scats in each sample unit.

The data were subdivided into temporal replicates (one month) for the survey in each sample unit “i”.

The number of one-month replicates surveyed per sample unit ranged from 12 to 48. The resulting

84 wolf haplotypes (69 found by dog and 15 found by FWN)

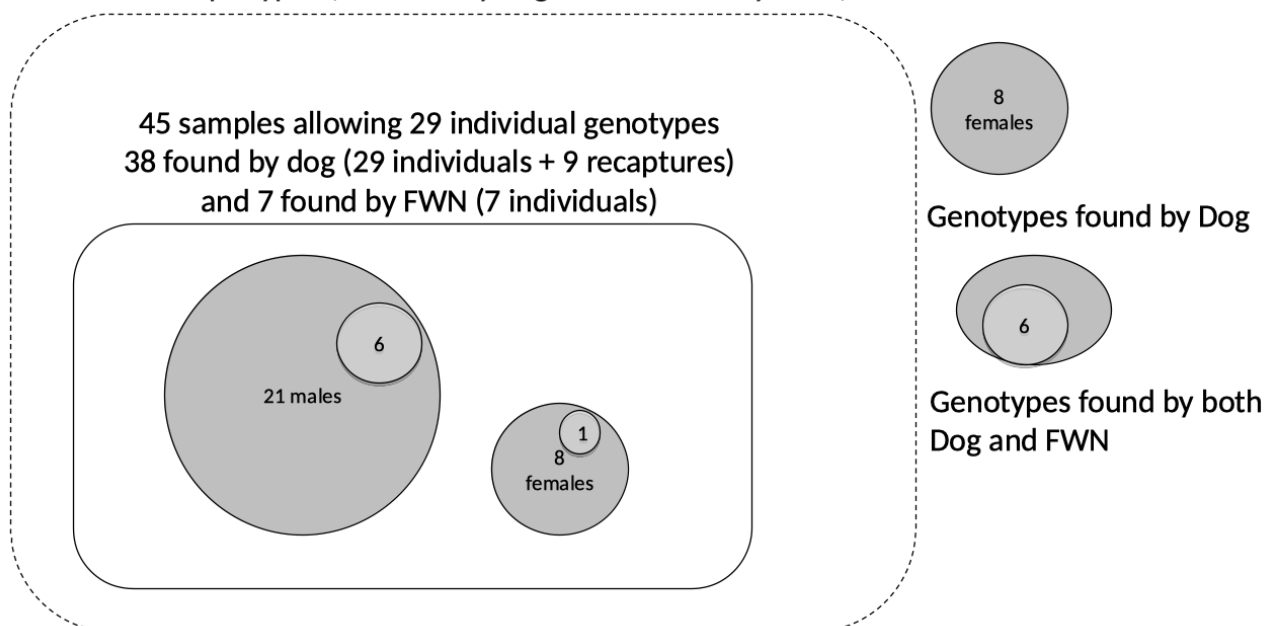


Fig. 5. Record of wolf haplotypes determined from analysis of scats found by FWN observers and by the dog-handler team. Dark grey circles show individual genotypes found as a result of the dog team surveys only, light grey indicates individual genotypes found by both the dog team and by FWN observers. A subset of the genotypes determined from scats ($n = 7$ of 29) found by the dog team were the same as those determined from scats recovered by FWN observers.

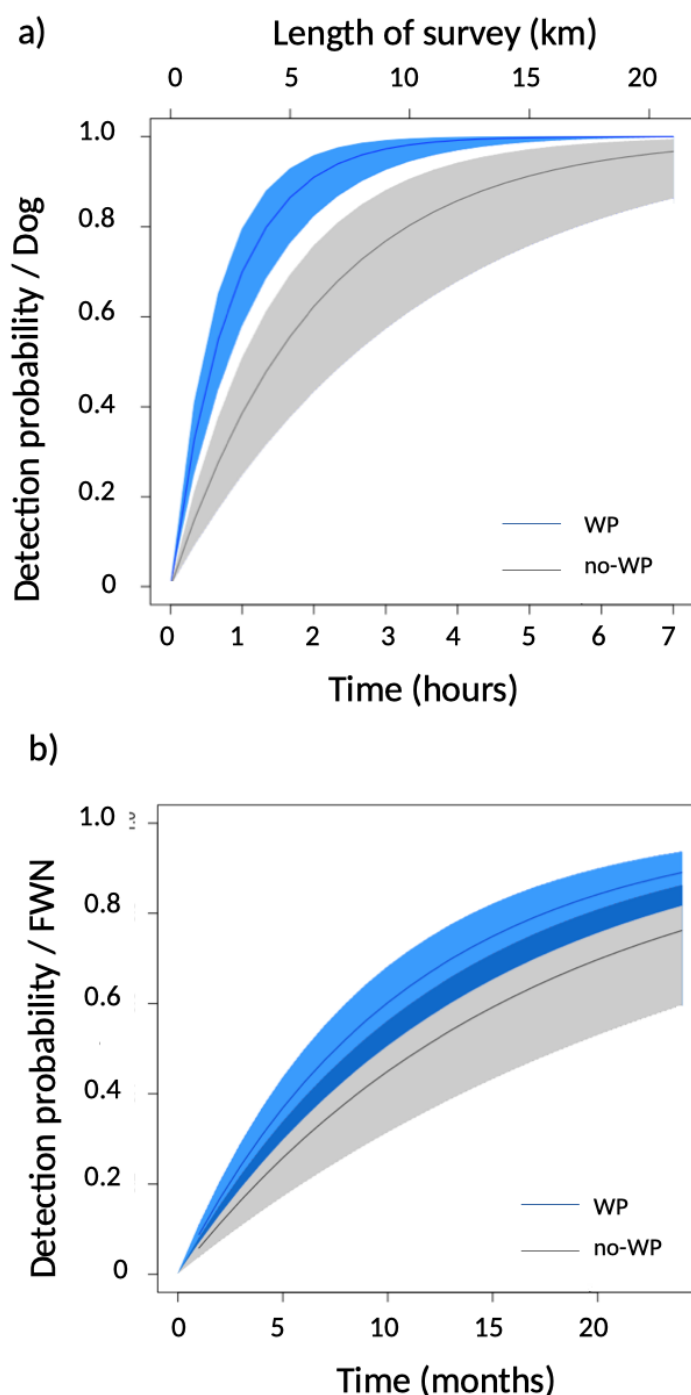


Fig. 6. Cumulative probabilities of scat detection in territories held by a wolf pack (WP) or dispersers (no-WP). Lighter shades represent the estimated 95% confidence interval. a) Targeted monitoring using a trained detection-dog, b) Opportunistic monitoring by FWN observers.

data were treated as single detection histories for each of the sample units "i" (large cells). Each detection history corresponding to one season (i.e. 12 months) in the same sample unit "i" was grafted to the precedent to construct one unique detection history per sample unit "i" (Appendix S3c). Each detection history contained a "0" (no detected scat) or a "1" (detected scat) per month "j".

Results

From October 2018 to May 2019, the dog-team covered 283 km of survey transects and 12 grid cells (143 km in WP territories and 140 km in no-WP territories). The dog covered an average of 20% greater distance than the handler per survey. To our knowledge, no surveys were performed near den



areas or rendezvous sites (latrines), i.e. no clusters of scats were found in areas which would have led us to suspect rendezvous site vicinity. The use of a detection dog for targeted monitoring provided up to 99.6% time savings compared to opportunistic monitoring by FWN observers. A total of 126 scat samples were found. The dog found 93 scats over a period of 19 search days, whereas FWN observers found 33 scats over a period of two years. All scats were genetically analysed (see Fig. 2 and Table 2 for a summary of results). Altogether, we identified 98 of 126 (77.7%) scat samples to species. We identified 95.8% (69 of 72) of the successfully typed samples detected by dog-handler team as wolf, and 4.2% (3 of 72) as red fox, i.e. false positives/non-target detections. Visual inspection of “false positives” and “no species” showed that these scats exhibited wolf scat characteristics. In contrast, we identified 57.7% (15 of 26) of the successfully typed samples detected by FWN observers as wolf and 48.2% (11 of 26) as false positives (domestic dog or red fox). Overall, the dog-team found 82.1% (69 of 84) of all the wolf scats (Fig. 3, Table 2). Two days of field survey resulted in the collection of fourteen times more genetic samples than one year of monitoring by FWN observers (Fig. 4). This represented a time savings of 99.6%.

Two wolf scats found by the dog team were determined to include haplotype w1, all other wolf scats found by the dog team or by FWN observers included w22 haplotype (sensu Pilot et al. 2010). In one of 12 sample units (in Camargue, agricultural lowlands, no-WP area) only the dog team found genetically confirmed wolf scats (two scats including one allowing individual genotyping). Genotypes from 45 samples (38 found by dog and seven found by FWN observers) were suitable for individual discrimination using microsatellite markers and yielded 29 individual wolves (Fig. 5). The scats found by the dog team enabled the genetic identification of 29 different wolves, including eight females. These 29 wolves represented 100% of the different wolves identified in this study. Without the work of the dog team, 22 of these 29 (75.9%) genotyped wolves would not have been identified at all.

We confirmed wolf presence in 61 of 283 surveyed segments covered by the dog team (25.1%; Appendix S3a). The number of scats found varied according to whether the territory was held by a wolf pack or not. The mean quantity of scat found per km was significantly higher in WP (0.40/

km) than in no-WP areas (0.10/km, $t = 5.7758$, $df = 7.3904$, $P < 0.001$). Opportunistic monitoring by FWN observers represented 288 cumulated months of monitoring (12 sample units \times 24 months of monitoring). Fourteen out of 288 months of opportunistic monitoring led to confirmation of wolf presence (Appendix S3c). The mean quantity of scat found per month (as opportunistic monitoring) did not differ between WP and no-WP areas (0.05/month in both types of area).

Based on our occupancy model, the chance for successful detection of genetically confirmed wolf samples was 0.33/transect km in WP territories and 0.15/transect km in no-WP areas (Table S1). We calculated that 10 km of transect search was necessary to obtain a 98% probability of detecting wolf presence in territories exploited by a wolf pack, due to “imperfect” detection (Fig. 6a). In territories without a confirmed wolf pack, 20 km of transect search was necessary to reach a 96% probability. Based on our occupancy model the chance for encountering genetically viable wolf scat samples using opportunistic detection by FWN observers was 0.09/month in WP territories and 0.06/month in no-WP areas (Table S2). Due to “imperfect” detection, we calculated that 24 months of opportunistic monitoring by FWN observers was required to obtain a 90% and 76% probability of detecting wolf scat in WP and no-WP areas, respectively (Fig. 6b).

Discussion

Due to the history of wolf colonization in France, the FWN monitoring system is well developed in the Alps and considered one of the best in Europe: the strength of several independent methods (geographical distribution and expansion, CMR data based on DNA samples, number of reproductions) supporting each other is important, especially for determining the growth rate of the population (Liberg 2012). However, several factors decrease the efficiency of the FWN observer network, as shown by our results and noted by Liberg (2012). In newly colonized areas, a lack of observers may lead to sampling bias as highlighted in Louvrier et al. (2018a, b). The lack of snow events can hamper reliable estimation of the MNW. In these areas, the MNW is thus estimated from visual observations and photographs. The use of a scat detection dog and handler team greatly improved the collection of samples for genetic analysis in newly wolf-colonized areas. As the dog team used



non-randomly selected transects trails previously surveyed by FWN observers, the two methods of survey (FWN observers *vs.* dog team) allowed comparison. Two days of field survey resulted in the collection of fourteen times more genetic samples than one year of monitoring by FWN observers. Interestingly, the wolf scats located by the dog represented all seven of the individual wolf genotypes in samples collected by humans, but also a further 22 individual wolf genotypes, which were found in considerably less time. Based on our occupancy model, ten km of transect trail survey (one day in the field) with a detection dog was required to reach a 98% detection probability in WP territories and 20 km (two days in the field) was required to reach 96% in no-WP areas. Ten to more than 55 FWN observers, the number of observers varying depending on the sample units, performing opportunistic surveys needed at least 24 months of monitoring to achieve comparable results. For site occupancy models to apply, several assumptions must be met (MacKenzie et al. 2003, MacKenzie 2006). First, the species should not be detected when absent from a site. Second, detection histories in all sampling units are assumed to be independent. Third, the closure assumption assumes that the status of a site should not change through occasions (or in our dog detection design, through spatial replicate, i.e. surveys segments “j”). These assumptions and model limitations are discussed in Appendix S4. Each outing with or without the scat-detection dog-handler team is estimated at an average cost of €300/day/agent. Cost benefit from FWN non-professional observers is more difficult to assess as there is no count of the sampling effort. As each professional FWN observer dedicated at least two days per year to wolf scat search, it represents a total cost of €2,400 to €7,800 per year and per sample unit, depending on the number of FWN professional observers active in each unit. As one dog-handler team can monitor 16 PPZ per year (two PPZ per month during fall-winter-spring), the cost saving may be between €28,000 to €115,000 per year, and allows better genetic results (human observers missed what equated to 75.9% of individual genotypes attributed to dog team finds). The French Bear Network estimated that the use of a scat-detection dog for brown bear scat collection has saved €736,200 between 2014 and 2019 (Sentilles et al. 2020). Finally, the training of a scat-detection dog takes a few months, compared to several years needed for humans to become experts in wolf monitoring. The use of the scat-detection dog is thus particularly cost-efficient.

Genetic samples are necessary to implement the CMR model and get reliable estimations of wolf population. This is particularly important in newly colonized wolf areas as people are not accustomed to dealing with sheep depredation (Chapron et al. 2014) and sheep breeders may focus on wolf population estimation when considering livestock protection measures. Indeed, French authorities allow farmers to prevent wolf depredation by lethal control (i.e. legal shooting) and the CMR population estimation is the base of this control (Duchamp et al. 2017).

Detection heterogeneity

Comparison of dog detection results in WP and no-WP areas suggest that the probability of detection seems to be correlated with the number of scats present (the higher the number of scats, the higher the probability of finding them). In areas where we suspected only dispersers, we found that using a dog team led to a detection probability that was half of that in wolf pack territories. Dispersers do not mark their territories as resident paired mates do (Mech & Boitani 2010). It is therefore logical that wolf faeces identified by the dog team were four times more frequent in WP territories than in no-WP. Despite that, the observed difference could also result more from the smaller potential quantity of faeces found in no-WP areas, rather than the difference in marking behaviour.

Complex biological phenomena may influence detection probability, and one should remain cautious when trying to interpret differences in detection probability. Some factors affecting detection heterogeneity by FWN observers include seasonal variation, as higher detection probabilities occur in winter (Marescot et al. 2011, Louvrier et al. 2018b). However, it is important to note that these different detection rates arise because of the reliance on visual factors influencing wolf sign detection by human observers. This may be especially true in the French Alps where the sampling effort is very high, and areas of historical wolf packs are well known (see map in Appendix S3b). Indeed, wolf sign probability detection by FWN observers is relatively high during winter in the French Alps core, where the sampling effort (defined as the number of observers per site and per year) is highest. In our study, detection probabilities by FWN observers did not differ between WP and no-WP territories. We suspect that since snow events were rare in these areas, sampling effort and experience of FWN trained observers was the main

factor determining wolf scat detection in newly colonized areas. This is in accordance with results found in previous studies (Louvrier et al. 2018a, b).

Detection dog team performance and scat collection

The detection dog found 82.1% of the total wolf scats detected by all survey methods. Trained dogs have been used to find scat samples from numerous species (Engeman et al. 2002, Smith et al. 2003, Wasser et al. 2004, Cablk & Heaton 2006, Sentilles et al. 2016, Richards et al. 2018, Statham et al. 2020). The presence of nontargets within the surveyed environment may introduce complexity. For example, when looking for otter (*Lutra* sp.) faecal matter, handlers must be particularly vigilant if a communal latrine is encountered, wherein numerous other species may have defecated upon and/or overmarked target scats, to avoid reinforcing the dog to co-occurring nontarget scat (Richards et al. 2018). If the handler unintentionally rewards the dog for a nontarget, e.g. because it is visually indistinguishable from the target or has been overmarked, the dog may then seek it out in addition to their target, which can in turn decrease accuracy (Smith et al. 2003, MacKay et al. 2008).

The proportion of false positive responses (i.e. detection of nontargets) using scat detection dogs has previously been reported to be highly variable, ranging from 3.7 to 44.6% (DeMatteo et al. 2018). False positives were low (4.1%) in the current study and therefore did not pose a problem. The non-target scat found in our study were visually characteristic of wolf scats, we suspect these were target scats that had been urine-marked by other species, mainly red fox. According to Wikenros et al. (2017), meso-predators such as red foxes tend to urinate or defecate on the scat of apex predators. Systematic DNA analysis of recovered samples is key for effective monitoring (Hollerbach et al. 2018) and genetic confirmation to species is typically also recommended by detection professionals (see Statham et al. 2020). We note that many factors, including the age of the sample, exposure to elements and diet, influence the success of DNA amplification (Murphy et al. 2003, Piggott 2005, Panasci et al. 2011), which can also reflect on the performance of dog-handler teams and human-only teams. Collection and storage conditions of the samples also influences their viability for genetic analyses (Waits & Paetkau 2005, Statham et

al. 2020). In the future, we could improve sample storage using cotton swabs in the field to isolate the mucous layer on the surface of fresh wolf scats (Rutledge et al. 2009).

In this work, the use of a detection dog and handler team helped improve the genetic monitoring of wolves in newly colonized areas of France. The team surveys enabled an improvement in quantitative (i.e. more genetic samples) and qualitative (individual genotyping) data collection. We showed that the use of the dog team allows considerable time savings (up to 99.6%) in comparison to opportunistic monitoring by human-only teams. As information sharing is an important aspect for mitigating conflict over wolves (Treves et al. 2009), detection dog utilization (and the time gain it represents) may be particularly useful in rapidly disseminating information to stakeholders. Moreover, detection dogs may play an ambassadorial and stakeholder engagement role, as livestock farmers often accompanied researchers in the field and were interested by our work.

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Supplementary online material

Appendix S1. Dog training (<https://www.ivb.cz/wp-content/uploads/JVB-vol.-69-3-2020-Roda-et-al.-Appendix-S1.pdf>).

Appendix S2. WinBUGS code for model 1 (<https://www.ivb.cz/wp-content/uploads/JVB-vol.-69-3-2020-Roda-et-al.-Appendix-S2.pdf>).

Appendix S3a. Detection histories (dog team) (<https://www.ivb.cz/wp-content/uploads/JVB-vol.-69-3-2020-Roda-et-al.-Appendix-S3a.pdf>).

Appendix S3b. FWN observers and sampling effort (<https://www.ivb.cz/wp-content/uploads/JVB-vol.-69-3-2020-Roda-et-al.-Appendix-S3b.pdf>).

Appendix S3c. Detection histories (French Wolf Network observers) (<https://www.ivb.cz/wp-content/uploads/JVB-vol.-69-3-2020-Roda-et-al.-Appendix-S3c.pdf>).

Appendix S4. Discussion on the model assumptions and limitations (<https://www.ivb.cz/wp-content/uploads/JVB-vol.-69-3-2020-Roda-et-al.-Appendix-S4.pdf>).

Table S1. Model-averaged estimates, standard errors, and 95% confidence intervals for p , the dog-team probability of detecting, when present, at least one wolf in wolf pack territory (WP) and in territory frequented only by disperser(s) (no-WP).

Table S2. Model-averaged estimates, standard errors, and 95% confidence intervals for p , the FWN observer's probability of detecting, when present, at least one wolf in wolf pack territory (WP) and in territory frequented only by disperser(s) (no-WP).

(<https://www.ivb.cz/wp-content/uploads/JVB-vol.-69-3-2020-Roda-et-al.-Table-S1-S2.pdf>)