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ISOLATION AND IDENTIFICATION OF THREE-DAY SICKNESS VIRUS IN EGYPT

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SUMMARY

In summer 2000 several outbreaks among imported cattle with clinical manifestations similar to Bovine Ephemeral Fever (BEF) have been recorded in different Governorates in Egypt. 186 heparinized blood samples were collected for virus isolation and identification and 213 serum samples for serological examination.

Suckling baby mice were intracerebrally (I/C) inoculated and Vero, BHK21 cells were also infected with buffy coat for viral isolation then indirect fluorescent antibody test (IFAT) was applied to identify the BEF virus in impression smear of baby mice brain and inoculated Vero and BHK21 cells.

Serological investigation was carried out to clear up the immune status in infected cattle.

INTRODUCTION

Bovine ephemeral fever "BEF" is an arthropod-born viral disease of cattle and water buffalo characterized by short duration of fever, stiffness with a shifting lameness affecting one or more legs. The disease occurs widely across Africa, Asia and Australia Loses (1986). The disease was firstly described in Egypt by Piot (1896) and Rabagliati (1924). Then BEF have been secondary recorded in Egypt (Hassan, 1991). Since that time no publications about the occurrence of BEF in Egypt could be traced.

Certain clinical manifestation appeared in cattle in eight dairy farms in lower and Upper Egypt during May to September 2000 referring to infection with bovine ephemeral fever disease (BEF). So the aim of the present study was to identify the agent incriminated in this problem.

MATERIALS AND METHODS

MATERIALS:

Outbreaks of a disease similar to Bovine Ephemeral fever (BEF) disease was recognized during June, July, August and September 2000 in dairy cattle farms located at different Governorates in Egypt as shown in Table (1).

1- Samples:

a- Blood samples on EDTA were collected from dairy cattle showing early clinical signs of the disease with body temperature of 40 - 42°C for virus isolation and detection.

b- Blood samples were collected from the clinically infected cattle in the investigated areas to estimate the antibody titer against BEF virus.

2- Antisera

a- Reference antiserum against BEF was kindly supplied by Plum Island USA.

b- Fluorescent antibovine immunoglobuline prepared in rabbit (Difco, USA).

3- Virus:

Reference BEF virus was kindly supplied by Virology Dept. Fac. Vet. Med. Cairo University.

4- Laboratory animals:

Suckling mice 1-3 days old were used for viral isolation.

5- Tissue culture:

VERO and BHK_{21} cells were used for virus isolation and serum neutralization test.

METHODS:

I- Blood samples preparation:

Buffy coats were separated from the blood samples, which was collected on EDTA according to Davis and Walker (1974).

Blood smears were made from buffy coats for indirect fluorescent antibody technique [IFAT] which was carried out according to Gardner and Quillin (1980).

II- Virus Isolation and Identification:

a- Baby mice inoculation:

White blood suspension diluted 1:10 in minimal essential medium "MEM" containing Penicillin and Streptomycin were intracerebrally I/C inoculated (0.25 ml) into suckling mice (1-3 days old). The mice were observed daily for any nervous manifestation or death. Impression smears were made from their brains for IFAT. After seven days the mice which had not shon nervous manifestation were killed and their brains were homogenized in 10 % W/V in MEM containing Penicillin and Streptomycin for second and third I/C inoculation in baby mice.

b- Tissue Culture:

The same prepared white blood suspensions were

inoculated three blind passages into confluent sheet of Vero and BHK21 cell lines, then observed daily for evidence of any cytopathic effect. Tissue culture cover slips were inoculated with the isolated virus and collected after 48 - 72 hours for IFAT.

c- Serological Examination:

Serum neutralization test was carried out according to Carbery and Lee (1966) using Vero cells.

RESULTS

Table (1) shows the incidence and distribution of the Bovine Ephemeral fever disease in dairy cattle farms at eight Governorates in Egypt. The first appearance of BEF disease was in Lower Egypt such as Dakahlia Governorate then rapidly spread to Sharkia, Gharbia, Monofia and Kalubia Governorates then reached to upper Egypt in Fayoum, Menia and Assuit Governorates.

• Isolation of the virus:

The virus was isolated from 27 % of buffy coats when inoculated intracerebrally I/C in suckling baby mice which showed nervous manifestation such as tremors, convulsions and paralysis.

The virus also was isolated from 13.44 % and 18.81 % of buffy coats when inoculated into Vero and BHK21 cells respectively as shown in Table (2).

Table (1) geographical distribution of BEF outbreak in dairy cattle farms in Egypt.

Governorate	Locality	Cattle breed	Samples	
			Blood on EDTA	Serum
El-Dakahlia	Mansora Talkha	Frisian-Brown Swiss Friesian	10 9	10 9
El-Sharkia	Belbes Zagazig	Semenital Holstein	11 28	9 30
El-Gharbia	Kotor	Holstein	47	67
EL- Monofia	Tala	Friesian	7	10
El-Kalubia	ElKanater	Friesian	15	18
El-Fayoum	Tamia Salhia	Friesian Friesian	18 4	16 4
El-Menia	Malawy	Holstein-Brown Swiss	7	5
Assuit	Bani-Mour Abnoub	Friesian Friesian	20 10	20 15
Total	-	-	186	213

• **Identification of the isolates:**

The indirect fluorescent antibody test (IFAT) was applied on smears which was made from the buffy coats of the brain of injected mice and infected tissue culture cover slips show the presence of the granular fluorescence in the cytoplasm of the leukocytes 11.3 %, brain cells 27 % and BHK21 as shown in Table (3) and Figs. (1, 2 &3) while Fig. (4) showed the negative control of non infected cells.

• **Serological results:**

The serological results showed that the antibody titer range from 1/4 to 1: 64 in different Governorates. The highest titer was found in EL-Gharbia, EL-Fayoum and Assuit and the total percentage of positive sera was (55.39 %).

Table (2) Results of BEF virus isolation from the buffy coats of clinically infected cattle in baby mice brain, Vero and BHK21 cell lines.

Governorate	No. of Samples	Baby mice brain		Vero cell		BHK21 cell	
		+ve No.	+ve %	+ve No.	+ve %	+ve No.	+ve %
El-Dakahlia	19	4	21.5	2	10.52	3	15.78
El-Sharkia	39	10	25.64	6	15.3	7	17.94
El-Gharbia	47	12	25.53	6	12.76	8	17.02
EL- Monofia	7	3	42.85	1	14.28	2	28.57
El-Kalubia	15	5	33.33	2	13.33	3	20.0
El-Fayoum	22	7	31.81	2	9.09	4	18.18
El-Menia	7	2	28.57	1	14.28	1	14.28
Assuit	30	9	30	5	16.66	7	23.33
Total	186	52	27	25	13.44	35	18.81

Table (3) Results of IFAT either applied on buffy coat smear "BCS" or baby mice brain impression smear "BMBIS"

Governorate	No. of Samples	+ve IFAT on "BSC"	+ve %	+veIFAT on BMBIS	+ve %
El-Dakahlia	19	2	10.5	4	21.5
El-Sharkia	39	4	10.2	10	25.64
El-Gharbia	47	5	10.6	12	25.53
EL- Monofia	7	2	28.6	3	42.85
El-Kalubia	15	1	6.7	5	33.33
El-Fayoum	22	2	9	7	31.81
El-Menia	7	2	28.6	2	28.57
Assuit	30	3	10	9	30
Total	186	21	11.3	52	27

Table (4) Neutralizing antibody titer against BEFV of infected cattle.

Governorate	No. o tested sera	No. of +ve sera	+ve %	Neutralizing antibody titer				
				1:4	1:8	1:16	1:32	1:64
El-Dakahlia	19	12	63.15	3	5	4	-	-
El-Sharkia	39	16	41.02	4	9	3	-	-
El-Gharbia	67	36	53.73	18	2	4	4	8
EL- Monofia	10	6	60.0	2	3	1	-	-
El-Kalubia	18	11	61.11	5	3	3	-	-
El-Fayoum	20	15	75.0	4	5	2	3	1
El-Menia	5	2	40.0	-	2	-	-	-
Assuit	35	20	57.14	5	2	1	8	4
Total	213	118	55.39	41	31	18	15	13

Fig. (1) showing intracytoplasmic fluorescence in leukocyte cells of buffy coat.

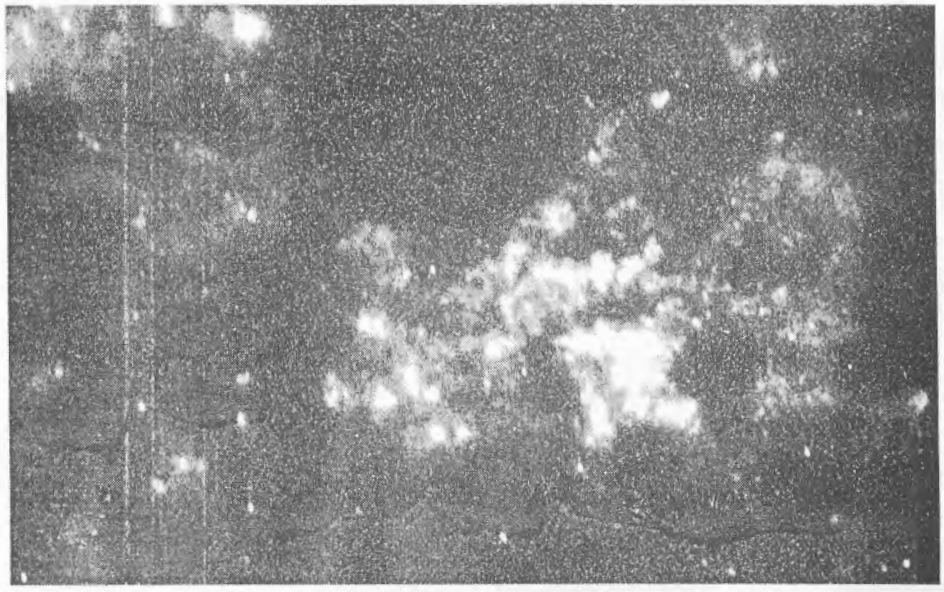

Fig. (2) Showing intracytoplasmic fluorescence in baby mice brain cells

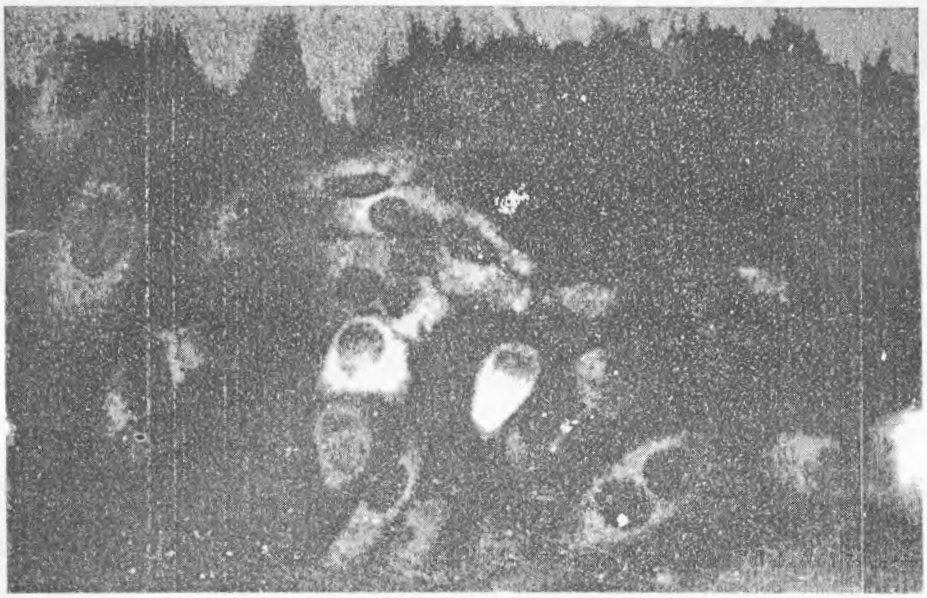

Fig. (3) Showing intracytoplasmic fluorescence in Vero cells

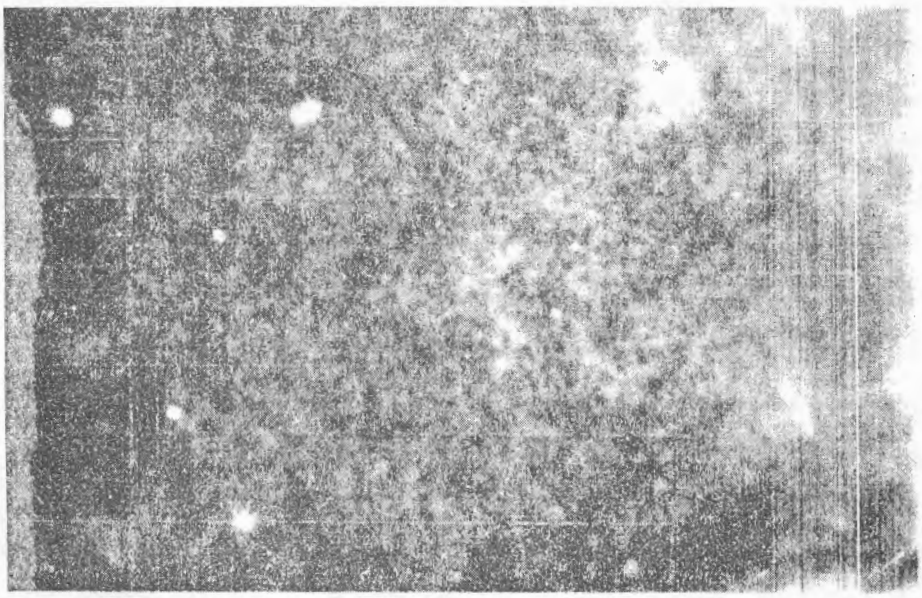

Fig. (4) Showing negative fluorescence in Vero cells

DISCUSSION

BEF outbreaks occurred in Egypt during the summer months (May to September 2000) in eight Governorates with initial stagnant water suitable for the production of the vectors and also probably linked to the effect of the Nile's water level on vector breeding.

Rabagliati (1924) reported that in 1909 the BEF disease moved progressively down the Nile valley from Aswan to Cairo in the summer months.

In 1991 the BEF disease appeared in five dairy cattle farms located in lower Egypt and did not extended to upper Egypt but in the present study the BEF disease appear firstly in five Governorates in lower Egypt then extended to the upper Egypt. The spreading of the disease is probably correlated with the distribution of vectors in addition to the rice fields.

The infected dairy cattle in different Governorates in Egypt showed clinical symptoms referred to BEF disease which characterized by sudden onset of fever in the range 41 - 42°C, nasal and ocular discharges, hurried respiration, stiffness and lameness and pulmonary emphysema with accumulation of air under the skin of backline was noticed in severe cases.

In Fayoum and Assuit Governorates more severe cases still become prostrate in sternal or lateral recumbency.

The observed clinical signs in this report simulate that described by Theodoridis and Coetzers (1979) and Burgess (1971).

There was sudden drop in dairy milk yield which reach in some farms to 50 %. This attributed by Loses (1986) to the subclinical mastitis developed in the febrile stage of the disease. The BEF virus was isolated and detected from buffy coats samples where the virus affects the endothelium of small blood vessels (Mackerras et al., 1940). The virus is contained in the leukocyte fraction of the blood during fever (Theodoridis, 1969) and more particularly in neutrophils (Young and Spradbrow, 1980 and 1985).

The results of attempts to isolate BEF virus from the buffy coat (prepared from blood of febrile cattle by intracerebrally I/C inoculation of the suckling mice 1-3 day-old led to obtain 52 isolates of 27.0 % . The results coincided with those reported by Van Der Westhuizen (1967), Inaba et al. (1968) and Doherly et al. (1969).

The results showed that BEF virus was also isolated in Vero cells (13.4 %) and BHK21 (18.8 %) and this recorded before by Snowdon (1970).

The number of isolates in tissue culture is less than that obtained by inoculation of suckling mice, this may be attributed to the fact that not all strains of BEF virus can be readily adapted to Vero or BHK21 cells (Standfast et al.,1976). The result of the detection of BEFV by IFAT applied on buffy coat smears showed that the number of positive cases is less than that obtained by IFAT applied on the infected baby mice brains impression smears.

The serological results showed that the antibody titer range from 4-64 in different Governorates. The antibody titer was very low or absent in the early stage of the disease then followed by rising in the titer in 5 to 14 day from the beginning of the disease. These findings agree with Burgress (1974).

The investigation proved the occurrence of BEF disease in Egypt and throw light on some pathogen aspects of the disease.

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