Article

Investigating the Sensitivity of Cadaver-Detection Dogs to Aged, Diluted Decomposition Fluid

Rebecca C. Buis [1]
LaTara Rust [1]
Katie D. Nizio [1]
Tapan Rai [2]
Barbara H. Stuart [1]
Shari L. Forbe [1]

Abstract: Cadaver-detection dogs (also known as human remains detection dogs) are used worldwide to locate deceased victims and human remains. Ethical restrictions often prevent the dog handlers from using cadavers as training aids, resulting in a reliance on pseudo-scents or human tissues, such as blood, bone, and decomposition fluid. Often these aids must be re-used many times because of the difficulty in obtaining new materials. The aim of this study was to investigate the dogs' sensitivity to aged human decomposition fluid samples that are used as a training aid. Human decomposition fluid was collected and serially diluted to 1 part-per-trillion (10^{-12}) and aged up to two years. The samples were presented throughout the aging process to three police accredited cadaver-detection dog teams under standard indoor training conditions. The dogs were capable of detecting the oldest and lowest dilution levels of decomposition fluid samples. Ongoing training to retain this level of sensitivity is recommended. The results of these trials indicate human decomposition fluid is a valid training aid for cadaver-detection dogs.

[1] Centre for Forensic Science, University of Technology, Sydney, NSW, Australia
[2] Graduate Research School, University of Technology Sydney, NSW, Australia

Received February 4, 2019; accepted February 21, 2019

Introduction

Cadaver-detection dogs (also referred to as human remains detection dogs) are specially trained scent-detection dogs that are used by law enforcement agencies, emergency services, and volunteer groups to help locate human remains in disaster situations, missing persons cases, and potential homicides where a body is concealed or may have been moved. Ideally, these dogs should be trained using human cadavers, but ethical and legal restrictions often prevent this from occurring in many countries. Instead, the dogs may be trained using porcine remains; synthetic scents; and human blood, bone, decomposition fluid, or grave soil.

However, human tissues such as decomposition fluid (the liquid formed during the autolysis and putrefaction of a cadaver [1]) have not yet been validated as training aids for cadaver-detection dogs. Prior studies investigating the dogs' responses to various training aids have reported that dogs are able to detect diluted volumes of fresh decomposition fluid from 10^{-7} to 10^{-12} [2, 3]; textiles that had been in contact with recently deceased bodies [4]; gauze pads saturated with the odor of fresh human remains, cremated human remains, decomposition fluid, adipocere, and blood [5]; human teeth [6]; 1 µL of blood diluted up to 4000 times [7]; decomposing human blood [8]; residual blood on clothing washed up to five times [9]; and grave soil samples [10]. However, it has not yet been determined whether the odor profiles of these training aids accurately reflect the odor profile of decomposed human remains.

Furthermore, because these types of training aids can be difficult to obtain, the aids are often re-used by the dog handlers for months, or even years, until new materials become available. To date, the majority of samples and training aids that have been studied have been relatively fresh. However, a study by Oesterhelweg et al. [4] investigated textiles exposed to recently deceased bodies and then aged for 65 days (the oldest samples reported in the literature), to which their dog teams' sensitivity (positive responses to the samples) ranged between 75% and 100%. Notably, there are no reported studies that have examined the effect of long-term use and storage on the odor profiles of decomposition fluid used as a training aid. This poses a potential issue for trainers and handlers, because decomposition odor is dynamic, and the odor profile of both cadavers and training samples can change over time. Hence, it is possible that the odor profile could change to such a degree that it no longer represents decomposition odor, causing false negative responses by cadaver-detection dogs.

It is important to understand the degree to which cadaver-detection dogs can detect aged fluid (i.e., their sensitivity) because they will likely be deployed to scenarios where the remains are decomposed or where only remnant fluid is present at the scene (e.g., in cases of scavenged and scattered remains or postmortem relocation of a body). Only one study has attempted to systematically calibrate cadaver-detection dogs' sensitivity to decomposition fluid and investigate whether their sensitivity increases with regular exposure to the training aid [3]. This study demonstrated that the cadaver-detection dogs in the trial were capable of detecting dilutions of up to 10^{-12} (i.e., 1 part-per-trillion) of "fresh" decomposition fluid (i.e., no older than 3 months), but additional aging of samples was recommended.

The aim of this study was to investigate the sensitivity of cadaver-detection dogs to aged samples of decomposition fluid and to determine whether their sensitivity increases with regular exposure to these training aids. This study was carried out with the intention of validating the use of aged human decomposition fluid as a training aid used by police canine handlers. The study will also assist in validating the cadaver-detection dogs' response to this training aid and provide scientific evidence of their capabilities if challenged in court [11].

Materials and Methods

A detailed method has been previously published [3] and is summarized below.

Decomposition Fluid Samples

Human decomposition fluid was obtained, following ethical guidelines, from the licensed Surgical and Anatomical Science Facility at the University of Technology Sydney. Decomposition fluid samples were collected from tissue samples obtained from recently donated, unembalmed bodies. A previous study by the authors used fresh decomposition fluid samples serially diluted to 10^{-12} in tap water [3], and the intent of the current study was to test these diluted training aids as they aged over two years. Serial dilution of the decomposition fluid produced 13 samples for use during training sessions. All samples were stored in cold refrigeration (4 °C) in 20 mL glass scintillation vials between training sessions, mimicking the conditions under which the police dog unit stored its training aids.

The diluted decomposition fluid samples were aged for 6, 9, 12, 15, 18, 21, and 24 months. A set of fresh decomposition fluid samples (i.e., aged between 0 and 3 months) were used as a positive control; tap water was used as a negative control. The positive control is a standard requirement for accreditation of the teams. Tap water was used as the negative control because it is a common distractor scent that is used in the line-ups and was the fluid chosen for the dilution of the decomposition samples.

Training Sessions

At the time of this study, there were only three accredited police cadaver-detection dog teams in Australia. Training sessions were completed at the training facility where the teams were based. These teams had previously been trained on blood, bone, and fresh decomposition fluid (i.e., from 0 to 3 months old). The aged decomposition fluid samples were included during training sessions every three months for two years and although the exact combination of dog and handler teams varied, depending on the teams' availability, each trial involved a minimum of two teams.

Concrete bricks (cinder blocks) were placed in a standard scent line-up formation around the training facility and 1 L metal cans with perforated lids were placed within each block. For each trial, approximately 40 cans contained tap water as a distractor odor (a negative control sample) whereas only 6 or 7 cans included the target odor (0.1 mL of the diluted decomposition fluid placed randomly in each line-up). This formation minimized the cross-contamination of target scents within the line-up. Samples were tested sequentially by age (i.e., 6 months at one session, 9 months at the next session, etc.), and the dilution levels were included in random order (for example, a line-up could contain the stock sample, 10^{-3} and 10^{-12} samples from the 15-month aged sample, or any variation thereof). This study was conducted only as a single-blind trial to align with the current training protocol of the police dog unit. For this reason, the training coordinator was aware of the location of the target odors, but the handlers were unaware of the location of these cans.

Each team conducted an initial search of the scent line-up, then following the standard training protocol, the cans were wiped clean, rearranged, and a new set of target odors was placed in the cans for the teams to perform a second scent line-up. Because of time constraints and the number of dilutions of the aged samples, each team was only able to complete a line-up of

the full sample set once per training session. This meant that each dog was typically exposed to all dilution levels of a particular set of aged samples (e.g., 6 months aged samples from stock to a dilution of 10^{-12}) during each trial.

Following the standard training procedures, the training coordinator would reward the dogs with their toy for positive alerts, rather than the handlers, which minimized (but did not completely remove) the risk of bias in the study. Responses were recorded in one of four categories as outlined below:

- positive alert (the dog correctly alerted to the target odor; the handler correctly called the alert)
- partial positive alert (the dog displayed a behavior change at the target odor, but did not give a true positive alert; the handler did not call an alert)
- false positive alert (the dog incorrectly alerted to a distractor odor; the handler called the alert)
- false negative response (the dog did not alert to the target odor, or the dog alerted to the target odor, but the alert was not recognized or called by the handler).

The teams were evaluated both on the response given by the dog and on the handler's interpretation of that response. The researcher and the training coordinator would discuss the responses after each trial to ensure agreement in the recording of responses.

Results

Throughout the study, the number of teams that were present for each trial varied based on the availability of the teams on training days. The results recorded below are presented as overall percentages, averaged across all the dogs (e.g., 50% of the dogs responded positively to the particular dilution), which allowed the results across all of the trials to be compared, even if one of the teams was unable to attend a training session.

The emphasis in this trial was on the dogs' ability to locate fluid that had been aged for various periods. Table 1 summarizes the cadaver-detection dog team responses to diluted decomposition fluid that had been aged for up to 24 months (2 years).

	Sample Age (Months)							
Dilution Level	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**
Stock	100%	100%	100%	100%	50%	100%	100%	100%
10^{-1}	100%	100%	100%	100%	100%	100%	100%	100%
10^{-2}	100%	100%	0%	100%	50%	100%	100%	100%
10^{-3}	50%	100%	100%	100%	100%	67%	100%	100%
10^{-4}	100%	100%	50%	100%	0%	100%	50%	50%
10^{-5}	100%	100%	100%	100%	50%	67%	100%	50%
10^{-6}	100%	100%	50%	67%	0%	100%	50%	100%
10^{-7}	50%	100%	100%	100%	0%	100%	100%	50%
10^{-8}	50%	100%	50%	100%	0%	100%	100%	100%
10^{-9}	100%	100%	100%	100%	0%	100%	100%	100%
10^{-10}	0%	100%	50%	100%	50%	100%	100%	100%
10^{-11}	100%	100%	100%	100%	50%	67%	50%	100%
10^{-12}	100%	100%	100%	33%	100%	50%	50%	50%

Table 1

The percentage of dogs responding correctly to diluted samples of decomposition fluid aged up to 24 months. Green indicates that all dogs correctly located the sample; red indicates that no dogs correctly located the sample, and shades of orange indicate the degree to which the dogs were able to locate the sample.

The teams correctly located an average of 90% of the diluted samples aged for 6, 9, and 12 months. There was a distinct decrease in the dogs' ability to locate the samples aged for 15 months (42%). This initially appeared to be the limit of their capability to detect aged decomposition fluid; however, after discussion with the training coordinator, it was decided to continue presenting older diluted samples to the teams in subsequent training. The teams demonstrated an average detection rate of 86% for all the subsequently aged samples (i.e., 18 to 24 months). Overall, the detection rates for the aged, diluted fluid were higher than those reported for the fresh, diluted fluid (77%) [3], with an overall average correct sample alert rate of 81%.

The number of correct and incorrect responses to the target odors over the course of two years is shown in Figure 1 for each team. There were relatively few incorrect (partial positive, false positive, or false negative) responses to the dilutions, and this trend remained consistent throughout the trials for all aged decomposition fluid samples. There was an increase in incorrect responses when a dog had not attended the previous training session, as shown in the 12- and 18-month trials for Team B, and the 24-month trials for Team C (Figure 1). However, no overall increasing trend was observed in the number of incorrect responses that were recorded with increasing age of the sample. This represents an ideal outcome for this study, although

both teams that were present for the 15-month aged trials had relatively high levels of incorrect responses. There is also no clear decreasing trend in the number of incorrect responses to the aged samples.

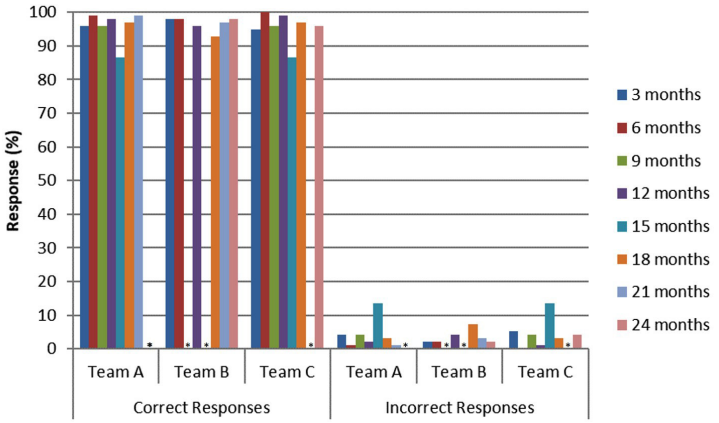

Figure 1

A comparison of correct and incorrect percentage responses across all trials. The colors represent the age of the decomposition fluid used in each trial as shown in the legend.

Note: any missing bars with an asterisk (*) indicate that the team was not available for that particular trial.

Discussion

The methods used in this study were based on the standard training protocols used by the police dog unit in this study. Samples were tested only at 3-month intervals because of the availability of the cadaver-detection dog teams for training sessions. This frequency was also selected to accommodate time and scheduling constraints on the instrumentation that was used to analyze the chemical profiles of the samples (results not presented here).

The teams demonstrated a strong positive response to almost all of the aged samples that were presented during the training sessions (with the exception of the 15-month samples). The study by Oesterhelweg et al. [4] determined that cadaver-detection dogs could detect decomposition residue on textiles for up to 65 days (approximately 2 months). Notably, a study by Lasseter et al. [12] confirmed that cadaver-detection dogs could detect

both human and animal remains that had been skeletonized for over 20 years. These results indicate that cadaver-detection dogs can detect decomposition odor from aged sources, and that the decomposition odor is still recognizable many years later in certain scenarios. These studies support the findings in this study, however, they do not explain the poor responses to the diluted samples aged for 15 months. This represented the oldest sample the dogs had been exposed to at the time of their introduction during training. It is hypothesized that the odor had changed distinctly at this point and the dogs did not initially recognize the odor as decomposition fluid. Chemical analysis of these samples (data not shown here) demonstrated a variation in the compound classes present but it was not deemed statistically significant when compared to the other aged samples. At the time, the training coordinator hypothesized that the exposure to subsequent samples of aged fluid (i.e., 18- to 24-month samples) may have led to the dogs associating this odor with their reward. Whether there was truly a change in the odor profile, or there was another reason for the poor response to the 15-month aged samples, this reinforces the need for multiple training aids from various stages of the decomposition process. The dynamic nature of decomposition odor will have an effect on the ability of the dogs to recognize and alert to their target odors. Hence, cadaver-detection dogs should be exposed to the widest possible variety of odor profiles in their training aids to enhance their capability in the field.

The incorrect responses that were produced during the training sessions were minimal and appeared to be influenced by the dogs' temperament and method of searching. The cadaver-detection dog in Team A was energetic and would often miss cans in its rush to scent the next can in the line-up, causing a higher number of false negative responses. Dog B demonstrated a slow and methodical search method; this included "self-checking" behavior, where it would scent the adjacent cans again before alerting to the target can, producing a higher number of false positive responses. The dog in Team C was both quick and methodical, which generally produced a low rate of incorrect responses, although there was no consistency to the type of false response provided. Dog B was also absent for two of the trials in this study and upon its return to training sessions, the false responses were higher than those of the previously attended sessions; this trend was also seen following the session that Dog C missed (21 months), though the increase in erroneous responses was not as noticeable as for Dog B (Figure 1). This

suggests that the dogs may require regular exposure to decomposition fluid in order to maintain their sensitivity to the lower dilutions and older samples. The samples were also presented in a sequentially aging pattern (i.e., 6 months, then 9 months, then 12 months, etc.). Presenting various ages to the dogs in a single training session may help to lower the number of incorrect responses and increase their sensitivity to the fluid.

The response rate data presented here cannot be analyzed for statistical significance because of the small sample size available. However, it is evident that the correct responses for each team are considerably greater than the incorrect responses. It is important to note that the cadaver-detection dogs are a biological detection system, and as such, will not yield entirely reproducible results. Although there will always be variations in their responses from session to session, this does not suggest that they are ineffective search tools, rather that their limitations should be recognized.

Overall, the dogs were able to detect diluted samples of human decomposition fluid that had been aged up to two years. All teams showed consistently high detection rates and low incorrect response rates for these samples in standard training conditions. Future investigations into the cadaver-detection dogs' sensitivity levels should continue with smaller volumes of decomposition fluid, as well as with decomposition fluid that has been aged for several years. Trials should also be carried out under nonstandard training conditions, such as an outdoor mock crime scene, in order to determine whether climatic variables (e.g., wind, rain) or various surface types (e.g., soil, textiles) will affect the dogs' sensitivity levels in operational scenarios.

Conclusions

The aim of this study was to examine the dogs' capability to detect the odor of aged decomposition fluid at diluted concentrations. The results suggest that the dogs may require regular exposure to the decomposition fluid. With regular exposure, the cadaver-detection dog teams were able to reliably detect a range of dilutions with the lowest being 0.1 mL of 1-part-per-trillion (10^{-12}) decomposition fluid that had been aged for 24 months. These represent the lowest sensitivity levels and oldest sequentially tested samples reported in the scientific literature for cadaver-detection dog teams. Although further testing is required to determine the dogs' limit of detection to decomposi-

tion fluid, these results support the use of human decomposition fluid as a valid training aid for cadaver-detection dog teams.

Acknowledgments

The authors wish to thank the police cadaver-detection dog teams who aided in this study, as well as Amanda Troobnikoff and Karlijn Stouten, who also assisted.

For further information, please contact:

Rebecca Buis
Centre for Forensic Science
University of Technology Sydney
P.O. Box 123, Broadway, NSW, 2007
Australia
Rebecca.C.Buis@gmail.com

References

1. Stadler, S.; Stefanuto, P.; Byer, J. D.; Brokl, M.; Forbes, S.; Focant, J. Analysis of Synthetic Canine Training Aids by Comprehensive Two-dimensional Gas Chromatography-time of Flight Mass Spectrometry. *J. Chromatography A* **2012**, *1255*, 202–206.
2. Buis, R. An Analysis of Putrefactive Liquid from a Pig (Sus Scrofa), Focusing on Cadaver Dog Responses to Chemical Changes Throughout the Bloat Stage of Decomposition. Master's Thesis. University of Bradford, 2011.
3. Buis, R. C.; Rust, L.; Nizio, K. D.; Rai, T.; Stuart, B. H.; Forbes, S. L. Investigating the Sensitivity of Cadaver-Detection Dogs to Decomposition Fluid. *J. For. Ident.* **2015**, *65* (6), 985–997.
4. Oesterhelweg, L.; Kröber, S.; Rottmann, K.; Willhöft, J.; Braun, C.; Thies, N.; Püschel, K.; Silkenath, J.; Gehl, A. Cadaver Dogs–A Study on Detection of Contaminated Carpet Squares. *For. Sci. Int.* **2008**, *174*, 35–39.
5. DeGreeff, L. E.; Furton, K. G. Collection and Identification of Human Remains Volatiles by Non-contact, Dynamic Airflow Sampling and Spme-GC/MS Using Various Sorbent Materials. *Anal. Bioanal. Chem.* **2011**, *401*, 1295–1307.
6. Cablk, M. E.; Szelagowski, E. E.; Sagebiel, J. C. Characterization of the Volatile Organic Compounds Present in the Headspace of Decomposing Animal Remains, and Compared with Human Remains. *For. Sci. Int.* **2012**, *220* (1–3), 118–125.

7. Schoon, A. A Comparison Between Canine Detection of Blood Residue and Some Blood Presumptive Tests. *J. For. Ident*. **2013**, *63* (3), 255–262.
8. Rendine, M.; Fiore, C.; Bertozzi, G.; De Carlo, D.; Filetti, V.; Fortarezza, P.; Riezzo, I. Decomposing Human Blood: Canine Detection Odor Signature and Volatile Organic Compounds. *J. For. Sci*. **2018**, doi: 10.1111/1556-4029.13901.
9. Rust, L.; Nizio, K. D.; Wand, M. P.; Forbes, S. L. Investigating the Detection Limits of Scent-detection Dogs to Residual Blood Odor on Clothing. *For. Chem*. **2018**, *9*, 62–75.
10. Alexander, M. B.; Hodges, T. K.; Bytheway, J.; Aitkenhead-Peterson, J. A. Application of Soil in Forensic Science: Residual Odor and HRD Dogs. *For. Sci. Int*. **2015**, *249*, 304–313.
11. Florida v Harris, Supreme Court of the United States, 2013 No. 11-817.
12. Lasseter, A. E.; Jacobi, K. P.; Farley, R.; Hensel, L. Cadaver Dog and Handler Team Capabilities in the Recovery of Buried Human Remains in the Southeastern United States. *J. For. Sci*. **2003**, *48* (3), 1–5.