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# AN EPIDEMIOLOGICAL STUDY ON RIFT VALLEY FEVER IN SHEEP IN EGYPT.

(With 5 Tables)

By

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## دراسة وبائية عن حمى الوادى المتصدع فى الأغنام فى مصر

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تم جمع ١٣٥٠ عينة سیرم من اغنام حية ومذبوحة من محافظات القاهرة - الجيزة - القليوبية - الشرقية - البحيرة - الفيوم - المنيا مطروح واسوان بالإضافة الى مزرعة اغنام كوم اوشيم بمحافظة الفيوم.

فحصت هذه العينات لوجود الاجسام المناعية لفيروس مرض حمى الرفت فالى باختبارات المثبت المكمل والترسيب فى الاجار وكذلك اختبار التعادل المصلى وذلك لتحديد مدى كفاءة التحصين المحلى لمرض حمى الرفت فالى المیت ، كذلك للحكم واكتشاف مدى وجود عدوى تحت اكلينيكية بالاغنام فى مصر. فى هذا البحث توجد نتائج الفحص المبدئى لوجود الاجسام المناعية لهذا الفيروس، كذلك تمت معايرة بعض العينات الايجابية لتحديد مستوى هذه الاجسام المناعية بالإضافة إلى التحليل الاحصائى وتحليل النتائج التى تم التوصل اليها.

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## RIFT VALLEY FEVER, SHEEP &amp; EGYPT

## SUMMARY

1350 serum samples were collected from slaughtered sheep from 9 governorates (Cairo, Giza, Qalubia, El-Sharkia, El-Behara, Fayoum, Minia, Matrouh and Aswan) as well as from 140 living sheep at kome Osheim, Fayoum province. The sera were subjected to CFT, AGPT and SNT to reveal the efficiency of the locally produced inactivated RVF vaccine in EGYPT and/or to detect the subclinical infection of RVF virus in sheep. The results of the screening with CF, AGP and SN tests as well as the titration of some positive samples with CFT and SNT are presented in this work together with its explanation and interpretation.

## INTRODUCTION

Rift Valley Fever (RVF) is an acute infectious, arboviral disease affecting mainly ruminants and man, it causes a high mortality rate among animals and abortion of pregnant ones (DAUBNEY *et al.*, 1931). It is characterized by a short incubation period, fever, leucopenia and necrotic changes in the liver.

Until 1977, RVF virus was geographically limited to subsaharan Africa. In 1977 an outbreak of RVF disease was recorded for the first time in Egypt causing high mortality and abortion rates among domestic animals with extensive human involvements (MEEGAN and MOUSSA, 1979). In this outbreak, RVF was considered to have possible origins through either importation of infected ruminants or camels from the Sudan, through infected insects carried in transportation vehicles or by wind (SELLERS *et al.*, 1982).

The aim of this work was to investigate the immune status of sheep during the 1989-1990 where a serosurvey was done in nine governorates representing a great part of the country including the fore western governorate (Matrouh) and the fore southern one (Aswan) to clarify the immune status of animals of the Egyptian borders.

## MATERIAL and METHODS

## 1- Rift Valley Fever Virus:

The original virus was isolated from a human patient at Zagazig, Sharkia governorate and identified as RVF virus. It was then twice passaged intra-cerebrally into suckling mice,



and has a final titer of  $2 \times 10^7$  MICID<sub>50</sub>/ml.

## 2- Cell Cultures:

2.1 CER Cells: These cells were supplied by Wister Institute Philadelphia, USA and used for virus titration.

2.2 BHK Cells: Baby Hamster Kidney Cells (BHK) were grown and maintained according to MACPHERSON and STOCKER (1962) and used in serum neutralization test.

## 3- Sera Samples:

A total of 1350 sheep serum samples were collected from farms and abattoirs of 9 governorates including Cairo, Giza, Qalubaia, El-Sharkia, El-Beheria, Matrouh, Fayoum, Minia and Aswan. In addition, 140 samples were collected from Kome Osheim sheep farm at Fayoum province after vaccination with RVF inactivated alum adjuvant vaccine.

## 4- Serological Tests:

4.1 Complement fixation test: The microtechnique of CFT was carried out according to EDWIN (1964), negative and positive control, antigens as well as R.B.Cs. controls were included in the test.

4.2. Agar gel precipitation test: The antigen used was prepared in BHK cells and finally treated with Tween 80 which acts as surface agents for dispersing clumped particles. The test was done following the technique described by AYOUB and ALLAM (1981).

### 4.3 Serum neutralization test:

The technique was that described by WALKER *et al.* (1970) where constant serum - virus dilution method was used. The neutralization index was calculated according to REED and MUENCH (1938).

## RESULTS

### 1- Screening of serum samples:

The 1350 serum samples were tested serologically using CF, AGP and SN tests for the detection of antibodies against RVF virus. The results as in tables (1 & 2) revealed that Matrouh province had the highest positive rates (58% by SNT, 56.6% by AGPT and 41.3% by CFT) while the upper Egyptian provinces having the lowest rates as in Aswan province had 14% by CFT, 22% by AGPT and SNT. From the tables it is obvious that results

## RIFT VALLEY FEVER, SHEEP &amp; EGYPT

Table (1): Comparative results of different serological tests in various provinces.

Province	No. of tested sera	Results of serological tests					
		CFT		AGPT		SNT	
		No. of +ve samples	rate (%)	No. of +ve samples	rate (%)	No. of +ve samples	rate (%)
Cairo	150	25	16.6	42	28	44	29.3
Giza	150	38	25.3	64	42.6	64	42.6
Qaluobia	150	30	20	41	27.3	43	28.6
El-Sharkia	150	48	32.0	56	37.3	56	37.3
El-Beheria	150	40	26.6	61	40.6	63	42
Matrouh	150	62	41.3	85	56.6	87	58.0
Fayowm	150	23	15.3	31	20.6	32	21.3
Minia	150	22	14.6	26	17.3	28	18.6
Aswan	150	21	14	33	22.0	33	22.0
Mean results	1350	309	22.8	439	32.5	450	33.3



Table (2): The results of different serological tests in addition to Age and season in different provinces

Province	Season	* Age		CFT		AGPT		SNT	
		Group No.		+	-	+	-	+	-
Cairo	Winter	1	118	19	99	31	87	33	85
		2	32	8	24	11	21	11	21
Giza	Summer	1	97	22	75	37	60	37	60
		2	53	16	37	27	26	27	26
Qalucbia	Summer	1	82	14	68	22	60	22	60
		2	68	16	52	19	49	21	47
El-Sharkia	Summer	1	93	29	64	35	58	35	58
		2	57	19	38	21	36	21	36
El-Beheria	Summer	1	117	32	85	49	68	51	66
		2	33	8	25	12	21	12	21
Matrouh	Summer	1	79	29	50	42	37	44	35
		2	71	33	38	43	28	43	28
Fayoum	Winter	1	85	13	72	17	68	17	68
		2	65	10	55	14	51	15	50
Minia	Winter	1	82	12	70	13	69	15	67
		2	68	10	58	13	55	13	55
Aswan	Winter	1	58	8	50	12	46	12	46
		2	92	13	79	21	71	21	71

\* Age: group 1: means lower than 2 years  
 2: means higher than 2 years.

## RIFT VALLEY FEVER, SHEEP & EGYPT

of AGP and SN tests are quite similar to each other. It is also evident that the rates detected by both tests are much higher than the analogous rates detected by the CF test.

### 2- RVF virus isolation:

Under the conditions of the present field investigation, no virus could be isolated from all the serum samples collected in this study.

### 3- Quantitative test on positive screened serum samples:

The results of quantitative CFT (Table 3) revealed that the titer of CF antibodies ranged between 8 - 32 and in some cases 64 especially in Matrouh province. The results of quantitative SNT on some positive sera sample (Table 4) revealed that at least 50% of the samples had an index higher than 1.7. Higher SN indices ranging from 2.2 up to 3.5 were noticed in all tested sera from Matrouh province.

### 4- Results of Kome Osheim farm:

Due to the insufficient data about the vaccination of the 1350 sheep examined above, a sheep farm (Kome Osheim farm at Fayoum province) was selected where animals are vaccinated to study the immune response of the animals in the farm. The results of different serological tests shown in table (5) indicated that in non vaccinated group of lambs of less than 2 months old, 20% of its samples had antibodies against RVF virus while lambs of more than 2 months old did not possess antibodies against RVF virus. In the second group (2 months post-vaccination with RVF alum gel vaccine), the rate of positive cases was very high reaching 76.6% by SNT. In the third group (8 months post-vaccination), the rate of positive cases declined reaching 11.6% by CFT, 16.6% by AGPT and 21.6% by SNT.

## DISCUSSION

Since the last epizootic of RVF in Egypt 1977, strict quarantine measures and vaccination programmes were adapted by the Veterinary Service Organization using a locally produced tissue culture inactivated vaccine produced at Abbassia, Cairo (EL-MIMR, 1980). It is important to follow the immune status of sheep to determine the efficiency of the applied vaccination programmes. Since the last outbreak, very little work was done in this concern. The latest investigation was carried out in the east part of delta at Dakahiliya governorate (BOTROS *et*



Table 3  
Quantitative results of complement fixation  
test on sera samples from various provinces

Province	Number of positive sera	Titer of CFT (1/x)				
		4	8	16	32	64
Cairo	25	2	11	7	4	1
Giza	38	5	14	10	6	3
Qalubia	30	1	12	11	4	2
Matrouh	62	5	18	21	11	7
El-Sharkia	48	4	18	16	9	1
El-Beheria	40	1	13	16	8	2
Fayoum	23	4	8	8	3	-
Minia	22	5	7	6	3	1
Aswan	21	4	9	7	1	-

# RIFT VALLEY FEVER, SHEEP & EGYPT

Table 4  
Quantitative results of serum neutralization test  
on some selected sera samples from various provinces

Province	No. of tested sera per pro- vince	SN Index		Rate (%)
		Range	No. of sera with an index above 1.7	
Cairo	10	0.5 - 3.5	7	70
Giza	10	0.5 - 3.5	5	50
Qaluobia	10	0.5 - 3.5	6	60
Matrouh	10	2.5 - 3.5	10	100
El-Sharkia	10	1.17- 3.5	9	90
El-Beheria	10	0.5 - 3.5	8	80
Fayoum	10	0.5 - 3.5	5	50
Minia	10	0.5 - 3.0	5	50
Aswan	10	0.5 - 3.0	6	60



Table 5

No. of sera samples	Age of the animal	Results of various serological tet.					
		CFT		AGPT		SXT	
		No. of +ve sera	rate (%)	No. of +ve sera	rate (%)		No. of +ve sera
10	non vaccinated less than 2 M	-	0	2	20	2	20
10	" more than 2 M	-	0	-	0	-	0
60	2 M. post vaccination	31	51.6	43	71.6	46	76.6
60	8 M. post vaccination	7	11.6	10	16.6	13	21.6

M.M. EL-SHINNAWY et al.

al., 1989). The present results of screening of serum samples from the selected governorates showed that Matrouh province has the highest positive rates with all serological tests (41.3% by CFT, 56.6% by AGPT and 58% by SNT). While Minia and Aswan had low titers of antibodies. Statistical analysis of these results showed that Matrouh province had a significantly higher rate in different serological tests than other provinces. The cause may be attributed to either efficient vaccination programmes or due to a sort of subclinical infection with RVF virus or other antigenically related viruses as phlebotomus virus; a matter which cannot be settled unless further investigations are done. However, the low percentage of positive sheep sera in other provinces which was mostly below 30% would give indication for irregular or inefficient vaccination programmes applied in these provinces (especially in Minia and Aswan). From statistical analysis, by applying CHI square method, there was no significant effect of age in different provinces. On the other hand, there was a significant effect of seasonal variation since sera collected during the summer season gave higher positive rates than those collected during the winter season. This is due to the prevalence of Culex pipens during summer months which is the main vector in the transmission of RVF in Egypt as recorded by MEEGAN et al. (1980). The results of quantitative CFT revealed that most of sera examined in this study had a low level of antibodies indicating that this test is not as efficient as the other tests. However, using the quantitative SNT, more than 50% of tested serum samples had an index higher than 1.7 which would be considered protective (randell ET AL., 1962). higher sn indices ranging from 2.2 up to 3.5 were noticed in all sera tested from Matrouh province which might be either due to recent vaccination or exposure of the animals to infection as suggested by SMITHBURN et al. (1949). The marked difference noted between the results of the CF test on one hand and those of the AGP and SN tests on the other hand may be attributed to that the latter two tests could detect the presence of a small amount of antibodies where as the CF test could not.

Serological results obtained from Kome Osheim farm showed that no antibodies could be detected in non vaccinated group except in two cases of less than 2 months old tested by AGP and SN tests which might mainly be due to residual maternal immunity. Such finding is in agreement with those results obtained by REDA et al. (1987). Sheep sera collected 2 months post vaccination showed better immune response than those obtained from 8 months post vaccination with RVF vaccine. From



## RIFT VALLEY FEVER, SHEEP &amp; EGYPT

these results it is concluded that a booster dose of the vaccine would be needed 6-8 months post vaccination which was suggested before by EL-NIMR (1980) and HARRINGTON *et al.* (1980).

As regards the sensitivity of the applied serological tests it was found that AGPT was nearly as good as SNT when used for screening serum samples. These results are supported by the findings of SWANEPOEL *et al.* (1986) who detected precipitation lines 6 days after the initial vaccination and reported that SNT was more sensitive than CFT. Besides SHOPE and WALKER (1980) reached the same conclusion since they considered SNT as the most specific and recommended test to evaluate the immune status of animals against RVF virus.

Due to the insufficient available information about the vaccination programmes, one cannot deduce a clear picture about the epidemiology of RVF virus infection in sheep. However attention should be directed towards governorates at Egyptian borders specially those of the southern part as Aswan which is close to Sudan where the disease is known to be enzootic, such area would create at least a buffer zone preventing the entry of the disease to our country in the future.

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