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SPECIAL ISSUE: DOGS AND CONSERVATION: CURRENT AND EMERGING CONSIDERATIONS

Training methodology for canine scent detection of a critically endangered lagomorph: a conservation case study

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Abstract. Efficient and systematic survey methods are essential for wildlife researchers and conservationists to collect accurate ecological data that can be used to make informed conservation decisions. For endangered and elusive species, that are not easily detected by conventional methods, reliable, time- and cost-efficient methodologies become increasingly important. Across a growing spectrum of conservation research projects, survey outcomes are benefitting from scent detection dogs that assist with locating elusive species. This paper describes the training methodology used to investigate the ability of a scent detection dog to locate live riverine rabbits (*Bunolagus monticularis*) in their natural habitat, and to determine how species-specific the dog was towards the target scent in a controlled environment. The dog was trained using operant conditioning and a non-visual methodology, with only limited scent from roadkill specimens available. The dog achieved a 98% specificity rate towards the target scent, indicating that the dog was able to distinguish the scent of riverine rabbits from the scent of other lagomorph species. The dog has already been able to locate ten of these elusive individuals in the wild. The training method proved successful in the detection of this critically endangered species, where scent for training was only available from deceased specimens.

Key words: endangered species, detect, olfactory, positive reinforcement, sniffer dog

Introduction

Wildlife surveys are fundamental components of field biology research that lead to the acquisition of data required to answer research questions in the fields of taxonomy, ecology, and conservation science (Goldsmith 1991, Buckland et al. 2000, McDonald & Thompson 2004). The selection of survey methods will directly influence the accuracy and comprehensiveness of survey outcomes (Nussear et al. 2008). It is well known that dogs have a remarkable olfactory sensory system (Jezierski et al. 2010, Lord 2013), and have been used for a variety of scent related assignments, such as the detection of drugs and explosives in forensics (criminal investigation) and for hunting (Browne et al. 2006, Hurt & Smith 2009, Adamkiewicz et al. 2013). Domestic dogs are also used to screen for cancer (Walczak et al. 2012) and to locate larger wildlife (like bears) by means of detecting scat (Wasser et al. 2004). The use of detection dogs in wildlife conservation, particularly to combat illegal wildlife trade (Braun & Stuart 2018) is becoming increasingly important.

When utilised with systematic search tactics, domestic dogs, that have been formally trained

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in scent detection methods, offer biologists efficient, alternative tools for locating wildlife (Wasser et al. 2004, Hurt & Smith 2009, Stevenson et al. 2010). Canine olfactory detection of wildlife scent is based on the principle that organisms produce characteristic volatile organic compounds (VOCs) that are detected by the canine olfactory system (Eisenberg & Kleiman 1972, Lesniak et al. 2008). Depending on the breed and individual characteristics, a dog's sense of smell is estimated to be up to 10,000 times greater than a human's (Craven et al. 2007). Consequently, conservation dogs (dogs trained to detect wildlife scents, e.g. wild animals and/or scat) have been used to locate a range of species, including mammals (Arnett 2006, Beckmann 2006), reptiles (Vice & Engeman 2000, Cablk & Heaton 2006), birds (Homan et al. 2001, Paula et al. 2011), and insects (Lin et al. 2011). Although Reed et al. (2011) found that the extent (area covered) and efficiency (detection rate) of a survey can be greatly improved using scent detection dogs, as opposed to conventional methods (such as visual surveys) or other non-invasive methods (e.g. trap cameras and scat collection), the use of detection dogs remains relatively unexplored for the detection of rare, endangered or cryptic species (Dahlgren et al. 2012).

Training dogs for scent detection typically requires a method called operant conditioning, whereby the consequences of an initially spontaneous behaviour, may reinforce or inhibit recurrence of that behaviour (Blackman 1974). Operant conditioning can be implemented using reward-based training, which is widely regarded as the best way to train a dog (Blackman 1974, Hiby et al. 2004, Geller 2008). As part of operant conditioning, it is also common to use clicker training, where the "click" sound, followed by a reward, is used to reinforce the dog's behaviour (Yoon et al. 2000, Fjellanger et al. 2002, Cornu et al. 2011).

The riverine rabbit (*Bunolagus monticularis*) is an endemic, habitat specialist found in the semi-arid Nama and Succulent Karoo biomes of South Africa. It is largely restricted to seasonal river vegetation (riparian habitat), particularly in the Nama Karoo (Duthie 1989, Duthie et al. 1989). According to the International Union for Conservation of Nature's Red List of Threatened Species, the riverine rabbit is classified as critically endangered (Collins et al. 2019) and is recognised as one of the most threatened terrestrial mammals in southern Africa. It is considered the 13th most endangered mammal globally and is listed as an evolutionarily distinct species by the Zoological Society of London (2020). The species' poor conservation outlook can largely be attributed to the fact that two-thirds of its habitat has been fragmented or destroyed in the past 50 years due to anthropogenic disturbance in the Central and Upper Karoo regions of South Africa's Northern and Western Cape provinces (Hughes et al. 2008).

Prioritising, mapping and conserving key areas for riverine rabbit conservation is critical to their survival (Hughes et al. 2008), thus it is necessary to obtain accurate distribution maps of the species. Conventional methods that are often recommended as standard field techniques for locating riverine rabbits, such as surveying an area with people to flush rabbits and camera-trapping, are often costly, inefficient, and time consuming, while covering a limited geographic area. Conservation planning is further compromised by both the behavioural ecology of the species, and the fact that they are sparsely distributed over a vast area (Duthie 1989, Duthie et al. 1989). The riverine rabbit is solitary, mostly nocturnal, and elusive, often spending daylight hours camouflaged in the shade of scrub riparian vegetation (Duthie 1989). Presently, there is no comprehensive, current distribution map for the riverine rabbit. Given that riverine rabbits are extremely difficult to detect by sight and will often remain concealed to avoid detection (Duthie 1989), it is expected that scent detection dogs could assist in the location of new individuals, which would lead to improved ecological understanding of this elusive species. Furthermore, this technique could become a valuable field technique for the detection and conservation of other threatened and cryptic species.

To ensure the species' survival into the future, a more effective, economical, and scalable approach is required to locate these rabbits. Recent research by Moser et al. (2020) demonstrates that it is possible to use scent from dead specimens to locate live insects. However, very little quantitative data and tangible results from field trials showing successful mammal species detection by dogs trained on the scent of dead specimens are currently available. The aim of this study was to evaluate the training methodology by measuring the success of the training, both in laboratory (controlled) and field conditions. The most important variables during laboratory setups were sensitivity, effectiveness, and how species-specific the scent detection dog was towards the target scent. We also investigated the ability of the dog to locate live riverine rabbits in their natural habitat. In this paper, we discuss some of the practical considerations to account for when formally training domestic dogs to detect elusive and endangered species in the wild, using limited scent and non-visual training techniques.

Material and Methods

Scent collection

Scent is caused by one or more VOCs, which are perceived by an animal using its sense of smell. As different bodily materials are composed of varying VOCs, it is important to obtain a range of samples from different individuals of the target species (as described in the protocol below). The scent detection dog is trained on each individual's scent sample separately, providing the dog with an opportunity to determine a unique combination of compounds (VOCs) that represents the target species' scent (Oldenburg et al. 2016).

Riverine rabbit samples were banked from different geographical areas, as it remains unclear whether the scent differs between distinct populations. The protocol below describes a standardised, repeatable methodology for the collection of scent for use by partners, such as universities and government departments, who may be able to assist in banking riverine rabbit scent samples. The scent library (or bank) represents both the Succulent and Nama Karoo regional riverine rabbit populations. It is vital that the rabbit is correctly identified before scent samples are collected and that the sample is labelled accurately. As riverine rabbits are elusive, critically endangered, and there are no captive individuals, all samples were limited to roadkill or frozen specimens from museum and university collections.

Similar to the research on insects for biosecurity detection dogs (Moser et al. 2020), we hypothesised that any odour variation between deceased samples and live specimens would not be substantial enough to prevent the dog from detecting live individuals in the wild. Valid provincial permits were obtained for the collection and use of riverine rabbit scent for training purposes (Cape Nature – permit number: CN44-30-4165, Northern Cape – FAUNA0271/2017). The field protocol for the collection of riverine rabbit hair scent samples from deceased specimens is described below: 1) Clean plastic was placed on the working surface and sprayed with disinfectant (70% ethanol). Once the disinfectant was dry, the rabbit carcass was placed onto the plastic; 2) Latex surgical gloves were worn by all involved in handling the carcass. Gloves were disinfected when contaminated (by blood, soil, or human scent), and new gloves were used with each new rabbit sample that was handled. Gloves were discarded after use; 3) The carcass was photographed. Photographs of the tail and head were captured as evidence of accurate species identification; 4) Dry, disinfected scissors were used to clip hair from the back and/or tail of the rabbit. When collecting the samples, areas contaminated by mud or blood were avoided; 5) Using tweezers, the hair sample was placed and sealed inside a glass container, while ensuring contamination with human scent was avoided; 6) The sample was labelled with the name of the species, sample type, date, location, and the GPS coordinates of where the carcass originated. The label also contained the name and contact details of the person who collected the sample; 7) The sample was then stored in a freezer at –20 °C.

For the skin swab scent collection step 4 and 5 were altered as follows: 4) Using tweezers to manoeuvre the carcass, dry, clean cotton wool pads were rubbed over the neck, stomach, and axillae of the rabbit for 1 minute. When collecting the samples, areas contaminated by mud or blood were avoided; 5) The cotton pad was placed and sealed inside a glass container, while ensuring contamination with human scent was avoided.

Detection dog

The dog used in this study was selected from a litter of sheep herding Border collies based on her temperament, strong motivation to learn, boldness, and high drive; all qualities that indicate trainability for scent detection work. A "drive" (prey drive) can be described as an inborn and almost overpowering predator behaviour (Spurway 1953, Marschark & Baenninger 2002), which is a valuable characteristic for working dogs. The boldness test by Svartberg (2002), indicates that bolder dogs can achieve higher performance as working dogs. The dog was trained previously, prior to this project, to locate amphibian species using similar scent detection training methods. The dog took two weeks to master detection of riverine rabbit scent in a controlled environment before *ex situ* training and field trials commenced. This timeframe will vary based on the strength of the target scent and the breed, age, and "drive"/ motivation of the detection dog. Our detection dog was four years old when this research was conducted. As this research was initiated as a case study to test the methodology, only one dog was used as an experimental tool, but all tests were designed to be replicable. We recommend that the duration of initial scent training (in a controlled environment) should be at least four weeks and the dog should be at least six months old. A younger dog may struggle with extended periods of mental concentration required for scent work. However, it is recommended that one starts with obedience and agility training during the first six months of the dog's life, as this will improve the dog's performance (Alexander et al. 2011). Our detection dog worked no more than four hours per day during experiments. This was divided into a 120-minute morning session and a 120-minute afternoon session, with at least a one-hour break between sessions. The detection dog worked a maximum of two days a week between the months of March and September. The working season was influenced by weather conditions and the target species' seasonal behaviour, as riverine rabbits are more active in the winter months (June to August). Working during this period also ensured that the dog-handler team avoided the extreme heat and potential snake bites in the study region's arid summer. Throughout the training, experiments, and field trials, the dog always had access to clean water. The dog received a food or toy reward for indicating on a positive target scent. The detection dog also received high quality food daily and lived with the handler.

Our detection dog has been certified, based on performance towards the target scent, by the Genesis K9 Group (Certificate number: GK9s19/446). Additionally, our detection dog handler received accreditation from Genesis K9 Group (SASSETA registration number: 695/ CERT/007636) and UK College of Scent Dogs (OCN London Certificate number: 6376726(1)) during this study.

In accordance with South African legislation on the use of animals in research, no animals were harmed during this study. This study was approved by the Ethics Advisory Committee on 23 January 2017.

Phase 1: Scent detection training – indoor experiments (controlled environment)

Conditioning training of dogs for scent detection requires the consideration of certain terminology, that have a specific meaning in the context of dog training. This study contains components that differ slightly from average detection dog training

Table 1. Terminology related to training of a scent detection dog as used in this study.

Term	Description
Indication	An operant conditioned response (behaviour) presented by the detection dog, such as sitting, pointing, or lying down, directed at the location of a target.
Targets	All possible locations where samples could be hidden, including negative, control, and positive targets.
Positive target	A target location containing the scent sample the dog is being trained on (example: riverine rabbit). The dog is trained to show an indication at this target.
Negative target	A target location containing scent that does not match the positive target nor that of the controls. In our study, negative targets were scents from other lagomorph species. These can also be considered a type of disturbance.
Control	Empty (clean) target location (or container).
Distraction	Anything that is seen as a distraction, disturbance, or obstacle for the dog during training or testing. These include other scent samples (such as negative targets), unsuitable/ extreme weather conditions, or the presence of other humans or animals.
Miss	A lack of indication on a positive target.
Incorrect indication	An indication made on a negative target or control; also referred to as false indication.
Run	A session during which the dog examines 10 targets on the platform, traveling in one direction only. This provides the dog with only one opportunity to indicate on each positive target.

techniques. Therefore, a unique set of terms relevant to dog training, as used in this study, is outlined in Table 1.

The training was conducted in three phases. The first phase included indoor experimental setups (controlled environment), which produced detailed quantifiable results. The second phase involved outdoor and field training sessions, while phase three consisted of field trials in the natural habitat of the target species. Only successful indications were recorded for phase two and three. During all phases, the scent detection dog was presented with sample scent at the start of each search. This process is known as "matching scent detection" (described in Schoon 1997), and aims to assist the dog with matching the associated scent with the positive target.

We used the principles of operant conditioning as a mechanism for the scent detection dog training (Blackman 1974). The dog was taught to search for, and indicate on, target scents, starting with food rewards. Later, the dog was introduced to the target species odour (riverine rabbit scent). The scent detection training involved the placement of targets and controls along a platform for the dog to investigate. Using a reward-based training, the dog was then reinforced to indicate on a specific scent (or scent complex) within the setup (Schoon 1997, McCulloch et al. 2006). A scent complex is a combination of VOCs, linked to a specific scent sample. The training process, where the dog is trained to distinguish between scents, is called scent discrimination, as the dog needs to identify specific components of the presented scents and differentiate between those components (Williams & Johnston 2002). Scent discrimination forms a vital part of many scent detection training techniques, and various types of equipment (such as our training platform) can be used to train a dog to discriminate between species (or even individuals). The training methodology used, and described below, was similar to that explained in (Matthew 2016). To avoid subconscious cues from the handler, as described by the Clever Hans effect (Pfungst 1911), both the dog and handler were stationed outside of the laboratory while targets were switched by experimental assistants.

A raised platform – used as a training aid (see Johnen et al. (2013) – with ten evenly spaced, 6 cm diameter holes was used to create a "false bottom", which concealed the positive, negative, and control target containers below the work surface. The equipment was specifically designed to train the dog on the scent of burrowing species, and thus the platform did not provide the dog with any visual confirmation of a positive target. The use of ten target spaces allows for easier data quantification and was consistent with other studies that made use of similar training aids (Fischer-Tenhagen et al. 2011, Johnen et al. 2013). For experimental purposes, the holes were numbered one to ten, and one run was performed by navigating the scent detection dog over the platform from the first hole to the tenth hole.

The positive target scent was placed in one (or two) of the containers while the remainder of containers were left empty or contained negative targets (such as scent from other lagomorph species). The dog was guided to investigate each opening of the raised plank and encouraged to smell, using a trained command (Figure 1 and 2). The platform included additional 50 cm planks on either side of the raised plank, which allowed the dog to step onto the equipment before the first target was reached. The original design (without these planks) caused a physical obstacle, which could have resulted in a mental distraction for the dog.

Clicker training was used, as a conditioned secondary reinforcer, in conjunction with the reward, to increase the dog's precision when indicating at the positive target during training

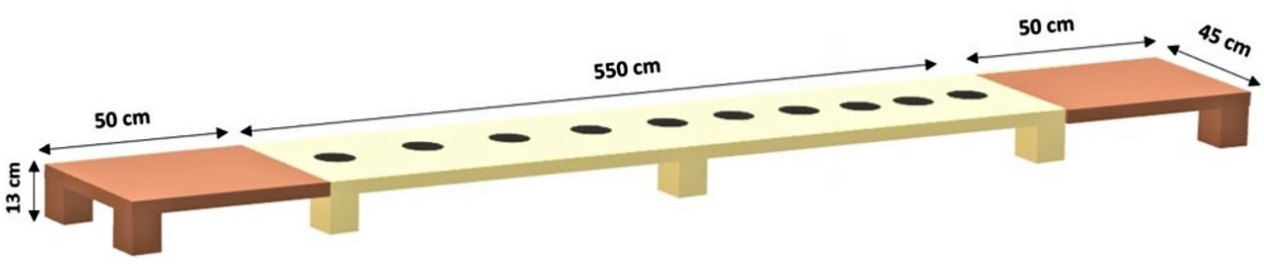

Fig. 1. Platform plank structure, measurement, and design. The holes for target containers were evenly spaced (approximately 53 cm apart).

Fig. 2. The scent detection dog conducting an off-lead search on the platform equipment.

(Fjellanger et al. 2002, Cornu et al. 2011). The dog indicated by lying down at the target. Concurrently, negative punishment (no reward) was employed when the dog indicated on a negative target. This way the dog learns to associate only the target species' scent with the allocation of a reward, which strengthens motivation for the dog to locate the scent again (Wasser et al. 2004). A sample of riverine rabbit fur was presented to the dog at the start of each run.

Phase 1 consisted of five different experimental designs, which included: 1) Riverine rabbit hair as the only scent in the setup (on-lead); 2) Riverine rabbit hair as the only scent in the setup, but with multiple targets, i.e. two positive target scents per setup (on-lead); 3) Riverine rabbit skin swab sample as the only scent in the setup (on-lead); 4) Riverine rabbit hair and hair from other lagomorphs as scent disturbances in the setup (on-lead); 5) Riverine rabbit hair and hair from other lagomorphs as scent disturbances in the setup (off-lead).

We decided to use hair (or fur) samples, instead of skin, to reduce the influence of scent from blood and epidermal components, as the study aimed to determine whether the detection dog could locate live rabbits, rather than dead specimens.

Experimental design 1, 3, 4 and 5 were replicated over 40 runs (each run contained ten concealed targets), while experimental design 2, which included two positive targets, was replicated over 20 runs. In total, all experimental designs consisted of 40 positive targets and a total of 360 negative targets (apart from experimental design 2 with 160 negative targets). A run was performed with the dog handler navigating the scent detection dog over the ten target locations along the length of the platform, one (or two) of which was the positive target (riverine rabbit scent). The target sample was switched to a randomised location by an experimental assistant between each run. The handler was never aware of the location of the positive scent. This is considered a double-blind approach and minimises subconscious bias from the handler towards the target scent. Experimental assistants took care to touch all ten containers during target switches, while wearing disposable gloves. This ensured that the target scent would not be recognised for any reason other than the scent it contained. On-lead and off-lead trials were conducted and compared. The inclusion of off-lead setups ensured that the dog was not receiving unwanted queues through the tension of the lead, and thus results may be considered more reliable. On-lead training sessions were still necessary, as permits required the dog to work on-lead when in the field due to the critically endangered status of the target species.

Containers were cleaned with soap and water, followed by 90% ethanol between each experiment. The platform equipment and containers were wiped clean with ethanol between every five test runs. Any fragments of food that may have fallen through the gaps between the containers and plank equipment were removed to reduce distraction and possible false identification. Experiments took place over a five-week period (two days per week), where no more than 20 runs were completed in one day. The dog was rested after ten runs and was not worked for more than two hours at a time.

The test for species specific detection (experimental design 4 and 5) included one container with the positive target scent (hair from a riverine rabbit) and a randomly-selected negative target with scent from one other lagomorph species (hair from *Lepus capensis*, *L. saxatilis* or *Pronolagus saundersiae*). Samples of other lagomorph species were also obtained from roadkill specimens and collected (and stored) using the same protocol described for riverine rabbit scent collection. All remaining containers (8) were left empty as controls. Using the same containers, the positive target's position was switched to a new randomised location between each test run. The other containers were also switched. This experiment consisted of 40 runs for both on-lead and off-lead experiments.

Phase 2: Scent detection training – outdoor training sessions (Part 1)

Before field trials were initiated, the dog was trained with scents hidden in a semi-natural outdoor space, hereafter referred to as outdoor training sessions. The outdoor space consisted of a two-hectare fenced area, with natural vegetation (i.e. natural aromas) that the dog would encounter during field trials in riverine rabbit habitat, without the presence of the target species. The outdoor training sessions took place over a four-week period, with different experimental setups (in terms of difficulty, duration, and degree of distraction), and both on- and off-lead sessions. Difficulty was increased by increasing the size of the search area, as well as by altering the location of the search area. Duration of training was increased by performing more setups in one day, and the distractions were increased by including contrasting environmental factors, such as weather conditions and scent of other lagomorphs. During the outdoor training sessions, positive targets (riverine rabbit fur) and negative targets (fur from other lagomorphs) were hidden by experimental assistants in the search area. In total, 40 different search setups were completed in this controlled outdoor environment. During these outdoor training sessions, the dog was permitted to continue searching until a correct indication was made (no time limit was enforced). The search setup was altered only once the dog successfully located the target scent. Drawings were made of the search area, which included notes on weather conditions, duration of search, perceived distractions, and search area size. These datasets could not be quantified, but were used to access factors that could influence the dog's performance during field trials. Assistants were requested to cross over their walking paths when placing scents, and to walk additional routes to prevent the dog from tracking their movements to help locate the target.

Phase 2: Scent detection training – field training (Part 2)

Once the outdoor training sessions were complete (40 successful indications on target scent), field training took place in the known habitat of the riverine rabbit (where presence of the species had been confirmed with camera traps). Valid provincial permits were obtained for the training and use of a scent detection dog in the search for riverine rabbits (Cape Nature – Permit number: CN44-30-4165, Northern Cape – FAUNA0271/2017). Using a similar methodology to that described above for the outdoor training sessions, 40 search setups were completed in an unfenced search area of six hectares. During these field training sessions, the dog was also permitted to continue searching until a correct indication was made (no time limit was enforced). The dog was deployed into the field on a 5-10 m lead. The length of the lead was controlled by the handler and was kept shorter when the vegetation density increased. This prevented the lead from becoming tangled and allowed the dog to move more freely. The use of a lead during all field experiments was necessary to control the dog's speed and ensure sufficient scanning of the search area, and was compulsory due to restrictions within the provincial permits, which aimed to prevent the dog from inflicting harm on a rabbit. A Survey123 for ArcGIS application was used to record sightings of riverine rabbits.

During field training, occasionally live riverine rabbits were flushed. We would use these opportunities to reinforce the scent of the target species. The dog was trained to indicate, by lying down at the base of the bush (from which the rabbit was flushed), when the target scent was located. The detection dog was only rewarded if the target was correctly identified by the handler as a riverine rabbit. If a positive identification could not be confirmed, the search effort continued as usual (no reward). This aimed to help the dog associate the scent of the live animal with the sent from the dead specimens. By rewarding the dog when fresh scent of a live rabbit was available, we suspect that the dog was able to identify a similarity (common VOCs) between the trained scent (from dead specimens) and the scent from the live animal; thus making it easier for the dog to locate the live target species in future. Once the dog was able to successfully locate the target scent within all 40 setups and had indicated on the scent of the live target animal that had been encountered during searches, scent detection field trails in new search areas (where the presence of riverine rabbits was unconfirmed) were initiated.

Phase 3: Scent detection training – field trials

The field trials took place within the restricted distribution of the riverine rabbit, in the riparian vegetation adjacent to seasonally dry rivers in the Nama Karoo biome of South Africa. Here, riverine rabbits generally remain concealed under cover of dense riparian vegetation.

As in all training sessions, the scent detection dog was presented with sample scent at the start of the search, to assist the dog with matching the scent to the target animal. The dog was rested for at least ten minutes every hour during a work session (more frequently, if required). The dog was not worked during the hottest or coldest times of the day and seasonal changes were taken into consideration. Fresh water was available for the dog during resting periods. The handler also carried a fully equipped canine first aid kit during field trials.

The team aimed to ensure that disturbances and distractions were kept to a minimum to allow the dog and handler to work and communicate as effectively as possible. As a result, the number of field assistants was limited, and all assistant were instructed to remain silent during the trials. On average, only two individuals are recommended: the dog handler and one assistant, who walks behind the dog-handler team, recording information, and ensuring the team remain on track.

Study areas, consisting of seven properties (approximately 4,500 ha in total, of which riparian vegetation covered approximately 500 ha), were selected for field trials. Properties were chosen in data deficient areas of the riverine rabbit's distribution and where the landowner was willing to allow access. The dog-handler team moved through selected areas in a zig-zag formation (coursing), along transects no more than 20 m apart. Wind direction influenced the direction teams travelled along the transects (see Fig. 3). The dog was encouraged to walk against the wind to allow scent to be blown towards the dog-handler team, rather than away from the team. This was expected to enhance detection probability and improve detection distance. On average the dog could effectively cover a 5-hectare area in a search session of three hours, considering a conservative detection distance of 5 m in each direction. The team was only allowed to deviate from the formation when the dog picked-up the target scent. The handler determined this by observing a change in the dog's body language, such as a stiffened upright tail and/or accelerated movement. The dog was trained to

Fig. 3. Example of a field trial search area (green outline) and the proposed survey route (red lines) for the dog-handler team within the search area. Transects were spaced 20 m apart. The direction of movement was decided according to the wind direction upon arrival at the site.

indicate, by lying down, when the target scent was located. If the rabbit remained concealed beneath vegetation (a behaviour commonly observed during foot surveys), the dog was expected to indicate at the base of the bush. The detection dog was only rewarded if the target was correctly identified by the handler as a riverine rabbit. If a positive identification could not be confirmed, the search effort continued with no reward. This technique is recommended when working on a moving target where the scent detection dog may never be able to get close enough to the target to indicate (J. van Straaten, pers. comm.). Riverine rabbit sightings were recorded using a Survey123 for ArcGIS application.

Data analysis

Sensitivity and efficacy were calculated for all five experimental designs in Phase 1 (Scent detection training in a controlled environment) as a proxy for the success of the dog's effort (Marschark & Baenninger 2002). A "correct indication" was recorded if the dog alerted the handler by indicating at the positive target. A "miss" was recorded if the positive target was not detected, while an "incorrect indication" consisted of any indication made at a negative target location ("distraction") or control. "Sensitivity" was defined as the accomplishment of the purpose, namely, to locate the positive target. Sensitivity was determined by the number of correct indications compared to the number of positive targets, calculated as: sensitivity = correct indications/positive targets. "Misses" were accounted for by the number of correct indications compared to total positive targets. This is also seen as an indication of the reliability of the dog to detect the target scent. Effectiveness was defined as the degree to which the dog is successful in achieving the purpose of the search. It was calculated by including the number of incorrect indications, where the dog indicated on a control or negative target, into the equation. Thus, comparing the total number of indications (correct and incorrect) with the number of positive targets (total possible correct indications). Effectiveness was calculated as: effectiveness = correct indications/(positive targets + incorrect indications).

Finally, specificity towards the positive target scent, when negative targets/disturbances (such as scents from other lagomorphs) were present, determined how well the dog was able to indicate on the positive target, without getting distracted by negative targets. Specificity was calculated as: specificity = correct indications/(correct indications + incorrect indications).

Results

Phase 1: Scent detection training – indoor experiments (controlled environment)

This phase consisted of five different experimental designs. All experimental designs consisted of a total of 400 possible targets, where 40 were positive

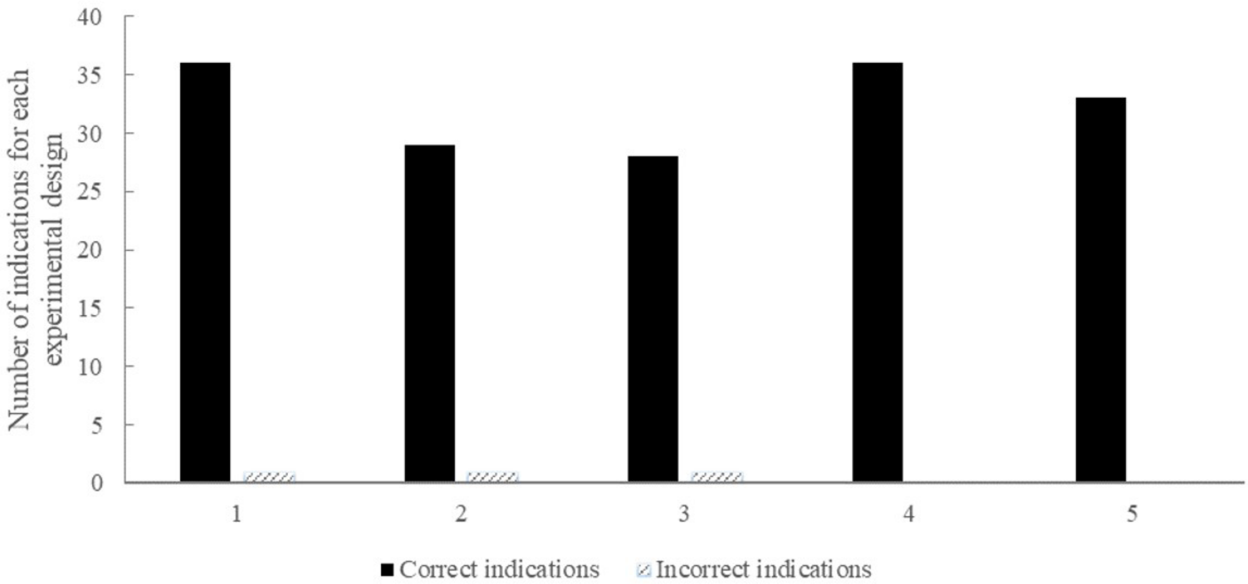

Fig. 4. Number of correct and incorrect indications made by the detection dog within each experimental design. Each design contained a total of 40 positive targets. Correct indication refers to indications on positive targets, while incorrect indication refers to indications on negative targets or controls.

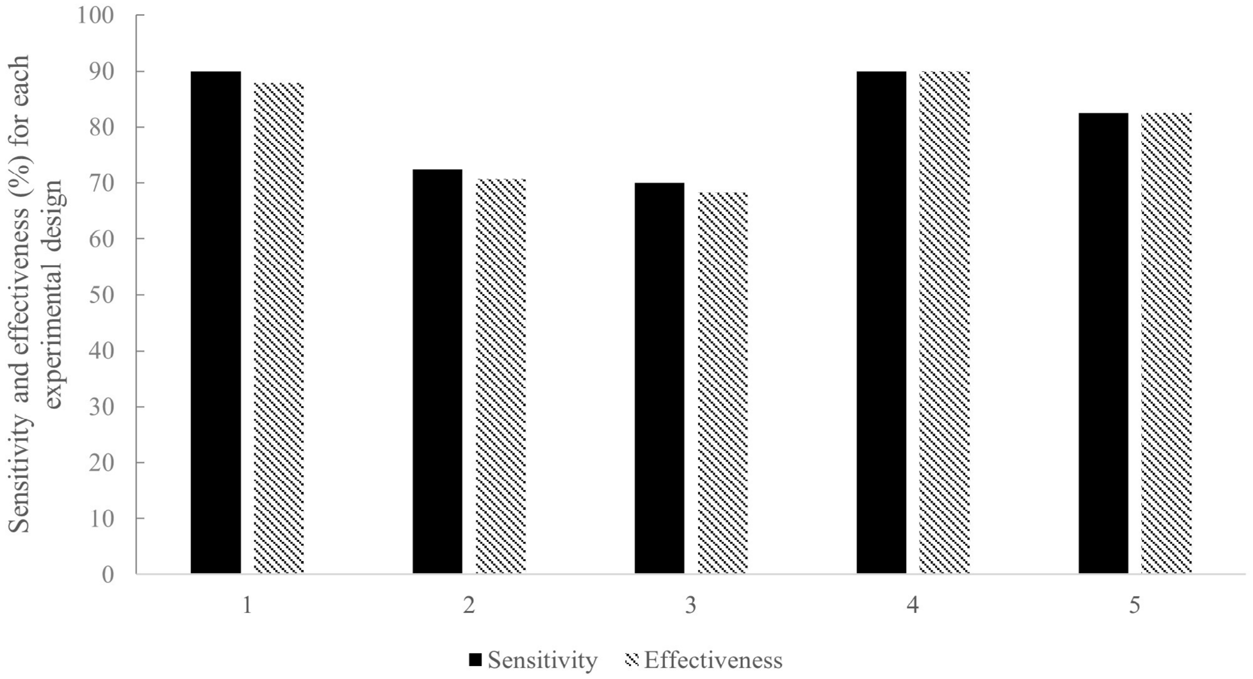

Fig. 5. Percentage sensitivity and effectiveness achieved by the detection dog in each experimental design.

targets and 360 were controls/negative targets. Experimental design 2 also consisted of 40 positive targets, but only 160 were controls/negative targets, as this experimental design contained two positive targets per run. Indications (correct and incorrect) for each of these experimental designs are displayed in Fig. 4.

The sensitivity of the dog was highest (90%) for experimental design 1 and 4 (Fig. 5). The highest effectiveness of the dog (90%) was achieved where riverine rabbit hair and hair from other lagomorphs (as scent disturbances) were present in the setup. During plank experiments, the dog-handler team showed an overall sensitivity of 81% for the detection of riverine rabbit scent. The overall effectiveness of the dog across all experimental designs was 79.9%.

Specificity was determined by calculating the number of positive targets correctly identified by

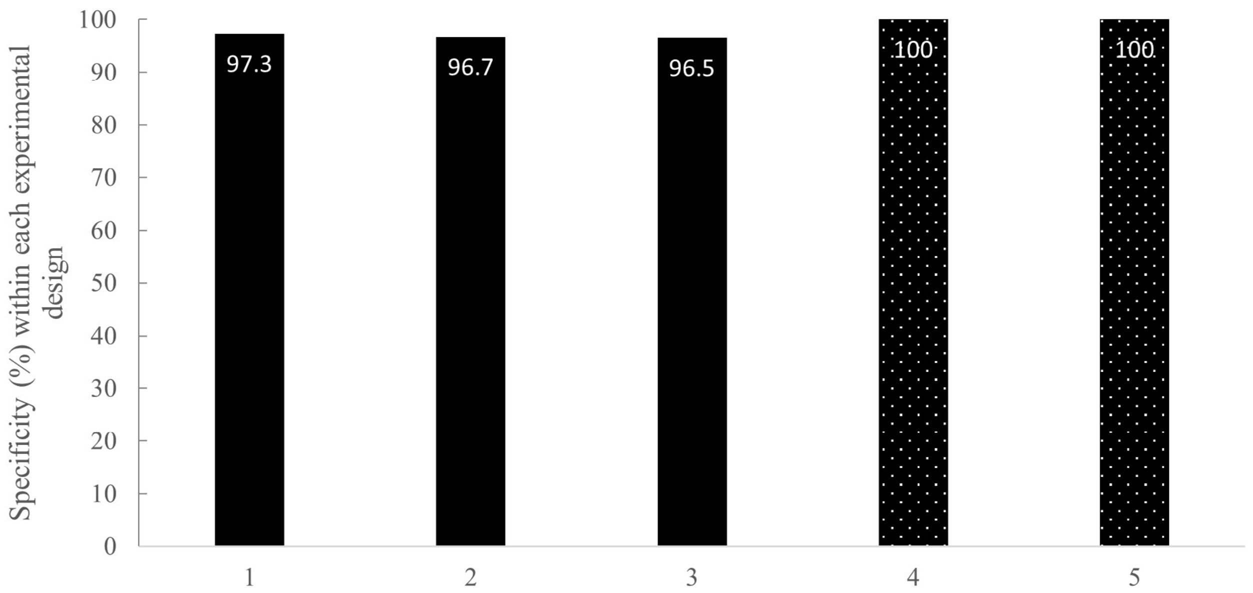

Fig. 6. Specificity (%) achieved by the detection dog for each experimental design. Dotted columns indicate where scents from other lagomorphs were present, thus specifically referring to species specificity.

the dog (Fig. 6). Because experimental design 4 and 5 contained scents form other lagomorphs, the specificity value reflects species specificity, as the dog was required to locate a specific target species between scent from other species. All results were higher than 96%, while an average specificity towards the target species was 98.1%.

Phase 2: Scent detection training – outdoor training sessions and field training

Although no quantitative data for outdoor training was obtained, the dog-handler team completed 40 outdoor and 40 field training sessions, and in all sessions, the dog was able to locate the target scent consistently. Following field training sessions at locations where camera traps had confirmed the presence of riverine rabbits, the methodology was tested at new sites.

Phase 3: Scent detection training – field trials

The scent detection dog was deployed at seven properties to investigate species presence and test the methodology. The dog correctly indicated more than 30 times during a six-month search period. As many of the indications occurred in close proximity to one another, it is possible that the dog detected the same individual rabbit more than once. Based on the approximated home range size of the species (±15 ha), one can assume that the dog was able to detect at least ten distinct individuals across the seven properties. Detections were only recoded if the dog indicated. No incorrect indications were observed during field trials, even though *L. capensis* was encountered on three occasions. Riverine rabbit presence was recorded on four of the seven properties. Riverine rabbits had not been sighted on three of the four properties since 1999, and one site had no historic recordings of riverine rabbit.

Discussion

The results of the indoor experiments showed that the sensitivity of the dog was highest for trials where only one positive target fur scent was used (experimental design 1 and 4). Both of these experimental designs were conducted on-lead, thus the dogs' speed was controlled while moving over the platform. We suspect that, when multiple target scents are placed too close to one another in the setup, it could become confusing for the dog. As a result, it is recommended that the targets are spaced further apart in future studies. The lowest score for sensitivity occurred during experiments where scents from skin swabs were used. Thus, one could assume that hair samples of riverine rabbits contain more VOCs compared to skin swabs. More experiments are necessary to confirm this assumption, as it may provide valuable information pertaining to the type of tissue that is best to use when training a dog on limited scent from rare or elusive species. Furthermore, the highest effectiveness of the dog was achieved when riverine rabbit hair and hair from other lagomorphs (as scent disturbances) were present in the setup, compared to the lowest effectiveness, which was observed when skin swab scent was used (experimental design 3). This indicates that it may be easier for the dog to locate the target, when other negative targets (scents from non-target species) are present in the setup. This may attribute to the fact that the dog was never rewarded for the non-target (negative target) scents and, because they have a different scent profile, they are easier for the dog to avoid compared to control containers with no specific scent. An alternative hypothesis is that the scent emitted from the skin swabs is weaker than that of the hair samples, making the skin samples more difficult to distinguish from the controls. During indoor experiments, the dog made very few incorrect indications and showed a high sensitivity for the detection of riverine rabbit scent. The success of this training methodology was demonstrated during field experiments, when the dog was able to detect over 30 live riverine rabbits (ten distinct individuals) over a six-month search period. This demonstrates that it is possible to train a scent detection dog to locate a live, cryptic, and rare target species in the wild, using only limited scent from deceased samples. This finding may be particularly valuable for the detection of other endangered and/or elusive species.

During future trials in other geographic areas where the species is known to exist, such as the Succulent Karoo biome, the team is cognisant that the dog may experience some difficulty in locating the target species. As the dog was not trained in those environments, it is likely that new and contrasting environmental conditions, such as vegetation type, could cause increased disturbance, and thus decrease the dog's performance. The Succulent Karoo biome hosts a greater biodiversity than the Nama Karoo biome. Additionally, all field trials in the Nama Karoo took place on livestock (mostly sheep) farms, while most Succulent Karoo properties are game farms that host a variety of wildlife. These factors should be considered during *ex situ* training, and the dog should be trained in the environment in which it is deployed (Johnen et al. 2013).

The use of a detection dog expands the arsenal of tools that conservation scientists could utilise when conducting research on elusive and endangered species. When performing critical functions with scent detection dogs, such as searching for a rare species in the wild, it is important for the handler to consider the effectiveness of the dog to detect the target scent (Greatbatch et al. 2015). It may be difficult to predict how specificity values will be affected when simultaneously presented with the scent of more closely-related species if it is assumed that related species (e.g. species within the same genus) share a greater percentage of odour signature elements than unrelated species (e.g. species within separate genera). Studies have documented that conservation dogs used for a variety of tasks can discriminate between related species with an accuracy of 85-100%. These studies included controlled setups of two species of foxes (Smith et al. 2003, Hurt & Smith 2009, Kerley 2010), two species of bears (Smith et al. 2003, Hurt & Smith 2009, Kerley 2010), and even individual Amur tiger (*Panthera tigris altaica*) scats (Smith et al. 2003, Hurt & Smith 2009, Kerley 2010). However, Lit & Crawford (2006) caution that if scents are too similar or too contradicting, it can lead the dog to indicate when no target scent is present (false indication); for example, it is difficult to operationalise a cadaver scent detection dog for search and rescue of live individuals, as the dog will most likely only search for dead individuals, and the dog's confusion or frustration could result in incorrect indications. How this will translate to scent detection in lagomorph communities, remains to be investigated. An important future consideration is whether the same dog could be utilised for both scat and live animal detection.

Based on other unpublished research, foot surveys for the detection of riverine rabbits are not invariably effective and can result in the collection of false absence data, as the target species is often missed using this visual method. Camera traps, on the other hand, are the most effective in determining activity patterns, presence, and absence of riverine rabbits, but the process is very time consuming. Aside from only covering small geographic areas, the camera surveys need to be deployed for at least six weeks, after which the data needs to be sorted and analysed. In contrast, the use of a detection dog could be a valuable, rapid method for confirming presence (and distribution) of the species in an area. The olfactory abilities of domestic dogs have aided wildlife biologists in locating protected native species, searching for introduced pest species, finding nests, and many other applications (Zwickel 1971, Browne et al. 2006). Some of the limitations experienced with conventional surveys have been addressed with canine detection of wildlife scents through increased individual recordings and sample acquisition rates (rapid detection) with reduced collection biases (Hurt & Smith 2009, Dahlgren et al. 2012). Having successfully displayed olfactory sensitivity towards riverine rabbits during laboratory tests and field trials, this methodology demonstrates promise for advancing endangered species research and conservation.

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