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Identifying environmental reservoirs of *Clostridium difficile* with a scent detection dog: preliminary evaluation

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SUMMARY

Background and aim: Prompted by an article describing a dog trained to detect *Clostridium difficile* in patients, our institution evaluated a dog's ability to detect *C. difficile* scent from equipment and surfaces to assist in strategic deployment of adjunctive cleaning measures.

Methods: An expert in drug and explosives scent dog handling trained a canine to identify odours from pure cultures and/or faecal specimens positive for *C. difficile*. Methods used to assess explosive and drug detection dogs were adapted and included evaluation of (i) odour recognition, using containers positive and negative for the scent of *C. difficile*, and of (ii) search capability, on a simulation ward with hidden scents. After demonstration that the canine could accurately and reliably detect the scent of *C. difficile*, formal assessments of all clinical areas began.

Findings: Odour recognition ($N = 75$ containers) had a sensitivity of 100% and specificity of 97%. Search capability was 80% sensitive and 92.9% specific after removal of results from one room where dog and trainer fatigue influenced performance. Both odour recognition and search capability had an overall sensitivity of 92.3% and specificity of 95.4%. The clinical unit sweeps over a period of five months revealed a sensitivity of 100% in alerting on positive quality control hides. These clinical unit sweeps also resulted in 83 alerts during 49 sweep days.

Conclusion: A dog can be trained to accurately and reliably detect *C. difficile* odour from environmental sources to guide the best deployment of adjunctive cleaning measures and can be successfully integrated into a quality infection control programme.

Introduction

Clostridium difficile infection (CDI) is a known risk for patients, particularly in acute care facilities. The organism is usually transmitted via the hands of caregivers or through indirect and/or direct contact with contaminated surfaces and equipment. *C. difficile* has the ability to form spores and consequently can persist in the environment for long periods of

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time. Environmental reservoirs have been implicated in outbreaks and two recent articles have documented that the risk of acquiring CDI increases if a previous occupant of the room was colonized or infected with *C. difficile* [1–4]. The ability to quickly assess clinical areas for reservoirs of *C. difficile* in order to direct additional disinfection efforts would therefore be useful.

A recent article from the Netherlands reported that it was possible to train a beagle to detect *C. difficile* in patients, first as a proof of concept followed by an evaluation during a hospital outbreak [5,6]. We elected to train a springer spaniel to detect *C. difficile* odours on equipment and environmental surfaces, rather than focusing on patients. This article details the training, evaluation process, examination results, and integration of a *C. difficile* scent detection team in a tertiary care facility.

Methods

Qualifications of the certified trainer and dog lineage

The trainer was a validated Explosives and Narcotics K9 Detection handler having completed the Advanced K9 Handler Course and the Detection Handler Course from the largest working K9 training centre in Western Canada (Obedience Plus Inc., Maple Ridge, BC, Canada). She was also a judge for the sport of Nosework under the National Association of Canine Scent Work (Los Angeles, CA, USA). The canine was a springer spaniel from a well-established line of hunting dogs (Hellfire Springer Spaniels, Anaconda, MT, USA), selected because of his intense work drive, biddable happy nature, and non-intimidating appearance. The dog was taught to sit to alert and/or to tap the container if he detected the odour of *C. difficile*. The hospital's Patient Safety and Quality Division approved the project as a Quality Initiative, the health authority's pet policy was followed, and the dog wore a jacket to identify it as a working canine.

Preparation of training and examination materials

Positive training and test materials consisted of odours from clinical toxigenic strains of *C. difficile* and from *C. difficile*-positive faeces as determined by polymerase chain reaction (PCR). *C. difficile* culture odours were prepared using a 10 μL loop of *C. difficile* colonies from clinical toxigenic strains suspended in 1 mL of normal saline and deposited on the bottom of a sterile urine container. The open container was then placed in a large glass jar with cotton gauze squares suspended above (Figure 1). *C. difficile* faecal odours were obtained by exposing gauze to 30 g clinical samples of faeces (collected within 24 h) confirmed as *C. difficile* positive. Negative training and test materials consisted of cotton gauze suspended above either 1 mL of normal saline or *C. difficile* PCR-negative faeces (the latter is known as an associated odour). Separate jars were used to expose gauze squares for 4, 8, 24, or 48 h to obtain

Figure 1. The *Clostridium difficile* (CD) scent dog-training process.

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different odour strengths. These training materials were hidden initially in wooden sniff boxes, then in large PVC containers and finally in smaller 'hides' commonly used in scent detection (Figure 1) [7]. Positive and negative samples were processed entirely separately and fresh faecal preparations from different patients were used for training and each examination to avoid the dog focusing on an individual's scent rather than the unique scent of *C. difficile*.

Training

Search and play techniques were initially used at 11 months of age where a favourite toy was paired with a positive *C. difficile* culture sample (gauze exposed to *C. difficile* cultures). Once the canine accurately and reliably found the paired odours, the toy was removed, and the dog rewarded with his toy for correct detection of the *C. difficile* culture odour. When the dog was confidently finding the odour from *C. difficile* cultures, *C. difficile*-positive faeces, gauze only (negative control), and *C. difficile*-negative faeces (negative control with an associated odour) were sequentially introduced. The canine was trained in various environments: at home, at K9 training centres, and on a clinical unit at Vancouver General Hospital.

Evaluation

Evaluations were two types: a container 'run' to assess odour recognition (discrimination) and a simulation unit assessment that tested search (detection) capability. Examination techniques were adapted from the UK Defence Science and Technology Laboratory validation testing for odour discrimination, the Scientific Working Group on Dog and Orthogonal Detector guidelines, and the Nevada narcotics detection K-9 team certification processes [8–10]. Both types of evaluation were conducted at Kwantlen Polytech University (Surrey, BC, Canada) in a controlled environment with a separate staging area for samples. Container runs consisted of *C. difficile* odour-positive and -negative gauzes in sterile containers that were placed inside 25 PVC containers with perforated lids by the investigators. The latter containers (which had been washed in an industrial washer, dried, and then wiped with accelerated hydrogen peroxide wipes prior to each evaluation) were placed in a single line about 2.4–3.0 m apart, with positive and negative specimens placed by different individuals to avoid cross-contamination. Two individuals with experience in training sport, explosive, or drug detection dogs were blinded as to which canisters held positive samples; they served as judges and impartial observers. Consistent with examinations for explosive and drug dogs, the handler was given an approximation as to the number of positives in the three container runs prior to each test. The dog was moved down the line, interrogating each container once. The handler would clearly announce an alert with true positive alerts acknowledged for reward of the dog. False-positives did not result in a reward and the dog would continue the search – the handler and judges were not informed of the total number of true positives until completion of the run, in order not to bias the rest of the search. No training run immediately before the examination was permitted, to minimize container recognition (handler bias) or odour recognition (dog bias).

Search capability was assessed on the simulation wards used to teach nursing students at Kwantlen Polytechnic University. These wards consist of mock clinical units fully stocked with healthcare supplies and equipment but never house patients. Four to seven positive and negative gauzes were hidden by the investigators in each of three mock clinical units. The handler and the judges were told the number of positive rooms but not which rooms were positive; neither were they informed as to the number of positive or negative hides placed. Positive and negative test samples were strategically hidden at different heights, and under and inside equipment, nursing supplies, and furniture. Similar to the odour recognition test, the handler systematically worked the dog through the room announcing alerts; true positives were acknowledged for reward of the dog. No time limits were set for each clinical space and the handler was permitted to recheck areas of the room provided she had not formally indicated that the search was complete.

True and false alerts were recorded for each clinical evaluation and sensitivity and specificity of search as well as positive and negative predictive values calculated. A questionable alert was counted as no alert. All evaluations (container and clinical examinations) were filmed and results recorded.

Hospital implementation

Following an initial introduction period and communiqués outlining the role of the canine team, a daily schedule was established. The first sweeps were in clinical areas with the highest rates of CDI, followed by a systematic check of all clinical and support service areas. Immediately prior to each sweep, a positive sample was hidden by a third party (infection preventionists or the ward managers) and the team was evaluated for their ability to find the sample with no false alerts. Results from this quality check as well as areas searched, and positive alerts, were recorded in an Access™ database (Microsoft, Seattle, WA, USA). Positive alerts resulted in immediate notification of housekeeping for priority recleaning of the room or piece of equipment and use of ultraviolet C light whenever possible.

Results

Three container (75 samples) and three clinical runs (53 samples) were conducted at Kwantlen Polytechnic University on September 3rd, 2015, April 26th, 2016, and May 31st, 2016. Statistical results for all container runs, ward evaluations, and the cumulative results are detailed in Table I. The time to successfully navigate a container run was 8 min for the first examination, 5 min for the second, and 83 s for the third. The time to search a room with a positive hide varied between 5 and 16 min while a negative room took between 4 and 13 min. Results of the quality control checks and the clinical sweeps from November 1st, 2016 to March 31st, 2017 in the facility are detailed in Table II.

Discussion

Clostridium difficile is a spore-forming bacterium that is the most common cause of hospital-associated diarrhoea in developed countries. An estimated 450,000 cases a year cost the US economy about $3.2 billion per year, and the annual

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Table I
Summary of results for odour recognition and search capability examinations

Examination	Total no. of samples	Sensitivity	Specificity	False positive	False negative	PPV	NPV
Container runs[a]	75	100%	97%	3%	0%	80%	100%
Simulation units[b]	53	67%	91.5%	8.5%	33.3%	50%	95.5%
Containers and simulation units	128	85.7%	94.7%	5.3%	14.3%	67%	98.2%
Simulation units without room 4, exam 2[c]	47	80%	92.9%	7%	20%	57%	97.5%
Containers and simulation units without room 4, exam 2	122	92.3%	95.4%	4.6%	7.7%	70.6%	99.0%

PPV, positive predictive value; NPV, negative predictive value.
[a] Container runs assess odour recognition/discrimination.
[b] Simulation units assess search capability.
[c] During the final leg of the second examination (room 4), both dog and handler displayed clear signs of fatigue.

burden of illness is increasing [11]. The organism is acquired in hospitals via contaminated hands or the environment. Because it is a spore-forming bacterium, it can survive in the environment for long periods of time and is more resistant to regular hospital disinfection protocols; therefore many facilities employ adjunctive disinfection methods such as bleach, ultraviolet C radiation or hydrogen peroxide vapour systems when outbreaks occur or when individual patients are identified with the organism [12]. However, there is no rapid or reliable method to detect the presence of *C. difficile* in the environment that would assist in directing these adjunctive disinfection measures in an efficient manner.

Scent detection dogs have been shown to be more sensitive than technology for chemical detection of a range of substances and canines have demonstrated impressive sensitivity and specificity in detecting various types of cancer, significant bacteriuria and, more recently, the detection of meticillin-resistant *Staphylococcus aureus* [13–18]. In our study, odour recognition ability was excellent early on in training with the canine readily discriminating between positive *C. difficile* odours and negative controls. The rapidity and clarity with which he alerted on positive *C. difficile* odours increased with maturity – the first container run took 8 min compared to the last examination that was completed within 83 s. The container runs had an overall sensitivity and specificity of 100% and 97% respectively. Scent search capability improved with maturity in terms of the systematic nature of the search and the dog's stamina in examining multiple rooms. The dog was able to sweep areas more thoroughly as he matured, progressing to opening doors and searching cupboards, bins and dressers. Exclusion of the results from one room during the second examination when dog and handler were clearly fatigued increased the sensitivity of detection from 67% to 80% for search capability.

Table II
Results of quality control examinations and hospital unit sweeps: November 1st, 2016 to March 30th, 2017

Measures	Counts (%)
Quality control examinations in hospital	
Total no. of quality control (positive cultures or stools) hides	84
Detection sensitivity of the positive cultures or stool hides	100%
Hospital unit sweeps	
Total no. of search days	49
Total no. of wards searched	198
Average time to search a unit (min)	16.3
Total no. of clear positive alerts in unit sweeps	83
Alerts on patient rooms (including patient beds and furniture, excluding patient care equipment)	24 (28.6%)
Alerts on common or shared areas (e.g. hallways and handrails, and washrooms and shared patient bathtubs)	34 (41.0%)
Alerts on patient care equipment (e.g. crash carts, commodes)	25 (30.1%)

There are variations in the literature about what is an acceptable pass or failure for detection dogs. Nevada, for example, requires 100% accuracy to pass the odour recognition test but permits up to two false-positive alerts [10]. The Florida guidelines require that canine teams only locate the training material at a 2 m radius from the source [9]. The lower overall positive predictive value reflects the canine's tendency to 'over-call' negatives as positive in the simulation wards which could result in unnecessary cleaning of a room when the team is sweeping a clinical area. Conversely the high negative predictive value means that a room can be confidently declared to be free of *C. difficile*. It should be noted that for both odour recognition and search capability, the positive predictive value was 80% and the negative predictive value was 100% for the final examination, illustrating the canine team's increasing competence. It could also be that we placed too high a demand on dog and handler by requiring that they physically locate the positive hides for the search capability phase of the examinations. It may be more pragmatic to simply require that the dog identify which areas contain a *C. difficile* reservoir.

There was no other local dog or trainer to cross-validate results and we did not standardize the odours apart from an attempt to use similar inocula of *C. difficile*, the same amount and similar collection times (i.e. age) of faeces, and positive PCR samples that were judged to have similar concentrations of *C. difficile* based upon PCR test results. We blinded the handler and judges as to which containers and/or rooms were positive and how many samples were planted, only informed her as to approximately how many positives were planted in the containers, and used impartial expert observers to confirm results. However, false-negative or -positive results could be due to canine or handler bias [19,20]. Similarly, the training of

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detection dogs is linked with a reward for correct alerts and the potential exists for the dogs to be biased. Ongoing quality assurance tests were therefore embedded in the canine team's work schedule and included both odour recognition (discrimination) as well as the ability to conduct reliable systematic searches.

The only other documented detection dog for *C. difficile* was a beagle that had been trained to detect faecal samples and acute care patients with CDI [5,6,21]. Interestingly, when the dog visited patients with CDI on a long-term care facility where individuals spent less time in bed, the dog was more challenged to detect true positives. The authors suggested that differences in detection between acute and long-term care patients might be because the bed or mattress was the primary strong source of odour, indicating that the dog may be detecting an environmental reservoir. Alternatively, the handler could have been providing inadvertent cues to the dog during the acute care evaluations, as she would have been aware of the isolation status of the majority of patients [5].

Limitations to using a dog to detect *C. difficile* in patients include the possibility of inadvertent detection of *C. difficile* carriage in HCWs, and the potential for the dog to be distracted or for patients to be afraid of a canine encounter. Further, clinical evaluation may be compromised due to handler bias as patients with suspected or known CDI are frequently in isolation rooms [5]. We therefore elected to have the canine team focus on the detection of environmental sources that could act as reservoirs for transmission that are often overlooked and seldom sampled for *C. difficile*.

The canine team was introduced into the hospital setting with a focus on detecting environmental reservoirs of *C. difficile* and implementing 'real-time' deployment of housekeeping to disinfect the contaminated surfaces or equipment. It is important to emphasize that the canine team was an adjunctive measure to pre-established intensive cleaning and disinfection on high burden wards and/or cluster events.

The team is also used to evaluate isolation rooms after post-discharge cleaning to ensure adequate disinfection. Positive alerts are analysed for trends — this enables environmental services to more strategically and rapidly deploy adjunctive measures such as ultraviolet C light. Areas without patients are more thoroughly evaluated as the dog is permitted to search without restriction (i.e. off-leash). Hides containing gauzes with no odour or the odour from a *C. difficile*-positive culture or stool are planted prior to each sweep of a clinical area both as a quality control measure for the dog and handler and as a way to periodically reward the dog for detecting a *C. difficile* odour — particularly attractive features as no second dog to confirm a positive test currently exists in British Columbia. It is possible that the highly visible presence of dog and handler will also improve compliance with other ancillary measures such as hand hygiene, attention to disinfecting personal items, and use of personal protective equipment, and it will be difficult to prove that the use of a scent-detection dog by itself decreases the incidence of CDI. The canine team has been well accepted by patients and staff and a positive consequence of this has been a renewed interest in the mechanisms of disease transmission by healthcare workers. The ability to provide immediate feedback upon a positive alert has resulted in opportunities for in-the-moment education and the canine handler is currently taking a university-accredited online infection control course. Analysis of the types and locations of the alerts will be used to improve our current policies and protocols for environmental services and infection prevention.

We are currently training a second canine unit which will enable us to assess inter-rater reliability between the teams. Canine teams will be certified annually and subjected to impromptu assessments by the medical microbiology team throughout the year. The goal is to develop a quality programme consisting of a set of standardized operating procedures for the selection, training, evaluation, and systematic implementation of *C. difficile* detection canine teams, a consistent environmental services process when positive alerts occur, and regular inspection and recertification of the teams. In addition, the dogs' health will be monitored four times annually [22].

In conclusion, a canine team can be trained to detect environmental sources of *C. difficile* and can be successfully integrated as part of an ongoing quality infection control environmental assessment programme. The authors hope that others are persuaded to pursue this option. This could lead to future sharing of methodologies, evaluation techniques, results, implementation protocols, and assessments of sustainability that could lead to a standardized and objective canine scent detection model from training to implementation.

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Conflict of interest statement
None declared.

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