SPECIFICITY OF A HAND-HELD IMMUNOCHROMATOGRAPHIC ASSAY FOR ANTHRAX IN CATTLE

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IN

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ABSTRACT

The purpose of this study was to determine whether the hand-held immunochromatographic assay was a reliable method for field based diagnosis of anthrax. This study was designed and conducted with an emphasis on obtaining an estimate of performance accuracy, in terms of specificity for the protective antigen (PA) component of the anthrax toxin. To examine specificity the hand-held assay had to be assessed under similar circumstances to that experienced in the field from typical animals in which anthrax may be suspected.

To achieve this, blood samples were collected post-mortem from 240 cattle at the Stanhope and Camperdown knackeries in Victoria. Blood smears were prepared, hand-held assays were performed on-site and a sample of blood transported back to the laboratory for bacterial culture. All 240 samples gave negative results in the hand-held assay and *B. anthracis* was not detected in any sample by culture or blood smear, which were considered the definitive diagnostic tests. Thus the hand-held assay was regarded as 100% specific (98.5-100%; 95% CI) for these cattle examined in Victoria.

The purpose of the second study was to determine whether the live Sterne strain $34F_2$ vaccine for anthrax would result in false positives arising in the hand-held assay in cattle recently vaccinated. Ten cattle were vaccinated with the $34F_2$ vaccine and monitored for 15 days. No PA was detected in the blood of vaccinated cattle in the hand-held assay or on culture within this time. These results show that the hand-held assay does not give false positive test

results in cattle post-vaccination with live $34F_2$ *B. anthracis* vaccine. The hand-held assay can be used with confidence on samples from recently vaccinated cattle that have died when it is necessary to know whether they had succumbed to anthrax or not.

The hand-held assay has the potential to be adopted as a routine test for the preliminary assessment of sudden death in cattle. The simplicity of the assay enables it to be used by unskilled lay people, which means that it could be used by knackery workers for surveillance in areas with a previous history of disease or by veterinarians as a preliminary routine tool in investigating sudden death in cattle. However the study described in this thesis only assesses the specificity of the assay and a further study, involving a similar number of cattle affected with anthrax, needs to be conducted to assess the sensitivity of the assay.

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B. anthracis	*Bacillus anthracis*
B. mycoides	*Bacillus mycoides*
cfu	colony forming units
CI	confidence interval
cm	centimetre
CO_2	carbon dioxide
°C	degree celcius
D+	disease present
D-	disease absent
EDTA	ethylenediamine tetraacetic acid
EF	oedema factor
ELISA	Enzyme Linked Immunosorbent Assay
ELLA	Enzyme-Linked Lectinosorbent Assay
ET	oedema toxin
FP	false positive
g	gram
h	hour
IgG	Immunoglobulin G
IHA	indirect haemagglutination assay
kDa	kilodalton
km	kilometre
LF	lethal factor
LT	lethal toxin

mm	millimetre
mL	millilitre
ng	nanogram
NPV	Negative predictive value
P	pretest probability
PA	Protective antigen
PCR	Polymerase chain reaction
PLET	polymyxin-lysozyme-EDTA-thallous acetate
PPV	Positive predictive value
rpm	revolutions per minute
s	seconds
SBA	sheep blood agar
Se	sensitivity
Sp	specificity
T+	test positive
T-	test negative
TN	true negative
TNF	tumour necrosis factor
TP	true positive
%	percent
μg	microgram
μm	micrometre