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Comparative Analysis of Lateral Flow Assay with Indirect ELISA for Detection of Anti-NSP Antibodies of Foot and Mouth Disease

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ABSTRACT

Foot-and-mouth disease (FMD) was an exceedingly infectious disease that spread to Indonesia in May 2022. A reliable diagnostic serologic test that can distinguish between infected and vaccinated animals was an important part of FMD (serotype O) control programs in affected areas in Indonesia. For this reason, a non-structural protein (NSP) serological test based on 3ABC proteins has been used. The indirect ELISA serological test requires time, skill, and specialized equipment. An alternative method that can be employed is the lateral flow assay (LFA), which offers the advantages of simplicity and portability, enabling rapid acquisition of results. The objective of this study was to validate the efficacy of a user-friendly anti-NSP antibody LFA for rapid diagnostic purposes. This was done by assessing its sensitivity and specificity in stored samples that had previously been tested using indirect ELISA. There were 32 preserved biological materials from dairy and beef cattle in three provinces in Indonesia that were examined with developed LFA. The results of each sample on LFA were compared to the ELISA result for its sensitivity and specificity according to positive and negative values on both tests. The test had a sensitivity of 95.2% and a specificity of 100%, compared to the indirect ELISA. The measured kappa value is also very good at 0.93, so LFA can be optionally used when examining anti-NSP FMD antibodies. Therefore, the LFA anti-NSP for detecting FMD is considered reliable because of its simplicity and the accuracy of the test results.

Keywords: Antibody, Bovine serum, Foot and mouth disease, Indirect ELISA, Lateral flow assay

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious disease that affects cloven-hoofed mammals (World Organization for Animal Health, 2022). The first FMD outbreak was observed in Indonesia in 1887, and the country has been declared FMD-free without inoculation since 1986. However, in 2022, there was a recurrence of FMD outbreaks in cattle and goats in Indonesia. The virus's serotype O/ME-SA/Ind-2001e sublineage was found and identified (Blacksell et al., 2019; Susila et al., 2022). The disease-causing virus, FMDV, is in the genus Aphthovirus and is in the family Picornaviridae. Its genome is 8.4 kb long and is made up of positive-sense, single-stranded RNA ([(+)] ssRNA). The virus produces ten non-structural proteins (Lpro, 2A, 2B, 2C, 3A, 3B1, 3B2, 3B3, and 3Cpro) from four precursor polypeptides including L, P1, P2, and P3 (Knowles et al., 2012). The virus contains four structural proteins (VP1–VP4).

In the oropharynx of some ruminant animals, such as cattle, the virus can persist for several months after recovering from infection, while pigs do not develop into carriers of the virus (WOAH, 2022). Detecting disease-carrying animals is crucial in controlling FMD, as they can serve as a reservoir of infection for other vulnerable animals. The majority of commercially available inactivated FMD vaccines only consist of the viral structural protein (SP). Uninfected animals, regardless of whether they have been vaccinated or not, should not possess antibodies to nonstructural protein (NSP) based on theoretical considerations. Hence, the presence of antibodies targeting NSP can serve as a means of distinguishing between animals that have received vaccination against FMDV and those that have not (Doel, 2003; WOAH, 2022). Some serological detection techniques include lateral flow assay (LFA), solid phase competition (SPC) ELISA, liquid phase blocking (LPB) ELISA, and virus neutralization assay (VNT, Wong et al., 2020).

The current serology test, the indirect ELISA, requires time, skill, and specialized equipment so this test can only be performed in select laboratories (Wong et al., 2020). The LFA strips that identify antibodies against NSPs are needed for

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differentiating infected from vaccinated animals (DIVA), just like they are for ELISA. The LFA is used in the current study to find NSPs. This assay may be a useful option for field FMD surveys as it can identify antibodies in cattle and goat serum. The purpose of this study is to determine how effective LFA is as a field substitute for indirect ELISA serological testing.

MATERIALS AND METHODS

Ethical approval

This study has complied with all relevant ethical guidelines of Universitas Airlangga, Indonesia.

Sera sample

The samples used in this research were collected from preserved biological materials and evaluated using indirect ELISA for monitoring purposes at the Tekad Mandiri Citra Laboratory, Indonesia. The study used 32 samples in total. The beef cattle and dairy that were chosen for sampling were from the province of East Java (14 samples), Banten (4 samples), and Lampung (14 samples) Indonesia, which historically had not received previous vaccination of FMD.

Indirect ELISA nonstructural protein

In this study, the commercial kits CHEKIT IDEXX FMD-3ABC bo-ov (IDEXX Laboratories, USA) were used. This kit detects antibodies against FMDV NSP 3ABC, which is a produced recombinant protein in the *Escherichia coli* expression system. This kit can only be used for bovine and ovine serum (Fukai et al., 2013). The test and control sera were diluted at a ratio of 1:100 using a diluent buffer. A microplate coated with FMD antigen is loaded with 100 µl of the diluted serum, followed by incubation at 37°C for 60 minutes. After washing, 100 µl of anti-ruminant IgG peroxidase conjugate was added to the wells of the microtitration plate. The plates were then incubated at 37°C for 60 minutes. After washing, each well was filled with 100 µl of TMB (3, 32, 5,52-tetramethylbenzidine) substrate, which was incubated at room temperature for 15 minutes in the dark before being filled with 100 µl of stop solution. Microplate read using an ELISA reader at 450 nm (Yousaf et al., 2021). The manufacturer recommended interpretation was < 20% is negative, 20–30% is ambiguous, and >30% is positive.

Lateral flow assays

The FMD commercial kit PMKit Ab (Tekad Mandiri Citra, Indonesia) was used for this assay. Bovine-ovine serum was put into a reagent tube (30 µl), homogenized, and left for 10 minutes. Then two drops of diluent were added and homogenized. The solution was dripped onto the test device marked "S" and the result was read in 10-15 minutes. The interpretation is negative if both the T line and the C line show a wine-red color reaction, and positive if there is no color reaction on the T line, just only on C line showing a wine-red color reaction.

Specificity and sensitivity tests

Compared to the gold standard, sensitivity and specificity are frequent statistical tools in evaluating the performance of alternative tests. This study compared the use of LFA as an alternative test to the gold standard of indirect ELISA (Table 1). In this study, "true positive" samples were determined using LFA and indirect ELISA. "False positive" samples were those that tested positive using LFA but negative using indirect ELISA. "False negative" samples were those that tested negative using LFA but positive using indirect ELISA. Both the "true negative" results obtained from LFA and indirect ELISA testing are negative. Sensitivity is the proportion of subjects with an actual positive outcome (true positives + false negatives) who are correctly assigned a positive assignment (true positives only). Sensitivity = TP/(TP+FN). Specificity is the proportion of subjects with an actual negative outcome (true negatives + false positives) who are correctly assigned a negative assignment (true negatives only). Specificity = TN/(TN+FP, Monaghan et al., 2021).

Table 1. The calculation for sensitivity and specificity of lateral flow assay

		Indirect ELISA	
		+	-
Lateral Flow Assay	+	TP	FP
	-	FN	TN
		Sensitivity: TP/(TP+FN)	Specificity: TN/(TN+FP)

*TP: True positive, FP: False positive, FN: False negative, TN: True negative

Statistical analysis

The collected data were analyzed using Microsoft Excel 2018 (US). The hypothesis of conditional independence was examined using the Kappa (κ) statistic (IBM SPSS 25, US).

RESULTS AND DISCUSSION

The samples used in this research were collected from preserved biological materials and evaluated using indirect ELISA for monitoring purposes at the Tekad Mandiri Citra Laboratory (Indonesia). The study used 32 samples in total. The cattle and dairy that were chosen for sampling were from the province of East Java (14 samples), Banten (4 samples), and Lampung (14 samples), which historically had not received previous vaccination of FMD. Serum samples tested using indirect ELISA resulted in 21 positive, 3 ambiguous, and 8 negative samples. Serum samples tested using lateral flow assay resulted in 20 positive and 12 negative samples. The details of each province can be seen in Table 2. All samples that were negative and ambiguous in the indirect ELISA, when tested with LFA were negative. This suggests that the negative cutoff of LFA includes both negative and ambiguous values in indirect ELISA. Only sample SL9 tested by indirect ELISA was positive, but tested by LFA was negative. The LFA demonstrated an overall sensitivity of 95.2% and a specificity of 100% when compared to the indirect ELISA. To determine the compatibility between standardized tests and new tests kappa statistical analysis is performed (Thrusfield, 2005). The tested LFAs had their kappa statistical values calculated with the indirect ELISA test as a comparison. The kappa value obtained was 0.93, indicating excellent agreement with the comparative test.

Table 2. Results of anti-nonstructural protein antibody testing by lateral flow assays and indirect ELISA on bovine serum samples from East Java, Banten, and Lampung provinces, Indonesia

Serum code	Breed	Province	Result		
			LFA	ELISA	
			(+/-)	(% Value)	Interpretation
JB1	FH	East Java	+	92.82	P
JB2	FH	East Java	+	81.75	P
JB3	FH	East Java	+	96.96	P
JB4	FH	East Java	+	99.38	P
JB5	FH	East Java	+	122.48	P
JB6	FH	East Java	+	60.92	P
JB7	SC	East Java	+	41.22	P
JB8	SC	East Java	-	23.26	S
JB9	SC	East Java	-	13.49	N
JB10	SC	East Java	+	68.31	P
JB11	OC	East Java	+	36.54	P
JB12	OC	East Java	-	25.34	S
JB13	OC	East Java	-	10.02	N
JB14	OC	East Java	-	3.81	N
BK1	OC	Banten	-	3.69	N
BK2	SC	Banten	-	10.51	N
BS1	SC	Banten	-	17.86	N
BS2	SC	Banten	-	15.97	N
SL1	SC	Lampung	+	116.53	P
SL2	SC	Lampung	+	121.22	P
SL3	SC	Lampung	+	104.99	P
SL4	SC	Lampung	+	132.06	P
SL5	SC	Lampung	+	153.94	P
SL6	OC	Lampung	+	109.8	P
SL7	OC	Lampung	+	70.77	P
SL8	OC	Lampung	+	47.61	P
SL9	OC	Lampung	-	35.11	P
SL10	OC	Lampung	+	110.59	P
SL11	SC	Lampung	+	60.26	P
SL12	SC	Lampung	+	44.94	P
SL13	SC	Lampung	-	23.85	S
SL14	SC	Lampung	-	14.66	N
Number of positives			20	21	

* FH: Friesian holstein, OC: Ongole, SC: Simmental-crossbreed, P: Positive, S: Suspect/ambiguous, N: Negative, LFA: Lateral flow assay

Since the FMD serotype O breakout in May 2022, Indonesia has been on high alert for the growth and spread of this case (Susila et al., 2022). In the event of an FMD outbreak, early and exact FMDV diagnosis allows for efficient FMD surveillance and response by implementing adequate controls and prevention measures. The availability of high-throughput machinery and highly skilled individuals is crucial for the diagnostic assays to check and diagnose FMD. Additionally, the poor samples that came from moving materials from a field to a lab may impede or postpone the early detection of the illness (Wong et al., 2020). Therefore, rapid LFA may serve as a promising on-site diagnostic method for rapid FMD detection and enable timely control measures.

In places where vaccination is used to reduce FMD, sero-surveillance should be carried out using a test that can distinguish between infected and vaccinated animals (Rout et al., 2014). The DIVA has effectively identified antibodies targeting specific NSPs of FMDV (Paton et al., 2006). To identify anti-NSPs antibodies in pigs, Chen et al. (2009) employed recombinant FMDV serotype O 3ABC protein. Later, Wu et al. (2011) developed an LFA strip based on FMDV serotype O recombinant 2C′3AB protein, in which 3C was removed due to low immunogenicity and substituted with a fragment of 2C protein fused to the N-terminus of 3AB. Despite the high sensitivity and specificity of the test, the serotypes of positive and tested vaccination serum samples were not disclosed. The use of LFA strip technology in DIVA has been hypothesized despite the absence of any reports to that effect.

The LFA kit in the assay operates in a competitive format. The competitive format of the assay is the target binds to the ligand and blocks the ligands from binding to the reporter (Qian and Bau, 2004). Because macromolecules are challenging to immobilize directly on solid phases, these competitive formats are frequently used for the detection of micromolecules. For macromolecules and whole-cell immunoassays, the competitive format can also be developed as an optional technique. The LFA kit used NSP of FMD as a ligand. Target analytes in this configuration interfere with the reporter's ability to bind to the test ligands. A reporter such as carbon black, dye-encapsulating liposomes, colored polystyrene, colloidal gold, and phosphor is combined with the target analyte in a solution that dissolves on the strip. Therefore, in the absence of any target analytes, a signal would be generated at the test line.

CONCLUSION

In this study, the results of the LFA analysis showed a sensitivity of 95.2% and a specificity of 100% compared to indirect ELISA. The compatibility of LFA with ELISA was demonstrated to be excellent, indicating its potential use as a differentiating infection from vaccinated animals test. Compared to ELISA, which requires time, equipment, and expertise, LFA is more practical and faster to use, and also easier to carry to the field. Further tests need more samples and groups to evaluate the effectiveness and reliability of the LFA in the field.

DECLARATIONS

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Availability of data and materials

The datasets generated during the current study are available from the corresponding author upon reasonable request.

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Authors' contributions

Akmal Jauhari did samples testing and manuscript preparation. Siti Munawaroh did the manuscript preparation. Wyanda Arnafia did manuscript preparation and revision. Denniswara Sibit did the analysis and funding. Jola Rahmahani designed the research and supervision. Suwarno Suwarno designed the research and manuscript revision. All authors read and approved the final version of the manuscript.

Competing interests

The authors declare that they have no conflicts of interest concerning the work presented in this article.

Ethical considerations

All the authors have thoroughly checked and confirmed the ethical concerns regarding the originality of collected data, analyzed data, and written sentences of this article before submission to the journal.

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