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Sensitivity of Lateral Flow Technique for Evaluation of Inactivated Rift Valley Fever Virus Vaccine in Comparison with Serum Neutralization Test

Abousenna Mohamed Samy[1*], Sayed Rafik Hamed[1], Darwish Darwish Mahmoud[1] and Saad Mohamed Ahmed[2]

[1]*Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), Agriculture research center, Cairo, Egypt, 11381*
[2]*Veterinary Serum and Vaccine Research Institute (VSVRI), Agriculture research center, Cairo, Egypt, 11381*
*Corresponding author's Email: mohamedsamy2020@hotmail.com, ORCID: 0000-0003-2202-9544

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ABSTRACT

Rift Valley Fever (RVF) is a zoonotic mosquito-borne bunyaviral disease associated with a high abortion rate, neonatal death, fetal malformations in ruminants, and mild to severe disease in humans. The vaccination has significantly reduced the abortion of ewes and mortality of newborn lambs during an outbreak, and induced immunity in cattle. The evaluation of inactivated RVF vaccine required in vivo and in vitro techniques. The present research aimed to evaluate the sensitivity of the Lateral Flow Device (LFD) in comparison with Serum Neutralization Test (SNT) by reference sera to determine the humoral immune response of the sheep vaccinated with an inactivated RVF vaccine. Three batches of inactivated RVF vaccine were inoculated in three sheep groups. Then samples of their sera were collected weekly, and tested by SNT and LFD. It was found that the sensitivity of LFD at a serum dilution of 1:128 was 95%, while SNT carried out at the fourth week after the vaccination showed that antibody titers was 32,64 and 32. On the other hand, LFD had positive results at dilutions of 1:32, 1:128 and 1:64 for the vaccine batches 1, 2 and 3 respectively. These findings suggest the possibility of using LFD for detection of the immune response of vaccinated sheep to the inactivated Rift Valley Fever Virus vaccine, and it could be improved to be more quantitative in future.

Key words: Lateral flow device, Rift valley fever virus, RVFV inactivated vaccine, Vaccine evaluation

INTRODUCTION

Rift Valley Fever (RVF) is a zoonotic arboviral disease accompanied high abortion rate, neonatal death, and fetal malformations in ruminants, and mild to severe clinical symptoms in human (Baptiste et al., 2018). RVFV has tri-segmented single-stranded RNA genome, which is composed of Large (L), Medium (M), and Small (S) segments (Ikegami and Makino, 2011). RVFV caused recurrent outbreaks in African among ruminants and humans, and has caused additionally outbreaks in the Arabian Peninsula (Pepin et al., 2010).

Over the past forty years, RVFV has been detected in African countries outside its traditional enzootic regions, like Egypt in 1977 (El Akkad, 1978). In 1990, a RVF outbreak outside of Africa was confirmed on the Indian Ocean island of Madagascar for the first time. In 2000, Saudi Arabia and Yemen also reported RVFV infection (Morvan et al., 1991), and until 2007, its geographical coverage included the French island of Mayotte in the Comoros Archipelago (Sissoko et al., 2009). Rift Valley Fever Virus was classified as a Category A priority agent by NIAID/NIH, and was selected as an overlap agent by the US Department of Health and Human Services (HHS, 2005) and US Department of Agriculture (USDA, 2005). Vaccination was the most effective countermeasure against RVFV. An ideal vaccine for livestock should be safe, rapid, long-lasting potent with a single dose, and it should sufficiently prevent viremia to be transmitted by competent vectors (Kortekaas et al., 2011).

For the batch release of inactivated vaccines, practically indirect tests have been developed to minimize the use of laboratory animals, which indicated validity of the correlation with a degree of protection percentage of the susceptible animals. Indirect potency tests often include serological tests for post-vaccination of suitable species. Alternative methods such as antigen mass, could be used if it was suitably validated (OIE, 2018). Therefore the present study was conducted to detect the protective antibody titer in vaccinated sheep with inactivated RVFV vaccine as alternative indirect potency test in comparison with Serum Neutralization Test (SNT).

MATERIALS AND METHODS

Ethical approval

The institutional Animal Care and Use Committee of the Central Laboratory for Evaluation of Veterinary Biologics, Cairo, Egypt hereby acknowledges the research manuscript and it has been reviewed by research authority and is considered to be compliant with bioethical standards in good faith.

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Lateral flow device

According to Sayed et al. (2019), Lateral Flow Device (LFD) was developed and prepared in the Central Laboratory for Evaluation of Veterinary Biologics (CLEVB) for detection of IgG against RVFV as a part of an international scientific project funded by U.S. Civilian Research and Development Foundation (CRDF Global), USA.

Cell line

Baby Hamster Kidney (BHK-21) cell line was supplied by the Department of Rift Valley Fever Vaccine Research Department (DRVFVRD), Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo. The cells were grown and maintained according to Macpherson and Stocker (1962), and used in the SNT.

Vaccine

Three batches of locally manufactured gel adjuvant inactivated RVFV vaccine which were prepared from a local isolate serotype were supplied by VSVRI which were selected for the present study. The existing vaccines were previously evaluated by the CLEVB with satisfactory safety and sterility results.

Live virus

Baby Hamster Kidney (BHK-21) cell culture which adapted strain of RVF at a titer of 10^7 $TCID_{50}$/ml (Elian et al., 1996) was supplied by the DRVFVR, and used in SNT to follow up the levels of induced antibodies in vaccinated sheep.

Sheep and experimental design

Fifteen native sheep aged three to six months old were allotted randomly into four groups (four animals in three vaccinated groups and three animals in the control group), and kept in separate insect-proofed stables at animal facility house of CLEVB, Cairo, Egypt. Serum samples of these sheep were previously screened by SNT, and found to be free of specific RVF antibodies (Seronegative). Groups 1, 2 and 3 were vaccinated with RVFV vaccine batches number 1, 2 and 3 respectively, while group 4 was kept without any vaccination as a control group.

Serum neutralization test

Serum samples were collected from all sheep groups at 7, 14, 21 and 28 Days-Post Vaccination (DPV) and three samples of each animal were tested to determine the RVF antibody levels by using the micro titer technique as described by Ferreira (1976). According to Singh et al. (1967), the antibody titer was calculated as the reciprocal of the final serum dilution which was neutralized and inhibited the cytopathic effect in 100 tissue cultures with infective dose 50 ($TCID_{50}$) of RVFV.

Sensitivity test

The test was carried out according to Thrusfield (2007) on Ovine RVFV antiserum obtained from Viral Large and Pet Animal Vaccines Evaluation department in CLEVB (Validated Serum). The serum samples were diluted by using two-fold dilutions (1, 1/2, 1/4. 1/8, 1/16, 1/32, 1/64,1/128, 1/256, 1/512, and 1/1024) to determine the minimal concentration of antibodies, which indicated the positive results with the prepared LFD in comparison with antibodies which were detected by SNT. Each serum dilution was tested by 20 strips of prepared LFD for detection of sensitivity percentage.

$$\text{Sensitivity} = \frac{T+}{(T+)+(F-)} = \frac{T+}{(T+)+(F-)} \times 100 \text{ (Stated as \%)}$$

T+= true positive; F- = False Negative

Potency testing of inactivated Rift valley fever virus vaccine utilizing Lateral Flow Device

All blood samples of the sheep groups were taken at 0, 7, 14, 21 and twenty eighth Days Post-Vaccination (DPV); and the sera were tested by SNT and LFD using two folds of serial dilutions, then the results were recorded and analyzed for interpretation.

RESULTS

Detection of the prepared LFD sensitivity by using standard positive ovine RVFV antiserum in comparison with SNT showed that the minimal concentration of antibodies which showed the positive results with prepared LFD was at dilution 7 $\log_2$ (1/128) with sensitivity percentage of 95 as indicated in table 1. The humoral immune response in vaccinated sheep (groups 1 and 2) indicated that the protective RVFV serum neutralizing antibody titer (1.5 $\log_{10}$) started from the third week of post-vaccination using SNT and red test line of LFD (positive result) at the same dilution, while these findings in vaccinated sheep (group 3) started from fourth week of post-vaccination as showed in table 2 and figure 1.

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Table 1. Sensitivity of prepared lateral flow device using standard positive ovine Rift valley fever virus antiserum in comparison with serum neutralizing test in CLEVB[1], Cairo, Egypt in 2019

Variable		Tested dilutions of standard positive Ovine Rift Valley Fever Virus antiserum										
		1	**1/2**	**1/4**	**1/8**	**1/16**	**1/32**	**1/64**	**1/128**	**1/256**	**1/512**	**1/1024**
*Serum Neutralizing Test		+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
**Lateral Flow Device (20 strips)	T+	20	20	20	20	20	20	20	19	0	0	0
	T-	0	0	0	0	0	0	0	0	15	0	0
	F-	0	0	0	0	0	0	0	1	0	0	0
	F+	0	0	0	0	0	0	0	0	5	0	0
Sensitivity % of LFD***		100	100	100	100	100	100	100	95	25	0	0

*-ve = result of SNT refer to CPE while +ve = refer to neutralization of virus by serum Antibody. **T+ = true positive, T- = true negative, F+ = false positive, F- = False negative; ******* LFD: Lateral Flow Device; [1]Central Laboratory for Evaluation of Veterinary Biologics.

Table 2. Evaluation of the humoral immune response of vaccinated sheep with inactivated Rift valley fever virus vaccine using lateral flow device and serum neutralizing test in CLEVB[1], Cairo, Egypt in 2019

Serum dilutions	*Serum Neutralizing Test (SNT)				**Lateral Flow Device (LFD)			
	Group 1							
	1wpv* **	**2wpv**	**3wpv**	**4wpv**	**1wpv**	**2wpv**	**3 wpv**	**4 wpv**
1/2	*+ve	+ve	+ve	+ve	**+ve	+ve	+ve	+ve
1/4	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
1/8	*-ve	+ve	+ve	+ve	**-ve	+ve	+ve	+ve
1/16	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve
1/32	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve
1/64	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
1/128	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	Group 2							
	1wpv	**2wpv**	**3wpv**	**4 wpv**	**1wpv**	**2wpv**	**3 wpv**	**4 wpv**
1/2	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
1/4	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
1/8	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
1/16	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
1/32	-ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve
1/64	-ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve
1/128	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
	Group 3							
	1wpv	**2wpv**	**3wpv**	**4 wpv**	**1wpv**	**2wpv**	**3 wpv**	**4 wpv**
1/2	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
1/4	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
1/8	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
1/16	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve
1/32	-ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve
1/64	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
1/128	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

*-ve = result of SNT refer to cytopathic effectwhile +ve refer to neutralization of virus by serum antibody. **+ve = result of LFD refer to the presence of antibodyin serum while –ve means absence of antibody. ***wpv = week post-vaccination; [1]Central Laboratory for Evaluation of Veterinary Biologics.

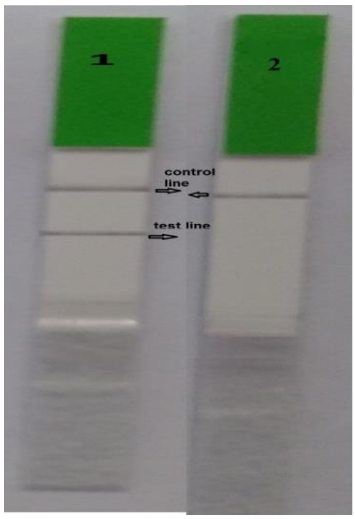

Figure 1. Interpretation of the prepared lateral flow device for detection of IgG antibodies against Rift valley fever virus. 1: Positive result (Test and control lines are seen); 2: Negative result (Control line is only seen)

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DISCUSSION

The sporadic reports of Rift Valley Fever (RVF) outbreaks in neighbor countries in addition to Inter-epizootic periods of RVFV in Egypt illustrated that there was a continuous risk of infection in Egypt. Control of RVF disease in Egypt depended mainly on the vaccination of cattle, sheep and goats. Two types of inactivated RVF vaccines were produced in Egypt (gel and oil) limited a great extent the possibilities of RVFV outbreaks in Egypt (Ahmed, 2011; GOVS, 2008).

The evaluation of inactivated RVFV vaccine in sheep was performed to assess the vaccine's safety and efficacy, while sheep are very sensitive domestic animals, and to demonstrate the specific antibody titer in the sera of vaccinated animals using SNT, indicated 5 log2 (32) or a neutralizing index which is not less than 1.5 after 28th days post-vaccination, confirm the identity of the vaccine virus, and the protection margin (Heba et al., 2020).

The present study was aimed to evaluate the efficacy of existing local commercial inactivated RVFV vaccine batches in sheep using SNT and LFD for the development of an alternative indirect potency test. The sensitivity of the LFD was assessed by comparison to the technique considered as a reference in the present study. The new prepared LFD indicated a diagnostic sensitivity of 95% at the dilution of 7 $\log_2$ (1/128) for detection of RVFV antibodies compared with SNT. Thus, the sensitivity test for LFD compared to SNT was agreed with Sastre et al. (2016) who compared the sensitivity of SNT and the Enzyme-Linked Immunosorbent Assay (ELISA) for simultaneous detection of antibodies against African and classical swine fever viruses using developed duplex lateral flow assay.

The humoral immune response in vaccinated sheep (group1, 2 and 3) with local commercial inactivated RVFV vaccine batches (1,2 and 3) respectively indicated a protective neutralizing antibody titer (1.5 $\log_{10}$) 5 Log_2 at third Week Post-Vaccination (WPV) for groups 1 and 2, but at fourth WPV for group 3, while the LFD showed positive results at 5 $\log_2$ at third WPV for groups 1 and 3, and at second WPV for group 2. Regarding the use of LFD for this purpose, Anouk et al. (2016) applied quantitative user Lateral Flow Assays (LFAs) for four immune markers in the whole blood samples from a longitudinal *Bacillus Calmette*–Guérin (BCG) vaccination. On the other hand, Ibrahim et al. (2017) developed Lateral flow immunochromatographic test to detect *Salmonella enteritidis* by specific antibodies in the chicken sera.

CONCLUSION

This study has shown that the LFD was appropriate for semi-quantitative evaluation of serum antibodies induced by RVFV vaccine, and was urgently needed to instantly assess the sera efficacy in the dubious batches of the vaccine. It is possible to use the LFD to detect the immune response of vaccinated sheep to the inactivated RVFV vaccine, and it could be improved in the future to be more quantitative.

DECLARATIONS

Authors' contribution

Dr. Mohamed Abousenna and Dr. Rafik Sayed performed in vitro tests (LFD sensitivity, SNT, and potency using LFD) and interpretation of results. Dr. Darwish Mahmoud conducted in vivo trial (inoculation, sampling and monitoring) while Professor Dr. Mohamed Saad supervised all the research processes, experimental design, and revision. All authors approved the final manuscript.

Competing interests

The authors declare that they have no conflict of interests.

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