Using dogs to find cats: detection dogs as a survey method for wide-ranging cheetah

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Short Title: Using Detection Dogs for Large-scale Cheetah Surveys

Abstract

Rapid global large carnivore declines make evaluations of remaining populations critical. Yet landscape-scale evaluations of presence, abundance, and distribution are difficult, as many species are wide-ranging, occur only at low densities, and are elusive. Insufficient information-gathering tools for many large carnivore species compounds these challenges. Specially-trained detection dogs have demonstrated effectiveness for carnivore surveys, but are untested on extremely sparse, wide-ranging species, such as cheetah (*Acinonyx jubatus*). In this study we conducted the first rigorous cheetah survey using detection dogs in a key transboundary area in the remote Liuwa-Mussuma Transfrontier Conservation Area (LMTFCA) in Western Zambia. We proposed to: 1) evaluate the effectiveness of detection dog versus spoor surveys in detecting cheetah presence; (2) extract and analyze DNA from scat samples to estimate minimum population size and genetic effective population size; and (3) determine the extent of cheetah occurrence in the unprotected transboundary corridor. Two detection dog teams surveyed 2,432 km^2 containing 74 randomly located transects in the transfrontier area. Twenty-seven cheetah scats were detected and confirmed by genetic analysis, while no cheetah spoor was detected, clearly demonstrating the superiority of detection dogs in detecting cheetah presence. Combining scat samples with opportunistically-collected samples we estimated 17-19 cheetahs, an effective population size of 8-14, and a density of 5.9-6.6/1000km^2. Cheetah utilized key transfrontier areas outside of the national park; however, because utilization appears low, improved connectivity and protection for these areas is critical. Approximately 1/3 of Africa's estimated cheetah resides in protected areas, with 84% in transboundary areas. Our study demonstrates the efficacy of detection dog survey methods in providing information on cheetah across large landscapes. It will have particular value in areas where other survey means may be impossible, such as TFCAs, where size, remoteness, and lack of accessibility often make traditional survey methods difficult or cost prohibitive.

Keywords: Cheetah, *Acinonyx jubatus*, Detection Dogs, Transboundary, Liuwa Plain

Introduction

Rapid human-induced ecological change has driven global large carnivore declines and the consequent trophic downgrading of planet earth (Estes et al. 2011). Halting these declines requires sound information on remaining populations to focus scarce conservation resources efficiently. Landscape-scale evaluation of large carnivore presence, abundance, and distribution is a daunting task: carnivores are wide-ranging, occur in low densities only, and are often declining; in addition, they are elusive and vulnerable to human impacts. Large carnivore ranges often transcend political boundaries, requiring international cooperation in management and conservation actions. We currently lack sufficient tools to gather reliable information on many species of large carnivores, particularly those that are most wide-ranging (Belbachir et al. 2015). This lack of reliable tools substantially hinders ongoing conservation efforts, preventing (1) accurate information on distribution and abundance for conservation decision makers (Durant et al. 2014); (2) evidence-based assessment of the effectiveness of a range of conservation interventions (Walsh, Dicks and Sutherland 2015); and (3) adaptive conservation management (Durant 2013).

Reliable information on large carnivores and their prey in recently developed Transfrontier Conservation Areas (TFCAs) is urgently needed, as TFCA development has led to development of international agreements and removal of barriers such as fencing to maintain and to restore large-scale wildlife movements, carnivore populations, and ecological processes (WCS 2008; Lindsey, Romañach & Davies-Mostert 2009). As the widest-ranging of all African large carnivores, often shy and elusive, and declining throughout its range, the cheetah (*Acinonyx jubatus*) is an iconic TFCA species and presents a significant

challenge in gaining reliable information on its presence, abundance, and distribution (IUCN/SSC 2007a). Southern Africa and Eastern Africa are the largest remaining strongholds of cheetah with a limited number of well-defined populations in Tanzania, Namibia, and South Africa (Marker 2002, Durant et al. 2007, IUCN/SSC 2007a, b, Mills and Mills 2014). Less than 40% of Africa's entire estimated cheetah population resides in protected areas, and 87% of cheetah live in transboundary areas (Durant et al. In Press). However, for more than 40% of historical cheetah range there is no reliable presence/absence data (IUCN/SSC 2007a,b, 2012). An array of research and monitoring techniques has been employed for cheetah, including long-term intensive studies (Caro 1994, Durant, Kelly and Caro 2004, Durant et al. 2007), spoor surveys (Funston et al. 2010), camera traps (Marnewick, Funston and Karanth 2008, Belbachir et al. 2015), and tourist photographs (Marnewick et al. 2014). Much of Africa's remaining potential cheetah habitat and populations for which no reliable data exists lies in remote, poorly described environments of key countries such as Angola, Chad, Ethiopia, and South Sudan. These environments are often seasonally inaccessible and poorly-developed, with little road access and infrastructure to facilitate many traditional survey and monitoring methods; they may also suffer from past or ongoing political insecurity. There is thus an urgent need for suitable methods across a broad range of habitats and in logistically challenging regions.

Detection dogs have emerged as a useful large carnivore research and monitoring technique, given the superior ability of dog olfaction to detect species presence in the form of scat, hair, scent-marks and other sign. Genetic data from scat can identify species, gender, and individual animals (Smith et al. 2003). However, rare species pose a particular challenge in detection, and the method has yet to be tested for density estimation for species below 10/100km^2; hence its utility for such species remains promising but poorly understood. Cheetah are routinely found at densities below 1/100km^{2,} and have never been recorded at densities much above 2/100km^2 (Durant et al. In Press). Recognizing the

potential for detection dogs to effectively provide data for poorly-described cheetah populations, the Cheetah Regional Strategy in 2010 created the Transboundary African Conservation Dog Working Group to promote the use of detection dogs, help ensure methods were standardized, and adhere to an accreditation process for developing programs (Bashir et al. 2004). Current cheetah distribution in Zambia is poorly understood, and the group identified Western Zambia's Greater Liuwa Ecosystem as a priority ecosystem for conducting the first detection dog survey. The area is suitable given its remoteness, its unsuitability for traditional road-based spoor survey methods (given the prevalence of vegetated substrate year-round and a limited dirt road network), its connectivity to Angola's Mussuma National Park, and its poorly described but regionally important cheetah population (Fig. 1).

We conducted what, to our knowledge, is the first rigorous survey of cheetah using detection dogs, focusing on a key transboundary corridor to address three objectives: (1) evaluate dog effectiveness in detecting cheetah compared to spoor surveys; (2) extract and analyze DNA from cheetah scat samples to estimate minimum population size and genetic effective population size (Ne); and (3) determine the extent of cheetah occurrence in the corridor, particularly on unprotected land.

Methods

Study Area

Our study occurred in Western Zambia's Greater Liuwa Ecosystem, comprised of Liuwa Plain National Park (LPNP) and adjacent West Zambezi Game Management Area (Fig. 1). Liuwa comprises the Zambian portion of the Transfrontier Conservation Area with Angola and habitats consist primarily of seasonally-flooded grasslands combined with scattered woodland (M'soka et al 2016). Wildlife was severely depleted by poaching during the Angolan civil war, human-wildlife conflict, and poorly-regulated

hunting. In 2003 the African Parks Network (APN) assumed management of LPNP in collaboration with the Department of National Parks and Wildlife (formerly ZAWA) and the Barotse Royal Establishment. Wildlife, particularly wildebeest (*Connochates taurinus*), have since recovered significantly (Viljoen 2013, M'soka et al. 2016). Cheetah are resident and breeding in Liuwa, but in unknown numbers, with the degree of transboundary exchange unknown. Lion (*Panthera leo*) were functionally eliminated, and the guild is dominated by high numbers of spotted hyena (*Crocuta crocuta*) with a small wild dog (*Lycaon pictus*) population (Purchase 2004, M'soka et al. 2016).

Detection Dogs

Detection dog teams consisted of a dog, handler, and orienteer (hereafter referred to as dog teams). We selected two dogs to conduct the survey, both adult male Belgian Malinois, sourced and trained by Working Dogs for Conservation, and Green Dogs. Collectively dogs had 9 years of scat detection experience spanning 17 projects in 4 countries on 15 species, facilitating the effective addition of cheetah scat into their scent repertoire. Six months prior to the survey, the dogs underwent training on wild cheetah scat obtained opportunistically from field research in Liuwa and South Africa. More extensive descriptions of training methods for scat detection dogs are described in Smith et al. (2003).

Survey Design

The survey area was 2,432 km^2, spanning the national park's northern half and portions of the Game Management Area (Fig. 2), a potential corridor between LPNP and Angola. The area is also heavily used by wildebeest seasonally (M'soka et al. In Press). The survey was sectioned into 8 × 8 km grids aligning with minimum cheetah home range sizes in similar savannah ecosystems (Laver, 2005), which facilitated

high detection probability while ensuring systematic and adequate coverage. Within each grid cell, rectangular transects were randomly located, each comprising a 1 km northbound leg, a 500 m perpendicular offset, a 1km southbound leg, and a return to the original starting point. Longer legs were oriented north-south to maximize scent interception on prevailing easterly winds. Most grid cells contained two transects; some contained only one if the other fell in unsuitable terrain (e.g. wetlands, open water); one grid cell contained four transects as it was the first cell surveyed while calibrating the number of transects which could effectively be surveyed in the time allotted. The survey was originally designed to provide a spatially replicated mark-recapture estimate (Thompson, Royle and Garner 2012) but sample sizes of recaptures were insufficient (see results). Concurrent with the detection dog survey, transects were walked for spoor (defined as tracks, sign and scat) and sightings of all carnivores.

Transects were surveyed morning (0600-1000 hrs) and evening (1600-1800 hrs) when temperatures were low and spoor detection probability was high. Dogs were worked off leash on transects to approximately 500 m strip width, with GPS tracker devices both on the dog and carried by the dog team, with one team per transect. A two-person team responsible for spoor counts surveyed ahead of the dog team to enable concurrent data collection just prior to dog surveys. Cheetah use trees for scent-marking and shade; hence dog teams also checked woodlands adjacent to transects for spoor. Scats were collected using sterile latex gloves and placed into paper bags for storage with desiccant.

Analyses

Samples were analyzed at the Zoological Society of London's genetics laboratory to determine species, sex, and individual identification. Ongoing intensive studies of cheetah in southern LPNP (approximately

450 km^2 not included in the survey area) provided additional scat samples from known individuals which were included to assist with population estimates.

DNA extraction

Fecal DNA was extracted using the QIAamp DNA Stool kit (QIAGEN), with minor protocol modifications to enhance removal of impurities/inhibitors and increase DNA yield, and a final elution volume of 120µl was used to improve DNA concentration. As DNA is not spread uniformly through fecal samples (Goossens et al. 2003) multiple independent extractions were performed to maximize probability of obtaining DNA. Two tissue samples collected from carcasses in Serengeti, Tanzania, were used as positive controls. Tissue DNA was extracted from control samples using the DNeasy Blood & Tissue Kit (QIAGEN). Rigorous standard procedures were employed to minimize contamination likelihood throughout extraction, including stringent cleaning of all surfaces and equipment (with 10% bleach and/or exposure to ultraviolet radiation).

Genotyping

DNA was amplified using domestic cat microsatellite primers with a cross-species amplification approach (Menotti-Raymond *et al.* 1999, Goossens *et al*. 2000b). Thirteen microsatellite loci previously tested for cheetahs (Gottelli *et al* 2007, Marker *et al* 2008, Charruau *et al* 2011) with allele sizes smaller than 250bp were screened in 5 multiplex mixes (Table 1); larger loci are problematic when amplifying DNA from non-invasive samples, (Goossens et al. 2000b).

Samples were amplified using fluorescently-labelled forward sequences for each primer pair via PCR in a reaction volume of approximately 7µl containing 2µl (≤50ng) template DNA, 1.5µl (0.3µ*M*) primer,

0.02µl bovine serum albumin (New England Biolabs), and 3.5µl Multiplex PCR Mix (QIAGEN, final concentration 3m*M* $MgCl_2$). The thermal profile for all PCR reactions consisted of: initial denaturation and enzyme activation at 95°C for 15 minutes; 30-35 cycles of denaturation at 94°C for 30 seconds; annealing at relevant temperature for 90 seconds; extension at 72°C for 60 seconds; and final extension at 72°C for 30 minutes. PCR products were visualized on an ABI PRISM 3130xl Genetic Analyser (Life Technologies) together with GeneScan 500 LIZ Size Standard (Life Technologies). Alleles were scored using GeneMapper V.4.1 (Life technologies) against the internal size standard to derive individual genotypes at each locus. Consensus genotypes were derived for each sample using a multi-tube, multi-sample approach and a strict set of *a priori* allele-scoring rules (Taberlet et al. 1996, Goossens et al. 2000a). A minimum of five independent PCR replicates were genotyped for each extraction to minimise genotyping errors associated with low-quality template DNA (e.g. false alleles, allelic dropout) (Taberlet et al. 1999). To ensure standardized allele sizes between samples/replicates, PCRs were prepared in physically-isolated areas to prevent cross-contamination but amplified simultaneously (same PCR), and reference samples (high quality DNA extracted from a tissue sample) were included in every PCR. Negative controls from every stage (extraction blanks & PCR blanks) were also included during genotyping to monitor potential contamination and PCR failure.

Sex-determination

All samples were genotyped using felid specific primers, ZN, developed for sex identification on the zinc finger region that has a three base pair deletion in males (Pilgrim *et al.* 2005). This deletion results in a single allele in females (163bp) compared to two alleles in males (163bp and 160bp). The forward primer was fluor-labelled (6-FAM) and the PCR product run through the ABI 3130 capillary sequencer. Amplification took place in 6µl PCR reactions using 1µl DNA (1:5 dilution), 3.5µl Qiagen Multiplex PCR

Master Mix and 1.5µl of forward and reverse primer mix (10 pM/µl). Thermal cycling was performed in a Gene Amp® PCR system 9700 (Life Technologies) and consisted of denaturing and hot-start enzyme activation for 15 min at 95°C then 35 repeating cycles of 30s denaturing at 94°C, 1 min at 56°C and 30s extension at 70°C. The final extension period was for 10 min at 72°C. PCR replication and genotype scoring rules were applied as above to obtain consensus genotypes for each individual.

Genetic Analysis

Genotyping errors due to false alleles, allelic dropout, stutter and null alleles were checked using MICRO-CHECKER V.2.2.3 (Van Oosterhout et al. 2004). Genetic diversity was measured by the number of alleles per locus (*A*), the inbreeding coefficient (F_{is}), observed heterozygosity (H_o) and expected Heterozygosity (H_e) under Hardy-Weinberg assumptions. The analyses were performed using *GENEPOP 4.2* (Rousset 2008), *FSTAT 2.9.3.2* (Goudet 2002)

We used a likelihood clustering method implemented in COLONY V.2.0.4.5 (Jones and Wang 2010) to identify distinct individuals and recognition of duplicated individuals and their relatedness. COLONY was also used to estimate the current effective population size (N_e) of this cheetah population using the full-likelihood sib-ship assignment (Wang 2009).

Results

Seventy-four transects were surveyed within 38 grid cells from 30 August-23 September 2012. Fifty scats were found by detection dogs. An additional 17 scats were found by humans in southern LPNP during intensive carnivore field work prior to the detection dog survey (2010-2012). Of the 50 dog-

detected samples, 35 were from planned survey transects and 15 were found opportunistically at other locations, primarily scent-marking trees. Scats were found by the dogs at 32 unique locations, some with multiple scats, and 17 of these were from transects (Fig. 2). No sightings of cheetah or cheetah spoor detection occurred on transects or during the course of the survey. Of 67 samples collected, 52 (77.8%) yielded DNA genotyped for 13 loci with various degrees of success, and of these, only 26 samples (38.8%) successfully amplified for 6 or more loci. Only this latter subset was used on the final analysis. Sample sizes, particularly of recaptures in this latter subset, were insufficient for mark-recapture population estimates.

Genetic diversity and estimation of population size

A summary of Genepop results are shown in Table 1. The average observed heterozygosity across loci was 0.47 (SD 0.03). This value is low for a wild population and is half of the estimate average expected under Hardy-Weinberg equilibrium (HWE) heterozygosity 0.81 (SD 0.025). Including all samples, we identified an upper bound of 19 individuals, comprised of 7 males and 12 females (Table 2), for an estimated density of 6.6 cheetah/1000 km^2. Including only samples identified by dogs in the survey area, we estimated an upper bound of 14 cheetahs (5.8 cheetah/1000km^2). Lower estimates would be obtained if we assumed that two pairs of apparent full siblings were in fact just two individuals sampled twice each; in this case, the total count would be 17 cheetahs (5.9 cheetah/1000km^2) and the count from the survey by detection dogs would be 12 cheetahs (4.9 cheetah/1000km^2). Analyses of effective population size (Ne) by COLONY using the full likelihood method and assuming random mating was 14 (95% CI=7,30), while estimates assuming non-random mating were 8 (95% CI= 4,24).

Discussion

We successfully conducted the first rigorous survey of cheetah using detection dogs and genetic techniques, providing the first quantitatively-derived cheetah density estimate for this transfrontier area. We detected cheetah presence throughout the survey area, including the unprotected corridor, consistent with findings elsewhere in Africa where the majority of cheetah persist in unprotected areas (IUCN/SSC 2007a, b, and IUCN/SSC 2012). Cheetah detection was low outside LPNP; thus additional protections of the GMA corridor though inclusion into LPNP, should receive strong support. At present, rapid land conversion for rice farming is occurring in the corridor, reducing the available habitat, degrading corridors, and encouraging bushmeat poaching. Should such trends continue, connectivity between Angolan and Zambian cheetah populations will be unlikely, and effective and enforced land use planning is urgently needed in Liuwa and throughout cheetah range in Zambia (Watson et al. 2014).

While mark-recapture population estimates were not possible, we were still able to estimate a minimum population size, and it is likely that a viable resident breeding population of cheetah exists throughout Liuwa and potentially into Angola (Fig 2). A minimum estimated population size of 17 cheetah suggested a genetic effective population size (Ne) of 8 (95% CI=4-24) to 14 (95% CI=7-30) individuals, assuming non-random and random mating respectively. The relatively low Ne could suggest that either this population does not border a large population on the Angolan side, or that there is limited exchange between these populations. The average observed heterozygosity across loci was 0.47 (SD 0.03); this value is low for a wild population and half of the average expected under Hardy-Weinberg equilibrium. This is potentially indicative of a genetic problem like inbreeding with a small isolated population, but could also simply be an artefact of sample degradation and laboratory limitations. In this case the discrepancy is probably due to the presence of null alleles (Table 1) that have artificially

increased the homozygosity and lowered the observed heterozygosity. The status of both countries' populations and the connectivity between them needs further investigation.

While detection dogs were effective, we identified an array of factors that should be evaluated when considering the application of this technique versus traditional techniques to survey cheetah and other large, elusive carnivores (Table 4). Particularly relevant to our study was scat persistence and the subsequent quality of DNA derived from it, which likely varies seasonally and by ecosystem. Carnivore scat losses to dung-beetles were observed during the survey and potentially had a pronounced impact on detection of cheetah scats, as well as on the quality of scats detected. We were unable to account for scavenger loss on detectability, but scats are more likely to be older and DNA quality lower in areas with fewer invertebrate scavengers. However in a less systematic survey in the Algerian Sahara ecosystem where scavengers are fewer and scats subjected to severe heat, DNA could still be extracted from 75% of carnivore scat samples, and of all eight cheetah samples identified, it was still possible to type eight loci (Busby et al. 2006, 2009). Where scats can be collected fresh, a 100% success rate can be expected (Gottelli et al 2007). In addition, with the advance of genetic techniques such as single-nucleotide polymorphisms (SNPs), the feasibility of more rigorous population estimation through methods such as pedigree reconstruction (Creel and Rosenblatt 2013) could be employed with DNA from scats, should SNP methodology be developed for cheetah scat samples. Lastly, a key factor for detection dog surveys in Africa is likely to be the presence of tsetse flies and potential Trypanosomiasis, or sleeping sickness, transmission to domestic dogs. Additional research to address this threat is high priority, as the use of detection dogs to survey and compare cheetah across different habitat types makes this method of significant value to conservation efforts.

The effectiveness of scat detection dogs (27 samples) compared to spoor-based surveys (0 detections) demonstrates the utility of this method for monitoring cheetah and other elusive and low density carnivores. While the method has demonstrated effectiveness there is an array of factors to consider and address before adopting it as a large scale alternative to traditional methods (Table 4). Nevertheless this method should be considered as a suitable alternative whenever possible, especially across vast landscapes when substrates are not suitable for track detection and only infrequent surveys are possible. Surveys using only spoor would have inaccurately concluded that cheetah were absent in the study area, when, in fact, the site supports a moderate density. We recommend detection dog surveys, coupled with concurrent use of spoor surveys, to allow further validation and potentially calibration of the two methods. In areas where adequate numbers of cheetah exist or multiple surveys can be conducted, rigorous mark-recapture estimates should be possible and study designs should facilitate this whenever possible.

As available habitat and connectivity for cheetah continue to decline continent-wide, urgent conservation actions for cheetah are required. At present we are limited by our ability to monitor how cheetah respond to both threats and benefits from conservation actions. Our study confirms detection dogs are a viable survey and monitoring technique for low density, wide-ranging large carnivores in TFCAs as well as in other remote, large-scale survey areas. While we recovered sufficient samples for our analysis, survey design could be improved to deliver additional samples by considering the various factors identified in Table 4. In many areas, such as Liuwa, there are not viable alternative methods for surveying cheetah, and this method provides an important option that could be used across a variety of different habitats throughout remaining cheetah range. As such, it represents an important step forward in our ability to monitor and conserve elusive large carnivores in the face of rapid human-induced environmental change.

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Tables

Table 1. Measure of diversity of 13 microsatellites loci. Showing results from GenePop: Observed *Ho* and expected *He* heterozygosity, Number of alleles per locus and Inbreeding coefficient *Fis* and Potential genotyping error identified in Microchequer

	Genepop				Microchequer			
Loci	*Ho*	*He*	*allele#*	*Fis*	Homozygote excess?	Possible stutter error?	Possible allelic dropout?	Likely Null Alleles?
Fca133	0.65	0.792	10	0.2603	y	y	n	y
Fca205	0.38	0.801	8	0.6212	y	n	n	y
Fca247	0.736	0.879	11	0.1421	n	n	n	n
Fca152	0.3	0.82	12	0.7647	n	n	n	n
Fca214	0.09	0.793	9	0.1656	n	n	n	n
Fca339	0.142	0.595	6	0.8879	y	n	n	y
Fca212	0.615	0.807	11	0.2417	y	n	n	y
Fca96	0.69	0.926	12	0.5308	y	n	n	y
Fca107	0.388	0.876	11	0.1892	y	n	n	y
Fca193	0.75	0.919	17	0.6404	y	n	n	y
Fca117	0.352	0.677	5	0.1801	y	n	n	y
Fca391	0.733	0.85	10	0.4866	y	n	n	y
Fca52	0.304	0.792	9	0.5633	n	n	n	n
	0.47 SD 0.03	0.81 SD 0.025	10 SD 2.99	0.4382				

Table 2. Location, samples and identification of individuals following likelihood clustering and pairwise likelihood relationship implemented in COLONY.

location	samples#	ID
	LS12021	Male 1
southern	LS12023	Female 1
LPNP	LS12031	Female 2
	LS12025	Female 3
	LS12020B	Female 4
	P0915_309_A_4	Male 2
	F0911_336A_4	Male 3
	F0911_336B_1	Male 4
	P0915_309B_1	Female 6
	F0919_OPP_A	Male 5
UWZGMA	F0919_OPP_B	Female 9
	P0919_OPP_2	Female 5
	F0911_336B_2	Male 6
	F0911_OPP_1	Female 7
	P0918_276_A_3	Female 8
	P0919_305B_1	Male 7
	F0919_OPP_E	Female 10
	F0911_336B_4	Female 11
	F0911_336A_2	Female 12

Table 3. Probability of Fullsib relationships between samples from COLONY.

sample#	ID	sample#	ID	Prob
F0911_336A_4	Male 3	F0911_336A_2	Female 12	1
F0911_336A_4	*Male 3*	*F0911_336B_1*	*Male 4*	*0.854*
P0918_276_A_4	*Female 8*	*F0911_OPP_1*	*Female 7*	*0.96*
F0919_OPP_B	Female 4	F0919_OPP_A	Male 5	0.954
LS12023	Female 1	LS12021	Male 1	1
LS12023	Female 1	LS12031	Female 2	1
LS12023	Female 1	LS12025	Female 3	0.993
LS12021	Male 1	LS12031	Female 2	1
LS12021	Male 1	LS12025	Female 3	0.989

Table 4. Considerations for implementing detection dog surveys of cheetah

Variable	**Consideration**
Permits	Special permission may be needed to take detection dogs into protected areas. The acceptance of detection dogs has increased with efforts to combat international wildlife crime which increasingly make use of dogs.
Access	Remote environments are typically rugged, seasonally inaccessible and have limited or absent road networks, conditions favoring foot-based surveys using dogs to increase detection.
Logistics	Large-scale surveys will typically require mobile camps and survey teams, and special considerations for dog travel, bedding, water and feeding will be required. Travel to and from study sites can be difficult and require similar considerations.
Expense	Most detection dog teams currently require regional or international sourcing.
Borders	Countries have differing requirements on domestic dog vaccination and health screening procedures which need to be addressed well in advance when moving dogs across borders.
Seasonality	Scavenger abundance and distribution is likely higher in wet seasons and more tropical environments while fires are more frequent in the dry season. These factors may mean scat persistence could vary greatly across seasons.
Habitat	Thick vegetation, topography, water and other environmental aspects will lengthen survey times. Such conditions may also preclude vehicle-based work and thus make the use of detection dogs the most feasible survey option.
Scavengers	Scavenger diversity (invertebrate and vertebrate), abundance and distribution can have strong impacts on scat persistence and vary depending on seasonality and habitat.
Prey Abundance, Distribution and Movements	Prey are likely to be more widely distributed in wetter environments, and distribution can widely vary in seasonal environments and with migrations. Whether carnivores are likely to mirror prey movements and whether carnivore abundance and distribution is strongly correlated with prey abundance should be considered in survey design.

Heat	Hotter temperatures will reduce daily survey time for dogs and makes travelling with them more difficult.
Disease	Presence of disease vectors such as tsetse flies and trypanomiasis can threaten health and lives of detection dogs.
Sympatric Carnivores and Large Herbivores	Presence of dangerous sympatric carnivores (i.e. lion, leopard) and herbivores (i.e. elephant, buffalo) can restrict survey range, require protection for survey team, and usually require leashed dogs.

Figures

Figure 1. Map of study area. The Liuwa-Mussuma Transfrontier Conservation Area (LMTFCA) includes Liuwa Plain National Park (LPNP), Upper West Zambezi Game Management Area in Zambia, and Mussuma National Park in Angola. Presence, abundance and distribution of cheetah outside LPNP prior to surveys was unknown.

Figure 2. Map of the Survey Area and spatial distribution of cheetah scat and sign from survey coverage. Surveys were conducted in 8 × 8 km grids, with two of a possible six transects of 1 km length each randomly selected within each grid for surveying by detection dog teams.