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Exploring olfactive performance in a non-specialized breed dog to detect parasites in lambs' feces

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Abstract: *Our aim was to explore the performance in a non-specialized breed, mixed-breed dog to find lambs' feces with high egg counts of gastrointestinal nematodes. A female 7-month-old dog of a cross breed of some type of Daschund and mestizo breed was used. Dog and trainer were inexperienced in training. The study was implemented in Mérida, México using a six stages program with 20 min. interactions in non-continuous days, using operating conditioning through positive reinforcement. Stages 1-3 were used to familiarization between dog and trainer and learning of basic obedience commands to sit, search a bait by sniffing and point position by sitting. Stages 4-6 were used to move the search of the bait to a positive sample (feces with 4000 eggs of gastrointestinal nematodes (EPG) mixed infection), then to find a positive sample among negative ones (feces with 0 EPG), and finally to find a positive sample among negative ones and positive controls (feces with < 150 EPG). The percentage of point correctly and incorrectly on first and last day on stage 3 to 6 were compared using Fisher test and resulted significantly accomplished on five days. Three to 17 sessions were required on the first three stages, learning to point position demanded higher number of commands (155). The dog accomplished the task of identifying the positive sample, among negative and positive controls. Six to 11 sessions were required during stages 4-6; the incorporation of negative plus positive controls required more commands (105). The results should be taken with caution due to the exploratory nature but suggest that using this type of dog could work on this specific task and that its worthing developing further studies.*

Keywords: mixed-breed dog; scent detection; operant conditioning; lamb' parasites; feces samples; inexperience trainer

HIGHLIGHTS

- Despite no training experience of the dog and handler the task was accomplished
- Identifying feces samples from parasitized ewes among control samples is possible under this context
- The results suggested it's worth it to perform further studies

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INTRODUCTION

In small ruminant production systems (sheep and goats) it is essential to detect those individuals with high parasitosis, i.e. with high loads of gastrointestinal nematodes. The latter is key to limit treatment only to those animals that deserve it and not to the entire herd. This is the basis of modern and sustainable control of gastrointestinal parasites in ruminants to avoid creating parasite strains with resistance to dewormers drugs (Torres-Acosta & Hoste, 2008). This need for detection of individual animals in a flock, is in part addressed with new approaches like scent detection dogs. The use of dogs to identify by sniffing biological samples coming from infected animals or persons has been largely investigated, showing their utility during the Covid-19 pandemia (Sakr et al. 2022). Thus, in the last decade some research with dogs trained in scent detection were oriented towards the olfactive identification of stool samples from parasitized sheep among samples from non-parasitized ones.

Those studies showed a high capacity of scent trained dogs to successfully smell and to identify signatures of specific odors. For example, dogs were very useful in the work of identifying feces of infected sheep with rates in detection of 92% (Richards et al. 2008). Still unknow is the chemical signature recognized by the dog; it could be something associated to the high presence of eggs of gastrointestinal nematodes in the feces. In any case, dogs can be used for this purpose, thus opening the potential of having a reliable, fast, and safe way of detecting infections for producers (Kokocińska-Kusiak et al. 2021).

Among the studies carried out, some have used a single dog such as Welch (1990), who use a dog of breed German Pointer. Other works used two animals as Richards *et al.* (2008) both animals of German Shepherd breed. The research about scent detection has been mainly produced using specialized breeds, for example Salamon et al. (2025) focused on olfactory performance between dog breeds and report differences such as Beagle breeds find food faster than Border Collie breeds. In general, lineages of Breeds like Basset hound, Beagle and German point are considered superior to other breeds and with performances closer to that of wolves (Polgár et al. 2016). Thus, the information about non-pure breeds or breeds not selected for their scent detection are scarce or not yet available.

Also, due to the critical task involved in some scent detection like potentially mortal diseases (diabetes, covid-19) or another risks (explosives, guns) to human life, the trainability of dogs' breeds like German shepherd or Labrador retriever and the expertise of trainers is mandatory and out of

question (Abdel and Gharib, 2020). That is the reason why the reported cost of training and maintaining that type of dogs, including their professional handlers has been estimated in thousands of dollars (Orkin et al. 2016). The latter limits their use for research purposes.

However, for other not so critical tasks like detection of biological samples of plants/wildlife, or nematode eggs in feces, it could be desirable to explore the use of non-selected breed of dogs, and novice volunteers in training. Some evidence available suggest such expansion as feasible, for example, a recent study using companion dogs and including breeds like chihuahuas showed that this type of animal can be trained and learn quick a scent-searching task (Adams, 2022). Concerning the trainers, Rutt et al. (2021) showed that novice trainers and their companion dogs following a 12-week program of scent detection were able to successfully detect a novel odor in controlled conditions at rates of 75%. Also, Salomon et al. (2025) founded that the owner style and reward could not be associated with the performance in a scent task between breeds of dogs. Thus, determining if a non-selected scent breeds like mixed-breed dogs with no defined breed could learn a scent task can contribute to boost the research about scent detection of parasites in feces.

In consequence the aim of the study was to explore the performance in a non-specialized breed, mixed-breed dog to find lambs' feces with high egg counts of gastrointestinal nematodes.

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MATERIAL AND METHODS

Non-specialized mixed breed dog

Due to the exploratory nature of the study, a dog without genotype and without any previous training was chosen. A female dog, cross breed with some type of Daschund or Tekke color piebald and mestizo dog was used (name Pandora). After a survey among students engaged on veterinary clinics, the dog was voluntary proposed by their tutor, a veterinarian for whom Pandora was her companion dog.

The dog used was not spayed and was 7 months old at the beginning of this study. As a companion dog, she spent her day indoors with access to the garden and a large terrace with shade and water and food availability. The animal was clinical healthy, did not have any surgery or previous treatments, it had the vaccination scheme and deworming up to date. All the general handling of the dog's usual diet (Purina Excellent kibble) and routine was respected. The temperament of the subject is playful, described by their owner as an active, non-aggressive dog, lives with dogs and cats,

although somewhat nervous to unknown places and reacts to people and animals. The owners gave their informed consent to use their pet, and they were free to withdraw her from the study at any moment. All procedures were approved by bioethics committee of the Veterinary Faculty of the Autonomous University of Yucatán (FMVZ-UADY), reference number CB-CCBA-I-2024-001.

Olfactory stimuli used

A semi-moist flavor highlighter in small pieces referred to from this section as "bait" (Bakers club mini beaf treats) was used as a reward. Vocal congratulations to the dog were also used as a reward, as well as petting.

As a negative stool sample or negative control, feces from sheep with a count of 0 gastrointestinal nematode eggs (GIN) per gram of feces (EPG) determined by coproparasitoscopic analysis with the modified MacMaster technique (Bauer et al., 2010) were used.

As a positive stool (fecal) sample, sheep feces with high mixed GIN infection (4,000 EPG) were used, this is the one that in practical terms is important to identify because it would be a sample from an animal with a high risk of being affected by parasitosis.

In addition, two other types of samples were used as a positive control, that is, sheep feces with low infection that in practical terms means infected animals but with low risk of being affected by parasitosis. These samples were feces from lambs with low monospecific infection (150 HPG) of *Haemonchus contortus* and sample of sheep feces with low monospecific infection (150 HPG) of *Trichostrongylus colubriformis*. This positive control is equivalent to the distractors of Rutter et al. (2021) commonly used in training sessions.

All positive samples were taken from several lambs, in all cases the lambs did not present signs indicative of anemia evaluated with the FAMACHA © card (Mendoza de Gives & Torres, 2023) regardless of their GIN count in feces.

Black 34 oz (7.28 in *2.36 in* 4.9 in) polypropylene containers with 2-millimeter diameter holes in the lid, and "Elite" paper napkins were used to move and deposit the bait and samples according to the stage, inside the container.

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Ethogram of behaviors during the training sessions

Four behaviors were considered to measure the progress in the process of conditioning the dog in the different stages of scent training, search after command, sitting, point correctly, point incorrectly (Table 1).

Behavior	Description
Search after command	Upon hearing the command "search", the canine approaches the containers and sniffs them.
Sitting	The canine rests its body on its flexed left and right pelvic limbs.
Point correctly	The canine positions itself on the side of the container where the target sample is located and shows the behavior "sitting", remaining in that position until indicated.
Point incorrectly	The canine is positioned on the side of the container where the target sample is NOT located and shows the "sitting" behavior, it remains in that position until it is instructed.

Table 1. Description of dog' behavior during training sessions, partially inspired from Rutter et al. (2021).

Training session

Court stages gradually increasing the complexity as recommended on guidelines to animal olfactory detection of human diseases (Edwards et al 2017), and Rutt et al. (2021) were implemented. In all, an auxiliary person put the samples and containers' position in random order to avoid influencing the dog or trainer.

1. Familiarization and basic training. Familiarization between handler and dog was planned to be carried out with 20-minute sessions a day in a frequency of 3 days per week. In this stage the training on basic commands "Sit" and "Stay" was simultaneously performed to the familiarization. Voice commands were executed, and if the animal responded to them, it was awarded offering a bait (semi-moist flavor highlighter) plus petting. When the animal reach 80% of success in a session the next stage was implemented.

2. Search by sniffing. 20-minute sessions a day, twice per week was used. When the command "search" was given, the bait was thrown while the dog

watched enabling him to go for the bait. Once this was achieved, hidden from the dog, the bait was placed 1 m away and the command was given by pointing with the finger to the floor until the dog reached the bait. Finger marking was gradually reduced and the distance to the bait was increased.

3. Point position. The dog was trained to sniff the containers to find among empty containers that one with the bait. The dog was placed 1 m. away from containers and the command stay was given. Then the "search" command was given and when the animal found the bait the command sit was given and rewarded with petting if the dog sat. In the first sessions the containers were open and were gradually closed (holed lids).

4. Transfer the bait to a positive sample. The same commands were used; the bait and a 1.5 g of positive stool sample were placed together in a container. Then the bait was removed and when the dog pointed to the non-empty container (positive sample, 4000 EPG) he was rewarded with the bait plus petting. The next stage was advanced when the dog reached ten consecutive attempts with success.

5. Inclusion of negative control. The same commands were used, containers with positive sample, with negative control and an empty container were used. This stage was considered accomplished when the dog pointed to the container with positive sample on ten consecutive attempts with success.

6. Inclusion of positive control. Like the previous stage, but containers used were with positive sample, control, and positive control. Positive samples were used alternating the two low (<150 EPG) monospecific samples of *H. contortus* and *T. colubriformis*. The stage was completed once reaching ten consecutive attempts with success.

Statistical analyses

The number of training sessions, days of session at each stage is reported with descriptive statistics. The percentage of points correctly and incorrectly performed on the first and last day on stages 3 to 6 were compared using Fisher Exact test. It was hypothesized that the percentage of points incorrectly performed will drop close to zero values, thus the Fisher test was chosen as recommended when 2x2 contingence tables contain expected values less than 5 (Agresti, 2002, Dickinson, 2004).

RESULTS

The training, since the familiarization of the dog to the handler (stage 1) to the identification of the positive sample among negative and positive control (stage 6) lasted in total 13 weeks. The stage where the dog learned to

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point the right container by sitting was the longest and with highest number (155) of commands performed (Table 2). The percentage on which the behavior point correctly was performed is shown in Table 3. The first two stages were reached in only four weeks.

Stage	Number of sessions	Number of commands	Number of weeks
1 Familiarization	3	15	1
2 Search by sniffing	7	50	3
3 Point position	17	155	5
4 Transfer to a positive sample	6	82	2
5 Inclusion of negative control	10	100	1
6 Inclusion of positive control	11	105	1

Table 2. Number of sessions and commands performed on each stage during the training of a non-specialized scent breed dog.

	Stage 3 Point position	
	Day 1	Day 5
Display the behavior targeted	40%	86%
Did not display the behavior targeted	60%	14%
	P<0.0001	

Table 3. Percentage of times where the dog performed or not the targeted behavior after the command was given during the last stage of basic training.

During the training stages four to six, the sample to be found by the dog was the feces with high infection of 4,000 EPG count whether among empty containers (stage 4), among containers with feces of 0 EPG (stage 5), or containers with feces of 0 EPG and with feces of < 150 EPG (stage 6).

The percentage of behaviors pointing correctly, performed by the dog after the command "search" when the feces samples were incorporated, are show in the Table 4. Percentages superior to 90% were achieved at day 5 in all the stages when using fecal samples.

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	Stage 4 transfer to a positive sample		Stage 5 inclusion of negative control		Stage 6 inclusion of positive control	
	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5
Point correctly	80%	100%	30%	95.6%	70%	100%
Point incorrectly	20%	0%	70%	4.3%	30%	0%
	P<0.0001		P<0.0001		P<0.0001	

Table 4. Percentage of behaviors point correctly and incorrectly performed by the dog after command "search" was given to find the positive sample in the different stages.

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DISCUSSION

In general, a fast familiarization and basic training

Despite that a non-pure breed dog was used and that this pet was untrained, the familiarization with the handler as well as the learning of the command "search" (by sniffing) were completed quickly (Table 2). A rapid training has been also reported using companion dogs (Rutter et al. 2021, Adams, 2022) and confirmed the well-known good performance when the learning form part of a social playing activity for the dog. Playing is complex in dogs and is considered a type of learning mechanism for dog interactions (Bekoff, 2014, Bradshaw et al. 2015). Thus, our results confirm the general good collaboration of dogs during the training as a social playing activity and explain partially the high percentages displaying targeted behaviors at day 5 of stages 1 to 3 (Table 3).

The dog used in this study was described by their owner as playful, a characteristic that could help to accomplish significant learning in short time. However, the stage 3 where the dog was trained to point the position by sitting, required higher number of commands than previous stages. With this result, the age of the dog used (7 months), and the inexperience of the handler could have had an impact. For search dogs, some studies shows that higher rates of success are associated to initial obedience training before 6 months of age in the dogs, also to more years of general canine training experience of trainers (Alexander et al. 2011). In our study both conditions were the opposite. Despite the inexperience of the handler and the age of the dog it was possible to reach the objective, the dog pointing the correct container (than one having the bait) and advanced in their training process.

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Search and found the fecal sample of high EPG by sniffing was accomplished

The stage four and five where the animal should pass the identification of the bait to find the positive sample (feces with 4000 EPG count) alone, then among negative samples (0 EPG count) was completed in five days. To compare such performance, works available focusing in using dogs to detect gastrointestinal parasites in feces from small ruminants are scarce. One of the few (Richards et al. 2008) used two German Shepherd dogs habituated to scent training with only three months of age and showed that their dogs obtained success rates from 80 to 90% to detect feces with EPG counts from 250 to 1500 EPG after two weeks of training. Thus, the dogs' performance of our study seemed comparable, but we recognize that due to the exploratory nature of this work and the use of one dog this result must be taken with caution and more research should be made. Despite that, this study suggests it is worth performing additional trials. Three factors could also help to explain the success on the identification of the positive sample. First, the dog used even if from a mixed non defined breed (a female dog, cross breed with some type of Daschund or Tekke color piebald and mestizo dog) it has an elongated nose (is not brachycephalic). A large nasal cavity where the olfactory epithelium is located allows a greater number of odor receptor cells, favoring dogs' ability to detect odors (Beebe et al. 2016, Lazarowski et al. 2020). Second, the cells of the olfactory bulbs are more active in females than in males, and in the present work the dog used was a female and third, it has been reported that a high environmental humidity, such as that present in the city of Mérida where the study was carried out, maintains an optimal nasal humidity that allows a better capture of odors and increasing olfactory abilities in canines (Kokocinska-Kusiak et al. 2021).

Compared to dog's breed of scent detection, the five days that took for the dog to reach high percentages displaying the targeted behavior (on stages four to six) are not faster. For example, Fischer-Tenhagen et al. (2011) trained dogs of breeds: Labrador retriever, Shetland Sheep, Berger de Pyrenean, Border Collie, Australian Shepherd. Those dogs were trained to detect among vaginal fluid samples of cows on dioestrus (negative samples) that sample from cows in oestrus (positive sample), those dogs reached high rates of success after 2 to 5 days. The advantage of using non pure breed scent dog lays on their availability, and even if the present work has limitations, it suggests that it is possible to train inexperience non scent breed dogs for such a task. It's probable that small ruminant feces could represent an instinctive signal of prey trace. While this does not form part of the objectives of this work, such hypothesis is in the same way of Adams (2022) who studied the instinctive significant of scent and learning in dogs

and explains in part the quick learning of companion dogs in scent search of biological samples.

CONCLUSION

The exploration of training a non-specialized breed, mixed-breed dog to find lambs' feces with high egg counts of gastrointestinal nematodes, implemented by an inexperienced trainer is possible. The results suggest that using this type of dog could work on this specific task and that its worthing developing further studies.

CONFLICT OF INTEREST STATEMENT

The authors confirm that no have any conflict of interest.

ETHIC STATEMENT

All procedures were approved by bioethics committee of the Veterinary Faculty of the Autonomous University of Yucatán (FMVZ-UADY), reference number CB-CCBA-I-2024-001.

DATA AVAILABILITY

The authors could provide the database associate to this manuscript under reasonable request.

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