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Seroprevalence and Economic Impact of Rift Valley Fever Among Small Ruminants

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ABSTRACT

Rift Valley Fever (RVF) is an acute, mosquito-borne viral disease that has a significant global threat to humans and livestock. The seroprevalence and economic impact of RVF among small ruminants in some localities in Egypt were studied. The antibodies against RVF were detected using three serological tests ELISA, AGPT and SNT. The obtained results showed high incidence of RVF in sheep and in El-Sharkia and Marsa Matrouh than in goat and in El-Dakhalia and El-Kaliobia governorates. Moreover, the SNT and ELISA showed higher sensitivity than AGPT. The SNT was sensitive and save the money in comparison with other test but less specific. Therefore, ELISA is most sensitive and economic test for detection of RVF among small ruminant.

Key words: RVF, small ruminant, serology, economic impact, immunefluorescent

INTRODUCTION

Rift Valley Fever (RVF) is arthropod-borne viral disease important in domesticated ruminants. This disease is characterized by high mortality rates in young animals and abortions in pregnant ruminants. Rift valley fever is endemic in Sub-Saharan Africa. Epidemics occur in this region when heavy rainfalls cause infected mosquito eggs to hatch and large numbers of susceptible animals are present (Peters, 1997; Bird *et al*., 2007; Gerdes, 2004).

Rift valley fever virus is a mosquito-borne virus related with epidemics in livestock and humans (Bird *et al*., 2007; Memish *et al*., 2015). The disease and the factors behind its emergence is something many African countries are working for to get a better understanding of epidemiological and surveillance studies in animal have been performed in some exposed countries (Jeanmaire *et al*., 2011). The studies have also evaluated risks (Anyamba *et al*., 2010), developed methods (Paweska *et al*., 2003a; Fafetine *et al*., 2007; Van Vuren *et al*., 2007; Boussini *et al*., 2014) and considered transmission to new areas (Balkhy and Memish, 2003).

Egypt suffers from several outbreaks, El-Sharqia in 1977-1978, Aswan in 1993, Assiut and Aswan governorates in 1997 (Abd El-Rahim *et al*., 1999; Paweska *et al*., 2008) and finally in Kafr El-Sheikh governorate in 2003 (Hanafi *et al*., 2011).

Diagnosis of RFV disease can be performed when serological tests such as CFT, ELISA, indirect immunefluorescent technique and virus neutralization tests are used in combination with clinical

observation and epidemiological history (Shawky, 2000; Ellis *et al*., 2014; Gerdes, 2004). ELISA is rapid and sensitive, specific and useful tool to reveal infected animal in endemic areas or during an epizootic (Paweska *et al*., 2003b, 2008) but the golden standard method for RVFV diagnosis in virus isolation with RT-PCR which is a specific sensitive tool for RVF diagnosis (Sall *et al*., 2002; Paweska *et al*., 2005; Li *et al*., 2015).

Serological survey is an effective tool of active surveillance and control of diseases. It is usually practiced to determine the distribution of infection, monitor herd immunity (Chevalier *et al*., 2005; Kamal, 2011; Chevalier, 2013; Glancey *et al*., 2015).

The exact situation of the circulation of the RVF virus among farm animal is still open question. The primary aim of this study was to evaluate the seroprevalence of Rift Valley Fever Virus (RVFV) antibodies in sheep and goats in some localities in Egypt with special reference to its economic importance and comparing between different tests for determination the highly sensitive and economic test which can save the costs of detection.

MATERIALS AND METHODS

Samples: About 640 blood samples were collected from sheep and goats from some governorates (El-Kaliobia, El-Dakahlia, El-Sharkia and Marsa Matrouh), from apparent healthy animals, showing fever and other's showing history of abortion in different seasons as shown in Table 1.

Serological examination

Agar Gel Precipitation (AGP) test: The antibodies against RVF was detected by AGP test according to method described by (Eisa, 1984).

Serum Neutralization Test (SNT): It was conducted according to a previously described method (Woods *et al*., 2002) with minor modifications. Virus stocks consisted of the supernatant fluids from vero cell cultures infected with the AR 20 368 isolate of RVFV stored in 0.5 mL volumes at -70°C. Sera were inactivated at 56°C for 30 min and serial twofold dilutions were prepared in 50 μL volumes of culture medium in flat-bottomed 96-well tissue-culture microplates (Nunc, Roskilde, Denmark). Equal volumes of the virus suspension containing a 100 $TCID_{50}$ were added to each well and the serum virus mixtures incubated at 36°C for 60 min before seeding with 2×10^5 vero cells per well in 100 μL Eagle's essential medium (Cambrex, East Rutherford, NJ, USA) containing 8% fetal calf serum (Delta Bioproducts, Johannesburg, South Africa), 100 IU penicillin, 100 μg streptomycin and 0.25 μg fungizone per milliliter of tissue culture medium. It was recommended that the protective titer of RVF neutralizing antibodies should be 1/40.

Enzyme Linked Immune Sorbent Assay (ELISA): IgM capture ELISA-the IgM capture ELISA was performed following a method previously described by Paweska *et al*. (2003a). Briefly, plates

Table 1: Number of examined animals for incidences of RVF among different localities

	Sheep		Goat		Total	
Locality	No.	%	No.	%	No.	%
El-Kaliobia	130	20.32	70	10.93	200	31.25
El-Dakahlia	69	10.79	28	4.37	97	15.16
El-Sharkia	100	15.62	99	15.47	199	31.09
Marsa-matrouh	82	12.81	62	9.69	144	22.50
Total	381	59.54	259	40.46	640	100.00

RVF: Rift valley fever

were coated overnight at 4°C with 100 mL rabbit anti-sheep IgM (Zymed Laboratories, Inc.) diluted 1:500 in PBS. After incubation plates were washed three times with the washing buffer (0.1% Tweeen-20 in PBS) and incubated with 10% skim milk in PBS in a moist chamber for 1 h at 37°C. Plates were washed 3 times with the washing buffer and duplicate volumes of 100 mL of test and control sera (NICD-SPU) diluted 1:400 added in rows A-D, 1-12, respectively to the corresponding wells in the bottom half of the plate (rows E-G: 1-12). After incubation at 37°C for 1 h and washing 6 times with washing buffer, 100 mL of the virus (NICD-SPU) and control antigen (NICD-SPU) diluted 1:200 in PBS containing 2% skim milk were added to both the rows of the top half of the plate (rows A-D: 1-12) and of the bottom half of the plate (rows E-G: 1-12), respectively. Plates were incubated for 1 h at 37°C, washed 3 times with the washing buffer and mouse anti-RVF serum diluted 1:3.000 added to each well of the plate. Plates were incubated again for 1 h at 37°C, washed 3 times with the washing buffer and goat anti-mouse IgG conjugated with peroxidase (Zymed Laboratories, Inc.) diluted 1:4.000 added to each well for 1 h at 37°C. Plates were washed 6 times with the washing buffer and the reaction was developed by the addition of ABTS (KPL Laboratories, Inc.). After incubation in a dark at room temperature for 30 min the reaction was stopped by the addition of 100 mL 1% SDS. Optical Densities (OD) were determined at 405 nm. The net OD values were first recorded for each serum as the value determined with the RVFV antigen minus the value determined with the control antigen and subsequently converted into percentage of the OD value of a high positive control serum as previously mentioned. Threshold PP values of sheep and goat sera producing PP values $\geq$7.9 and $\geq$9.5, respectively, were considered to be positive.

Statistical analysis: Statistical and economic analysis was performed using the SPSS software package (SPSS., 2004). Chi-square analysis was used to test the significance of differences in the incidence of RVF in sheep and goat , the data from diseased male and female were also compared. The means for costs of samples were recorded as Least Squares Means±Standard Errors (LSM±SE), p-values of less than 0.01 were considered to represent a statistically significant difference.

RESULT

Incidences of Rift valley fever among sheep: The results cleared in Table 2 and 3 indicated the significant differences in the incidences of RFV among different localities ($p<0.01$). In addition,

Table 2: Incidences of RVF among examined sheep of different localities using different tests

Locality	Test	Total number	Positive	Negative	Incidence (%)
El-Kaliobia	SNT	130	7	123	5.83
	ELISA	130	7	123	5.83
	AGPT	130	5	125	3.84
El-Dakahlia	SNT	69	10	59	14.49
	ELISA	69	10	59	14.49
	AGPT	69	6	63	8.69
EL-sharkia	SNT	100	39	61	39.00
	ELISA	100	38	62	38.00
	AGPT	100	30	70	30.00
Marsa-Matrouh	SNT	82	14	68	17.07
	ELISA	82	14	68	17.07
	AGPT	82	8	74	9.75

Chi-square: 33.35**, **: Significant at $p<0.01$, RVF: Rift valley fever, SNT: Serum neutralization test, ELISA: Enzyme linked immune sorbent assay, AGPT: Agar gel precipitation

Table 3: Incidence of RVF among examined sheep using different tests and costs of tests

Locality	Test	Total number	Positive		Negative		Incidence (%)		Cost of test/sample (L.E)
			Male	Female	Male	Female	Male	Female	
El-Kaliobia	SNT	130	5	2	2	96	3.85	1.54	15±2.55[b]
	ELISA	130	5	2	2	96	3.85	1.54	20±3.22[a]
	AGPT	130	4	1	28	97	3.08	0.77	7±0.55[c]
El-Dakahlia	SNT	69	7	3	23	36	10.14	4.35	15±2.22[b]
	ELISA	69	7	3	23	36	10.14	4.35	20±3.12[a]
	AGPT	69	4	2	26	37	5.79	2.89	7±0.21[c]
EL-sharkia	SNT	100	7	32	13	48	7.00	32.00	15±2.56[b]
	ELISA	100	7	31	13	49	7.00	31.00	20±0.22[a]
	AGPT	100	5	25	15	55	5.00	25.00	7±0.12[c]
Marsa-Matrouh	SNT	82	7	7	19	49	8.54	8.54	15±1.55[b]
	ELISA	82	7	7	19	49	8.54	8.54	20±2.23[a]
	AGPT	82	4	4	22	52	4.88	4.88	7±0.15[c]
Chi-square			25.55**		36.37**		33.47**		

**: Significant at p<0.01, For different tests of each locality: Means within the same column of different litters are significantly different at (p<0.01), RVF: Rift valley fever, SNT: Serum neutralization test, ELISA: Enzyme linked immune sorbent assay, AGPT: Agar gel precipitation

Table 4: Incidence of RVF among examined goats of different localities using different tests

Locality	Test	Total number	Positive	Negative	Incidence (%)
El-Kaliobia	SNT	70	3	67	4.28
	ELISA	70	3	67	4.28
	AGPT	70	1	69	1.42
El-Dakahlia	SNT	28	4	24	14.28
	ELISA	28	4	24	14.28
	AGPT	28	2	26	7.14
EL-sharkia	SNT	99	20	79	20.20
	ELISA	99	20	79	20.20
	AGPT	99	18	81	18.18
Marsa-Matrouh	SNT	62	3	59	4.83
	ELISA	62	3	59	4.83
	AGPT	62	1	61	1.61

Chi-square: 35.12**, **: Significant at p<0.01, RVF: Rift valley fever, SNT: Serum neutralization test, ELISA: Enzyme linked immune sorbent assay, AGPT: Agar gel precipitation

there a significant (p<0.01) differences in the sensitivity of the examined tests to RFV virus (p<0.01), ELISA is most sensitive test to detect antibodies against RVF in small ruminant.

The results cleared that, the higher incidences observed in El-Sharkia, Marsa Matrouh while, the incidence of the disease was lower observed in El-Dakahlia and El-Kaliobia.

The result of incidence of RFV in sheep revealed that, the higher incidences in male higher than that of female except in El-Sharkia and the differences in male and female incidence of RVF differ significantly at (p<0.01).

Incidences of Rift valley fever among goat: The obtained results in Table 4 indicated the significant differences in the incidences of RVF among different localities (p<0.01). Also, there a significant (p<0.01) differences in the sensitivity of the examined tests to RVF virus (p<0.01), ELISA have high sensitivity to detect antibodies against RVF in small ruminants.

The results cleared that, the higher incidences of the disease observed in El-Sharkia and El-Dakahlia while, the lower incidence observed in Marsa Matrouh and El-Kaliobia.

The obtained results in Table 5 showed that, the higher incidences of RVF in female than in male and the differences in male and female incidence of RVF are significantly at (p<0.01).

Table 5: Incidence of RVF among examined goats using different tests and costs of tests

Locality	Test	Total number	Positive		Negative		Incidence (%)		Cost of test/sample (L.E)
			Male	Female	Male	Female	Male	Female	
El-Kaliobia	SNT	70	1	2	9	58	1.42	2.85	15±2.55[b]
	ELISA	70	1	2	9	58	1.42	2.85	20±3.22[a]
	AGPT	70	1	-	9	60	1.42	-	7±0.55[c]
El-Dakahlia	SNT	28	1	3	9	15	3.57	10.71	15±2.22[b]
	ELISA	28	1	3	9	15	3.57	10.71	20±3.12[a]
	AGPT	28	1	9	1	17	3.57	32.14	7±0.21[c]
EL-sharkia	SNT	99	2	18	18	61	2.02	18.18	15±2.56[b]
	ELISA	99	2	18	17	62	2.02	18.18	20±0.22[a]
	AGPT	99	2	16	18	63	2.02	16.16	7±0.12[c]
Marsa-Matrouh	SNT	62	-	3	8	51	-	12.90	15±1.55[b]
	ELISA	62	-	3	8	51	-	12.90	20±2.23[a]
	AGPT	62	-	1	8	53	-	1.61	7±0.15[c]
Chi-square			27.56**		35.33**		35.22**		

**: Significant at ($p<0.01$), For different tests of each locality: Means within the same column of different litters are significantly different at ($p<0.01$), RVF: Rift valley fever, SNT: Serum neutralization test, ELISA: Enzyme linked immune sorbent assay, AGPT: Agar gel precipitation

DISCUSSION

Rift Valley Fever (RVF) is a mosquito-borne viral disease that typically occurs in various areas of Africa, where virus activity may be vary from a low-level enzootic cycle to explosive outbreaks covering large areas. Usually, RVFV spreads to other areas, including northward into Egypt in 1977 and Eastward across the Red Sea into Saudi Arabia and Yemen in 2000 (Andriamandimby *et al*., 2010; Hanafi *et al*., 2011; Nanyingi *et al*., 2015). How RVFV travels is unclear but probably involves movement of infected livestock or mosquitoes. The serological study of antibodies against RVF in sheep and goat in some localities in Egypt revealed that high incidence rate of the disease in El-Sharkia and Marsa Matrouh but the lower incidence rate obtained in El-Dakahlia and El-Kaliobia.

The obtained result may be attributed to the higher level of mosquitoes that act as an intermediate host for transmitting the RVF among sheep and goats than other governorates. The seroprevalence in this study is higher in sheep than in goat and hence show the same tendency as the studies of (Fafetine *et al*., 2013; Sindato *et al*., 2015) in Zambezia. From past epizootics, the experience is that sheep are the most susceptible species (Woods *et al*., 2002). Seroprevalence study in Gaza, Mozambique in 2011 was showing the opposite (Engstrom, 2012). In that study 11% of sheep and 21% of goats were positive, Engstrom mentions that the higher seroprevalence in goats might have to do with the higher number of goats in the sampled area.

The higher incidence of RVF in female than male may be due to it commonly under stress conditions than male especially during pregnancy that causes the female of a high sensitivity to RVF than male.

The result of different serological test for detection of antibodies against RVF virus revealed that SNT and ELISA have higher sensitivity than AGPT, these results are in agreement with Eweis *et al*. (2008).

The SNT is considered as high specific and sensitive but requires cell culture and animal facilities that can be only performed with live virus with special containment to laboratories. Furthermore, it needs long time to be performed, so that the ELISA is considered as sensitive and specific test for detecting different antibodies which gave result in parallel with SNT, also ELISA used safe, robust and accurate diagnostic tool in disease surveillance and control programmers (Swanepoel *et al*., 1986; Madani *et al*., 2003; Gerdes, 2004).

Moreover, AGPT gave poor result compared with other serological tests performed in all serum samples, this finding is in agreement with Swanepoel *et al*. (1986), who proved that the AGPT is considered very specific but not very sensitive.

In addition, the cost of the sample for detection of RVF using SNT was 15 LE while, for ELISA 20 LE and for AGPT was 7 LE/sample but AGPT of lower sensitive for detection of RVF. The AGPT will save the costs than ELISA by about 1300 LE/100 samples and SNT save cost by 500 LE/100 samples in comparison with ELISA test but they are not preferable as mentioned before.

Therefore, our results cleared that the using of SNT for detection of RVF of highly sensitive and economic methods and can save the costs of detection over ELISA by (5 LE/head).

CONCLUSION

The SNT is sensitive test for detection of RVF in small ruminant and save the money in comparison with other test but it needs long time and less specific, Therefore, ELISA is considered as sensitive and specific test for detecting different antibodies which gave result in parallel with SNT.

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