Ability of Bed Bug-Detecting Canines to Locate Live Bed Bugs and Viable Bed Bug Eggs

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ABSTRACT The bed bug, *Cimex lectularius* L., like other bed bug species, is difficult to visually locate because it is cryptic. Detector dogs are useful for locating bed bugs because they use olfaction rather than vision. Dogs were trained to detect the bed bug (as few as one adult male or female) and viable bed bug eggs (five, collected 5–6 d after feeding) by using a modified food and verbal reward system. Their efficacy was tested with bed bugs and viable bed bug eggs placed in vented polyvinyl chloride containers. Dogs were able to discriminate bed bugs from *Camponotus floridanus* Buckley, *Blattella germanica* (L.), and *Reticulitermes flavipes* (Kollar), with a 97.5% positive indication rate (correct indication of bed bugs when present) and 0% false positives (incorrect indication of bed bugs when not present). Dogs also were able to discriminate live bed bugs and viable bed bug eggs from dead bed bugs, cast skins, and feces, with a 95% positive indication rate and a 3% false positive rate on bed bug feces. In a controlled experiment in hotel rooms, dogs were 98% accurate in locating live bed bugs. A pseudoscent prepared from pentane extraction of bed bugs was recognized by trained dogs as bed bug scent (100% indication). The pseudoscent could be used to facilitate detector dog training and quality assurance programs. If trained properly, dogs can be used effectively to locate live bed bugs and viable bed bug eggs.

KEY WORDS *Cimex lectularius*, pseudoscent, dog, *Camponotus floridanus*, *Blattella germanica*

Archaeological evidence shows that the obligate hematophagous bed bug, *Cimex lectularius* L., has been disrupting the sleep of humans for at least the past 3,500 yr (Panagiotakopulu and Buckland 1999). The decline of bed bug numbers in developed countries after the end of World War II was caused by multiple factors, such as novel house designs, improvements in cleaning appliances, and the widespread use of synthetic insecticides such as DDT (Kruger 2000, Gangloff-Kaufman and Schultz 2003). The resurgence of bed bugs in the developed world was detected in the late 1990s, and calls to pest control professionals for bed bug infestations have increased as much as 4,500% in Australia (Doggett and Russell 2007).

Bed bugs hide in cracks and crevices during the day where they remain unseen; they come out during the night to feed (Usinger 1966). The variety of bed bug harborages makes visual detection challenging (Cooper and Harlan 2004). Their cryptic nature especially makes it difficult to discover small, early infestations (Pinto et al. 2007). Because many pest control operators will not apply insecticide if they cannot visually locate the pest, inspections are essential but they can be time-consuming (St. Aubin 1981). Also, many people have delayed reactions to bed bug bites or even no reaction at all (Sansom et al. 1992), making it difficult to correlate reactions with a specific time frame a person could have been exposed to an infestation. The difficulties of confirming bed bug infestations cause most early infestations to go unnoticed until the populations are overwhelming (Pinto et al. 2007). Early control of infestations is more likely to succeed, and these infestations are less likely to spread and are cheaper to control (Doggett 2007). Therefore, a method that complements visual location of bed bugs would be valuable in live bed bug detection, especially for small and early infestations.

Dogs rely on olfaction rather than vision, and they have been used to detect a variety of materials, such as gases that are odorless to humans (Johnson 1977), black-footed ferrets (Reindl-Thompson et al. 2006), brown tree snakes (Engeman et al. 1998), explosives, and even missing people (Ashton and Eayrs 1970). There are also accounts of dogs trained to locate insects, such as gypsy moths (Wallner and Ellis 1976), screwworm pupae and larvae (Welch 1990), and termites (Brooks et al. 2003). Bed bug-detecting canines are currently being used at least in the United States and Australia (Cooper 2007, Doggett 2007). The quality of bed bug-detecting canines depends on the efficiency of their training and what the dogs are trained to do (Cooper 2007). A high accuracy for bed bug dogs is essential because people want bed bugs to be eliminated, not just a reduction in population (Pinto et al. 2007).

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For bed bug-detecting canines to achieve a high level of accuracy, they should be able to differentiate bed bugs from other cryptic pests and environmental factors commonly found in the same location, such as ants, cockroaches, termites, and mold. Also, they should be able to differentiate live bed bugs and viable eggs from bed bug debris (feces, cast skins, and dead bed bugs) because the presence of bed bug debris does not necessarily indicate a live infestation (Pinto et al. 2007). Therefore, bed bug-detecting dogs are usually trained using target odors (live bed bugs and viable eggs) that are separated from nontarget odors (e.g., other general household pests and bed bug debris). However, because bed bugs defecate and shed their skins inside training apparatuses, nontarget odors (debris) must be removed or the dogs would be inadvertently trained to respond to them (USCS 1979). For example, a dog that was trained on both termites and wood debris had a false positive indication rate of almost 75%, meaning the dog indicated the presence of termites when only termite-damaged wood was present (Brooks et al. 2003). To simplify training, a termite pseudoscent was developed for trainers and handlers of termite-detecting canines, reducing the possibility of training dogs on nontarget odors (Brooks 2001).

The purpose of our study was to determine the ability of canines to detect bed bugs when trained with live adult bed bugs. The first objective was to determine whether trained dogs are able to differentiate bed bugs from other general household pests, such as Florida carpenter ants, *Camponotus floridanus* Buckley; German cockroaches, *Blattella germanica* (L.); and eastern subterranean termites, *Reticulitermes flavipes* (Kollar). Second, we wanted to determine whether dogs could be trained to discriminate live bed bugs and viable eggs from other bed bug materials, such as fecal deposits, cast skins, and dead bed bugs. We also wanted to verify that, in a controlled experiment, trained dogs could locate hidden bed bugs in hotel rooms. Finally, we wanted to test different solvent extractions to see whether a bed bug pseudoscent could be recognized as live bed bugs by trained dogs.

Materials and Methods

Bed Bugs. The Harlan strain (Harold Harlan, Armed Forces Pest Management Board, U.S. Department of Defense, Washington, DC) of the bed bug was reared at the University of Florida's Department of Entomology and Nematology (Gainesville, FL). The insects were maintained in 240-ml glass rearing jars (Ball Collection Elite, Jarden Home Brands, Muncie, IN) with a 90-mm filter paper circle (Whatman no. 1, Whatman, Clifton, NJ) on the bottom of the rearing jar. Harborages were made from rectangles of manila folder (90 × 60 mm) folded in a fan-like manner and placed inside each jar.

Bed bugs were separated with feather-tipped forceps and placed into rearing jars according to life stage (≈200 bed bugs in each jar). As adults laid eggs, the eggs were placed into new rearing jars weekly. This was done by placing the rearing jar on ice to knock down the adults and by transferring the filter paper and harborage with the eggs attached into a new rearing jar. New paper and harborage were added to the rearing jar containing the adults. To prevent insect escape, organdy fabric was placed over the mouth of the rearing jar and secured by a screw-on lid. Bed bugs were maintained at 23–24°C with a relative humidity of ≈50% and a photoperiod of 12:12 (L:D) h.

Bed bugs were fed to engorgement once a week on chickens (Institutional Animal Care and Use Committee [IACUC] protocol E876). The chickens were bound at the feet and hooded, and the feathers on the side of the chickens' breasts were shaved to expose skin. The rearing jars of bed bugs were placed upside down on the shaved skin and the bed bugs fed through the organdy cloth. Bed bugs were harvested with a camel's-hair paintbrush ≈2 h before working with the dogs.

General Household Pests. Orlando strain German cockroaches were reared in large glass utility jars containing cardboard harborages. Dry food (23% crude protein; Lab Diet 5001 rodent Diet, PMI Nutrition International, Inc., Brentwood, MO) and water were provided ad libitum. The cockroaches were maintained at 23–24°C with a relative humidity of ≈50% and a photoperiod of 12:12 (L:D) h.

Eastern subterranean termites were collected from a single colony (Gainesville, FL). They were given damp cardboard and maintained at 23°C with a relative humidity of 55% and a photoperiod of 12:12 (L:D).

Florida carpenter ants were reared at the USDA–ARS laboratory in Gainesville, FL, at a temperature range of 26–28°C. They were fed crickets five days a week, hard boiled eggs once a week, and given 10% sugar water and water ad libitum. All general household pests were handled with feather-tipped forceps to prevent damage to the insects.

General Household Pests, Bed Bug Debris, and Hotel Field Experiment Scent Vials. Filter paper (90 × 40 mm) was folded in a fan-like manner and placed in a plastic snap-cap vial (18.5 ml, Thornton Plastic Co., Salt Lake City, UT). A hole (≈15 mm in diameter) was cut into the cap. Organdy fabric (60 × 60 mm) was placed over the vial opening and held in place with the cap. Multiple vials were prepared and five of either live adult bed bugs (mixed sexes), carpenter ants, termites, cockroaches, viable bed bug eggs, dead adult bed bugs, or bed bug cast skins were placed in the vials. For the hotel field experiment, six scent vials were prepared containing one, five, or 10 male-only or female-only adult bed bugs. Vials also were prepared with filter paper that was taken from the rearing jars and contained bed bug feces deposits of various ages. Control vials were prepared with only filter paper inside them. All scent vials were used within 2 h of preparation.

Pseudoscent Extracts and Scent Vials. Fifty live, mixed sex, adult bed bugs were placed in each of four glass vials (15 ml, Fisher Scientific, Pittsburgh, PA). Ten milliliters of either pentane, methanol, acetone, or water was added to the vials. Vials with solvent and

bed bugs were swirled for 10 min. Solvents were then pipetted out of the vials and placed into separate clean glass vials. Vials containing the different solvent extractions were then sealed until use later the same day.

Snap-cap vials with filter paper and organdy fabric were prepared as in the general household pest and bed bug debris experiments. Fifteen minutes before the experiment, 1 ml of the extract (equivalent to five bed bugs) was placed on the filter paper inside separate snap-cap vials. A snap-cap vial containing only filter paper was used as a control. It was determined previously that dogs do not indicate on pentane, methanol, acetone, or water.

Scent-Detection Stations. A scent-detection station consisted of a capped polyvinyl chloride (PVC) pipe (50 mm in diameter by 150 mm in height) that was secured onto a recycled plastic board (17 by 48 by 4 cm). A hole (30 mm in diameter) was drilled into the center of the PVC cap to allow scent to escape the station after scent vials were placed inside the PVC tube and on top of the plastic board, ≈ 10 cm from the opening of the PVC tube.

Canines. Seven dogs were used in the following experiments (IACUC protocol E732). Dog A was a 10-yr-old spayed female beagle. Dog B was a 4-yr-old spayed female Chinese crested. Dog C was a 2-yr-old spayed female beagle mix. Dog D was a 2-yr-old spayed female beagle mix. Dog E was a 1-yr-old neutered male Jack Russell terrier. Dog F was a 1-yr-old spayed female beagle. Dog G was a 2-yr-old neutered male beagle.

Canine Training Method. Scent vials containing live bed bugs and viable bed bug eggs were prepared as described above, and they were placed in scent-detection stations. Dogs were trained to scratch at a scent-detection station containing either the live bed bugs or viable eggs by a modified food and verbal reward method (Brooks et al. 2003). During training, other scent vials containing distracting substances (e.g., dog food, human scent, German cockroaches, and bed bug cast skins) were placed in stations to ensure that the canines were alerting only to the odor of the live bed bugs or viable bed bug eggs. Once the bed bug scent was associated with the reward, the canines were fed only after they indicated on the scent of live bed bugs or viable bed bug eggs. All dogs went through 90 d of initial training before being used in the experiments. After the initial training was completed, dogs were maintained by feeding them twice daily only after locating the target odor. To ensure optimal performance, individual dogs were never worked in any experiment for >40 min/d (Brooks et al. 2003).

General Household Pest Experiment. Five scent-detection stations were used in this experiment, each containing a scent-detection vial of either live bed bugs, cockroaches, termites, ants, or a control vial. Vials were placed inside the scent-detection stations. Scent-detection vial contents were written on the PVC cap with invisible ink that could only be seen using an UV light. This was done to prevent the dog handler from knowing which insect was in the station. All stations were marked with invisible ink to prevent the dogs from detecting the presence of the ink.

The five stations were placed in a line ≈1 m apart from each other. The dog handler walked the dog down the line, allowing the dog to sniff each station. If the dog missed a station, the handler was allowed to turn the dog around and walk it past the station again. If the dog did not indicate on any station, the dog and handler were allowed to walk down the line of stations a second time. The order of the stations was chosen randomly for each repetition. In total, four dogs (A, B, C, and D) using one handler were evaluated with 20 repetitions each. The data were taken over a 10-mo period.

As the dogs were evaluated, one of three outcomes was recorded depending on the performance of the dog: a positive indication, a false positive, or no indication. If the handler interpreted an indication by the dog at a station, the handler checked with the evaluator to determine whether bed bugs were present. If bed bugs were present, the indication was scored as a positive indication, and the dog was rewarded. If bed bugs were not present, the indication was scored as a false positive, and the dog was not rewarded. If the handler did not interpret an indication by the dog at any station, it was recorded as no indication.

Bed Bug Debris Experiment. Six scent-detection stations were used in this experiment, each one containing a scent-detection vial with five of either bed bug cast skins, dead bed bugs, bed bug feces, viable eggs (collected 5–6 d after adult feeding), live adult, mixed sex bed bugs, or a control vial. The labeling, positioning, and randomization of the stations were completed as described previously in the general household pest experiment. Dog evaluation and scoring procedures also were as described previously, except dogs were rewarded for positive indications on live bed bugs and viable eggs. Three dogs (A, B, and D) using one handler were evaluated with 20 repetitions each. The data were taken over a 10-mo period.

Hotel Room Field Experiment. Six scent vials were used in this experiment, each containing one, five, or 10 male-only or female-only adult bed bugs. Two double queen bed hotel rooms were used, one room containing only scent vials with female bed bugs, and the other room containing only scent vials with male bed bugs. Both hotel rooms were identical in size and had similar furniture with the same pattern of arrangement (Fig. 1). For each repetition, the scent vials were randomly hidden in any of 17 possible locations in each room; the four corners of bed one, the two corners of the nightstand, the four corners of bed two, the two corners of the arm chair, the desk chair, the two corners inside dresser drawer one, or the two corners inside dresser drawer two. All vials were hidden from view of both the dog and the dog handler. Scent vials hidden in the bed were placed between the mattress and boxspring ≈5 cm from the edge. In the nightstand, scent vials were placed in the inside front corners of the open face. Scent vials hidden in the sitting chair were placed under the cushion ≈5 cm from the edge. In the desk chair, scent vials were placed in the crevice where the backrest and seat join. All four dresser drawers were opened slightly to allow the dogs access

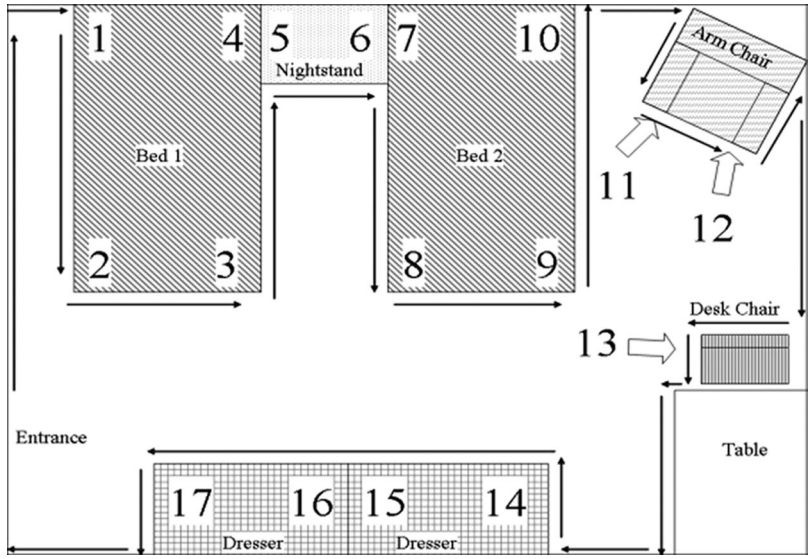

Fig. 1. Layout of furniture in hotel rooms, locations where bed bugs were hidden, and the path followed for searching the rooms.

to the scent. Because of this, scent vials were only placed in the bottom two drawers so the handler would not be able to see them.

The dogs were walked through the rooms following the same path for each repetition. The dog–handler team passed the possible locations of hidden bed bugs in the order stated in the previous paragraph. Dogs were allowed two passes in the room if needed. Scent vials were randomly moved to new locations between each run. Fifteen minutes elapsed between runs to allow the scent at the old locations to dissipate and to allow the scent to accumulate at the new locations. Three dogs (A, B, and G) using one handler were evaluated with six repetitions each. Data were taken over a 1-wk period.

Pseudoscent Extracts Experiment. Five scent-detection stations were used in this experiment, and they contained a scent-detection vial of either pentane, acetone, methanol, or water extracts, or a control vial. The labeling, positioning, and randomization of the stations were completed as described previously. Dog evaluation and scoring procedures also were as described previously, except dogs were rewarded for indication on any of the scent-detection stations except the control. An additional 1 ml of each extract was added to the appropriate scent-detection vial before a new dog–handler team was evaluated. Three dogs (D, E, and F) using one handler were evaluated with 20 repetitions each, and the data were taken over a 1-wk period.

Statistical Analysis. The percentage of positive or false positive indications was calculated for each scent based on 20 repetitions with each dog except for the hotel room experiment, which had six repetitions with each dog. Data were then arcsine square root transformed and analyzed by two-way analysis of variance (ANOVA), with main effects as the dogs and the scents in the scent-detection vials. Means were separated with Student–Newman Keuls ($P < 0.05$; SAS Institute 2003).

Results

General Household Pest Experiment. Two-way ANOVA determined the scent of household pests in scent-detection stations significantly affected the dogs' responses ($F = 3211$; df = 4, 3, 8, 380; $P < 0.0001$). There were no significant differences among the four dogs ($F = 2.11$; df = 4, 3, 8, 380; $P = 0.098$). There was a significant interaction between household pest scents and the tested dogs ($F = 2.11$; df = 4, 3, 8, 380; $P = 0.0156$), because one dog was less accurate in finding bed bugs when the insects were present. Dogs trained to locate the scent of live bed bugs and viable bed bug eggs were able to distinguish live bed bugs from other household pests, including carpenter ants, cockroaches, and termites (Table 1). When live bed bugs were present in scent-detection stations, the dogs averaged ≈98% accuracy in locating them. There were no false positives for any of the dogs; dogs did not indicate at any scent-detection station that did not contain bed bugs. With dogs A, B, and D, there were no false positives, no missed indications, and the dogs found the bed bugs every time the insects were present. The positive indications for dogs A, B, and D were significantly higher than the positive indications for dog C as well as the false positives for all dogs ($F = 1897.47$; df = 7, 392; $P < 0.0001$). There were no false positives for dog C either, but it failed to detect the bed bugs twice during 20 repetitions.

Bed Bug Debris Experiment. Two-way ANOVA determined the scent of bed bug materials in scent-detection stations significantly affected the dogs' responses ($F = 677$; df = 5, 2, 10, 342; $P < 0.0001$). There

Table 1. Percentage of indication (mean % ± SE) by dogs at scent-detection stations containing live general household pests and live common bed bugs

Dog	% indication					Indication	
	Bed bugs	Ants	Cockroaches	Termites	Blank	Positive[a]	False Positive[b]
A	100 ± 0a	0 ± 0c	0 ± 0c	0 ± 0c	0 ± 0c	100 ± 0x	0 ± 0z
B	100 ± 0a	0 ± 0c	0 ± 0c	0 ± 0c	0 ± 0c	100 ± 0x	0 ± 0z
C	90 ± 6.88b	0 ± 0c	0 ± 0c	0 ± 0c	0 ± 0c	90 ± 6.88y	0 ± 0z
D	100 ± 0a	0 ± 0c	0 ± 0c	0 ± 0c	0 ± 0c	100 ± 0x	0 ± 0z
Mean	97.5 ± 1.76m	0 ± 0n	0 ± 0n	0 ± 0n	0 ± 0n		

Means in a treatment block followed by the same letter are not significantly different ($P = 0.05$; Student–Newman–Keuls; SAS Institute 2003).
[a] Positive indications are indications by dogs on bed bug scent.
[b] False positive indications are indications by dogs on any scent other than bed bugs.

were no significant differences among the three dogs ($F = 0.53$; df = 5, 2, 10, 342; $P = 0.59$), and there was not a significant interaction between bed bug debris scents and the tested dogs ($F = 0.53$; df = 5, 2, 10, 342; $P = 0.87$). Dogs trained to locate the scent of live bed bugs and viable bed bug eggs were able to distinguish the live bed bugs and viable eggs from other bed bug debris, including bed bug feces, dead bed bugs, and cast skins (Table 2). Dogs were significantly more accurate in locating live bed bugs than they were in locating viable bed bug eggs ($F = 267$; df = 5, 174; $P < 0.0001$), but their mean positive indication rate on viable bed bug eggs was still high at 90%. The dogs had an average false positive rate of 3% on bed bug feces, with no false positives on any other scent. All three dogs located the live bed bugs every time the insects were present, giving them a perfect positive indication rate on live bed bugs. Each of the three dogs missed the viable bed bug eggs two times out of 20 repetitions. The overall positive indication rate was the same for each dog at 95%, which was significantly higher than the false positive rates ($F = 657$; df = 5, 354; $P < 0.0001$). When live bed bugs and viable bed bug eggs were not present, there was no significant false positive rate although dog A did have two false positives on bed bug feces.

Hotel Room Experiment. Two-way ANOVA determined the source of the scent (whether the vials contained male or female bed bugs at densities of one, five, or 10) did not significantly affect the dogs' responses ($F = 1.0$; df = 5, 2, 10, 36; $P = 0.4317$). There were no significant differences between the three dogs ($F = 1.0$; df = 5, 2, 10, 36; $P = 0.3779$). The interaction between the dogs and the scent vials was also not significant ($F = 1.0$; df = 5, 2, 10, 36; $P = 0.4618$). Dogs trained to locate the scent of live bed bugs and viable bed bug eggs were able to shift that ability from the experimental scent-detection stations to the more realistic hotel room situation, with a 98% average accuracy (Table 3). Dogs A and B were 100% accurate in locating live bed bugs, whereas dog G was 94.4% accurate. Dog G had one missed indication on one of six possible scent vials out of six repetitions; it did not indicate once on the vial containing five female bed bugs. There were no false positives for any of the dogs; dogs did not indicate anywhere that bed bugs were not present.

Pseudoscent Extracts Experiment. Two-way ANOVA determined the extract in the scent-detection station significantly affected the dogs' responses ($F = 3571$; df = 4, 2, 8, 285; $P < 0.0001$), but again there were no significant differences among the three dogs ($F = 1.0$; df = 4, 2, 8, 285; $P = 0.369$). There was not a significant interaction between the tested dogs and the extracts ($F = 1$; df = 4, 2, 8, 285; $P = 0.436$). Dogs trained to locate the scent of live bed bugs and viable bed bug eggs always indicated on the pentane extract (Table 4), but they averaged only ≈2% on the methanol and had no indications on the acetone, water, or blank scent-detection stations. All dogs averaged 100% indication on the pentane extract, which was significantly higher than all other extracts. Dog B had a 5% indication rate on methanol extract. The pentane pseudoscent we used was stored in a refrigerator at a temperature of 3.3°C. Three months later, the dogs still

Table 2. Percentage of indication (mean % ± SE) by dogs at scent-detection stations containing bed bug materials, live common bed bugs, and viable bed bug eggs

Dog	% indication						Indication	
	Live bed bugs	Viable bed bug eggs	Feces	Cast skins	Dead bed bugs	Blank	Positive[a]	False positive[b]
A	100 ± 0	90 ± 6.88	10 ± 6.88	0 ± 0	0 ± 0	0 ± 0	95 ± 3.49a	2.5 ± 1.76b
B	100 ± 0	90 ± 6.88	0 ± 0	0 ± 0	0 ± 0	0 ± 0	95 ± 3.49a	0 ± 0b
D	100 ± 0	90 ± 6.88	0 ± 0	0 ± 0	0 ± 0	0 ± 0	95 ± 3.49a	0 ± 0b
Mean	100 ± 0x	90 ± 6.88y	3.33 ± 2.34z	0 ± 0z	0 ± 0z	0 ± 0z		

Means in a treatment block followed by the same letter are not significantly different ($P = 0.05$; Student–Newman–Keuls; SAS Institute 2003).
[a] Positive indications include indications of dogs on live bed bug and viable bed bug egg scents.
[b] False positive indications include indications of dogs on any scent other than live bed bugs or viable bed bug eggs.

Table 3. Ability of dogs to locate varying numbers of live male and female bed bugs in hotel rooms

Dog	% indication (mean ± SE)						
	No. female bed bugs			No. male bed bugs			Positive[a]
	1	5	10	1	5	10	
A	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
B	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
G	100 ± 0	66.7 ± 33.33	100 ± 0	100 ± 0	100 ± 0	100 ± 0	94.4 ± 5.56
Mean	100 ± 0	88.9 ± 11.11	100 ± 0	100 ± 0	100 ± 0	100 ± 0	

There were no significant differences at all variables ($P = 0.05$; Student–Newman–Keuls; SAS Institute 2003).
[a] Positive indications include indications of dogs on live bed bug and viable bed bug egg scents.
[b] There were no false positive indications.

indicated on it, so as long as it is stored properly the pseudoscent has at least a 3-mo shelf-life.

Discussion

Detector dogs trained to locate live bed bugs and viable bed bug eggs have been used as a tool for pest control operatives. However, for them to be effective, the dog must be able to locate the target odor accurately. Dogs trained to locate live bed bugs and viable bed bug eggs had an overall accuracy of 97%, which is similar to previous studies on insect detector dogs. A German wirehaired pointer trained to detect screwworms had an accuracy of 99.7% (Welch 1990). Wallner and Ellis (1976) were able to train three German shepherds to detect gypsy moth egg masses at an accuracy of 95%. Six dogs that were trained to locate live termites had an overall accuracy of 96% (Brooks et al. 2003). Similarly, our dogs were able to discriminate bed bugs from other general household pests that may be found in the same locations, such as German cockroaches, Florida carpenter ants, and eastern subterranean termites. The dogs also were able to differentiate materials of an active infestation (live bed bugs and viable bed bug eggs) from materials of a possibly inactive infestation (dead bed bugs, cast skins, and bed bug feces). In a more realistic situation, dogs also were able to locate live bed bugs hidden throughout hotel rooms. The minimum acceptable standard proposed by Brooks et al. (2003) of a positive indication rate of ≥90% and a false positive rate of ≤10% was achieved by the bed bug-detecting canines we tested.

Although a high positive indication rate is a realistic expectation for detection dogs, a few studies showed that some dogs had a positive indication rate less than the proposed minimum acceptable standard (Brooks et al. 2003). Three dogs that were trained to identify off-flavor pond water compounds (2-methy-lisoborneol and geosmin) had an overall accuracy of 77% (Shelby et al. 2004). Dogs trained to locate brown tree snakes hidden in cargo on Guam had an overall accuracy of 70% (Engeman et al. 1998). These lower positive indication rates could be the result of a variety of different factors, such as dog training method, training apparatus used, training maintenance, and length of search time. Environmental factors such as temperature, air flow, handler misinterpretation, and scent accessibility also could have affected the accuracy of the dogs (Moulton 1972, Wallner and Ellis 1976, Ashton and Eayrs 1970, Welch 1990). In our study, the dogs had a high positive indication rate because we controlled as many of these influences as possible. The training method we used was modified from Brooks et al. (2003). Training was maintained twice daily and the length of search time was limited to 40 min or less. Airflow was minimal and temperature was constant due to the indoor test environment, and one handler was used in all experiments. If the training methods proposed by Brooks et al. (2003) are used, if training is maintained regularly, and if environmental and human factors are controlled, it is possible for dogs to have a positive indication rate equal to or higher than the proposed minimum acceptable standard.

Sometimes dogs do not indicate when the target odor is present; they show no indication. In our study, all dogs had a 10% no-indication rate on viable bed bug eggs. Dogs trained to respond to a target odor will react only if the target odor meets or surpasses a threshold concentration (Moulton 1972, Settles 2005). The relatively high no-indication rate of our dogs on viable bed bug eggs may be due to low concentration of target odor, although the 90% positive indication rate on the viable bed bug eggs was within the acceptable minimum standard. However, a dog's response also must be interpreted by the handler. No indications can be caused by the handler misreading dog behavior, emphasizing the importance of an experienced handler.

A high false positive rate also may be caused by faulty training or misinterpretation by the handler. Brooks et al. (2003) reported on a dog with a 75% false positive rate on termite-damaged wood, when the

Table 4. Percentage of indication (mean % ± SE) by dogs at scent-detection stations containing chemical rinses of live common bed bugs

Dog	% indication				
	Pentane	Methanol	Acetone	Water	Blank
A	100 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
E	100 ± 0	5 ± 5	0 ± 0	0 ± 0	0 ± 0
F	100 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Mean	100 ± 0a	1.67 ± 0b	0 ± 0b	0 ± 0b	0 ± 0b

Means followed by the same letter are not significantly different ($P = 0.05$; Student–Newman–Keuls; SAS Institute 2003).

target insects, termites, were not present. That particular dog was trained on termites and termite damaged wood, when the only target odor was termites. However, dogs trained only on termites had a considerably lower false positive rate. In our study, we believe the false positives recorded for dog A on bed bug feces may have occurred because of bed bug defecation in the scent-detection vials. The feces were removed every 2 or 3 wk from the scent-detection vials, which were used daily for training for dog A. Therefore, dog A was being trained on both target and nontarget odors. Feces were monitored and removed daily before training the rest of the dogs.

Training a dog only on target odors can be difficult, especially if handling of target insects is difficult, as with live bed bugs. The creation of a pseudoscent can make the training of bed bug-detecting dogs easier. A pseudoscent can eliminate the need for dog trainers to handle bed bugs while ensuring the dogs are only being trained on the target odor. The dogs did not indicate on the acetone or water extract. One dog indicated once on the methanol extract. Pentane seems like the most possible candidate for creating a pseudoscent because all dogs indicated 100% on the pentane extract. It seems that pentane has the ability to contain the target odor of the bed bugs because the dogs indicate on the pentane extract like they indicate on live bed bugs and viable bed bug eggs.

The pentane pseudoscent can be used in many different ways. It can be used to train dogs, replacing the live bed bugs that many people are uncomfortable handling. Also, quality control programs are necessary and usually required to evaluate whether trained dogs continue to work properly (Doggett 2007). The existence of a pseudoscent would be ideal in this situation. The pseudoscent would allow a technique for quality assurance that could be used in any building, without the possibility of accidentally creating infestations.

Bed bug-detecting canines can be a valuable tool to the industry. They can aid in the detection of early and established infestations. From an economic point of view, locating these infestations can reduce the number of possible lawsuits from customers (Doggett 2007). Instead of hotel managers learning of an infestation due to a customer being bitten, they can seek out the infestations and treat them before customers are affected. Also, because bed bug-detecting canines can be trained only to locate live bed bugs and viable bed bug eggs, the dogs can recheck previously treated rooms to confirm whether the treatment was successful.

Our study has shown that dogs can be trained to accurately locate live bed bugs and viable bed bug eggs at a positive indication rate $\geq$90% and a false positive rate $\leq$10%, as proposed by Brooks et al. (2003). Dogs can differentiate the live bed bugs from other general household pests, such as German cockroaches, eastern subterranean termites, and Florida carpenter ants. The dogs also can discriminate live bed bugs and viable bed bug eggs from other bed bug materials, such as cast skins, feces, and dead bed bugs. The hotel room experiment showed that dogs can locate as few as one bed bug in a hotel room. The production of a pseudoscent would make it easier to train dogs only on the target odor, possibly increasing the accuracy of the dogs. Dogs can be trained to locate cryptic insects that are difficult to uncover visually as long as dogs are trained in a similar manner to the method we used, training is maintained regularly, an experienced handler is used, and nontarget odors are separated from target odors. The ability of carefully trained dogs to accurately locate cryptic insects holds many possibilities; dogs could be used to locate and monitor populations of many important insects, such as Africanized honey bees or the emerald ash borer, *Agrilus planipennis* Fairmaire.

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