

VU Research Portal

A whiff of C-diff:

Bomers, M.K.

2017

document version

Publisher's PDF, also known as Version of record

[Link to publication in VU Research Portal](#)

citation for published version (APA)

Bomers, M. K. (2017). *A whiff of C-diff: using scent detection to diagnose Clostridium difficile infection*. [PhD-Thesis - Research and graduation internal, Vrije Universiteit Amsterdam].

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:

vuresearchportal.ub@vu.nl

CHAPTER 3

**Using a dog's superior olfactory sensitivity
to identify *Clostridium difficile* in stools and
patients: proof of principle study.**

**MK Bomers, MA van Agtmael, H Luik, MC van Veen,
CMJE Vandenbroucke-Grauls CM, YM Smulders.
BMJ. 2012;345:e7396.**

ABSTRACT

Objectives:

Timely recognition of *Clostridium difficile* infections (CDI) is important to control transmission. Diarrhoea due to *C. difficile* has a specific smell, and dogs have superior olfactory sensitivity compared to humans, which prompted us to investigate whether a detection dog could be used to detect CDI.

Design:

'Proof of principle' study in which a two-year-old beagle was trained to identify the smell of *C. difficile*. Diagnostic accuracy was tested on stool samples and hospitalised patients in a case-control design. The dog was guided along cases and controls by his trainer, who was blinded to participant's CDI status. The dog was trained to sit down when he smelled *C. difficile*.

Setting:

Two large Dutch teaching hospitals.

Test samples and participants:

First, the dog examined 50 *C. difficile*-positive and 50 *C. difficile*-negative stool samples. Subsequently, consecutive CDI cases were studied in detection rounds of 10 patients (1 case and 9 controls per detection round), totalling 300 participants with 30 CDI cases.

Main outcome measures:

Sensitivity and specificity for detection of *C. difficile* in stool samples and infected patients.

Results:

In stool samples, the dog's sensitivity and specificity for identifying *C. difficile* were both 100% (95% confidence interval (CI): 91 - 100%). In hospitalised patients, the dog correctly identified 25/30 cases (sensitivity 83%; 95% CI: 65-94 %) and 265/270 controls (specificity 98%; 95% CI: 95 - 99%).

Conclusion:

In this proof of principle study a trained detection dog was able to identify *C. difficile* with high estimated sensitivity and specificity, both in stool samples and in hospitalised CDI patients. This finding could have great potential for CDI screening in healthcare facilities and thus contribute to CDI outbreak control and prevention.

INTRODUCTION

Clostridium difficile infection (CDI) is a common health care-associated infection that mainly occurs after patients receive antimicrobial therapy. It causes toxin-mediated intestinal disease with symptoms ranging from mild diarrhoea to severe pseudomembranous colitis and toxic megacolon. *C. difficile* can be transmitted via personal contact or environmentally.⁽¹⁾ Over the past decades more frequent and severe disease has emerged and large hospital outbreaks have occurred requiring ward closures and extensive infection control measures.⁽²⁻⁴⁾ *C. difficile* infection rates seem higher in North America than in Europe.^(3;5) The Netherlands has a nosocomial CDI incidence rate that is comparable to other European countries (mean incidence rate 17.5 - 23/10,000 admissions^(6;7); the mean incidence rate in the United Kingdom is in the order of 50/ 10,000 admissions.⁽⁶⁾

Early and rapid identification of CDI cases is important to prevent transmission by initiating adequate isolation measures and treatment.⁽⁸⁾ Several (combinations of) tests are used for the diagnosis of CDI. The traditional gold standard is a cytotoxin assay, which demonstrates cytotoxicity of faecal eluate on cell lines if *C. difficile* toxins are present. This however requires cell cultures and takes at least 1-2 days.^(9;10) Toxigenic culture entails culturing the bacteria on selective media and subsequently testing isolates for the presence of toxin(s) or toxin genes: this is regarded as the most sensitive method, but is also time consuming.^(4;10) Easy and rapid enzyme immunoassays (EIA) to detect *C. difficile* toxins or antigens are frequently used, despite their limited sensitivity and/ or specificity.^(10;11) More recently, several nucleic acid amplification tests have been developed with high diagnostic accuracy and short turn- around time but these are more expensive and require specialised equipment and expertise.^(4;10-12)

In daily practice, a number of factors delay the identification of CDI patients. These include doctor's delay, inefficient sampling and the diagnostic process in the laboratory.^(13;14) As a result, the mean time from onset of symptoms of a first CDI episode to start of treatment in studies ranges from 2.8 to 7.7 days.^(13;14) Screening all patients at regular intervals could theoretically prevent diagnostic delay, but is costly and impractical.

In the 1970's *C. difficile* was identified as the cause of pseudomembranous colitis.^(15;16) Since then, *C. difficile* -associated diarrhoea has often been described as having a characteristic smell.⁽¹⁷⁾ Sensitivity and specificity of the odiferous detection of *C. difficile* by nursing staff are 55- 82% and 77-83%, respectively.^(18;19) Dogs, however, have a far superior sense of smell which is thought to exceed that of humans by a factor 100.⁽²⁰⁾ Hence, we reasoned that a detection dog could possibly be trained to recognise *C. difficile* in stool samples, or even in CDI patients. If so, this might prove a valuable screening tool for CDI in healthcare facilities.

METHODS

The canine training process:

The detection dog used in this study was a two-year-old male beagle called Cliff (figure 1). A professional detection dog instructor (HL) trained the dog with the objective to enable him to identify *C. difficile* in stool samples and, if this proved possible, also in patients with *C. difficile* infection. A reward-based training method was used in which the correct behaviour is reinforced, for instance with a snack. He was taught to indicate the presence of the specific scent by sitting or lying down. The dog had not been trained for detection purposes before.

We started training the dog with the specific odour of toxigenic *C. difficile* strains on culture plates. The scent emanating from the culture plates (often described as resembling horse manure⁽⁹⁾) was absorbed by small wooden sticks that were held above the sample. In the beginning the wooden sticks were left to absorb the scent overnight, but in time we decreased the strength of scent by shortening the time exposed to the culture plates to \geq five minutes. This way the smell fades, thus making the exercise of finding the stick more and more challenging for the dog. Early recognition of the scent was achieved by using simple search and find games, which were gradually replaced by exercises of increasing difficulty. For instance, the absorbed scent was presented to the dog on different materials (on wooden sticks as described, but also absorbed on paper, fabric, metal etc.) and in different environments (kitchen, forest, petrol station etc.) to vary background odours.

Next step in training was discriminating *C. difficile* positive stool samples from *C. difficile* negative stool samples. Again, rather than having direct physical contact with the stool sample, the scent was presented in various forms (absorbed on a wooden stick or on fabric, sample contained in a plastic vial etc.) as described previously.

After a two months training period, the diagnostic accuracy of the dog was formally tested on stool samples. Finally, we explored the dog's CDI detection abilities in hospitalised patients, as described below.

SAMPLES AND PARTICIPANTS:

C. difficile on culture plates

Clinical isolates of toxigenic *C. difficile* strains, cultured on standard media under anaerobic conditions, were used for training.

Stool samples

We used stool samples that were sent to the microbiology laboratory to test for *C. difficile* and other infectious causes of diarrhoea. Samples were considered *C. difficile* positive if a toxin enzyme immunoassay (EIA) (VIDAS® *Clostridium difficile* A& B) was positive and culture revealed a toxigenic strain of *C. difficile*. Negative stool samples had negative results in both tests. Samples with inconsistent results (negative toxin EIA but positive culture, or samples with an undetermined toxin EIA value etc.) were excluded.



Figure 1: detection dog Cliff on hospital ward

Selection of participants

We further explored the dog's CDI detection abilities on the wards of two hospitals: VU University medical centre (VUmc, Amsterdam: a tertiary clinical care centre) and St. Lucas Andreas Hospital (SLAZ, Amsterdam: a large community hospital).

Between September 2010 and May 2011, consecutive patients with a positive toxin EIA in their stool sample were screened for inclusion. We aimed to include 30 CDI cases in total. Both hospitals use an EIA plus a toxigenic culture to diagnose CDI; however, SLAZ uses an EIA by a different manufacturer (ImmunoCard® Toxins A&B, Meridian Bioscience®).

Eligible cases had symptoms of diarrhoea, and both a positive toxin EIA and culture with a toxigenic *C. difficile* strain (in a sample taken < seven days before the detection round). Diarrhoea was defined as three or more loose or watery stool passages per day. Patients from paediatric or adult intensive care units or haematology wards were not included. Patients suffering a first relapse after completing treatment for a previous CDI episode were eligible. Patients suffering subsequent relapses were considered ineligible. Patients whose positive test result became available in the weekend were not included, because it was not possible to have the dog and the trainer available every weekend.

For each CDI case we approached nine controls, who were on the same ward, close to the index patient. Control patients did not have diarrhoea, or had diarrhoea but with a negative *C. difficile* toxin EIA and culture (in a sample taken < seven days before). All participants (n= 300; 30 cases plus 270 controls) gave informed consent.

Canine testing experiment:

Diagnostic accuracy for detecting C. difficile in stool samples

After a two-month period of practice, the diagnostic accuracy of the dog was formally tested on 50 *C. difficile*-positive and 50 *C. difficile*-negative stool samples. The samples had not been used in the training phase to avoid the possibility of simply recognising the odour of the sample, instead of identifying the presence of *C. difficile*. The scent of each sample was again absorbed onto different materials, which were then repeatedly (10 times) presented to the dog in different environments and concentrations. A result was considered positive (or negative) when it consistently provoked the same positive (or negative) response. If a sample provoked a mixed response ($\leq 8/10$ consistency: e.g. 8 positive responses and 2 negative responses), it was classified as inconclusive.

Diagnostic accuracy for detecting C. difficile infection in patients

Next step was to evaluate the dog's CDI detection abilities in hospitalised patients on the wards of the two hospitals. We prospectively included 30 consecutive CDI cases and 270 controls as described under 'selection of participants'. For each case and corresponding nine controls on the ward a detection round was organised as soon as possible, preferably before starting treatment or within 36 hours. During this round the detection dog, his trainer and a member of the research team would simply walk past the beds of all ten participants. The trainer classified the dog's response as either positive (dog sitting down), inconclusive (dog showing excitement, taking extra time etc., without actually sitting down) or negative (showing no particular interest). In case of doubt, the round was repeated once. The trainer was not aware of which patient was CDI positive.

Statistical analyses

In the primary analyses, 'inconclusive' responses were interpreted as negative. Secondary analysis was performed interpreting these responses as positive. 95% confidence intervals were calculated using an approximation (according to the efficient-score method, corrected for continuity).⁽²¹⁾

Safety precautions

We consulted the hospital's infection control committee to discuss the potential hazards of allowing a dog to enter the hospital and come near patients. In accordance with recent guidelines, special attention was given to hand hygiene, making sure staff and patients washed their hands both before and after any animal contact.⁽²²⁾ During detection rounds, the dog had no physical contact with patients, and contact with their environment (bed, chair etc.) was avoided as much as possible. He did not visit food preparation areas and neonatal-, haematology or intensive care wards.

The dog receives a health evaluation by a licensed veterinarian four times a year. He is not fed raw meat. He is trained solely for the purpose of recognising *C. difficile*. When at work, he neither barks nor shows aggressive behaviour, he is easily recognised by his outfit (figure 1) and is continuously on a leash. The research protocol was approved by the institutional review boards in both hospitals.

RESULTS

Diagnostic accuracy for detecting C. difficile in stool samples

The detection dog examined a total of 50 *C. difficile* positive and 50 *C. difficile* negative stool samples. He gave all 50 positive samples a positive response and 47 of 50 negative samples a negative response. An inconclusive response was recorded in the remaining three negative samples. In the primary analysis (interpreting inconclusive as a negative response), sensitivity and specificity are both 100 % (95% CI: 91- 100%). If an inconclusive response is considered a positive result (secondary analysis), the detection dog's sensitivity and specificity are 100 % (95% confidence interval (CI): 91- 100%) and 94% (95% CI: 83- 98%), respectively.

Patient characteristics

A video to illustrate how the rounds were conducted can be found on the BMJ website. One detection round took place on the paediatric ward, but caused considerable turmoil amongst the children and distraction for the dog. For this reason, the round was not included, and paediatric wards were excluded from the project.

A total of 30 CDI cases and 270 controls were included in the study. Table 1 shows patient characteristics. All CDI cases had diarrhoea on the day of the detection round versus 16 (5.9%) of the controls. Thirty-five controls (13%) had a

Table 1: Patient characteristics

	Controls n= 270	CDI cases n= 30	Total n= 300
Male (n, %)	152 (56.3%)	15 (50.0%)	167 (56.7%)
Age in years (median, IQR)	65 (54-78)	68 (51-75)	65 (54-78)
Ward:			
• Medical (n, %)	165 (61.1%)	19 (63.3%)	184 (61.3%)
• Surgical (n, %)	105 (38.9%)	11 (36.7%)	116 (38.7%)
Diarrhoea on day of detection round (n, %)* ¹	16 (5.9%)	30 (100%)	46 (15.3%)
CDI characteristics (n, %):			
• No test performed and no diarrhoea symptoms * ²)	235 (87.0%)	0 (0%)	
	35 (13.0%)	0 (0%)	
• Confirmed no CDI by neg. testing * ^{1, 2})	0 (0%)	30 (100%)	
	0 (0%)	3 (10.0%)	
• Confirmed CDI by diarrhoea plus pos. testing * ²)			
• Treatment for >36 hrs on day of detection round			

CDI= *Clostridium difficile* infection. IQR= interquartile range. Neg. = negative, pos. = positive.

*¹) All controls with diarrhoea on the day of the detection round underwent diagnostic testing for CDI and are included in the group 'Confirmed no CDI by neg. testing'.

*²) Testing refers to whether a *Clostridium difficile* toxin enzyme immunoassay (EIA) and culture was performed on a stool sample in the 7 days before the detection round took place.

stool sample tested for *C. difficile* on clinical grounds (i.e. diarrhoea) in the week leading up to the detection round; these were all negative, although in 2 controls a non-toxicogenic *C. difficile* strain was cultured. All but three cases formally met the CDI definition. One had CDI symptoms and a positive toxin EIA, but a culture was accidentally not performed. One had symptoms and a positive toxin EIA but an initial negative culture, which turned out positive when repeated. The third patient had a relapse with recurring symptoms and a positive toxigenic culture, but negative toxin EIA.

Diagnostic accuracy for detecting C. difficile in patients

The diagnostic accuracy of the detection dog is illustrated in figure 2. An inconclusive response was recorded in seven participants: three cases and four controls. In the primary analysis (interpreting inconclusive as a negative response), he correctly identified 25/30 cases (sensitivity 83%; 95% CI: 65- 94 %) and 265/270 controls (specificity 98%; 95% CI: 95 - 99%). If an inconclusive response is considered a positive result (secondary analysis), the dog correctly identified 28/ 30 cases (sensitivity 93%; 95 % CI: 76- 99%) and 261/ 270 controls (specificity 97%; 95% CI: 94- 98%).

Table 2 provides information on the occasions that the dog and the laboratory gave discrepant results (inconclusive dog responses, 'false positives' and 'false negatives'). In some instances the dog was clearly distracted by unrelated stimuli (for instance by being offered a cookie; see control #9). Other cases were less clear- cut and it cannot be ruled out that the dog responded to diarrhoea that was not caused by CDI (e.g. control #7) or asymptomatic carriage of a (non-) toxigenic strain (e.g. control #8). Out of all 16 participants with (non- CDI) diarrhoea, the dog gave a negative response in 13 controls and was inconclusive in three controls.

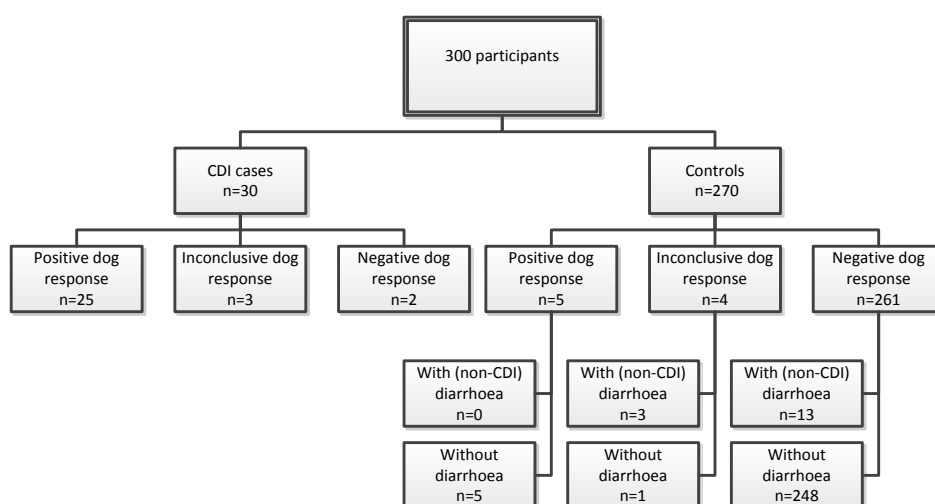


Figure 2: The use of a detection dog for identifying *Clostridium difficile* infection. CDI= *Clostridium difficile* infection.

DISCUSSION

This study demonstrates the feasibility of employing a detection dog for identifying *C. difficile* in stool samples and in patients. The dog's accuracy in stool samples suggests that immediate identification of *C. difficile* is possible. Moreover, our data suggest that the same may be true for rapid diagnosis of CDI on clinical wards. The dog did not need a stool sample, nor did he need to make physical contact with the patient. Apparently, he can smell *C. difficile* in the air that surrounds patients. In addition, he is quick and efficient: in less than ten minutes a complete hospital ward can be screened for the presence of patients with *C. difficile* infection.

Table 2: Participants with a discrepancy between laboratory results and dog response.

		Dog re- sponse	Laboratory results (EIA + toxigenic culture)	Diarrhoea on day of detection round	Comment
Participants with an inconclusive dog response					
1	Case	Inconcl.	Positive	Yes	Dog appeared distracted by a plastic cup on the floor
2	Control	Inconcl.	Negative	Yes	During this round there was a penetrating chlorine smell in several rooms, related to disinfection activities, which could have influenced the dog's response. Tests were performed on participants with an inconclusive response in this round.
3	Case	Inconcl.	Positive	Yes	'Chlorine round', see comment control 2
4	Control	Inconcl.	Not perf.	No	Case 5 had just changed beds; the dog seemed to have difficulty choosing between two neighbouring patients (control 4 and case 5) and the 3 rd (empty) bed across the room; he sat down exactly in the middle.
5	Case	Inconcl.	Positive	Yes	See comment control 4
6	Control	Inconcl.	Negative*	Yes	On the ward this round took place was a CDI patient that refused participation in the study. The dog was not allowed to enter this patient's room; however, he behaved very excited and tried to enter nonetheless. When forced to move away, the dog immediately sat down next to control 6, which was the first participant he encountered. *Because of symptoms, tests had been performed. The EIA was negative; however stool culture did show a non- toxigenic <i>C. difficile</i> .
7	Control	Inconcl.	Negative	Yes	No apparent explanation
'False positives': participants with negative laboratory results but a positive dog response					
8	Control	Positive	Negative*	No	'Chlorine round', see comment control 2. *Tests showed a negative EIA, however stool culture did show a non-toxigenic <i>C. difficile</i> .
9	Control	Positive	Not perf.	No	Dog was being offered a cookie by the participant.
10	Control	Positive	Not perf.	No	Dog was being beckoned by the participant
11	Control	Positive	Negative	No	Dog appeared distracted by a puddle of urine on the floor from a broken catheter bag.
12	Control	Positive	Negative**	No	This participant had been treated for CDI, diagnosed 11 days previously. Since his symptoms were resolved on the day of the detection round, he was included as a control. ** However, a week after the round his symptoms returned and retesting proved a CDI relapse.
'False negatives': participants with positive laboratory results but a negative dog response					
13	Case	Negative	Positive	Yes	No clear explanation
14	Case	Negative	Positive***	Yes	***The EIA was positive; accidentally culture was not performed

EIA= enzyme immunoassay, Inconcl.= inconclusive, perf.= performed, CDI= *C. difficile* infection

This is the first report of animal-assisted *C. difficile* detection. There are several studies and anecdotal reports on olfactory detection in the medical field, mostly by dogs, for instance for detection of malignancies like bladder, lung, breast, melanoma, prostate, ovarian and colorectal cancer.⁽²³⁻²⁹⁾ In nearly all studies, however, animals were exposed to biological samples obtained from patients, not to the patients themselves.

There are several limitations to our study. The small number of CDI patients limits the precision to which we can establish the detection dog's sensitivity and specificity. The design consistently included one CDI case per round of ten patients. This could have influenced the dog and the trainer's results by anticipating a single positive result in each detection round.⁽³⁰⁾ Furthermore, two-thirds of CDI cases were already moved to a single room when the dog arrived (for transmission control), and occupancy of a single room might have prejudiced the dog or trainer in favour of CDI diagnosis.⁽³⁰⁾

In this study culture has not routinely been performed on controls to screen for asymptomatic carriage of toxigenic and non-toxigenic *C. difficile*. This is a limitation since consequently we do not know the percentage of asymptomatic *C. difficile* carriers in our population and how the dog responds to them. Asymptomatic carriage of both toxigenic and non-toxigenic strains occurs in up to 18-30 % of hospital patients.⁽³¹⁻³⁴⁾ This argues against a positive dog response. Since the clinical relevance of detecting *C. difficile* infection rather than carriage is far greater (both for the individual patient and for prevention of transmission^(1;31)), this has been the focus of our study.

Another concern is that the results are not easily generalizable, since we used only one dog and one trainer. It could be that for another dog and/or another trainer, the findings may not be so optimistic. Although unlikely, we cannot rule out the possibility that our first and only experience was with a very exceptional dog-trainer combination. Should the future bring more *C. difficile* detection dogs, trained animals would need an individual performance assessment, and regular practice to maintain their skills. A second limitation of using an animal as a diagnostic tool is that, like in humans, their behaviour is not fully predictable. The dog's reaction to other stimuli (children's play, being beckoned, being offered a cookie etc.) illustrates that, despite a high level of training, dogs are still subject to distraction.

Another point to be made is that we trained the dog in the hospital setting. Outside the research protocol we visited a few CDI patients on long-term care facility wards. These CDI cases were found in a shared living room, not in their beds. This proved more difficult for the dog. We hypothesise that in the hospital setting the bed is a strong source of smell since the patients are often bedridden, and the mattress may have collected the scent. Outside of the hospital patients are often less confined to their room and bed. This could make the odour more diffuse and more difficult to pinpoint. Also, the dog may have been conditioned to respond to CDI in presence of a patient in a hospital room (usually in bed). This may have made him less suitable for other settings like nursing homes, at least without additional training.

The use of dogs in hospitals might pose a risk to the dogs, hospital personnel, and patients. Dogs can be carriers of *C. difficile* strains and other pathogens. Similarly to contact with hospital personnel, there is a possibility of spreading infectious micro-organisms via the dog. Use of strict preventative measures like avoiding physical contact with patients and their surroundings would minimise these risks.

Unanswered questions remain: what does the dog actually smell: is it a certain quantity of bacteria, toxins or other bacterial product? How does the dog respond to toxin EIA-negative stool samples that are positive in toxigenic culture, cytotoxicity assay or toxin PCR? And how does he respond to patients very early in the course of CDI, or those with asymptomatic carriage of toxigenic and non-toxigenic strains? Does a positive dog- response in an asymptomatic patient predict disease? Can a persistent positive dog response after clearance of CDI symptoms predict relapse, as suggested by a response in the single participant (control #12)? Will the dog perform equally well in a high-incidence setting, i.e. during an outbreak, when several patients in one room could be affected? We intend to address these issues in future studies.

How could a dog that detects CDI be used in daily practice? With regular surveillance rounds (for instance to screen all wards in a hospital with a high CDI incidence several times a week like a 'pet scan') CDI might be detected earlier. It could overcome common diagnostic delays (lack of clinical suspicion, delays in sampling stool and laboratory procedures) and lead to prompt hygienic measures and treatment. However, further studies will clearly have to examine whether surveillance can actually limit transmission and reduce CDI incidence. For instance, surveillance is principally different from the type of case-directed diagnosis in this study design, since the dog cannot immediately receive a reward after a positive identification, potentially extinguishing the trained alert.

In conclusion, in this proof of principle study a trained detection dog was able to identify *C. difficile* with high estimated sensitivity and specificity, both in stool samples and in CDI patients in a hospital setting. This finding could have great potential for CDI screening in healthcare facilities and thus contribute to CDI outbreak control and prevention.

REFERENCE LIST

- (1) Samore MH, Venkataraman L, DeGirolami PC, Arbeit RD, Karchmer AW. Clinical and molecular epidemiology of sporadic and clustered cases of nosocomial *Clostridium difficile* diarrhoea. *Am J Med* 1996; 100(1):32-40.
- (2) Loo VG, Poirier L, Miller MA, et al. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhoea with high morbidity and mortality. *N Engl J Med* 2005; 353(23):2442-2449.
- (3) Freeman J, Bauer MP, Baines SD, et al. The changing epidemiology of *Clostridium difficile* infections. *Clin Microbiol Rev* 2010; 23(3):529-549.
- (4) Rupnik M, Wilcox MH, Gerding DN. *Clostridium difficile* infection: new developments in epidemiology and pathogenesis. *Nat Rev Microbiol* 2009; 7(7):526-536.
- (5) Kuijper EJ, Coignard B, Tull P. Emergence of *Clostridium difficile*-associated disease in North America and Europe. *Clin Microbiol Infect* 2006; 12 Suppl 6:2-18.
- (6) Bauer MP, Notermans DW, van Benthem BH, et al. *Clostridium difficile* infection in Europe: a hospital-based survey. *Lancet* 2011; 377(9759):63-73.
- (7) Hensgens MP, Goorhuis A, van Kinschot CM, Crobach MJ, Harmanus C, Kuijper EJ. *Clostridium difficile* infection in an endemic setting in the Netherlands. *Eur J Clin Microbiol Infect Dis* 2011; 30(4):587-593.
- (8) Muto CA, Blank MK, Marsh JW, et al. Control of an outbreak of infection with the hypervirulent *Clostridium difficile* BI strain in a university hospital using a comprehensive "bundle" approach. *Clin Infect Dis* 2007; 45(10):1266-1273.
- (9) Delmee M. Laboratory diagnosis of *Clostridium difficile* disease. *Clin Microbiol Infect* 2001; 7(8):411-416.
- (10) Kufelnicka AM, Kirn TJ. Effective utilization of evolving methods for the laboratory diagnosis of *Clostridium difficile* infection. *Clin Infect Dis* 2011; 52(12):1451-1457.
- (11) Crobach MJ, Dekkers OM, Wilcox MH, Kuijper EJ. European Society of Clinical Microbiology and Infectious Diseases (ESCMID): data review and recommendations for diagnosing *Clostridium difficile*-infection (CDI). *Clin Microbiol Infect* 2009; 15(12):1053-1066.
- (12) Karre T, Sloan L, Patel R, Mandrekar J, Rosenblatt J. Comparison of Two Commercial Molecular Assays to a Laboratory Developed Molecular Assay for Diagnosis of *Clostridium difficile* Infection. *J Clin Microbiol* 2010.
- (13) Frenz MB, McIntyre AS. Reducing delays in the diagnosis and treatment of *Clostridium difficile* diarrhoea. *QJM* 2003; 96(8):579-582.
- (14) Scheurer D. Diagnostic and treatment delays in recurrent *Clostridium difficile*-associated disease. *J Hosp Med* 2008; 3(2):156-159.
- (15) Bartlett JG, Onderdonk AB, Cisneros RL, Kasper DL. Clindamycin-associated colitis due to a toxin-producing species of *Clostridium* in hamsters. *J Infect Dis* 1977; 136(5):701-705.
- (16) George RH, Symonds JM, Dimock F, et al. Identification of *Clostridium difficile* as a cause of pseudomembranous colitis. *Br Med J* 1978; 1(6114):695.
- (17) Bartlett JG, Gerding DN. Clinical recognition and diagnosis of *Clostridium difficile* infection. *Clin Infect Dis* 2008; 46 Suppl 1:S12-S18.
- (18) Burdette SD, Bernstein JM. Does the nose know? The odiferous diagnosis of *Clostridium difficile*-associated diarrhoea. *Clin Infect Dis* 2007; 44(8):1142.
- (19) Johansen A, Vasishta S, Edison P, Hosein I. *Clostridium difficile* associated diarrhoea: how good are nurses at identifying the disease? *Age Ageing* 2002; 31(6):487-488.
- (20) Issel-Tarver L, Rine J. The evolution of mammalian olfactory receptor genes. *Genetics* 1997; 145(1):185-195.
- (21) Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. *Stat Med* 1998; 17(8):857-872.
- (22) Lefebvre SL, Golab GC, Christensen E, et al. Guidelines for animal-assisted interventions in health care facilities. *Am J Infect Control* 2008; 36(2):78-85.
- (23) Willis CM, Church SM, Guest CM, et al. Olfactory detection of human bladder cancer by dogs: proof of principle study. *BMJ* 2004; 329(7468):712.
- (24) McCulloch M, Jezierski T, Broffman M, Hubbard A, Turner K, Janecki T. Diagnostic accuracy

- of canine scent detection in early- and late-stage lung and breast cancers. *Integr Cancer Ther* 2006; 5(1):30-39.
- (25) Ehmann R, Boedeker E, Friedrich U, et al. Canine scent detection in the diagnosis of lung cancer: Revisiting a puzzling phenomenon. *Eur Respir J* 2011.
 - (26) Sonoda H, Kohnoe S, Yamazato T, et al. Colorectal cancer screening with odour material by canine scent detection. *Gut* 2011; 60(6):814-819.
 - (27) Horvath G, Andersson H, Paulsson G. Characteristic odour in the blood reveals ovarian carcinoma. *BMC Cancer* 2010; 10:643.
 - (28) Cornu JN, Cancel-Tassin G, Ondet V, Girardet C, Cussenot O. Olfactory detection of prostate cancer by dogs sniffing urine: a step forward in early diagnosis. *Eur Urol* 2011; 59(2):197-201.
 - (29) Williams H, Pembroke A. Sniffer dogs in the melanoma clinic? *Lancet* 1989; 1(8640):734.
 - (30) Lit L, Schweitzer JB, Oberbauer AM. Handler beliefs affect scent detection dog outcomes. *Anim Cogn* 2011; 14(3):387-394.
 - (31) McFarland LV, Mulligan ME, Kwok RY, Stamm WE. Nosocomial acquisition of *Clostridium difficile* infection. *N Engl J Med* 1989; 320(4):204-210.
 - (32) Samore MH, DeGirolami PC, Tlucko A, Lichtenberg DA, Melvin ZA, Karchmer AW. *Clostridium difficile* colonization and diarrhoea at a tertiary care hospital. *Clin Infect Dis* 1994; 18(2):181-187.
 - (33) Clabots CR, Johnson S, Olson MM, Peterson LR, Gerding DN. Acquisition of *Clostridium difficile* by hospitalized patients: evidence for colonized new admissions as a source of infection. *J Infect Dis* 1992; 166(3):561-567.
 - (34) Shim JK, Johnson S, Samore MH, Bliss DZ, Gerding DN. Primary symptomless colonisation by *Clostridium difficile* and decreased risk of subsequent diarrhoea. *Lancet* 1998; 351(9103):633-636.

