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UNCLASSICAL PICTURE OF RIFT VALLEY FEVER (RVF) IN MAN AND ANIMALS IN ASWAN GOVERNORATE IN MAY 1993.

BY

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SUMMARY

After 15 year from the last epidemic of Rift Valley Fever (RVF) in Egypt, a mild wave of the disease began in Aswan governorate in May 1993, characterized by ocular form in human beings and abortion in cattle and buffaloes. The virus isolated from the liver and spleen of aborted foetus of a buffaloe (EDFU) and from the placenta of aborted cow (EL-Khatarra) by I/C and I/P inoculation of baby mice and in BHK 21 cells. Virus neutralization test in mice and Agar Gel Precipitation Test (AGPT) were applied on the above positive samples with reference (RVF-HIMAF) for identification of RVF virus. 65 serum samples were collected from different species (no history of vaccination), 16 of them were positive in AGPT by using reference (RVF-HIMAF), indicating the prevalence of RVFV in Aswan,

INTRODUCTION

Rift Valley Fever (RVF) is an acute febrile arthropod-borne zoonotic disease caused by a Bunyavirus of the Genus Phlebovirus. It causes high rates of abortion and neonatal mortality in sheep, goats and catle. The disease was limited to Subsaharan africa untill RVFV appeared for the first time in Egypt in 1977-1978 in an epizootic-epidemic of unprecedented size causing extensive morbidity and mortality rates in domestic animals. It was estimated that there were as many as 200.000 human cases and at least 600 deaths *Imam & Darwish; 1977.; Darwish, et al. 1978 and Meegan, et al. (1979). Four clinical syndromes have been described in infected individuals: undifferentiated fever, encephalitis with blindness or severe hamorrhagic fever and death. Man became infected by direct contact with sick animals, their excreations or foetuses and infected tissues. It is therefore essential to perform all manipulation with infective materials in condition of biohazard containment (Meegan, 1981.; Lupton, 1984 and O. I. E. 1989). In 1979 further sporadic cases of RVF in man and animals were recorded (Sellers, 1982). Since 1980 the situation became obviosly calm, but in fact the last recorded isolation was from a dead calf in El-Serw (Damiatta) in January, 1980 (Allam, 1987). After 1981, RVF seemed to have disappeared from Egypt, suggesting that it is not maintained in an enzootic cycle in this area (Allam, et al., 1986).

Again and after 13 year from the last RVFV isolation and about 15 years from the last epidemic, a mild wave of the disease began in May 1993 in Aswan governorate and characterized by ocular form in human beings and a considerable high number of abortion in cattle and buffalloes.

Samples were collected as aborted foeti, placenta of aborted animals and sera were collected also from places where abortions were recorded. This report documents the trial appiled to explore this condition.

MATERIALS & METHODS

MATERIALS:

(I) Samples:

a) animal sera: a total of 65 sera samples was collected from Aswan as follow (sheep 22, goats 12, cattle 16, and buffalloes 15. The samples stored at -70C°untill it could be tested for virus isolation or detection of RVF

antibodies.

b) portions from the liver, spleen, kidney, lymph node and brain of aborted foeti of one aborted cattle and three aborted buffaloes, also portions from the placenta of two aborted cattle and one aborted buffallo. these samples were taken aseptically and kept frozen at 70C° untill tested for virus isolation.

(II) RVF-antigen: It is sucrose-acetone extracted infected mouse liver prepared from Entebbe strain (HA-titer: 1024). Kindly supplied by U. S. Naval Medical Research Unite- 3 (NAMRU-3), Cairo - Egypt.

(III) RVF-hyperimmune sera: prepared in mice as Hyperimmune Mouse Ascaitic Fluid (HIMAF). Kindly supplied by (NAMRU-3).

METHODS

Virus isolation:

a) Baby Hamster Kidney (BHK 2: Cells : The methods of preparing and infecting the culture of BHK2: have been described (O. I. E.,89).

b) Baby mice inoculation : The methods of preparing and inoculation of mice either I/C or I/P have been described (O. I. E., 89).

Identification of isolated virus:

a) Virus neutralization test in mice : Tissue that produce paralysis and killed inoculated mice, each tissue suspension was mixed with equal volume of the diluted reference RVF-HIMAF, then inoculated into infant mice as (0.01 ml/mouse, I/C) ad 0.03ml/mouse. I/P .

b) Agar Gel Precipitation test (AGPT) : Brains and livers of mice showig paralysis or killed after inoculation, also the inoculated BHK21 cells that gave cytopathic effect (CPE) were tested against RVF-HIMAF by (AGPT) according to the method by (Ouchterlony, 1968).

RVF-Antigen detection: All tissues collected from aborted foeti or placenta of aborted cows and aborted buffalloes were homogenised as suspension in (PBS), and tested ag (RVF-HIMAF) by AGPT according to method described by (Ouchterlony, 1968).

FVF- Antibodies detection: All sera colle were screened against reference (RV antigen) by AGPT according to the me described by (Ouchterlony, 1968).

RESULTS

Virus inolation:

The liver and spleen suspensions from abo faetus of a buffallo (EDFU) causing paralysis mortality 2-3 DPI in mice (I/C or I/P) inducing (CPE) in BHK 21 cells within 36 h The placenta of the aborted (El-KHATARRA) produced the same results., other tissuce or blood sera produced any positi in mice or in BHK21.

Identification:

a) Virus neutralization in mice: No symptoms deaths produced in mice inoculated with samples after they were mixed w RVF-HIMAF. This specific neutralizat indicates that the tissues (liver & splee aborted foetus of a buffallo (EDFU) and placenta of aborted cow contained RVF viru

b) AGPT: By testing 10% suspensions of b and liver of mice showing paralysis or ki after inoculation by the above mentio tissues against (RVF-HIMAF), precipita bands were observed. The same results w recorded by using concentrated BH harvested virus by Polyethylen glycol (P that show (CPE) after inoculation by the s above mentioned tissues aga (RVF-HIMAF).

RVF-Antigen detection: By AGPT us reference RVF-HIMAF, the liver & the sp of aborted faetus of aborted (KOM-OMBO), gave precipitation bands, tissues that not producing positive isola either in BHK21 or in baby mice.

RVF-Antibodies detection: AGPT was applied to detect RVF antibodies in the serum samples against reference RVF-antigen. Sera were collected from non-vaccinated pregnant and non-pregnant differant animal species. Results were presented in table (1). From the results, it is clear that all animal species showed percentages of positive reaction which reached 24.6%. Cattle & Buffaloes show a nearly similar percentages of positive sera 37.5% and 26.7%, also sheep & goats 18% and 16.6% respectively but of a lower incidence.

tissues, including the aborted foeti and foetal membranes. Spleen of infected animal could be a source of RVFV for at least 21 day postinfection and the liver for slightly less time, with other meaning, the spleen was determined to be nearly 17 times better than the liver for RVFV isolation (Yedloutschnig, et al., 1981). Accordingly, we could isolate RVFV from the spleen and the liver of aborted faetus of a buffallo, as well as from the placenta of aborted cow. These tissues inducing (CPE) in BHK21 cells within 36 hour; and causing paralysis and deaths in baby mice 3-5

Table 1:Results of tested animal sera by (AGPT).

Species	Condition	Tested sera	AGPT		
			+ve	+ve ratio	total +ve%
Sheep	aborted	1	1		
	pregnant	14	1	4/22	18%
	non-pregnant	7	2		
Goats	aborted	1	1		
	pregnant	3	0	2/12	16.6%
	non-pregnant	8	1		
Cattle	aborted	8	3		
	pregnant	3	1	6/16	37.5%
	non-pregnant	5	2		
Buffalloes	aborted	6	2		
	pregnant	2	1	4/15	26.7%
	non-pregnant	7	1		
Total		65	16	16/65	24.6%

DISCUSSION

There is some evidence that cell cultures are slightly more sensitive for isolating RVFV than mice, therefore isolations made in 1977 - 1978 were obtained initially in cell cultures (Swanpoel 1981) as well as the identification of the agent can be done by neutralization test with known RVF-positive serum (O. I. E. 1989). Moreover, AGPT produces a highly specific results within 12-36 hour and constitutes a very convenient mean of confirming the identity of a virus isolated in mice (Ayoub & Allam, 1981). In the viraemic stage,RVFV can be isolated from nearly all

DPI. The virus identified by neutralization test in mice and by AGPT with reference RVF-HIMAF, producing no deaths or even paralysis in the first and a definite precipitin lines in AGPT. Concerning the detection of RVF-antigen in the liver and spleen of aborted foetus of aborted cow (KOM-OMBO) by using RVF-HIMAF, and that tissues not producing positive isolation either in BHK21 or in infant mice, this may be due to these tissues were collected when viraemia had disappeared and RVF-antigen could be present in the form of inactive antigen (Niklasson, et al., 1983). This finding proved that AGPT is a highly specific mean of diagnosis of recent infection

especially when tissue cultures or mice inoculations gave negative results. Also, the detection of RVF-antibodies in the sera of non-vaccinated different animal species (Table 1) pointing to the circulation of RVF virus in Aswan governorate.

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