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Early View

Research letter

# Training dogs to differentiate *Pseudomonas aeruginosa* from other cystic fibrosis bacterial pathogens: not to be sniffed at?

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**Training dogs to differentiate *Pseudomonas aeruginosa* from other cystic fibrosis bacterial pathogens: not to be sniffed at?**

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*To the editor*

The major cause of lung damage in cystic fibrosis (CF) is infection with bacterial pathogens, the most prevalent of which is *Pseudomonas aeruginosa* (Pa), chronically infecting ~60% patients by adolescence/ adulthood (https://www.cysticfibrosis.org.uk/news/registry-report-2017). Pa may be successfully eradicated, but frequently recurs and establishes biofilms resistant to antibiotics/ host defences[1]. Chronic Pa is closely linked with pulmonary exacerbation frequency, faster lung function decline and earlier mortality[2]. The huge antibiotic burden imposed upon patients and the resulting bacterial resistance, allergies and toxicities compound the detrimental impact of the infection itself. Chronic Pa should be avoided if at all possible; early detection and rapid treatment may be crucial in achieving this.

Currently, bacterial infections are detected by culture of airway samples at clinic attendance (2-3 monthly). In the absence of new symptoms, long periods could therefore ensue between cultures. Furthermore, whilst sputum is most commonly obtained from adults with established bronchiectasis, children and those with milder lung disease rely on cough/ throat swabs. These lack specificity and in some studies, sensitivity, so infections can be missed[3]. More reliable techniques such as sputum induction are time-consuming and expensive, whilst the gold standard, bronchoalveolar lavage, is invasive so neither technique can be undertaken regularly. Serology has been used with some success, but is not generally performed frequently as accuracy remains somewhat controversial and it requires invasive blood tests[4]. With the improving health of current CF cohorts, obtaining reliable samples from non-sputum producers will become an increasing challenge.

Several teams, including our own, have explored the utility of breath sampling for Pa detection. Carroll *et al* tested the head-space of culture plates in sealed bags with SIFT-MS demonstrating higher levels of hydrogen cyanide (HCN) with Pa cultures[5]. The group later reported that the presence of HCN in CF breath lacked the sensitivity to detect early Pa infection[6]. We used a similar technique to test a combination of VOCs in breath, also finding insufficient ability to distinguish infected from non-infected on an individual basis[7]. Within the Strategic Research Centre for Pseudomonas in CF, we continue to explore several of these technologies; however, in parallel, we have explored training the canine nose as a detection system.

Sniffer dogs are familiar in contexts such as airport security, and reports of their use in medical fields are established (diabetes)[8]and emerging : recognising seizure-related auras [9] and detection of cancers [10]. Given the natural odour of Pa, we hypothesised that dogs can be trained to detect this organism. One small study, available only in abstract form[11], would suggest this is a fruitful endeavour. As a first step we have assessed the ability of dogs to identify Pa from other CF bacteria in culture supernatants.

CF bacterial strains were obtained from the microbiology laboratory of the Royal Brompton Hospital and stored on beads at -80$^{O}$C prior to culture on agar plates. Each isolate was from an individual patient, but as isolates have not been typed or sequenced, some strains may have been shared. Following overnight culture at 37$^{O}$C in broth (~$10^9$ CFU/ ml), they were centrifuged at 3,900g (15 mins) and the supernatant removed and filtered through 0.2µm disc filter.

Dogs were trained and tested on customised sample presentation stands within which supernatants were presented just below head height under a grill (Fig 1). During training, correct identification of Pa samples was rewarded by an auditory click and food reward. Once trained, dogs were presented with Pa-positive samples, other bacterial controls or sterile broth in a random, computer-generated sequence. A positive indication (dog stopped or sat down) was rewarded if correct. In the double-blind testing, the indication was entered into an electronic spreadsheet, which immediately revealed the correct identification of the sample, allowing the dog to be rewarded if appropriate. Four blinded studies were undertaken: (i) Pa vs other bacteria familiar to the dogs; (ii) Pa vs previously unencountered bacteria; (iii) dilution testing (1:1,000 and 1:10,000); (iv) mixed, multi-organism cultures. Exact 95% confidence intervals were calculated for the sensitivity and specificity of each dog in each study, based on their first encounter with each sample. Analyses were undertaken using SAS V9.4.

(i) Pa was tested alongside *Staphylococcus aureus* (Sa), *Moraxella catarrhalis* (Mc) and sterile broth (598 samples/ dog; 3 dogs). Mean sensitivity (correct signalling at a Pa sample) was 94.2% with a specificity of 98.5% (Fig 2). (ii) We next tested the dogs' ability to identify Pa when the controls were bacteria to which they had not been previously exposed: *Achromobacter xylosoxidans*, *Burkholderia cepacia* complex, *Stenotrophomonas maltophilia* and *Serratia marcessens* (166 samples/ dog). Two of the 3 dogs maintained sensitivity >90%, but in the third it was lower at 62.5%. The dogs were most likely to give a false positive indication at Bcc (specificity 76.9%). (iii) Two dogs were tested on diluted broths (114 samples/ dog). At dilutions of 1:1,000 no substantial impact on sensitivity (93.8%) or specificity (94.9%) was seen, but at 1:10,000, sensitivity was lower (56.3%) although specificity was maintained (89.1%). (iv) Finally, when all four dogs were tested for their ability to detect Pa in a mixed culture with either 1 or 2 other organisms (*Haemphilus influenzae*, Sa, Mc, 104 samples/ dog), they still correctly identified Pa with a sensitivity of 86.5% and a specificity of 84.1%.

In this series of pilot studies, we have confirmed that, following training, dogs can detect the odour of *Pseudomonas aeruginosa* in broth supernatants with high levels of sensitivity, distinguishing them from other bacteria commonly encountered in the CF lung. This ability was generally maintained when the dogs encountered other organisms for the first time, or when Pa was present in mixed cultures. Sensitivity declined at dilutions of 1:10,000, equating to ~$10^5$ CFU/ ml.

The mode of growth of Pa in broth is significantly different from that in the CF airway and the volatile signals generated will also likely differ. Whilst it is unlikely training on broth will be sufficient

for detection on clinical samples, these studies provided useful proof-of-principle for the concept. Had we been unable to train dogs on this substrate further development into the clinic would likely have been futile. We note the reduced sensitivity of Pa detection once broth supernatant was diluted. Although the bacterial burden in the chronically-infected CF airway can be as high as $10^9$ CFU/ ml, the numbers of organisms in early infection will be much lower. This may pose a limitation to this technique which we will test at the next stage.

We are often questioned about the clinical utility of this method should it prove successful. Whilst the presence of dogs in our CF clinics might be viewed positively by some of our patients, particularly the children, this is clearly not the way forward. Rather, we aim to develop a non-sputum, non-culture based test which can be performed by people with CF on a frequent basis. This could involve breath, cough/ huff tissues, exhaled breath condensate or even urine. Samples could be collected at home by people with CF and sent to Medical Detection Dogs for screening, a positive indication leading to clinical assessment for conventional testing. Once we have optimised the next stage of training, we will perform a direct head-to-head comparison of this method with available 'electronic noses' or mass spectrometry-based techniques. Compared with new technologies, dogs may ultimately prove more sensitive or more affordable for screening lower airway infection in CF.

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**Figure legends**

**Figure 1**

During training and subsequent testing, samples are presented to dogs in a specially designed rig or row of metal arms, which allows them to sniff the headspace above the sample, move on if they consider it 'negative' and indicate at a 'positive'. A dog could indicate by sitting or standing still; the method of response was consistent within an individual. A negative sample was usually abandoned in a 1 or 2 seconds, the dog moving onto the next sample. When the dog gave a positive indication, the sniffing of that sequence was halted and they were rewarded if appropriate. An incorrect indication resulted in no reward. Any sample which had not been encountered in that run (ie. was after a correctly identified positive), was placed into a subsequent run so that each dog encountered every sample and control.

**Figure 2**

Exact 95% confidence intervals for the sensitivity and specificity of each dog (named) in each trial. Studies (i) Pa vs controls on which the dogs had been trained; (ii) Pa vs new controls (different organisms which the dogs had not previously encountered); (iii) Pa vs controls at 1:1,000 (blue) and 1:10,000 (red) dilutions; (iv) Pa mixed in culture with other organisms.


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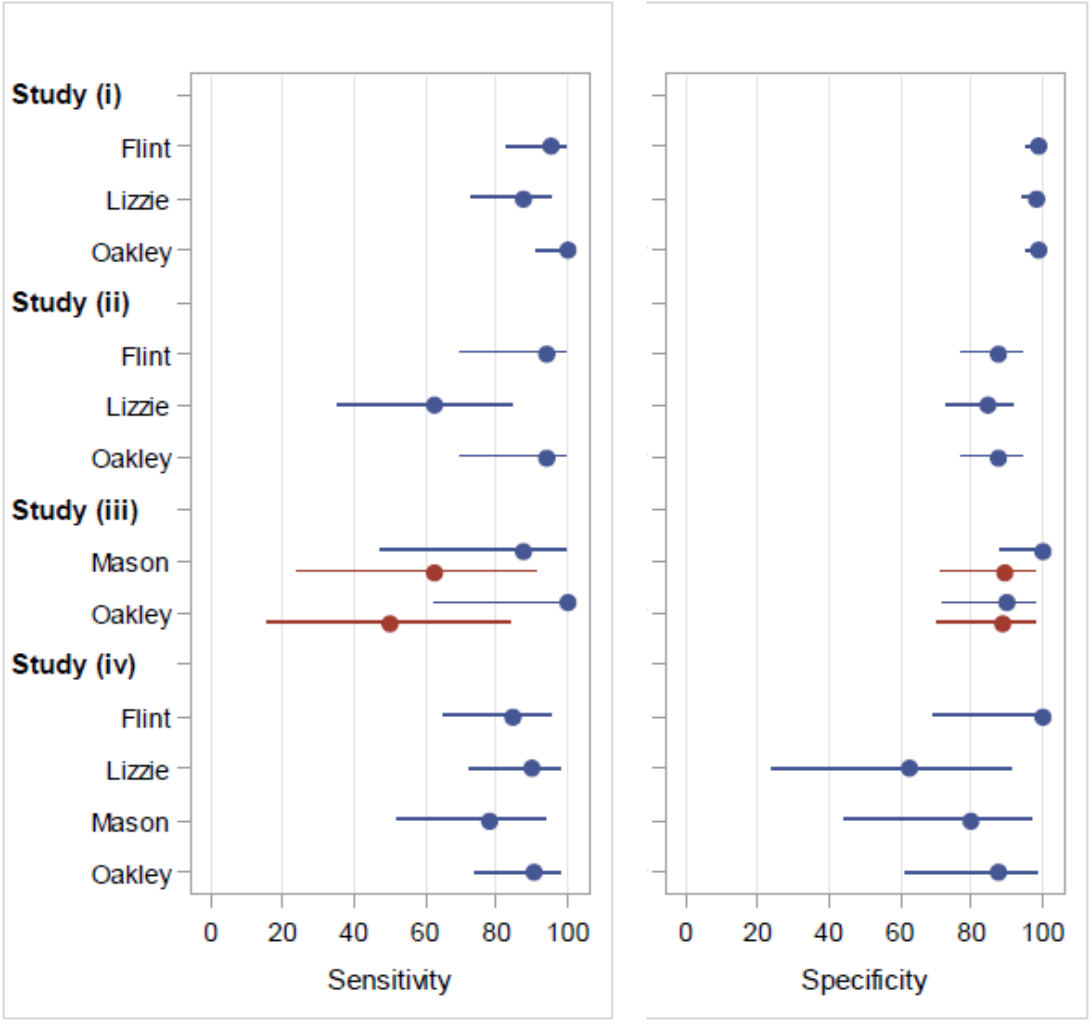
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