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ISOLATION AND IDENTIFICATION OF BOVINE EPHEMERAL FEVER VIRUS FROM CATTLE IN MIDDLE EGYPT

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SUMMARY

During 2000-2001 outbreaks of bovine ephemeral fever (BEF) occurred at Middle Egypt, 251 heparinzied blood samples from cattle and buffaloes with BEF symptoms were collected for virus isolation and identification and 549 serum samples were collected for serological examination. Suckling baby mice were intracerbrally (I/C) inoculation for virus isolation and identification, in the same time isolated virus was adapted to grow in both $Vero1_{21}$ and BKH_{21} cell line. Identification of isolated virus was achieved using both reference and prepared hyper immune serum against BEF virus in rabbit by using SPA agglutination test (SPA), Serum neutralization test (SNT), Mouse protection test (MPT) and Agar gel precipitation test (AGPT).

INTRODUCTION

Bovine ephemeral fever (BEF) is an arthropod borne disease of cattle and water buffaloes. It is caused by virus belonging to family rhabdoviridae, a member of the genus ephemeroviruses, (St. George 1988) it has a negative-sense, single stranded RNA genome (Walker et al., 1991 and Zakrzewski et al, 1992).

Bovine ephemeral fever is characterized clinically by sudden onset fever, stiffness lameness, nasal and ocular discharge and paralysis (St. George 1988, St. George, 1994, Nandi and Nagi, 1999 Radostitis et al. 2000). The virus can be grown by intracerebral inoculation of suckling mice and in cell culture (Van Der Westhuizn 1967 and Doherty et al., 1969).

The disease was firstly described in Egypt by (Rabagliati, 1924) since that time no publications about the occurrence of BEF in our country could be traced. Then BEF have been secondary recorded in Egypt (Hassan, 1991. Bounb., 1994).

In 2000 and 2001 the outbreaks of BEF reoccurred and spread widely all over the country. The present study aimed to isolate and identify the causative virus of the 2000-2001 outbreaks which occurred in Middle Egypt from native and foreign breed cattle.

MATERIAL AND METHODS

2.1- Virus:

BEF virus (Webster's Strain) was obtained from Virology Department Faculty of Veterinary Medicine, Cairo University.

2.2- Virus Vaccine:

Inactivated BEF virus vaccine prepared in $Vero_{[2]}$ cell culture and adjvanated with aluminum hydroxide gel was purchased from Veterinary Serum and Vaccine Research Institute (VSVRI) Abbasia, Cairo.

2.3- Sera and antis era :

2.3.1- Sera :

3.1.1- Serum samples ;

549 blood samples without anticoagulant were collected from diseased cattle and buffaloes that showed symptoms of BEF disease and located in small farms in different area in Beni-Suef and El-Fayoum Governorates. Their sera were separated and individually stored at -20C°, until used for serological studies.

3.1.2- Foetal Calf Serum (FCS); Approved (Virus and Mycoplasma Screened). It was purchased from GIBCO limited, Paesly, Scotland, and U.K.

3.1.3- Control negative serum; was kindly obtained from VSVRI Abbasia, Cairo.

3.1.4- Reference BEF virus anti serum; Reference BEF virus antiserum was obtained from (VSVRI) Abbasia, Cairo.

2.4- Specimens:

2.4.1- Organs:

Portions of lymph nodes, different parts of the lung, liver, spleen, synovial fluid were collected from emergency slaughtered animals during outbreaks of BEF virus in summer 2000 and 2001.

2.4.2- Blood Samples:

Heparinzed blood was taken from cows and buffaloes which showed clinical symptoms of BEF and subjected for separation of buffy coat as described by (Davies and Walker 1974).The buffy coat was used for virus isolation.

2.5- Experimental animals:

2.5.1- Rabbit three Newzland rabbits (Two months old) were purchased from local rabbits farms, reared in animal houses, of National Research Center (NRC), and used for preparation of specific hyperimmune serum against BEF virus.

2.5.2- Suckling mice:

Albino Swiss mice 1-3 days old, obtained from the animal house of National Research Centre (NRC), these used for virus isolation and virus titration.

2.6- Tissue Culture Media:

2.6.1- Eagle's Minimum Essential Medium (MEM): Commercial MEM (Flow laboratories).

2.6.2- Growth medium. Eagle's Minimum Essential Medium with Earle's balanced salt solution containing 10% foetal calf serum (FCS).

2.6.3- Maintenance Medium:

Was prepared as growth medium without lactalbumin hydrolysate and foetal calf serum (FCS) was reduced to 0.5%,

2.7- Media a for agar gel precipitation test (AGPT) was prepared according to (Beard 1982).

Sodium chloride EDTA tris (Net) buffer was prepared according to Kessler (1975) and used for treatment of SPA.

2.8- Staphylococcal protein A:

It was kindly obtained from Virology Dep. Cairo University Beni-Suef branch).

Methods:

2.2.1- Preparation of hyper immune serum against BEFV:

It was prepared according to (WHO. 1973).

2.2.2- Virus isolation:

2.2.2.1- Mice inoculation: it was done from Buffy coat according to Davis and Walker, (1974).

2.2.2.2- Tissue culture inoculation:

a-BHK_{21} inoculation :

BEFV was isolated from collected sample and BHK_{21} according to St. George (1980).

b-Vero $_{121}$ inoculation:

It was done according to Tzipari, (1975).

2.2.3- Titration of the isolated virus:

2.2.3.1- Mouse adapted BEFV:

It was done according to (Theodoridis 1973) and LD_{50} was calculated according to Read and Munch (1938).

2.2.3.2- Titration of the virus on $Vero_{121}$ cells :

It was carried out according to Theodoridis, (1973) and the end point was determined and virus titer was calculated according to the Reed and Muench (1938).

2.2.4- Identification of the isolated virus:

2.2.4.1- Serum Neutralization test (SNT):

It was carried out according to Carbrey (1971),

2.2.4.2- Agar gel precipitation test (AGPT):

It was carried out according to Cown and Graves, (1966) .

2.2.4.3- Staphylococcus protein A (SPA) agglutination test:

Rapid slide agglutination test: (Microscopic agglutination test (MAT).

It was carried according to Barrow and Feltham (1995).

Slow quantitative SPA. Agglutination test:

It was carried according to Kessler (1975).

2.2.4.4- Mouse protection test:

It was carried out according to Van-Der Westhuizen, (1967).

RESULTS

Table (1): Isolation of BEF virus in one day old baby mice.

Year	Animal	Sample inoculated in baby mice brain			
		Buffy coat	Organ extract pool* sample	Synovial fluid	Symptoms of the 4th passage
2000	1. Cattle 1.1. native breed cattle	+ve	-ve	-ve	Neurological signs convulsions, paralysis and loss of body weight then deaths
	1.2. foreign breed	+ve	-ve	-ve	
	2. buffaloes	-ve	-ve	-ve	
2001	1. Cattle 1.1. native breed cattle	ND	ND	ND	-
	1.2. foreign breed	+ve	-ve	-ve	Neurological signs convulsions, paralysis and loss of body weight then deaths
	2. buffaloes	ND	ND	ND	ND

ND = not done

Table (1) showed clearly that BEF virus was isolated in one day old body mice 4-5th passage during 2000 from Buffy coat of native and foreign breed cattle located in Beni-Suef and El-Fayoum Governorates but not isolated from any extracted pooled organs (lymph nodes, spleen, lung, and synovial fluid) the infected mice showed neurological disorder convulsion, paralysis, loss of body weight and death.

Table (2): Mortality and mean survival time in suckling mice with serial passage of BEF1 2000 & BEF3 2001

Passage	Isolate BEF1 2000		Isolate BEF3 2001	
	Survival time/days	Percentage of mortality	Survival time/days	Percentage of mortality
1	21	-	21	-
2	13.5	12.5	15.5	11.1
3	10.5	25.85	13.5	37.5
4	7.5	42.82	10.5	50
5	4.5	80	6.5	66.6
6	3	100	6.5	100
7	2	100	4	100
8	2	100	3	100

Table (2) showed clearly that the survival time post inoculation was decrease greatly from the fist passage to 8th passage and the morality rate was correspondingly increased. There is significant difference between both isolate in 2000 and 001 in their pathogen city to mice. The mortality rate in inoculated mice by second passage was 12.5% while it was 100% from 6th passage, in outbreak 200 while it was 11.1& 100% also in the same passage respectively during the outbreak 2001.

Table (3): Titration of reference and locally prepared hyper immune serum against BEFV by using SPA agglutination test.

Isolate	Log_2 antibody titer of hyper immune BEFV sera		
	Reference	Prepared in rabbit after 2nd dose	Prepared in rabbit after 4th dose
1. BEF1 2000	10	7	10*
2. BEF2 2000	10	7	10
3. BEF3 2000-2001	10	7	10
4. Reference BEFV	10	7	10

* Weak aggregation.

Table (3) showed clearly that both reference and locally prepared hyper-immune serum has higher antibody titer reacted to 10 log_2. The serum from rabbit injected with 2nd dose showed 7 log_2 but after injection with 4th dose 10 log_2.

Table (4): Identification of isolated BEF viruses by AGPT

Isolate	Brain suspension	Liver & spleen	Intestine	Carcass
1. BEF1 2000	++	--	-	-
2. BEF2 2000	++	-	-	-
3. BEF3 2000-2001	++	-	-	-
4. Reference BEFV	++	-	-	-

* Weak aggregation.

BEF 1 isolate from native breed cattle outbreak year 2000.

BEF 2 isolate from foreign breed cattle outbreak year 2000.

BEF 3 isolate from foreign breed cattle outbreak year 2001.

Table (4) showed that the brain suspension of inoculated mice after 6th passage of isolated viruses (passage no. 6th) gave clear precipitating line but liver and spleen suspension, intestinal content and carcass not reacted.

Table (5): Identification isolated viruses by mouse protection test.

Isolate	Pass aged No.	$Log_{10}MLD_{50}$ before treatment hyper-immune sera	$Log_{10}MLD_{50}$ after treatment hyper-immune sera
1. BEF1	6^{th} P	4.7	2.7
2. BEF2	4^{th} P	4.1	2.1
3. Reference BEF virus	3^{rd} P	5.3	3.3

Table (5) showed that the reference and isolated viruses were neutralized by using specific hyper-immune serum. The inoculated mice with a mixture of 4.1 to 5.3 log_{10} MLD_{50} and two neutralizing unit prevent appearance of deaths or clinical signs on inoculated mice. The neutralizing indices were 2.7, 2.1 and 3.3 in BEF BEF_2 and reference BEF virus respectively.

Table (6): Neutralization index (NI) of isolated BEF viruses

Isolate	Pass aged No.	TCID $_{50}$/ml	NI
1. BEF1 2000	6th P	4.5	2
2. BEF2 2000	4th P	4	2
2. BEF3 2001	4th P	3.6	1.6
3. Reference BEF virus	3rd P	5.5	3.5

Table (6) showed that the isolated BEF viruses 2000, 2001 and reference were neutralized by two neutralizing unit of hyper-immune serum. The neutralizing indices 2, 1.6 and 3.5 for the isolated and reference BEFV strain respectively.

DISCUSSION

Bovine ephemeral fever virus is one of the arthropod born virus that induces sever economic losses among cattle in areas showing epidemic of the disease. The disease has a high morbidity and low mortality rate and characterized by short duration, fever, stiffness, lameness and sometimes paralysis. Both onset and recovery are sudden and it has been occurring in summer and Autumn in subtropical and temperate region in Africa, Asia and Australia (St. George 1995) the virus mainly transmitted by arthropod vector and the severity of disease depends upon the vector abundance and distribution beside host susceptibility performance of infection and may be other stress factors (St. George 1993, Radostits et al., 2000).

In Egypt, the disease was first described by (Rabagliati 1924) and since this time and until summer 1991 no any publication about occurrence of the disease after that typical forms of the disease has been occur in different Governorate in Egypt (Hassan et al., 1991; Banoub 1994, , Sayed et al., 2001, Shehab et al., 2004).

Concerning virus isolation the result in table (1) showed clearly that BEF virus was isolated in one day old baby mice After 4-5th passage during 2000 and 2001 outbreaks from buffy coat native breed and foreign breed cattle located in Beni-Suef and El-Fayoum Governorates but not isolated from any extracted pooled organs (Lymph nodes, spleen, lung, and synovial fluid), the infected mice showed neurological disorder convulsion paralysis, loss baby weight and death the results concided with those reported by (Van - der - Westhuizen, 1967). Also the results of attempts to isolated virus adapted on mice brain 4-5th passage also adapted into tissue culture Vero1_{21} and BHK_{21}, all isolated virus need 5th passage to

appear CPE The virus growth in this type of cell line producing characteristic CPE began by cell vaceulation progressed gradually by appearance of granulation, rounded cell and elongation and tapering of infected cell ended with 120 hours by cell degeneration result cell free area by floating cell in medium these results were parallel to that obtained by (Van - Der - Westhuizen, 1967., , Doherty et al., 1969, St. George et al., 1995, Ali et al., 2001).

Results in table (2) showed clearly that the survival time post-inoculation was decrease greatly from the first passage to 8th passage and the mortality rate was correspondingly increased. There no significant difference between both isolate in 2000 and 2001 in their pothogenicity to mice mortality rate in inoculated mice by second passage was 12.5% while it was 100% from 6th passage forward, in outbreak 2000 while it was 11.1 - 111% also in the same passage respectively during the outbreak 2001 this finding agree with (Hassan et al., 1991 and Bounb., 1994).

Results in the tables (3&4) showed that the isolated viruses compared with reference BEFV strain by using SPA agglutination test, AGPT. The isolated viruses beside the reference BEFV strain, showed aggregation in SPA and precipitating line in AGPT by using positive BEF virus hyper-immune serum, these results were agreed with the results of (Van-Der Westhuzien, 1967, Hassan et al., 1991 and Ali et al., 2001).

These results were confirmed by the results obtained from neutralization test and Mouse protection test as in tables (5&6).

The investigation proved that the occurrence of BEF disease in Egypt with complete isolation of the virus from foreign and native breed cattle.

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