Preventive Veterinary Medicine 120 (2015) 277–282

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

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

# Diagnostic performance characteristics of a rapid field test for anthrax in cattle

Janine Muller[a], Jacek Gwozdz[a], Rachel Hodgeman[a], Catherine Ainsworth[a], Patrick Kluver[c], Jill Czarnecki[b], Simone Warner[a], Mark Fegan[a,*]

[a] Department of Economic Development, Jobs, Transport and Resources, Biosciences Research Division, AgriBio, Centre for AgriBioscience, 5 Ring Road, La Trobe University Research and Development Park, Bundoora, VIC 3086, Australia
[b] Biological Defense Research Directorate, Naval Medical Research Center-Frederick, 8400 Research Plaza, Fort Detrick, MD 21702, USA
[c] Livestock Biosecurity Networks, National Farmers Federation House, Canberra, ACT 2601, Australia

ARTICLE INFO

*Article history:*
Received 3 July 2014
Received in revised form 25 March 2015
Accepted 25 March 2015

*Keywords:*
Anthrax
*Bacillus anthracis*
Immunochromatographic test
In-field diagnosis
Test diagnostic sensitivity and specificity

ABSTRACT

Although diagnosis of anthrax can be made in the field with a peripheral blood smear, and in the laboratory with bacterial culture or molecular based tests, these tests require either considerable experience or specialised equipment. Here we report on the evaluation of the diagnostic sensitivity and specificity of a simple and rapid in-field diagnostic test for anthrax, the anthrax immunochromatographic test (AICT). The AICT detects the protective antigen (PA) component of the anthrax toxin present within the blood of an animal that has died from anthrax. The test provides a result in 15 min and offers the advantage of avoiding the necessity for on-site necropsy and subsequent occupational risks and environmental contamination.

The specificity of the test was determined by testing samples taken from 622 animals, not infected with *Bacillus anthracis*. Diagnostic sensitivity was estimated on samples taken from 58 animals, naturally infected with *B. anthracis* collected over a 10-year period. All samples used to estimate the diagnostic sensitivity and specificity of the AICT were also tested using the gold standard of bacterial culture. The diagnostic specificity of the test was estimated to be 100% (99.4–100%; 95% CI) and the diagnostic sensitivity was estimated to be 93.1% (83.3–98.1%; 95% CI) (Clopper–Pearson method).

Four samples produced false negative AICT results. These were among 9 samples, all of which tested positive for *B. anthracis* by culture, where there was a time delay between collection and testing of >48 h and/or the samples were collected from animals that were >48 h post-mortem. A statistically significant difference ($P < 0.001$; Fishers exact test) was found between the ability of the AICT to detect PA in samples from culture positive animals <48 h post-mortem, 49 of 49, $Se = 100\%$ (92.8–100%; 95% CI) compared with samples tested >48 h post-mortem 5 of 9 $Se = 56\%$ (21–86.3%; 95% CI) (Clopper–Pearson method). Based upon these results a post hoc cut-off for use of the AICT of 48 h post-mortem was applied, $Se = 100\%$ (92.8–100%; 95% CI) and $Sp = 100\%$ (99.4–100%; 95% CI).

The high diagnostic sensitivity and specificity and the simplicity of the AICT enables it to be used for active surveillance in areas with a history of anthrax, or used as a preliminary tool in investigating sudden, unexplained death in cattle.



* Corresponding author. Tel.: +61 3 90327274; fax: +61 390327604.
*E-mail address:* mark.fegan@ecodev.vic.gov.au (M. Fegan).

http://dx.doi.org/10.1016/j.prevetmed.2015.03.016
0167-5877/

## 1. Introduction

*Bacillus anthracis* (*B. anthracis*) is the aetiological agent of anthrax, an infectious bacterial disease that affects domestic livestock, game animals and occasionally humans worldwide. In Australia, anthrax is an endemic disease that causes sporadic outbreaks in ruminants in defined regions of Australia (Beveridge, 1983). The disease in Australia is largely confined to the south eastern states of Victoria and New South Wales with only occasional occurrence outside of these areas.

Identifying animals that have died from anthrax is reasonably straightforward in the laboratory provided that the animal is not in the advanced stages of decomposition. Traditionally, blood smears stained with aged polychrome methylene blue for the demonstration of encapsulated gram positive rods, and bacterial culture, are the recommended tests for identifying the bacterium as stated in both the World Animal Health Organisation (OIE) manual (Office International des Epizooties, 2008) and the Australian & New Zealand Standard Diagnostic Procedure (ANZSDP) (Hornitzky and Muller, 2010). Molecular based confirmation by the polymerase chain reaction (PCR) to identify unique genetic markers for *B. anthracis* is also becoming more accepted as a stand-alone laboratory test (Office International des Epizooties, 2008, Ramisse et al., 1996; Rao et al., 2010; Wielinga et al., 2011). However, field diagnosis in ruminants can be more difficult as anthrax classically runs a peracute course resulting in death before the expression of clinical signs. Furthermore, the availability of a portable PCR machine or a microscope, and the skills required to make and interpret a blood smear on-site, are not always practicable, resulting in significant time delays whilst testing is completed at a laboratory. For veterinarians faced with a sudden, unexplained death, the use of a rapid and simple test that can be performed in the field, to confirm the suspicion of anthrax, is of considerable value.

*B. anthracis* has two virulence plasmids; pXO2 which encodes the genes that synthesise the poly-D-glutamic acid capsule and pXO1 which encodes the genes for the tripartite exotoxin comprised of protective antigen (PA), lethal factor (LF) and oedema factor (EF) (Beall et al., 1962; Stanley and Smith, 1963). The exotoxins are binary combinations of PA with either LF to form lethal toxin or EF to form the oedema toxin (Brossier and Mock, 2001) both of which are responsible for the characteristic signs and symptoms of anthrax (World Health Organization, 2008). The action of *B. anthracis* toxins within the host result in necrosis of the lymphatic tissue and breakdown of blood vessels, resulting in internal bleeding, rapid and fatal septicaemia, and terminal haemorrhage (Radostitis et al., 2000). Production of anthrax toxin within an animal is dependent on the presence of viable *B. anthracis*, hence the detection of PA is indicative of active anthrax infection (Boyer et al., 2007). At death the anthrax toxin, and in particular PA, is widely distributed in tissues and blood at concentrations of 80–100 μg/mL although this may vary according to the species affected (Mabry et al., 2006).

For the past 10 years, the Department of Economic Development, Jobs, Transport and Resources (DEDJTR) Victoria has been collaborating with the United States Biological Defence Research Directorate, Naval Medical Research Centre (USNMRC), in the evaluation of a field test for anthrax. The resultant test, known as the anthrax immunochromatographic test (AICT), represents a major step forward in anthrax diagnosis. The AICT is a rapid and simple in-field test that detects PA in the blood of an animal that has died from anthrax. Here we report on an evaluation of the performance of the AICT, in terms of diagnostic sensitivity and specificity, as a tool for field veterinarians in Australia.

## 2. Materials and methods

### 2.1. Source population and sample collection

Estimates of diagnostic sensitivity and specificity of the AICT were determined using samples collected from the same animal species from the same geographic areas where anthrax is endemic within Australia.

Whole blood samples were collected from 680 cattle sourced from Victoria and New South Wales over a period of 10 years (2001–2010). Only one sample was collected and tested from each animal. Of the 680 cattle, 456 convenience samples were selected on an ad hoc basis from a knackeries[1] surveillance programme and the remaining 224 samples were obtained from samples submitted to the National Anthrax Reference Laboratory at DEDJTR for anthrax testing. Samples sourced from the surveillance programme were collected from animals that had no known cause of death. Generally the first sign of anthrax in a herd is a sudden unexplained death in one or more animals (Beyer and Turnbull, 2009). Therefore all samples used for testing were collected from animals that died suddenly on farm and all euthanized animals and animals where a cause of death was known were excluded from the study. The number of samples excluded based on these criteria was not recorded. Samples sourced through the National Anthrax Reference Laboratory were from investigations of animals that had died of an unexplained cause with the intent to identify if the animal had died of anthrax. No samples submitted to the National Anthrax Reference Laboratory were excluded from this study. Information on age, sex and breed were not recorded but as the AICT is designed to detect the PA component of the toxins produced by *B. anthracis*, and there is no known variation in the susceptibility of different breeds of cattle to the anthrax toxins, it is unlikely that these characteristics would affect the diagnostic sensitivity and specificity of the test.

All knackeries surveillance samples and the majority of samples submitted to the laboratory for anthrax testing (205 of 224) were tested in the field using the AICT. Where in-field testing was not possible AICT testing was performed in the laboratory after receipt of the samples. All 680 blood samples were sent to the National Anthrax Reference Laboratory at DEDJTR for bacterial culture and PCR to confirm the presence or absence of *B. anthracis* as outlined below. All 58 culture positive samples included in

[1] A knackery is a place for the disposal of dead, old or injured animals for use in non-human food chain.