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Development of a rapid immunochromatography test for detecting antibodies after anthrax vaccination in cattle: A preliminary study

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Abstract

Anthrax is a zoonotic disease of strategic importance with high mortality rate in ruminants. Vaccination and monitoring of antibody titer after anthrax vaccination have been carried out in an effort to prevent anthrax disease in cattle. Enzyme linked immunosorbent assay (ELISA) is a method commonly used in the monitoring of antibody titer after vaccination. However, the use of the ELISA method requires skilled laboratory personnel, specialized laboratory equipment, and relatively more expensive. This study aimed to develop a method to detect antibodies after anthrax vaccination in cattle using rapid immunochromatography detection. Colloidal gold as a marker were conjugated with protective antigen (PA), with a concentration of 0.2, 0.4 and 0.8mg/ml, then put on the conjugate pads as part of the immunochromatography test strip. A total of 13 serum samples of cattle after vaccination was used in this study. The sample consisted of seven positive and six negative sera samples based on the results of the ELISA test to detect the presence or absence of anti-PA antibodies in serum. The results using the rapid immunochromatography test indicate that the anti-PA antibodies in the serum can be detected within 10min. Antigen concentration of 0.2mg/ml in the conjugate pads showed the same sensitivity test as other antigen concentrations used in this study.

Keywords:anthrax, rapid test, immunochromatography, cattle, protective-antigen

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Introduction

Anthrax, which is caused by the bacterium *Bacillus anthracis*, is one of five strategic contagious animal diseases in ruminants in Indonesia. Siregar (2002) reported that there are still many anthrax endemic areas in Indonesia such as Jakarta, West Java, Central Java, Yogyakarta, West Nusa Tenggara (NTB), East Nusa Tenggara (NTT), West Sumatra, Jambi, Southeast Sulawesi, Central Sulawesi, South Sulawesi and Papua. The mortality rate due to anthrax is very high (at 80%), especially in ruminants such as cattle, sheep and goats. In NTB particularly the islands of Sumbawa, and in particular Flores island of NTT province, the case of anthrax almost repeated every year so that the two islands are classified as areas with high endemicity (Putra et al., 2011).

In an anthrax endemic areas, vaccination and good surveilance are considered as the most appropriate controlfor anthrax. Antibody responses after vaccination are routinely monitored in vaccine recipients. A quantitative enzyme-linked immunorbent assay (ELISA) has been developed and used for the serological detection of protective antigen (PA)-spesific IgG in cattle. However, the ELISA is impractical in field settings, as it requires laboratory equipment and higly trained personnel.

With advancing technology, the development and commercialization of screening tests are moving toward rapid point-of-care assays (Bienek et al., 2008). Rapid immuno-chromatography detection is a semiquantitative colorimetric test that is well establish for

detecting diseases or monitoring vaccination status. A specimen is suspended in sample buffer and added to the sample pad. The samples flow via capilarry action through a conjugate pad and across nitrocelulose membrane that has a test strip and a control strip. If disease-spesific antibodies are present in the specimen tested, they will react with the test strip containing the diagnostic target molecule and the result can be visualised after 10 to 15min with the unaided eye.

This study was conducted to determine whether positive reaction occurs in an immunochromatography test strip developed using the serum of cattle following anthrax vaccination.

Materials and Methods

Specimens

Blood samples were collected from 13 cattle after anthrax vaccination from Palibelo (PL) and Labuan Badas (LB) districts in Sumbawa island. Serum was then separated by centrifugation at 5,000 rpm for 15min. Serum samples used for the immuassays were frozen at -20ºC until needed. As a gold standard for comparison, an ELISA was used to characterise the specimens by their amounts of PA-spesific IgG. Based on ELISA test, seven samples (PL1, PL4, PL22, PL23, PL15, PL55 and PL63) were tested positive and six samples (LB43, LB12, LB6, PL3, PL70 and negative control) were negative.

Antigen source

Recombinant PA (rPA) purchased from Indonesian Research Centre for Veterinary Science was used as the target molecule in the immunoassay. The antigen was then run on 12% acrylamide gel electroforesis in order to determine its purity.

Preparation of colloidal gold and Rapid Immunochromatography

Colloidal gold probes measuring 40nm were prepared with citric acid by using a modified citric acid reduction method.The rapid immunochromatograhy test device consists of nitrocellulosa membrane, conjugate pad and adsorbent pad. The rPA, with the concentration of 0.2, 0.4 and 0.8mg/ml, were striped in the line test position while a goat anti-mouse antibody was applied on the control test position on nitrocellulose membrane. The membrane was then dried in a controllable drying chamber (Heraeus Instruments, USA) for 60min at 60ºC. Colloidal gold conjugated with the rPA (2.5µg/ml) were dispensed onto a conjugate pad, and the pad was then affixed to the test strip by overlapping the nitrocellulose membrane by its proximal end. The addition of a sample pad completed the assembly by overlapping onto the conjugate pad. Test strip, with size of 6cm by 4mm, was produced using Matrix 2360-programable shear (Kinematic Automation, USA). All samples were tested by placing 4µl of sera onto the sample delivery pad. The fluid was drawn across the antigen- coated membrane and within 10min a visible pink dot formed in the control line. A positive result, when anthrax anti-PA antibody is present, is evident by the formation of an aditional dot in the test line.

Results and Discussion

With regard to the gel electrophoresis analysis, the rPA used for coating the nitrocellulose membrane in this study were relatively pure (data not shown). Gel electrophoresis analysis also showed that protein with size of approximately 60kDa was the major component. Seven positive and six negative sera, based on the ELISA test, were tested using the rapid immunochromatography developed.The rPA concentration of 0.2mg/ml on the solid phase seems to be sufficient to detect anthrax anti-PA IgG present in the vaccinated cattle. Figure 1 shows the representative of negative (negative control) and positive(sample #PL1) immunochromatography test results.

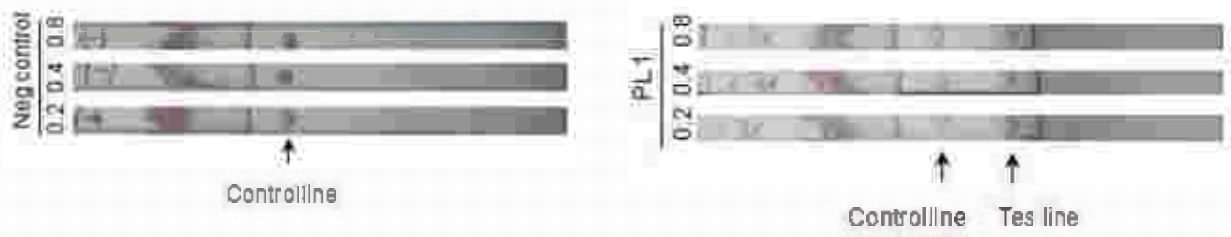

Figure 1. Reprensentative immunochromatography test results

All sera tested positive on the ELISA test showed positive results on the rapid test (Table 1). Whilst, 67% (4 out of 6) sera showed negative on the rapid test were also negative on the ELISA test. The sensitivity and specificity of the rapid test were 100% and 67% respectively.

Table 1. Diagnostic performance of rapid immunochromatography test to PA-spesific IgG present in vaccinated cattle in comparison to ELISA test.

ELISA	Sample No	rPA conc on immunochromatography test		
		0.2mg/ml	0.4mg/ml	0.8mg/ml
Negative	Negative cont	-	-	-
	LB43	-	-	-
	LB12	-	-	-
	LB6	-	-	+/-
	PL3	+	+	+
	PL70	+/-	+/-	-
Positive	PL1	++	++	++
	PL4	+	+	+/-
	PL22	+/-	+/-	+/-
	PL23	+	+	+
	PL15	+	+	+
	PL55	++	++	+
	PL63	+	+	+

Note: - (negative); +/- (weak positive); + (strong positive); ++ (very strong positive)

When estimating the specificity of diagnostic test, it is important to ensure that samples should represent the population for which the test is going to be used (Peruski & Peruski Jr., 2003; Muller et al., 2004). The limited number of samples tested in this study may contribute to relatively low level of specificity. The use of the ELISA as a gold standard in this study should also be taken into account as it is also a screening test method. This suggests that further works on validating a good gold standar is necessary.

Anti-PA antibody titres are not frequently measured after anthrax vaccination both in human (Bienek et al., 2008) and animals. The major reason for this is that anti-PA IgG mesurements are laboratory based, necessitating transport material to be tested to laboratory, skilled personnel and specific equipment. Therefore, a simple and rapid method would provide an important tool to screen or measure anti-PA antibody after anthrax vaccination.This study indicates that we have successfully developed a strip tes kit that can rapidly detect the anti-PA IgG on cattle sera following vaccination. The results imply that the strip assay is higly sensitive and and sufficiently specific to support the detection. The test is easy to operate, may suit for on-site testing and is much quicker than the time required for ELISA. Further study to examine the accuracy of the rapid test with respect to its specificity still needs to be undertaken.

Acknowledgements

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