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PHYSICO - CHEMICAL CHARACTERS OF LOCALLY ISOLATED BOVINE EPHEMERAL FEVER VIRUS (BEFV)

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SUMMARY

locally isolated strain of bovine ephemeral fever virus particles from outbreak in native breed 2000-2001 was examined by electron microscope and showed bullet or cone shaped, with an average length of 140-180 nm and diameter of 45-85 nm after passage (I/C) on baby mice or Vero121 and BHK21 cell line, the complete virus particles consisted of spikes, a membranous envelope and a helical nucleocapsid. This spike were easily disrupted. The harvest of BEF virus contained defective ñ interfering particles. These particles were short cone-shaped forms about one - third the length of the infectious particles and similar in morphology to infectious virions . These particles interfering with virus replications. The BEF virus was affected by storage at 4°C and -20 °C losing its infective titer1-1.1 log10 at 4°C for18 hours and 1.6 and 1.7 log10 at -20°C for 2 months. The virus lost it's infective titer within 20 min at 56°C, and was very sensitive to ether and chloroform as it decreased 4 and 2.6 log10 when treated , respectively. The isolated BEFV was not able to agglutinate any RBCs of different species.

INTRODUCTION

Bovine ephemeral fever (BEF) is an arthropod-borne viral disease of cattle and water buffaloes characterized by short duration, fever, stiffness, lameness, and sometimes paralysis. Both onset and recovery of infection are sudden. (St George1988 and 1994; Nandi and Negi, 1999 Radostits et al., 2000).

The disease has a variety of names including three days sickness, stiff sickness, dengue fever of cattle, bovine epizootic fever and lazy man's disease (St. George, 1994). However, the name of bovine ephemeral fever is most commonly used. (Comb 1978; Doherty, 1978).

The causative virus was registered in the international catalogue of Arboviruses by Doherty (1978) as Bovine ephemeral fever (BEF)virus. The morbidity may be high but the mortality is low. The disease causes great economic losses such as moralities, abortion, decreased body weight, disruption of markets, drop in milk production ranges from 34-94 with an average of 46% and cows milk yield did not reach to pre-illness level on convalescence and from lowered fertility of bulls as well as the expenses of care or treatment and vaccination (Davis et al., 1984; St-George, 1986 and 1996, Nandi and Negi, 1999, Sayed et al., 2001 and Yeruham et al., 2003) The disease was first described in Africa (Schwenfurth, 1867) it has been reported in many countries in Australia and Asia (St. George, 1986).

Although a viral aetiology was suspected, the cause of the disease remained unknown until Van-der westhuizen, (1967) succeeded in isolating BEF virus from defebrinated blood of cattle with syndrome of ephemeral fever by intracerebral inoculation of suckling mice. He also adapted the virus to grow in cell culture (BHK - 21, clone 13). The virus was isolated from cattle and mixed pool of culicoides spp in 1972 - 1973 (Davies and Walker, 1974).

The disease has been occurred in summer and autumn in subtropical and temperate regions of Africa, Asia and Australia. (St-George, 1984and 1986; Nandi and Negi, 1999). It spread by insect vectors viz., mosquitoes and culicoides by both biologically and mechanically (Burgess, 1971; Standfast and Dyce, 1972).

The dynamics of transmission depend on the vector's abundance, distribution, host preference, and susceptibility to infection (Standfast and Dyce, 1972; Burgess, 1977; St. George 1993 and Radostits et al., 2000).

The disease was firstly described in Egypt by Rabagliati (1924). Since that time no publications on the occurrence of BEF in Egypt could be traced until the summer of 1991, where typical form of the disease has been recorded in different Governorates (Hassan et al., 1991; Nagi et al., 1992; and Banoub 1994). Then reoccurred of the disease at summer 2000 as severe (Ali et al., 2001; Hamoda et al., 2002, Rahman et al., 2002 and Shehabe 2004).Isolation and identification of the virus was carried out from affected animals (Hassan et al., 1991;Banoub 1994;Ali et al., 2001;Rahman et al.,2002;Shehabe 2004; Madbouly et al.,2006)

The present work aimed to study physico-chemical characters of locally isolated bovine ephemeral fever virus .

MATERIAL AND METHODS

1-Virus:

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

b-Local isolated : Isolated BEFV from native breed cattle during outbreak 2000-2001 in middle Egypt Madbouly et al., (2006)

2-Sera and antisera:

2-1 Sera :

2-1-1 Foetal Calf Serum (FCS) Approved (Virus and Mycoplasma Screened). It is purchased from GIBCO limited, Paesly, Scotland, U.K.

2-1-2 Control negative serum. Was kindly obtained from VSVRI Abbassia, Cairo

2-1-3 Reference BEF virus anti sera :

Anti BEFV reference hyperimmune sera was obtained from Veterinary Serum and Vaccine Research Institute, (VSVRI) Abbasia, Cairo..

3-Experimental animals :

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.

4-Tissue Culture Media :

4-1 Eagle's Minimum Essential Medium (MEM): Commercially MEM (Flow laboratories, U.K)

4-2 Growth medium Eagle's Minimum essential Medium with Earle's balanced salt solution containing 10% foetal calf serum (FCS),

4-3 Maintenance Medium:

Was prepared as growth medium without loctalbumin hydrolysate and foetal calf serum (FCS) was reduce to 1-5%

Methods :

1-Preparation of hyper immune serum against BEFV: was done According to (Davies and Walker, 1974).

2-Propagation of the isolated virus: the isolated virus was prepared in mice and an BHK21 or Vero121 cells according to Van-Der Westhuezen, (1967).

3-Titration of the isolated virus:

a-Mouse adapted BEFV :

It was done according to Theodoridis (1973).

b-Titration of the virus on Vero121 cells:

It was carried out according to Theodridis (1973).

4-BEFV purification:

It was done according to (Della - Porta an Snowdon, 1979).

5-Virus characterization:

Locally isolated strain of BEF virus was studie

for effect of preservation at 4°C for 18 hours, freezing at- 20 °C , heat stability at 56°C according to Heushele (1970) , diethyl- ether sensitivity according to Van Der Westhuizen, (1967) and chloroform sensitivity according to Cybinski and Zakrzewski, (1983) .

Haemagglutiation test :

It was performed according to Kaneko, et al., (1985).

6-Electron Microscopy :

It was performed in a guidance of Della. Parta and Brown,(1978) .The shape and size of BEFV were determined by Electron microscope, with 4% ammonium mylbdate.

RESULTS

Table (1) : *Effect of heat on the infectivity titer of BEFV*

Time	*Heat*	*Log_{10} MLD50*		*Log_{10} Difference*
		Before treatment	*After treatment*	
5 min	56°C	4.3	2.3	2
10 min	56 °C	4.3	1.1	3.2
15 min	56 °C	4.3	0.7	3.6
20 min	56 °C	4.3	0	4.3

MLD : Mean lethal dose

Data presented in table (1) showed that the heat effect for BEF_1 virus at 56°C for 20 minutes. The virus lost it's infectivity titer. While it's infectivity titer decrease 2,3.2, 3.6 and 4.3 after heating at 56°C for 5, 10, 15 and 20 minutes, respectively.

Table (2) Effect of preservation at 4°C and -20°C on the infectively titer of BEFV.

Type of effect	Virus	Log 10 MLD 50		Log 10 difference
		Before treatment	After treatment or preservation	
preservation of BEFV1 at 4°C	6th passage of BEFV1	4.7	3.7	1
	Reference strain	5.5	4.4	1.1
preservation of BEFV1 at -20°C	6th passage of BEFV1	4.7	3	1.7
	Reference strain	5.5	3.9	1.6

MLD : Mean lethal dose

Data presented in table (2) showed clearly that the 6th passage BEF_1 virus was decreased one log_{10} after preservation at 4°C for 18 hours. While the reference one was decreased 1.1 log_{10} at the same time. Both viruses were decreased 1.7 and 1.6 log_{10} after preservation at -20°C for 2 months.

Table (3) Effect of Diethyl ether 25% and Chloroform on the infectively titer of BEFV.

Type of effect	Virus	Log 10 MLD 50		Log 10 difference
		Before treatment	After treatment or preservation at 4°C	
Chloroform(5%)	BEFV local isolate	3.9	1.3	2.6
	Reference strain	4.5	2.1	2.4
Diethyl ether 25%	6th passage of BEFV1	4.7	0.7	4
	Reference strain	5.5	1.1	4.4

Data presented in table (3)showed clearly that the BEFV and reference strain were decreased 2.6 and 2.4 log 10 after treatment with chloroform 5% for 30 minutes respectively . Both viruses were decreased 4 and 4.4 log10 when treated with diethyl either with 25% at 4°C for 18 hours.

BEF did not agglutinated RBCs of different species .

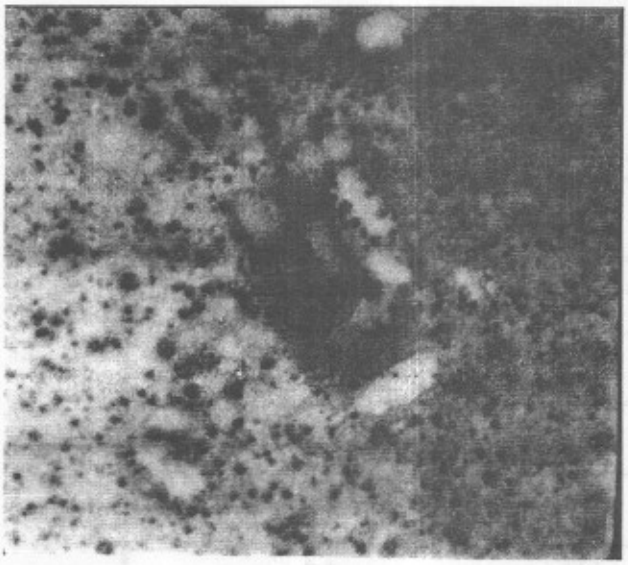
The local isolated BEFV and reference strain did not agglutinated % and 10% RBCs of human, different animal species and bird.

Electron microscopy BEF virus.

This was performed as Della Porta and Brown (1979),using a EM microscope in National Research Center(NRC), Dokki, Giza. Sample were stained , without fixation, with 4 % ammonium molybdate.

The virus shaped was cone like shape or small bullet spike shape with helical nucleocapside coated with enveloped with short spikes as in photo (1) and (2).

Different shaped of virus particles were seemed in field like cone shape. Circular like shape.



(1) Electron micrographs of BEFV stained with 4% ammonium molybdate X100.000 (bullet, cone shape, Truncated cone shaped particles.

(2) Electron micrographs of BEFV stained with 4% ammonium molybdate X80.000 (bullet, cone shape, Truncated cone shaped particles.

DISCUSSION

Bovine ephemeral fever remains as a viral disease of considerable importance to many countries including Egypt .The disease was first described by Rabagliati (1924) and this time and until summer 1991 no publication about occurrence of the disease, after that typical forms of the disease has been occurred in different Governorate in Egypt (Hassan et al .,1991; Banoub, 1994; Sayed et al., 2001; Shehab et al., 2004).

The isolated BEF virus from native breed cattle during outbreak at 2000 as well as the reference one, were characterized by studying the effect of heat, Diethyl ether and chloroform, the agglutina-

bility by RBCs, observation EM and purification by ultracentrifugation. The obtained result revealed that BEFV was inactivated at 56°C for 20 min table (1), sensitive to ether and chloroform table (3), not agglutinated human,duck, chicken,goose,pigeon, turkys, buffuloes,cattle, sheep, goat, and horse RBCs. This result agree with result agree with Kaneko el a., (1986)who reported that no hemagglutination was detected with ephemeral fever virus or anther Australian virus, Berrimah (Dpp 63).

Concerning the process of purification, BEF virus on 15 to 45% sucrose density gradient ultracentrifugation ,fractions of each grad from the upper and lower of the gradient tube to obtain BEFV, this result agrees with (Della - Porta and snowdon 1979 Dell Porta and Brown, 1980).

Concerning examination of purified BEF virus lower and upper peak in electron microscope by using 4% ammonium molybdate, it showed that lower peak contains bullet shape photo(1), cone shape photo (2) and a number of other different bullet shaped forms of virus. The upper grade contains a homogenous population of truncated cone shaped particles about one ñ third the size of the lower grade. The cause of variation from typical bullet shape virions may be due to the temperature at which the virus grown (Della Porta and Snowdon 1979) of to the growth of interfering virus particles Murphy et al., (1972). Or may be characteristic of BEF virus, varying slightly with different isolate like other rahabdoviruses, BEF virus produces a defective interfering (DI) particle. This DI particles is similar to the virion is beginning cone- shape and is about one ñ third the size of the infectious particles similar to vesicular stomatitis virus and rabies virus DI particles (Crick et al.,1969,Crick and brown 1974 and Della ñ Porta and Snowdon ,1979). These DI interfere with replication of BEF virus but not interfere with VSV (Della - Porta and Brown 1978).The BEF virus isolated strain during BEF outbreaks at 2000 and 2001 are cone or bullet shaped on morphology, that particles is similar of rabies and VSV this finding agree with (Della Porta and Brown 1979; Della porta and Snowdon 1980).

The BEF virus shape and size bullet or cone shapes 70X145nm and slight tapered toward the rounded end. The outer enveloped is closely opposed to electrons dense shell, a large particle ranged from 140-160 nm length and 85-90 nm diameter.

This finding agree with (Van-Der Westhuize 1967; Della Porta and Brown 1979; Della Port and Snowdon 1980; Cheng et al., 1992 and Nan and Negi 1999).

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