ANTIGENIC RELATIONSHIP BETWEEN BOVINE EPHEMERAL FEVER (BEF) AND RABIES VIRUSES

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ABSTRACT

The results achieved in the present study explore clearly that the used tcehniques were unable to indieate any antigcnic relationship between bovine ephemeral fever (BEF) and rabies viruses, although both viruses are of Lyssaviruses of the family Rhabdoviridae. Serum neutralization and agar gel precipitation tests were used. The results of both techniques showed that each of BEF and rabies viruses was able to generate a protective level of immunity in vaccinated mice and rats. Moreover, the challenge of vaccinated and boostered mice and rats revealed that such animals were unable to face the heterologous challenge virus while they withstood the challenge with the homologous virus resulted in protection percentage of 1.6-0 and 83.3-100, respectively.

INTRODUCTION

Bovine ephemeral fever and rabies viruses are belonged to Lyssaviruses of the family Rhabdoviridae (**Cybinski and Zakrzewski, 1983; Gard *et al.*, 1983 and Gard *et al.*, 1984**).

Bovine ephemeral fever (bovine epizootic fever, three day sickness, stiff sickness) is an arthropod-borne viral disease of cattle and water buffalo characterized by an acute febrile reaction, stiffness, disinclination of movement accompanied by lameness, high morbidity but with very low mortality and short course (**Burgess, 1971 and St. George, 1988**).

The disease was recorded first in Central Africa 1878 (**Schweinfurth, 1898**) and in Egypt by **Piot, (1896)**. It was also recorded in South Africa (**Vau der Westhuizen, 1967**), Kenya (**Davies and Walker, 1974**), Australia (**Doherty *et al.*, 1972**), Japan (**Ito *et al.*, 1969**), Saudi Arabia (**Abu El-Zein *et al.*, 1997**) and Egypt (**Hassan *et al.*, 1991; Soheir, 1994; Hassan, 2000 and Zaghawa *et al.*, 2000**).

George, (1986) stated that the BEF virus adapted to laboratory animals and tissue culture were enable to develop serum glycoprotein-G which is the major neutralizing and the protective antigen of BEF virus (**Kongsuwan *et al.***,

1998 and Uren *et al.*, 1994). The G-protein was found to be of a molecular weight 79 kDa (**Hertig *et al.*, 1996**). Also, **Atanasiu *et al.*, (1974) and Cox *et al.*, (1977)** reported that the glycoprotein-G of rabies virus, with a molecular weight of 80,000 dalton has been shown to be the only structural protein of the virus that induces the formation of virus-neutralizing antibodies and which confers immunity to animals.

Among the antigenic relationship between the members of the family Rhabidoviridae, **Snowdon, (1970)** stated that there is no evidence of antigenic or immunogenic diversity with the BEF virus population, although **Cybinksin, (1988)** showed that preliminary epitope mapping revealed some variations. In addition, **Sodja, (1986)** stated that an antigenic difference between different rabies isolates, was batch-specific and there was no correlation between protection and virus neutralization.

So, the present study aim to explore the relationship between BEF and rabies viruses using simple laboratory techniques.

MATERIAL AND METHODS

1. Viruses:

1.1. Bovine ephemeral fever (BEF) virus:

A local isolate of BEF virus adapted to mice brain was used in the present study as a challenge virus. It had a titre of 10^6 $MICLD_{50}$/ml (mice intracerebral lethal dose).

1.2. Rabies virus:

A virulent strain of rabies virus to mice (a challenge virus strain) "CVS" was used to challenge vaccinated mice and rats. It had a titre of 10^7 $MICLD_{50}$/ml.

The challenge viruses were used at a final dilutions of I00 $MICLD_{50}$ where each mouse and rat was challenged with 0.03 ml of such dilution inoculated intracerebrally.

2. Vaccines:

Inactivated cell culture vaccines were locally prepared against BEF and rabies viruses at the Department of Pet Animal Vaccine Research, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. The used dose of each vaccine was 0.1 ml injected intraperitonially in each of mice and rats. Two doses of each vaccine were administrated on two weeks interval according to the experimental design.

3. Animals and experimental design:

Forty two weaned mice and forty two weaned rats were used in the present study and supplied by the Department of Pet Animal Vaccine Research, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. They were apparently healthy and reared under hygienic measures.

Each animal breed was divided into 4 groups as follows:

Group (1): Consisted of 12 animals vaccinated with rabies vaccine.
Group (2): Including 12 animals vaccinated with BEF vaccine.
Group (3): Consisted of 12 rats or mice kept unvaccinated as test control.
Group (4): Containing 6 animals received 2 doses of the used vaccine with 2 weeks interval and kept under observation for further 2 weeks. All of such animals (rats and mice) were sacrified and the blood of each animal was collected aseptically and allowed to clot then the serum was separated and subjected to serological examinations.

Groups 1, 2 and 3 were challenged with homologous and heterologous viruses.

4. Serum neutralization test (SNT):

Microtitre SNT was carried out according to **Bass *et al.*, (1982)** to detect and estimate the developed antibodies in the sera of mice and rats. The antibody titres were calculated according to **Reed and Muench, (1938)**.

5. Agar gel precipitation test (AGPT):

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

RESULTS AND DISCUSSION

Bovine ephemeral fever appeared to be as one of the viral diseases which cause economical losses due to the decline in milk production and loss of body weight **(Sharma, 1992)**. Milk production was found to decrease 34-95% **(Davis *et al.*, 1984)**. Abortion was recorded in the late stages of pregnancy **(Parsonson and Snowdon, 1974)**.

So, vaccination against the disease could be considered a main base to control the disease. The presence of an alternative vaccine is essential and helpful especially in emergency cases.

The present studies could be considered as trials to detect an antigenic relationship between BEF and rabies viruses.

The results of serum neutralization test (Table 1) showed that all vaccinated rats and mice exhibited very good levels of specific neutralizing antibodies according to the corresponding vaccine (1.35 and 1.2 $\log_{10}$ for rabies in rats and mice and 1.0 and 1.16 $\log_{10}$ for BEF in rats and mice, respectively) using homologous virus. On the use of heterologous viruses in SNT, a very low (0-0.1 $\log_{10}$) level of cross neutralization. Parallel to SNT, the results of agar gel precipitation test (Table 2) showed strong positive reactions (++++) on the use of homologous virus and antisera while negative reactions were recorded on the use of heterologous components.

Also, Table (3) showed that the protection % was 98.3-100 in case of using of homologous challenge virus according to the inoculated vaccine while this ratio was 1.6-0 in case of heterologous challenge.

The results of the three illustrated techniques, revealed that there is no antigenic relationship between BEF and rabies viruses although they are two

members of the same family. These findings come in agreement with those of **Suowdon, (1970)** who stated that there is no evidence of antigenic or immunogenic diversity with BEF virus population. In addition, **Cybinksin, (1987)** detected some variations in the preliminary epitope mapping.

Among the developed antibodies in vaccinated rats and mice, the present results agree with **George, (1986)** who reported that BEF adapted to laboratory animals and tissue culture was able to develope serum neutralization antibodies in vaccinated laboratory animals. Similar results were obtained in case of laboratory animal vaccinated with rabies vaccine (**Edries, 1994 and El-Gallad *et al.*, 2001**).

From the recorded data, it could be concluded that there is very low or there is no antigenic relationship between BEF and rabies viruses. The study needs more investigation using more advanced techniques dealing with viral structures and genetic mapping.

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Table (1): Homologous and heterologous neutralization of the induced antibodies in mice and rats vaccinated with inactivated cell culture BEF and rabies vaccine.

Animals and vaccines	Mean neutralizing antibody titres (log_{10})	
	Rabies antibodies	BEF antibodies
Rats vaccinated with rabies vaccine	1.35	0.11
Rats vaccinated with BEF vaccine	0.0	1.0
Mice vaccinated with rabies vaccine	1.2	0.1
Mice vaccinated with BEF vaccine	0.12	1.16
Unvaccinated rats	0	0
Unvaccinated mice	0	0

Table (2): Results of agar gel precipitation test using homologous and heterologous viruses.

Immune sera	Rabies virus	BEF virus
Rabies serum from rats	++++	-
BEF serum from rats	-	++++
Rabies serum from mice	++++	-
sBEF serum from mice	-	++++
Negative rat serum	-	-
Negative mice serum	-	-

Table (3): Potency of inactivated BEF and rabies vaccines in mice and rats challenged with the homologous or heterologous viruses.

Animals and vaccines	Protection % in animals challenged with	
	Rabies virus	BEF virus
Rats vaecinated with rabies vaccine	100	0
Rats vaccinated with BEF vaccine	0	100
Mice vaccinated with rabies vaccine	98.3	1.6
Mice vaccinated with BEF vaccine	0	100
Unvaccinated rats	0	0
Unvaccinated mice	0	0

الملخص العربي

العلاقة الأنتيجينية بين فيروسى حمى الثلاث أيام وداء الكلِب

أحمد محمود داود عادل محمد حسن عزب محمد حسن خضير
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معهد بحوث الأمصال واللقاحات البيطرية-العباسية

تم حقن مجموعات مختلفة من الفئران السويسرية البيضاء الصغيرة والكبيرة بكل من لقاحي حمى الثلاثة أيام وداء الكلب النسيجين المثبطين، كل على حدة، وقد تبين سيرولوجيا أن هذه الفئران تكتسب مناعة جيدة ضد كل فيروس حسب اللقاح المحصنة به ألا انه عند إجراء اختبار التحدى باستخدام الفيروس الضاري المماثل للقاح المحصن به كانت نسبة الحماية ٩٨,٣٠%-١٠٠% بينما تكون هذه النسبة صفر%-١,٦% في حالة إجراء التحدي باستخدام الفيروس الآخر. هذا وقد أوضحت نتائج اختباري المصل المتعادل والترسيب في الأجار تفاعلات إيجابية قوية بين الأمصال المناعية والفيروسات المماثلة وتفاعلات سلبية في حالة استخدام مصل مناعي مع الفيروس المخالف. وعلى ذلك يعتقد أنه لا توجد علاقة أنتيجينية بين فيروسي حمى الثلاثة أيام وداء الكلب بالرغم من كونهما من عائلة واحدة والآخر يحتاج لمزيد من الدراسات التي تبين التشابه أو الاختلاف بين تركيب الفيروسين.