Sensors and Actuators B 171–172 (2012) 588–594

Contents lists available at SciVerse ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

# Detection of volatile organic compounds in cattle naturally infected with *Mycobacterium bovis*

Nir Peled[a,1], Radu Ionescu[b,1,2], Pauline Nol[c,1], Orna Barash[b], Matt McCollum[c], Kurt VerCauteren[c], Matthew Koslow[a], Randal Stahl[c], Jack Rhyan[c,*], Hossam Haick[b,**]

[a] *The Thoracic Cancer Research and Detection Center, Sheba Medical Center, Tel-Aviv University, Tel-Aviv 52621, Israel*
[b] *The Department of Chemical Engineering and Russell Berrie Nanotechnology Institute, Technion – Israel Institute of Technology, Haifa 32000, Israel*
[c] *The National Wildlife Research Center, US Department of Agriculture, Animal and Plant Health Inspection Service, Fort Collins, CO 80521, USA*

ARTICLE INFO

*Article history:*
Received 21 March 2012
Received in revised form 30 April 2012
Accepted 8 May 2012
Available online 18 May 2012

*Keywords:*
Bovine tuberculosis
*Mycobacterium bovis*
Breath analysis
Volatile organic compound
Chemical sensors array
Gas-chromatography/mass-spectrometry

ABSTRACT

We report here on a novel methodology in detecting *Mycobacterium bovis* (*M. bovis*) infection in cattle, based on identifying unique volatile organic compounds (VOCs) or a VOC profile in the breath of cattle. The study was conducted on an *M. bovis*-infected dairy located in southern Colorado, USA, and on two tuberculosis-free dairies from northern Colorado examined as negative controls. Gas-chromatography/mass-spectrometry analysis revealed the presence of 2 VOCs associated with *M. bovis* infection and 2 other VOCs associated with the healthy state in the exhaled breath of *M. bovis*-infected and not infected animals, yielding distinctly different VOC patterns for the two study groups. Based on these results, a nanotechnology-based array of sensors was then tailored for detection of *M. bovis*-infected cattle via breath. Our system successfully identified all *M. bovis*-infected animals, while 21% of the not infected animals were classified as *M. bovis*-infected. This technique could form the basis for a real-time cattle monitoring system that allows efficient and non-invasive screening for new *M. bovis* infections on dairy farms.



## 1. Introduction

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis* (*M. bovis*), is a serious global disease with an impact on animal health, public health, and international trade [1–3]. The transmission of tuberculosis to humans via infected milk was considered a significant cause of morbidity and mortality from Victorian times until the Second World War [4,5]. Milk pasteurization and intensive eradication programs led to sharp declines of bTB in domestic livestock and humans, especially in developed countries [6]. However, the challenge of eradication remains, largely due to unauthorized movement of infected animals and persistent wildlife reservoirs of the disease [7,8].

Accurate and efficient detection of bTB in animal populations remains of paramount importance to bTB control programs. Currently, tuberculosis testing in live cattle in the United States consists of a caudal fold test (CFT) as a screening test, with a comparative cervical test (CCT) or interferon gamma assay test as supplemental or confirmatory tests [3,9–11]. The CFT and CCT involve injecting tuberculin(s) intradermally and measuring any subsequent swelling at the site of injection 72 h later [12]. The interferon gamma assay is a confirmatory or supplemental blood test that relies on quantifying the amount of gamma interferon that is produced in animal blood samples cultured in the presence of tuberculin. The final diagnosis of bovine tuberculosis requires post mortem laboratory confirmation of disease via histopathology, polymerase chain reaction, and bacteriological culture [9,10].

Although these combined tests have good specificity and sensitivity depending on the stage of infection (over 80% sensitivity and over 90% specificity, respectively), conducting these tests at large dairies is expensive, time-consuming, logistically challenging, and must be performed by certified veterinarians [13]. Skin testing requires a second examination, and the interferon gamma assay is considerably more expensive in comparison with a skin test [14,15]. Both interferon gamma and skin testing results are delayed a minimum of 48–72 h [13,15]. The development of a

*Abbreviations:* bTB, bovine tuberculosis; *M. bovis*, *Mycobacterium bovis*; VOC, volatile organic compound; CFT, caudal fold test; CCT, comparative cervical test; GC–MS, gas chromatography–mass spectrometry; DFA, discriminant factor analysis; GNP, gold nanoparticles; TP, true positive; TN, true negative; FP, false positive; FN, false negative; CV, canonical variable.

* Corresponding author at: The National Wildlife Research Center, US Department of Agriculture, Animal and Plant Health Inspection Service, Fort Collins, CO 80521, USA. Tel.: +1 970 266 6126; fax: +1 970 266 6138.
** Corresponding author. Tel.: +972 4 8293087; fax: +972 77 8871880.
*E-mail addresses:* Jack.C.Rhyan@aphis.usda.gov (J. Rhyan), hhossam@technion.ac.il (H. Haick).
[1] These authors have contributed equally to the manuscript.
[2] Present address: Department of Electronics, Electrical and Automatic Engineering, Rovira i Virgili University, 43007 Tarragona, Spain.

0925-4005/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.snb.2012.05.038

sensitive, specific, non-invasive, and efficient method for detecting *M. bovis* infection, which can be performed on the premises, would be highly beneficial to both regulators and managers in the livestock industry.

An emerging approach for diagnosing, an infectious disease at its earliest stages relies on volatile organic compounds (VOCs) that are emitted from the infectious agent and/or the host. The successful analysis of infectious disease-related VOCs is based on the following principles of cell biology. The bacterial cell membrane consists primarily of amphipathic phospholipids, carbohydrates and many integral membrane proteins that are distinct for different cell types. In disease formation, both host and invading cells can undergo structural changes, one example of which would be oxidative stress, i.e., a peroxidation of the cell membrane that causes VOCs to be emitted [16]. Some of these VOCs appear in distinctively different mixture compositions [17–22]. What is particularly significant about this approach is that each type of disease has its own unique pattern of VOCs; therefore, the presence of one disease would not mask other disease types [23]. These VOCs can be detected directly from: (i) cultured cells (i.e., the mixture of VOCs trapped above the cells in a sealed vessel) [20–22]; (ii) urine [24]; or (iii) exhaled breath [17–19].

In regard to exhaled breath, the principle is that disease-related changes in blood chemistry are reflected in measurable changes to the breath through exchange via the lungs. In certain instances, breath testing offers several potential advantages, such as (a) breath samples are non-invasive and relatively easy to obtain, and (b) breath testing has the potential for direct, inexpensive and eventually real-time monitoring.

In this paper, we explore the utility of breath testing for the detection of *M. bovis* infection in cattle. We analyze breath samples collected from cattle using gas-chromatograph/mass-spectrometry (GC–MS) to identify the VOC patterns linked with the disease conditions. Based on the detected VOC patterns, a nanotechnology-based array of sensors, termed Nano Artificial NOSE (NA-NOSE) [25–30], was tailored for the detection of bTB disease from exhaled breath. NA-NOSE is an artificial olfactory system based on an array of cross-reactive, nanomaterials-based, chemical gas sensors, which can identify and separate different gaseous mixtures, even if their constituent analytes are present at very low concentrations and their differences are very subtle. The results obtained indicate that the NA-NOSE could efficiently detect *M. bovis* infection from breath samples of cattle.

## 2. Materials and methods

### 2.1. Breath collection from cattle

Breath samples were collected and tested from 14 cattle from an *M. bovis*-infected dairy in the southern part of the state of Colorado, USA. Ten of these animals were identified as bTB-positive based on conventional tests. Nine of 10 cattle were culture positive for *M. bovis* at necropsy. One animal was culture negative but had gross and microscopic lesions compatible with bTB which were polymerase chain reaction positive. Nine of 9 animals tested were positive on CFT, 9 of 10 animals tested were positive on CCT, 8 of 10 animals tested on interferon gamma assay were positive and 2 were suspect; all 10 animals had gross lesions and 8 animals had microscopic lesions compatible with bTB. The remaining four animals from the same dairy were deemed bTB-negative based on the following: none of the animals had gross lesions, three of three animals tested were negative on CFT, one of one animal tested was negative on CCT, and one of one was negative on gamma interferon. Only one animal was cultured and it was negative, whereas the others were not cultured because they were negative on skin tests and gamma interferon tests and they had no evidence of disease on post mortem examination. Additionally, breath samples from 13 cattle from two bTB-negative dairies located in northern Colorado were also tested. These animals served as negative controls, as well as to exclude confounders caused by farm and feed differences. These animals were not skin tested.

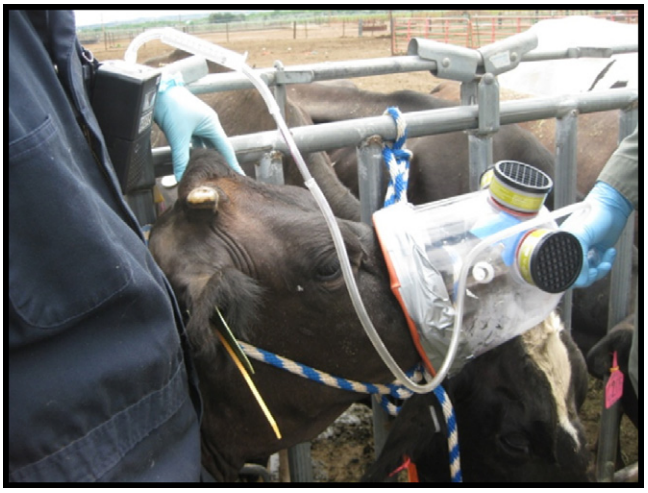

**Fig. 1.** Photo illustrating the system employed for breath sample collection in the cattle. Inspired air first passes into the mask through three charcoal filters and one-way valves to remove environmental VOCs. Expired air passes out of the mask through two one-way valves and through the tubing inserted into a hole in the front of the mask. Air in the tubing passes through a glass cartridge containing sorbent material (Tenax™) and is exhausted through the hand-held suction pump.

Breath specimens were collected by use of a mask designed to deliver nebulized medication to horses (Aeromask®, Trudell Medical International, London, Ontario, Canada) modified so that inspired air passed through charcoal filter cartridges (North Safety Products by Honeywell, Cranston, RI, USA) and air in the mask was pumped via Tygon® tubing (Saint-Gobain Performance Plastics, Akron, OH, USA) through a glass cartridge containing inert sorbent material (Tenax™ Catalog No. 226-35-03, SKC Inc. Eighty Four, PA, USA) by means of a handheld pump (Air Check XR5000, SKC) (Fig. 1). This approach is necessary to reduce as much as possible any confounders or contaminants that occur external to the animals we are targeting. Air was sampled from the mask at a rate of 1 L/min for 2 min. The sorbent material concentrated the VOCs in the 2 L gas sample that passed through the tube. Following exposure, the sorbent tubes were sealed and stored at −70 °C until shipment to Israel for GC–MS and NA-NOSE analyses. The experiment was performed in compliance with the U.S. laws for the humane treatment of animals and was done in conjunction with disease management procedures of the Colorado Department of Agriculture and the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services.

### 2.2. Breath analysis using the GC–MS

The chemical composition of the breath samples collected from all 27 cattle was analyzed employing a Gas Chromatography–Mass Spectrometry equipment (GC–MS-QP2010; Shimadzu Corporation, Japan), combined with a thermal desorption system (TD20; Shimadzu Corporation, Japan). The GC oven temperature profile used was (i) 35 °C, hold for 10 min; (ii) ramp of 4 °C/min until 150 °C; (iii) ramp of 10 °C/min until 300 °C; and (iv) hold for 15 min at 300 °C. VOCs were chromatographically separated using an SLB-5ms, 30m × 0.25 mm, 0.5 μm film thickness, with 5% phenyl methyl siloxane, capillary column (Sigma Aldrich Ltd., Rehovot, Israel). The injection port was configured in a splitless injection mode