

معهد بحوث ونتاج اللقاحات - بالعباسية .
وزارة الزراعة - القاهرة .
مد ير المعهد / أ . د . / سيد سلامة .

د راسة ك يف ية نتيج لمس ير المر ض وخواص الدم لعد وى الأغ نام صناع يا ب ف يروس حمى الواد ي المتصد ع (عد وى الدم وتخلصه من الف يروس

عا يد ه الد بيجي ، أحمد عبد الفتاح ، محمد طه ، فوزى عطا ، محب النمر*

تم عد وى الاغنام البالغة صناعيا ب فيروس حمى الوادى المتصدع بعد
حقنها في الوريد وتحت الجلد . ولقد ظهر ارتفاع درجة حرارة الأغنام في
الثلاثة أيام الأولى فقط بعد الحقن .

وأمكن عزل الفيروس من أمصال كل الحيوانات المحقونة في الوريد
(١٠٠ %) ولكن في بعض الأغنام المحقونة تحت الجلد (٥٥ %) وكانت القوة
العيارية للفيروس المعزول عاليه خلال الثلاثة أيام الأولى بعد الحقن .
ولقد تخلصت الحيوانات المحقونة تحت الجلد من الفيروس أسرع من تلك
المحقونة في الوريد .

وقد كونت كلتا المجموعتين أجسام صناعيه تعادلية ضد فيروس حمى
الوادى المتصدع حيث كان أعلى مؤشر تعادلى (Neutralization index)
في المدة بين اليوم الرابع عشر واليوم الواحد والعشرون .

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**THE MECHANISMS OF PATHOGENICITY AND BLOOD
CLEARANCE OF RIFT VALLEY FEVER VIRUS
IN SHEEP**

(with 4 Tables)

By

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SUMMARY

Adult sheep inoculated either intravenously (I/V) or subcutaneously (S/C) with Rift Valley Fever virus (RVFV) manifested a thermal reaction for 1-3 days occurring during the first three days post-infection (P.i), but not beyond the third day p.i.

RVFV could be isolated from the sera of infected animals at a higher rate in the I/V inoculated group (100%) than in the S/C inoculated group (55%). Higher titres were found in samples of the first three days p.i. Animals of the S/C group cleared themselves from the virus more rapidly than those of the I/V group.

Seroconversion in infected sheep was clear and the highest Neutralization Indices (NI's) were obtained at the 14th and 21st days p.i. (4.0).

INTRODUCTION

The pathogenesis of RVFV infection in sheep is a sequence of events including entry of the virus and the reaction of the host to the invading virus. Entry of the virus may occur through mosquito bites, subcutaneous (S/C), intratesticular, intracutaneous, intramuscular (I/M) and intravenous (I/V) inoculations as well as intranasal instillation, aerosol and the application of the virus to scarified skin and conjunctive (MURPHY and EASTERDAY, 1961; EASTERDAY *et al.*, 1962 b and IMAM *et al.*, 1978). Besides, contact infection was mentioned by WEISS (1957), WALKER *et al.* (1970 a); ABDEL-KARIM (1982) and SABER *et al.* (1982). During viral infection, multiplication of the virus at the site of entry may occur followed by generalization of the invading virus. Generally a second cycle of multiplication in the internal organs and a second phase of viraemia proceeds the signs of the disease and liberation of the virus into the environment.

In RVFV infection, the isolation of the virus from the sera as well as evaluation of the resulting immunity by means of the serum neutralization test (SNT) are of the most important criteria.

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Thus the purpose of the present study is to determine the rate of clearance of the virus after infection from the blood of infected sheep and to elucidate the role of specific neutralizing antibodies in the process of clearance.

MATERIAL and METHODS

1. **Virus:** RVF virus used in the present study was that mentioned by EL-NIMR et al. (1983).

2. **Sheep:** Adult female balady sheep of 2-3 years old were used. All the animals proved to be susceptible to RVF infection except two had a NI of 2.0-2.8.

3. **Virus isolation:** This was done according to EL-NIMR (1980).

4. **Serological tests:** Serum neutralization test was carried on sera of infected sheep collected at predetermined interval p.i. was performed following the technique of WALKER et al. (1970 a). The serum neutralization index (NI) was calculated using the formula of REED and MUENCH (1983).

5. **Experimental infection of sheep:** Seven animals were used for this purpose. Six were each inoculated with one ml. of virulent RVF virus containing log 8.5 TCID (2 I/V and 4 S/C). In addition, one animal was left without inoculation as contact control. The animals were kept under observation for twenty one days with daily temperature recording.

RESULTS

1. **Thermal reactions:** Results of thermal reaction manifested by experimentally infected sheep are shown in Table (1).

2. **Isolation of RVF virus:** Results of isolating RVF virus from sera of infected sheep are demonstrated in Tables (2 and 3).

3. **Seroconversion in experimentally infected sheep:** Results of this investigation are presented in Table (4).

DISCUSSION

Natural infection with RVE virus is mainly through mosquito bites or via the subcutaneous route. Moreover, this latter one is recommended as the post-vaccinal challenge test (BERNARD and BOTHA, 1977). However, it has been reported that the disease could be produced experimentally by other routes (DAUBNEY et al., 1931; MURPHY and EASTERDAY et al., 1962 b and IMAM et al., 1978).

In the present study, two routes of inoculation were used (I/V) and S/C). Following infection of adult sheep with RVF virus, a subacute form is a common sequelae characterized by a thermal reaction (DICKSON, 1951). Results as presented in Table (1) showed a rise of temperature between the first and third day post infection (p.i) but never beyond the third day. Animals infected by the S/C route showed a rise of temperature for only one day. Yet, those inoculated by the I/V route manifested a rise of temperature during the first three days p.i. There was no difference between the average peak temperature in both group (mean of 40.2°C). Other workers got the same result, i.e. a rise of temperature for not more than three days (EASTERDAY, 1965; McINTOSH, 1973; FAGBAMI, 1975 and

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YEDLOUTSCHNIG *et al.*, 1980 b). The saddle shaped type of fever mentioned by ABDEL-KARIM (1982) with the same strain of RVF virus was not noticed in this study.

With respect to virus isolation from the sera of infected sheep, we were restricted to the observation period of the experiment, i.e 21 days p.i RVF virus could be isolated from the sera of the I/V group at a rate of 95% and earlier than in the S/C group. Moreover, in this latter group, titres were very high in the first three days, then started to decline by the 7th day and could not be detected after 10 days. It was noticed that the rate of isolation in the S/C group was lower than that in the I/V group (52% versus 95%). In addition, there was no appreciable differences between the average titres of the two groups, except during the third day where the mean titre of the S/C group was two logs higher than that of the I/V group (6.8 versus 4.6). No explanation could be given for this observation, since the reverse was expected and highly supported by the data in Table 2, where the virus could be detected during the early hours in sera of the I/V group, but only in few samples of the S/C group. No relation could be noticed between the rise of temperature and virus isolation, since even after the drop of temperature, the virus could still be isolated from the serum.

Comparing our results with those of other investigators, WEISS (1975) mentioned that the virus circulates for 6-7 days. Yet, FAGBAMI (1975) could not find the virus beyond the 5th day p.i. On the other hand, ABDEL-KARIM (1982) reported that adult sheep had the virus in their sera for 11 days p.i. This may be due to difference in the route of inoculation and the amount of inoculated virus. Although we could not detect viraemia in contact animals, yet ABDEL-KARIM (1982) found a rate of viraemia of 22% in two out of three contact animals.

Finally, seroconversion in infected sheep was studied as well. The subcutaneous group started to reveal the presence of neutralizing antibodies from the 3rd day p.i. with the peak NI's between the 14th and 21st day p.i. On the contrary, seroconversion in the I/V group could not be accurately evaluated since the animals of this group had NI's of 2.0 and 2.8 prior infection, hence the inoculated virus behaved as a boosting dose. Our results agree with those of ABDEL-KARIM (1982) who reported that neutralizing antibodies appear after 7 day p.i. with the peak at 21 days p.i. In addition, viraemia is important for the development of higher neutralizing antibodies (BERNARD, and BOTHA, 1977). This fact was proved here, since all animals manifesting viraemia, revealed appreciable NI's although no difference was found between the indices at 21 days p.i.

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Table (1) : Thermal reactions in sheep experimentally infected with RVF virus.

No. of animal	Route of inoculation	Day of rise of temperature	Duration of rise of temperature	Peak temperature degree °C
5658	I/V	1st, 2nd, 3rd	3D.	40.6°C
8510	"	2nd	1D.	39.8
5397	S/C	3rd	1D	40.3
3620	"	1st	1D	40.5
5688	"	1st	1D	40.0
Without	"	3rd	1D	40.0
4117	Control	-	-	-

I/V : intravenous, S/C : subcutaneous, D. : day, - : No reaction.

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Table (2): Infectivity titres of RVP virus in sera of infected sheep.

No of animal	Route of inoculation	Titre at the following intervals post-inoculation (log ₁₀ TCID ₅₀ per ml.)											
		0	1h	2h	3h	4h	1D	2D	3D	4D	7D	8D	9D
5658	I/V	-	1.5	2.0	2.1	3.0	6.0	6.0	5.0	5.0	3.0	3.0	1.5
8510	"	-	1.0	2.3	3.0	4.3	5.1	5.3	4.3	3.1	2.3	2.2	1.1
5397	S/C	-	-	-	-	2.1	3.3	5.0	6.3	5.1	3.4	1.0	1.0
3620	"	-	-	-	-	-	5.0	6.0	7.0	2.5	1.0	1.0	1.0
5688	"	-	-	2.3	2.3	3.0	6.0	6.3	7.0	5.0	3.0	1.0	1.0
without	"	-	-	-	-	-	5.0	6.3	7.0	4.5	2.0	1.0	1.0
4117	control	-	-	-	-	-	-	-	-	-	-	-	-

I/V: Intravenous; S/C: subcutaneous, h: hour, D: day, -: Virus could not be detected.

Table (3). Isolation rates of RVF virus from sera of infected sheep.

No of animals.	Route of inoculation	Isolation of the virus at the following intervals										Rate of isolation/anim. (%)	Rate of isolation per group (%)			
		0	1h	2h	3h	4h	1D	2D	3D	4D	7D			8D	9D	
5658	I/V	-	+	+	+	+	+	+	+	+	+	+	+	+	11/11(100)	21/22 (95)
8510	"	-	-	+	+	+	+	+	+	+	+	+	+	+	10/11(91)	
5397	S/C	-	-	-	-	+	+	+	+	+	+	+	+	+	6/11(54.5)	23/44(52.2)
3620	"	-	-	-	-	-	+	+	+	+	+	+	+	+	4/11(36.3)	
5888	"	-	-	+	+	+	+	+	+	+	+	+	+	+	8/11(72.7)	
without	"	-	-	-	-	-	-	+	+	+	+	+	+	+	5/11(45.4)	
4117	control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rate of isolation/6		1/6	3/6	3/6	4/6	6/6	6/6	6/6	6/6	6/6	5/6	2/6	2/6	2/6		
lution per day (0)		(16)	(50)	(50)	(66)	(100)	(100)	(100)	(100)	(100)	(100)	(83)	(33)	(33)		

I/V: intravenous ; S/C: subcutaneous, h:hour, D: Day, Inoc: Inoculation.

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Table (4) Neutralization test on sera of infected sheep.

No of anim.	Route of inoc	NI ₅₀ at the following intervals (Days)								Range of NI per animal
		0	1	2	3	7	8	14	21	
5658	I/V	2.0	2.4	2.5	3.5	3.5	4.0	4.0	4.0	(2.4-4.0)
8510	"	2.8	1.8	2.2	3.5	3.9	4.0	4.0	4.0	(1.8-4.0)
5397	S/C	-	-	-	0.9	3.5	ND	3.3	4.0	(0.9-4.0)
3620	"	-	-	-	-	2.0	"	3.7	4.0	(2.0-4.0)
5688	"	-	-	-	0.3	2.2	"	3.8	4.2	(0.3-4.2)
without	"	-	-	-	-	1.2	"	1.9	2.6	(1.2-2.6)
4117 control	-	-	-	-	-	-	-	-	-	-

NI₅₀: neutralization index, I/V: intravenous, S/C: subcutaneous,

ND: not done.

