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STANDARDIZATION OF BOTH RIFT VALLEY FEVER CELL LYSATE AND SUPERNATANT ANTIGENS USED IN ELISA COMPARING WITH SERUM NEUTRALIZATION TEST AND AGAR GEL PRECIPITATION TEST.

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SUMMARY

To compare between different serological tests and in trial to standardize both RVF cell lysate and cell supernatant antigens, 1377 serum samples collected from different Egyptian Governorates from different species (sheep, goat, cattle and buffalo) were tested for the presence of IgG and IgM percent using ELISA. The result ranged from 61.06 : 63.1 for IgG and 13.73 : 15.3 for IgM. ELISA, NI and AGPT results were higher from 1 : 4 months than 1 : 9 months. At the same time ELISA gave higher figure of sensitivity than NI and AGPT.

INTRODUCTION

Rift Valley Fever (RVF) has been recognized exclusively in African countries, with an underlying association with high rainfall and dense populations of vector mosquitoes. Many epizootic outbreaks of RVF outside sub - Saharan Africa were recorded in animals and humans in Egypt in 1977 - 78, Mauritania in 1987 and again in Egypt in 1993. Also laboratory infections have been recorded in other parts of the world (OIE, 2000).

ELISA is the most broadly applicable assay for serological testing, and is recommended for widespread use in regions with suspected RVFV activity. Kits for testing human, sheep and cattle sera for the presence of both RVFV - specific IgG and IgM were used (WHO 1993).

ELISA provide to be more sensitive than HI or CFT and almost as sensitive as the Plaque Reduction Neutralization Test (PRNT) in detecting specific antibodies (Niklasson, et al ., 1984).

The specificity and sensitivity of ELISA were determined by the use of vaccinated sheep serum samples, compared with the virus neutralization

test (Paweska, et al., 1995).

(Taha, et al.., 2001) used ELISA technique to detect the incidence of both IgG and IgM antibodies to RVF virus in vaccinated sheep, goat, cattle and buffalo.

So the object of this study is to standardize both RVF cell lysate and supernatant antigens used in ELISA in comparison with NI and AGPT.

MATERIAL and METHODS

1. Virus:

Rift Valley Fever Zagazig Human strain (ZH501), isolated from patient in Zagazig, Sharqyia Governorate (1977) was used in this study. It was kindly supplied by RVF Vaccine Production Department, Serum and Vaccine Research Institute, Abbassia, Cairo.

2. Samples:

A total of 1377 serum samples were collected from different Egyptian Governorates 1 to 9 months post - vaccination with one dose of inactivated RVF vaccine, 425 sheep sera sample, 315 goat sera sample, 357 cattle sera sample and 280 buffalo sera sample were used after inactivation at 56°C for 30 minutes.

3. Neutralization Index:

According to Walker et al., (1970).

4. Agar Gel Precipitation Test:

According to Gihan (1990).

5. ELISA:

To detect IgG and IgM antibodies in sera sample according to Voller et al., (1976); Niklasson, et al ., (1984) and Meegan, et al., (1987).

6. RVF antigen (used in ELISA):

Lyophilized cell lysate and supernatant RVF antigen was used for detection for RVF antibodies using ELISA technique, it was prepared according to Ksiazek, et al., (1989) and Elian and Botros, (1997).

RESULTS

Table (1) : Sero - conversion of samples collected at different intervals post one dose vaccination using IgG and IgM ELISA technique

Animal species	Total No. of animals	ELISA		negative (both IgG & IgM)
		IgG + ve	IgM + ve	
Sheep	425	268 (63.1%)	65 (15.3%)	92 (21.6%)
Goat	315	198 (62.86%)	47 (14.92%)	70 (22.22%)
Cattle	357	218 (61.06%)	49 (13.73%)	90 (25.21%)
Bffalo	280	174 (62.14%)	39 (13.93%)	67 (23.93%)

Table (2) : Sero - conversion rate by day 30 till 9 months after one dose vaccination (using ELISA ìIgGî, NI and AGPT)

Species	Test		1-4 months	1-9 months
Sheep and Goat	ELISA	%	68.15	63.13
		Mean titer	238 - 250	222- 235
	NI	%	62.4	50.8
		Mean index	1.7 - 3.2	1.47 - 2.9
	AGPT%		47.6	38.24
Cattle and Bffalo	ELISA	%	71.9	61.2
		Mean titer	234 - 247	201 - 129
	NI	%	65.1	51.3
		Mean index	2.0 - 3.4	1.7- 2.4
	AGPT%		48.3	39.5

Table (3): Sensitivity of ELISA comparing with NI and AGPT

Species	Test	1-4 months	1-9 months
Sheep and Goat	ELISA / NI	91.5%	80.4%
	ELISA / AGPT	69.8%	60.5%
Cattle and Bffalo	ELISA / NI	91.4%	83.8%
	ELISA / AGPT	67.8%	64.5%

Table (1) indicated that the positive IgG percent ranged from 61.06 - 63.1 in all tested sera comparing with 13.73 - 15.3 for IgM.

Table (2) illustrated a high figure percent of ELISA or NI and AGPT in all tested sera at 1- 4 week period.

Table (3) ELISA test had given a high figure of sensitivity than NI and AGPT for all the experiment period

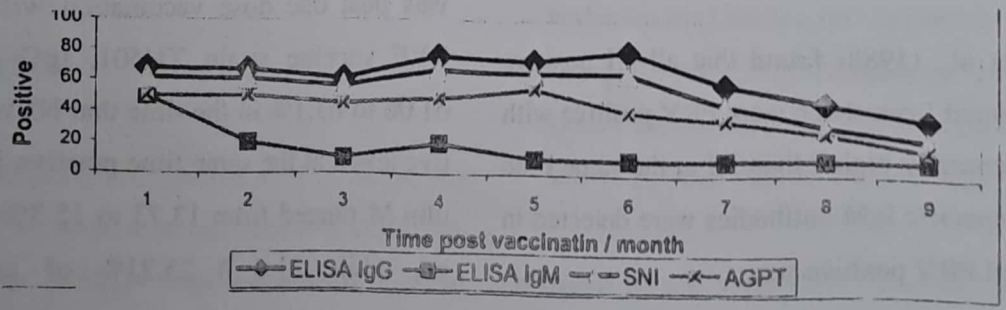

Fig. (1): Seroconversion of sheep and goat samples collected at different intervals post one dose vaccination

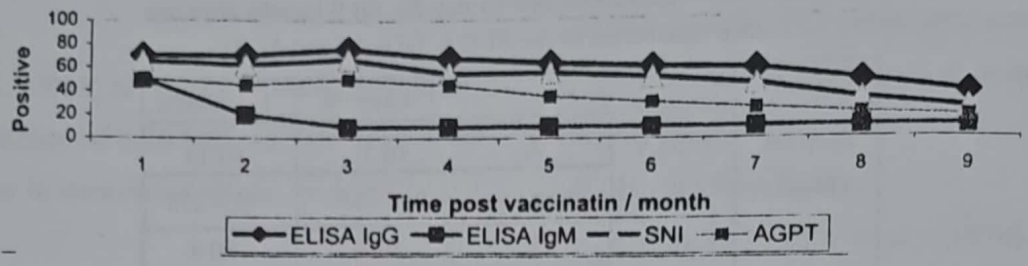

Fig. (2): Seroconversion of cattle and buffalo samples collected at different intervals post one dose vaccination

DISCUSSION

Rift Valley Fever (RVF) is an acute or peracute, mosquito - borne disease, it is most sever in sheep, cattle and goats, causing high mortalities in neonates and abortion in pregnant animals (Swanepoel & Coetzer, 1994). Different serological tests were done:

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Eisa (1984) used AGPT technique for preliminary survey of domestic animals for RVF virus in Sudan and he could detect an incidence of 34.3% in sheep, 33.2% in cattle, 22% in goats, 7.9% in camels and 4% in donkeys.

Niklasson and Gargan (1985) concluded that ELISA is useful in detecting arthropod infected with RVF in the field.

Botros, et al., (1988) found that all HI positive sera collected from sheep were PRV positive with a titers generally higher than HI at the same time no RVF specific IgM antibodies were detected in the HI and PRV positive sera.

While, Guillaud, et al., (1988) used ELISA technique to detect the prevalence of antibodies to RVF virus in sheep and goat in Senegal.

Swanepoel, et al., (1986) applied nine serological techniques to compare and monitoring the response to infection with RVF in sheep. All tests could be used satisfactory for the serological diagnosis.

Wilson, et al., (1994) used ELISA technique to investigate positive infection and transmission of RVF virus to human within an endemic focus in Senegal.

The results of the previously mentioned authors are in parallel with the results indicated in this study. Regarding to table (1) which explain sero - conversion of samples collected at different intervals post one dose vaccination with inactivated RVF vaccine strain ZH501, IgG ranged from 61.06 to 63.1% in the time that NI was in protective level, at the same time positive immunoglobulin M ranged from 13.73 to 15.3% were detected, while 21.6 ñ 25.21% of samples were

negative for both IgG and IgM that indicate possibility of these animals to be subjected for infection as it considered susceptible.

On the other hand table (2) and figure (1 & 2) illustrated high figure of positive percent of ELISA or NI and AGPT in all tested sera at 1 - 4 months period than 1 - 9 months, using booster dose which gave high figure as detected by these obtained results were correlated to studies done by Taha, (1980) and Gihan, (1990).

From the previous results it is clear that ELISA gives a higher figure of sensitivity than NI and AGPT for all the experiment period as shown in table (3), this agrees with (Paweska, et al., 1995) who proved that ELISA was highly sensitive and specific as compared with VN and HI tests.

Finally from the previous studies it is clear that ELISA considered as sensitive and specific test for detecting different antibodies against RVFV, and at the same time boostering is important to improve the immune system the animals and could be used as monitoring methods for vaccination programmes.

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