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Comparison Between two Commercial Kits used for Detection of Anti-Rift Valley Fever Antibodies in Sheep Vaccinated with Smithburn Vaccine

Samir Mohamed Ali[1]*, Mansour Hashim Abdel Baky[2], Mohamed Hamd Al-Blowi[3]

[1]Veterinary Serum and Vaccine Research Institute (VSVRI); [2]Central Laboratory for Evaluation of Veterinary Biologics; [3]Animal Resources Administration, Ministry of Agriculture, Riyadh, Saudi arabia.

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The Rift Valley Fever (RVF) virus is a mosquito-borne pathogen with high potential to cause explosive outbreaks of severe human and livestock diseases. Although historically limited to Africa and Madagascar, more recently severe outbreaks of the disease were recorded in 2000 on the Arabian Peninsula and in 2008 on the Archipelago of Comoros, including the French department of Mayotte (Sissoko *et al*., 2009). Rift Valley Fever infections in livestock are characterized by an acute hepatitis, abortion and high mortality rates, particularly in young animals. Human infected with RVFV typically develop a mild self limited febrile illness, but retinal degeneration, severe encephalitis, fatal hepatitis and hemorrhagic fever may also occur (Swanepoel and Coetzer, 2004). In this study, eight hundred serum samples were collected from eight sheep consignment imported from different African countries previously vaccinated with RVF live attenuated smithburn vaccine 3-4 weeks ago before transportation to kingdom of Saudi Arabia (KSA). In KSA, routine detection of RVF antibodies was done using indirect sandwich ELISA (Enzyme Linked Immunosorbant Assay) instead of viral neutralization test (VNT) for monitoring of RVF immunostatus in sera of imported sheep.

In this study we evaluate the diagnostic performance of two existing commercial serological assays in evaluation of antibodies against RVF post vaccination. An inhibition ELISA and Direct ELISA compared with Sandwich ELISA as a guide test. Sera from sheep vaccinated with smithburn vaccine of RVFV taken as 100 samples from different consignments of the imported African sheep to KSA according to the animal quarantine laws of Jeddah Islamic seaport as shown in table 1, were tested using different types of ELISA, as Sandwich ELISA according to (Paweska *et al*., 2003), the ELISA is based on a sandwich format in which the plates were coated with mouse anti-RVF serum and then reacted with antigen. Tested sera were applied and specific anti-RVF IgG antibody was detected with an anti-sheep IgG HRPO conjugate and ABTS substrate, the reagents have been irradiated to inactivate RVF virus, inhibition ELISA according to (Paweska *et al*., 2005b) using RVF C-ELISA (Inhibition ELISA) jointly produced by BDSL (biological Diagnostic Supplies Limited) flow Laboratories and special pathogen units, NICD, South Africa, and Direct ELISA using ID screen RVF Multi-species ED Kit from ID. Vet innovative diagnostic- France for the detection of anti-RVFV nucleoprotein antibodies in serum samples of bovine, ovine, caprine and equine. The direct ELISA method makes use of the presence of two Fab (on IgG) or ten Fab (on IgM) to allow for the early detection of anti-RVF antibodies. A first fab binds the immunoglobulins to the microplate and the other Fab a peroxidase antigen used as conjugate, so when samples and control added to the microwells. Anti-RVF antibodies, if present, form an antibody-antigen complex. Plates were washed and a RVF nucleoprotein peroxidase conjugate is added to the microwells. It fixes to the free Fab of bound serum anti-RVF antibodies

The statistical analysis of the study in table (2) showed that the specificity of inhibition ELISA was 99.3% while for Direct ELISA was 98.8 % in compared with Sandwich ELISA, also the sensitivity of Inhibition ELISA was 90.3%, while for Direct ELISA was 83.7% compared with Sandwich

*Corresponding author: Samir Mohamed Ali

E-mail address: samir.ali9@gmail.com

ELISA. The increasing demand for high-quality diagnostic assays worldwide aims to ensure that the best protection of human and animal populations and to facilitate the free circulation of animals and animal products in international trade. Diagnostic laboratories are suppliers of analytical test results that must be scientifically valid, quality-controlled and based on internationally recognized methods and standards (Caporale *et al*., 1998; Nannini *et al*.,1999; Wiegers, 2000). In KSA, the evaluation of immune response of the vaccinated animals with smithburn vaccine is done by detection of humeral immunity which is sufficient for protection against RVF (Niklasson *et al*., 1984), since VNT was laborious, expensive, and requires 5-7 days for completion. It can performed only when standardized stock of live virus and tissue cultures are available. Consequently, it is rarely used, and then only in highly specialized reference laboratories (Paweska *et al.,* 2005a), and since the sensitivity of ELISA diagnostic test (sandwich ELISA) in detecting IgG antibodies of RVF had been reported to be 99% to 100% and 99.1% to 99.9 % specificity for sheep (Paweska *et al*., 2003). So, in this study we depend up on using of sandwich ELISA for detection of IgG as standard or guide test instead of VNT in comparison with other types of ELISAs.

The present study showed that Inhibition ELISA

Table 1. Details of the vaccinated sheep consignments imported from African countries to KSA.

Consignment No.	No. of sheep per consignments	Origin of the consignments	Time of samples collection (W.P.V.)
1	8263	Eriteria	4 W.P.V.
2	16978	Somalia	4 W.P.V.
3	5860	Sudan	4 W.P.V.
4	7710	Djibouti	4 W.P.V.
5	10111	Somalia	3 W.P.V.
6	4153	Eriteria	4 W.P.V.
7	10491	Sudan	4 W.P.V.
8	5788	Djibouti	3 W.P.V.

W.P.V.: Week Post Vaccination

Table 2. Specificity and sensitivity of Commercial Inhibition and Direct ELISA Kits in comparison with sandwich ELISA Kit for detection of Rift and Valley Fever virus antibodies in sera of sheep vaccinated with Smithburn vaccine 3-4 weeks before shipping from Coast of Africa to Kingdom of Saudi Arabia.

Serial No. of consignment	Serological results			Specificity %		Sensitivity %	
	No. of +ve samples /100						
	Sandwich ELISA	Inhibition ELISA	Direct ELISA	Inhibition ELISA	Direct ELISA	Inhibition ELISA	Direct ELISA
1	65	58	55	97.2	97.2	90.3	86.7
2	70	65	56	96.8	96.8	95.3	83.3
3	40	35	30	100	100	88.9	80
4	51	45	41	100	100	89.5	83.6
5	45	37	33	100	100	89.4	82
6	59	53	50	100	98.3	90.8	85.6
7	47	43	40	100	97.7	92.2	85.5
8	45	39	36	100	100	88.2	83.3
	(52.4%)	(46.9%)	(42.6%)	(99.3%)	(98.8%)	(90.3%)	(83.7%)

(-): Arithmetic Mean

Statistical methods of (Greiner and Gardener, 2000) were used to estimate: sensitivity (D-Se) = [Tp / (Tp+Fn)] x 100, And specificity (D-Sp) = [Tn / (Tn+Fp)] x 100

Where Tp is the true positive sera, Fn the false-negative sera, Tn the true-negative sera and Fp the false-positive sera.

Kits gave 99.3% specificity and 90.3 % sensitivity when compared with sandwich ELISA, while Direct ELISA Kits gave 98.8% specificity and 83.7% sensitivity when compared with sandwich ELISA, that means our results agreed with Paweska *et.al*. (2005b), who said that inhibition ELISA can be used as highly accurate diagnostic tool in disease surveillance and control programs, import/export veterinary certification, and for monitoring of immune response in vaccine. As an accurate, robust and safe, it has the potential to replace traditional diagnostic methods using live antigens that pose health risk, necessitating their use being restricted to high containment facilities outside endemic areas. While for Direct ELISA Kits, it considered a new, rapid and specific tool in the detection of RVF antibodies in animal sera, consuming the least time between other commercial Kits in this study, but it was the least in sensitivity and specificity between the other ELISA Kits used in this study.

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