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EXPERIMENTAL INFECTION OF RAMS WITH VIRULENT RVF-VIRUS

(With Two Table and One Figure)

By

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عدوى الكباش صناعيا بفيروس حمى الرفت فالي الضارى

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بعد حقن كباش عمرها سنتان بفيروس حمى الوادى المتصدع لوحظ ارتفاع في درجات الحـــــراره وصلت الى ٥ر٤١م من اليوم الثاني الى اليوم السادس وقد وصل أعلى معدل للفيروس في المصل منذ اليـــوم الثاني الى اليوم الرابع وتم عزل الفيروس من افرازات الانف منذ اليوم الثالث الى الثاني عشر ووصـــل - أعلى معدل الفيروس في الافرازات الأنفية في اليوم السادس بعد الحقن · وقد تم عزل الفيروس أيضا من السائل المنوى للحيوانات المحقونة في الفترة من ١٠ ـ ٢١ يوما بعد الحقن ـ وقد وجدت إجسام مناعيـــة مضادة لفيروس حمى الوادى المتصدع في مصل الحيوانات المحقونة ابتداء من اليوم ٢٨ بعد الحقن ووصـــل المعامل التعادلي ٣ر١ ـ ٩ر١ وذلك بطريقة استخدام اختبار التعادل مما يؤكد الاستجابة المناعيـــــــة للحيوانات المحقونة بالفيروس مع عدم عدواها بالفيروس ·

SUMMARY

Rams of 2-years-old were experimentaly infected with Zagazig strain of RVF-virus at a dose of 10^6 $TCID_{50}$/ml subcutanouslly. There was a thermal reaction following animal inoculation from the second till the tenth-day post infection (DPI).

The virus could be isoalted from the blood of inoculated animals from the second till the 8th -day post-inoculation.

The virus could also be isolated from the nasal discharge from the third-day till the twelvth-day-post inoculation, and it was detectable in the semen between tenth day till the twelvth day-post inoculation.

The result of serum neutralization test (SNT) done on sera samples collectd from rams after 28 DPT gave a NI of more than 1 (1 3-1.9) during the period from 21 to 28 days post infection indicating that the virus could not produce the disease, yet giving an immune response.

INTRODUCTION

Rift valley fever (RVF) was reported by DAUBNEY; HUDSON and GRAHAM (1931) in Kenya on a farm Near Lake Nivasha. A heavy mortality occured among

calves and newly born lambs and the death rate reached 95% with respect to the latter. Easterday, Mc GAVRAN; ROONEY and MURPHY (1962), described RVF as an acute, viral, mild or sometimes inapparent infectious disease of sheep, cattle and other animals. The disease was characterised by a high rate of abortion among pregnant ewes and cows. In the summer of 1977, it took a rather severs form among humans,beside demostic animals especially sheep and calves for thefirst time in Egypt.

In Egypt, many workers were interested in inoculating laboratory animals as well as large animals with the virulent virus to follow the pathogenesis of the virus in these animals. ABDEL-KARIM (1982) and ABOU-BAKER (1982). Yet the question of infecting male animals (rams or bulls) with the virus and following the pathogenesis of the virus in these animals was not studied and deserves to be explorated.

Hence the purpose of the present work is to infect rams with virulent RVF-virus and to follow the pathogenesis of the virus including the most important parameters such as/virus isolation and seroconversion.

MATERIAL and METHODS

I- MATERIAL :

A) Virus : The orignial virus was that isolated from humen patient in Zagazig, Egypt (MEEGEN and MOUSSA, 1978). It was twice passaged intracerebrally into suckling mice and has a final titre of 10^7 $TCID_{50}$/ml. It was stored at -70°C till used.

B) Cell culture : Baby hamster kidney cells (BHK_{21}) (Mac PHERSON and STOCKER, 1962) was propagated in 199 medium supplemented with 10% RVF- antibody free calf serum which was replace by horse serum (3%) after inoculating the virus.

C) **Titration of RVF-Virus :** This was carried out as usual, and the titre expressed in $\log_{10}$ $TCID_{50}$ per ml. (REED and MUNECH, 1938).

D) **Animals :**

1. Sheep : Four non-vaccinated, non- infected 18-24 months - old rams, from a local breed at the New Valley (Barky) were used. They proved to be susceptible to RVF infection after carrieing the serum neutralization test (SNT) on their sera before virus infection.

2. Suckling mice : 3-5 days - old suckling mice were used for virus isolation, as well as, virus titation.

II- METHODS :

A) Animal inoculation : Rams of 2-years-old were experimentaly infected subcutanouslly with Zagazig strain of RVF-virus ata dose of 10^6 $TCID_{50}$/ml. Daily temperature recording was done during the period of the experiment (28-days).

B) collection of samples :

1) Nasal discharges : Nasal discharges were colleccted using sterile swaps and small sterile glass bottles containing Hank's solution with 20% antibiotics. They were kept at-70°C till used. ABDEL-KRIM (1982).

2. Semen sampls : It was collected according to ABOU-AHMED (1962).

3. Serum samples : It was collecled daily from the infected rams, then kept at -70°C for virus isolation. Another part of the serum samples was inactivated at 56°C for 30 minutes and stored at -20°C for detection of specific antibodies against RVF-virus (EL-NIMR, 1980).

C) Isolation of RVF-virus :

1. In suckling mice : It was conducted by using the method described by EL-NIMER (1980). The mice were Kept under observation for 7-days and dead mice were collected for virus re-isoaltion.

2. In tissue culture : Using test tubes containing BHK cells and according to the method of EL-NIMR (1980).

D) Serum neutralization test (SNT) : the technique was that of EL-NIMR (1980) to detect antibodies against RVF-virus in sera of animals.

RESULTS

There was a clear rise in the body temperature of infected rams, reaching 41.5°C which started form the second day till the sixth DPI (Fig. 1).

A) From the sera :

Table (1) shows the quantitative estimation of RVF-virus in the sera of rams in the respective days post infection. It was clear that the virus started to be detected by the second DPI in the first, second and the third ram. The virus attained its maximum titre on the third day, then started to decline on the eighth DPI. There was no appreciable difference between the three inoculated rams.

B) From the nasal discharges :

Virus could be detected from nasal discharge from the third day till the twelvth DPI. The virus attained its highets titre on the sixth DPI.

C) From the semen :

Table (2) shows the quantitative estimation of RVF-virus in the semen of rams in the respective days post infection. The virus started to be detectable from the eight day till the 20th the day, reaching its maximum on the sixteenth day-post-inoculation with RVF-virus.

It could be said that RVF-virus could withstand of the semen condition.

D) Immune response of rams infected with RVF-virus :

The results of SNT on serum samples collected from rams indicated that all rams had a neutralizing index (NI) more than 1.0 (1.3-1.9) indicating the presence of specific antibodies against RVF-virus in sera of rams following infection. ABDEL-KRIM (1982); ABOU-BAKER (1982) and WASSEL (1983) reported on the immune response of sheep maintaining a neutralizing index in their sera of more than 1.0 during the period from 21 day till 10 months post infection indicating that the virus could not produce the disease yet giving an immune response.

Table (1): Titration of RVF virus in sera of rams artificially inoculated with RVF firus.

No. of animal	Inoculation (Log_{10} $TCID_{50}$/ml)	Titre of thevirus expressed in log_{10} $TCID_{50}$/ml								
		2	3	4	5	6	7	8	9	10
1	6.0	6.5	7.0	6.2	5.5	4.2	2.0	1	0	0
2	6.0	6.0	6.9	6.2	5.1	4.1	2.0	1	0	0
3	6.0	6.4	7.1	6.2	5.3	4.3	3.0	1	0	0
Control	--	--	--	--	--	--	--	-	-	-

O: Titre is less than 10^1 $TCID_{50}$ per ml.

Table (2): Titration of RVF virus isolated from semen of artificially infected rams.

No. of	Inoculum ($1og_{10}$ $TCID_{50}$/ml)	Titre of virus expressed in log_{10} $TCID_{50}$/ml at the following days post-inoculation.										
		2	4	6	8	10	12	14	16	18	22	28
1	6.0	0	0	0	3.5	4.5	5.0	6.0	7.0	6.0	4.0	0
2	6.0	0	0	0	4.0	5.0	5.5	6.5	7.0	6.0	4.0	0
3	6.0	0	0	0	0	0	5.5	6.5	6.0	4.0	2.0	0
Control	-	-	-	-	-	-	-	-	-	-	-	-

O: See table (1).

DISCUSSION

The thermal response of rams inoculated with RVF-virus revealed a clear rise in body temperature reaching 41.5°C from 2-6 DPI with the virus. This results agrees with those of ABOU-BAKER (1982). This viraemic stage is more clear in table (1) where the virus was detectedduring this period at ahigher titre.

The quantitative estimation of RVF-virus in animals during the respective days post inoculation showed that the virus was detectable in the sera from the second day till the 8th DPI attaining its maximum titre on the second, third and fourth DPI, then started to decline, disappering by the ninth DPI. This finding was previusly reported by EASTERDAY et al. (1962 b) since RVF-virus remains detectable in the sera of artificially infected ovines for aperiod 28-days.

We could also isolat the virus from the semen, by the 8th till the twenth DPI reaching its maximun titre on the 16th DPI. It could be said that RVF-virus could with stand the natural resistance of the semen (ABOU-BAKER, 1982).

All the infected animals had a NI of more than 1-0 (1.3-1.9) indicating the presence of specific antibodies-againet RVF-virus in their sera. Other workers such as, EASTERDAY et al. (1962) and EL-NIMR (1980) previously reported on the immune response of sheep mentioining a NI of in their sera during the period from 21 day to 21 day to 12 months D.PI. indicating that the virus could not produce the disease but elicit an immune response.

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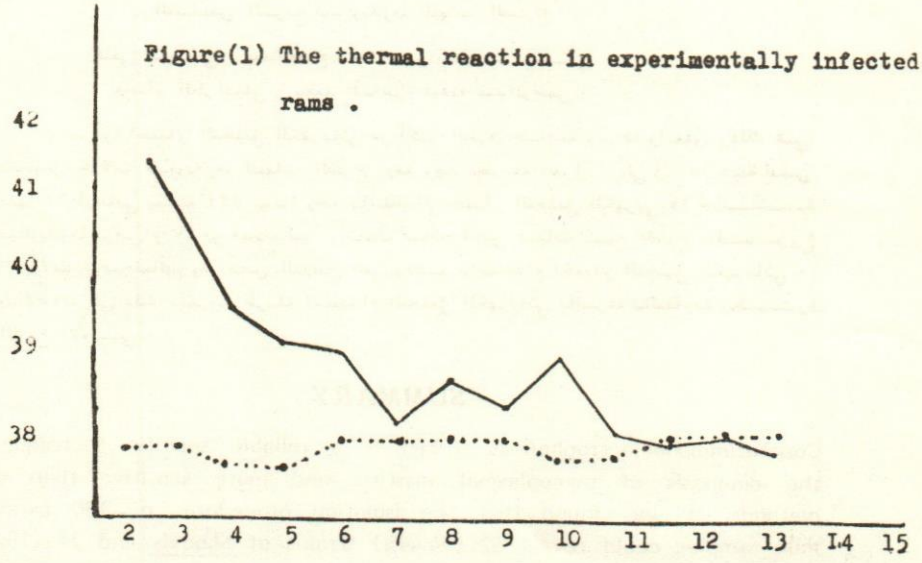

Figure(1) The thermal reaction in experimentally infected rams .

Fig. (1): Temperature ..... control rams.

°_____° Inoculated rams.