ISSN 1110-7219

COMPARATIVE EVALUATION OF THE HOMOGENECITY AND HETEROGENICITY BETWEEN RABIES AND BOVINE EPHEMERAL FEVER HYPER IMMUNE SERA WHEN USED SEROLOGICALLY

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P.B.131

ABSTRACT

Hyper immune sera were prepared in rabbits and cattle against rabies and bovine ephemeral fever (BEF) viruses with the conjugation of some portions of them with FITC. Neutralizing antibodies were estimated in each serum and it was found that rabies antibody titers were higher in rabbits than in cattle while those of BEF were higher in cattle than in rabbits. Homologous and heterologous application of SNT, AGPT and direct FAT revealed that the homologous tests resulted in higher titers and more clear reactions than in hetreogenous tests indicating that there is a little antigenic relationship between rabies and BEF viruses. This relation may be used in the diagnosis of any of the two diseases in the absence of its specific antiserum but with taking in consideration the case history of animal biting and history outbreaks of BEF in addition to clinical symptoms.

INTRODUCTION

Rabies and bovine ephemeral fever (BEF) are two viral diseases caused by single negative RNA viruses within the Rhabdoviridae family **(Hummeler and Koprwski, 1989)** although rabies belongs to genus lyssa while BEF belongs to genus ephemero virus **(Cybinski and Zakrzewski, 1983)**.

The serological link between BEF and lyssa viruses was investigated by **Yonghong et.al. (1995)**. The epitope mapping of rabies and BEF viruses revealed similarities in the location of neutralizing epitopes ,although preliminary epitope mapping within BEF virus population suggested some variation indicating that there is no evidence of antigenic or immunogenic diversity within them **(Kong Suwan et. al.,1998)**.

As BEF is a disease of economic importance and its rapid diagnosis is the first step to plan

a suitable program of control **(Nandi and Negi, 1999)** and rabies is a disease of great antiquity: production of hyper immune antisera against each of them to be used in different serological tests: is of a great demand **(Sikes,1973)**. It was suggested that rabies antiserum could be used in diagnostic purposes of BEF **(Zaghawa, et.al, 2002a)**. It is well known that to obtain an accurate diagnosis and identification of the causative agents, specific antiserum must be provided. So, the present work was planned in a trial to answer the question which is : To any extent rabies or BEF antisera could be used for diagnosis of any of them in the absence of its specific antiserum.

MATERIAL AND METHODS

1. Viruses:-

1.1. Cell culture adapted strains:-

Cell culture live attenuated rabies (ERA Strain) and BEF viruses were propagated in BHK cells and supplied by the Department of pet Animal vaccine Research, Veterinary serum and vaccine Research, Institute, Abbassia , Cairo . They had a Titer of 10^7 and $10^{6.5}$ $TCID_{50}$ /mal respectively. They were used for serum neutralization test (SWT).

2.2. Mice brain adapted strains:-

Rabies and BEF Mice adapted strains were supplied by the same department and used in Agar Gel Precipitation test and fluorescent Antibody Technique. They had a Titer of 10^5 and 10^4 $MICLD_{50}$ / ml (mice intracerebral lethal dose) respectively.

2. Vaccines:-

Inactivated cell culture Rabies and BEF vaccines locally prepared at the Department of pet Animal vaccine Research, veterinary serum and vaccine Research Institute, Abbasia, Cairo, were used for hyper immunization of Rabbits and cattle .

3. Animals:-

3.1. Rabbits:-

Ten adult healthy boscat rabbits of about three Kg body weight were used to prepare hyper immune sera against Rabies and BEF where five rabbits were used to each purpose.

3.2. Cattle:-

Six mixed breed calves of about 1-1.5 years old were used for preparation of Rabies and BEF antisera where three animals were used for each serum preparation.

All animals were kept under hygienic measures receiving balanced ration and adequate water.

4- Preparation of hyper immune anti-sera:

Preparation of rabies and BEF hyper immune anti-sera in rabbits and cattle was carried out according to the procedure described by **Benedict (1967).**

5-Titration of the prepared anti-sera;

The prepared anti-sera were titrated to estimate the levels of induced rabies and BEF neutralizing antibodies using the micro titer technique of serum neutralization test (SNT) according to **Bass, et.al. (1982)** while the antibody titer was calculated as the reciprocal of the final serum dilution which neutralized and inhibited the cytopathic effect of 100-200 $TCID_{50}$ of the used virus according to **Singh, et.al. (1967).**

6- Precipitation of immune globulins from the prepared anti-sera and their conjugation with flourecine isothiocyanate (FITC):

Precipitation of immune globulins from the prepared rabies and BEF anti-sera were precipitated using ammonium sulphate solution following the method adopted by **Narin and Marrack (1964)** then their values were estimated according **Weichselbaum (1946)** and adjusted to be 20mg/ml in normal saline.

Conjugation of these immune globulins with FITC was carried out according to the methods of **Narin (1969).**

7- Agar gel precipitation test (AGPT):

AGPT was carried out using the mice brain adapted rabies and BEF viruses with homologous and heterogonous anti-sera. The test was carried out according to **Cowan and Graves (1966).**

8-Direct fluorescent antibody technique (FAT):

FAT was applied on smears prepared from infected mice brains with rabies and BEF viruses according to **Soliman, et.al. (1989).**

*** N.B.**

Both of SNT; AGPT and FAT were carried out using the prepared anti-sera in homologous and heterogonous manners.

RESULTS AND DISCUSSION

Rabies and BEF are two viral diseases of non neglectable zoonotic and economic importance and their rapid diagnosis is the first step to start the control and eradication programs **(Nandi and Negi, 1999)**. Diagnosis of the two diseases depends on the detection and identification of the causative agent using serum neutralization test **(Diaz and Varela, 1976 and Tzipori ,et.al. 1975)**; agar gel precipitation test **(Ferguson and Schild, 1982 and Gard, and Melvile. 1984)** and fluorescent antibody technique **(Young and Spradbrow, 1985; Loza, et.al., 2000 and Ali, et.al., 2001)**.

The present study was planned to prepare rabies and BEF hyper immune sera in a trial to detect to any extent any of these sera could be used in the diagnostic purposes of rabies and BEF in the absence of its specific antiserum in addition to the conjugation of such antisera with FITC in order to provide local products to be available in need saving time and high cost of imported little amounts of such products.

The obtained results that tabulated in table (1) revealed that rabies hyper immune serum prepared in rabbits was of higher titer (1024) than that prepared in cattle (512) while BEF hyper immune serum prepared in cattle was of higher titer (2048) than that prepared in rabbits (1024). These findings could be attributed to the host specificity in case of BEF anti sera where cattle are the first specific host to BEF **(Davis et.al., 1993)** while rabbits were not recorded as host or even laboratory animals for the disease assay although suckling mice were laboratory animals of choice for such purposes **(Burgess and Spradbrow, 1977 and Nagi et.al., 1992)**. On the other side rabbits were widely used for preparation of different viral and bacterial anti sera and to a wide range in rabies diagnosis; preparation of hyper immune serum and even in preparation of old vaccines **(Dzhmukhadze and Girbencha, 1972 and Edries et.al., 1999)**.

The recorded results in tables 2;3 and 4 clarified that homologous reactions between the prepared anti rabies and anti BEF hyper immune sera and their specific viruses resulted in great differences in the estimated neutralizing and precipitating antibody titers and more clear positive fluorescent antibody reactions than in case of use heterogeneous ingredients as demonstrated by SNT; AGPT and FAT. These results indicate that there is a very little antigenic similarities be-

tween rabies and BEF viruses the thing which could be attributed to the little similarities in the location of neutralizing epitopes, although preliminary epitope mapping within BEF virus population suggested some variation between the two viruses the fact that supported by the findings reported by **Daoud, et.al. (2001); Zaghawa, et.al. (2002b) and El-Habbak (2005).**

So, it could be said that rabies or BEF anti sera may be use in the diagnostic purposes of each other in the absence of the specific antiserum but with an attention directed to the case history (i.e. exposure for animal biting; the affected animal species or a history of BEF outbreaks) and clinical symptoms.

In addition; the prepared hyper immune sera and conjugates resample very important products and enable the veterinary authorities to obtain accurate and rapid diagnosis of such important disease at time when need.

Table (1): Neutralizing antibody titers in the prepared anti-sera.

Tested anti-serum	Neutralizing antibody titer*	
	Prepared in cattle	Prepared in rabbits
Rabies antiserum	512	1024
BEF antiserum	2048	1024

*Antibody titer= the reciprocal of the final serum dilution which neutralized and inhibited the cytopathic effect of 100-200 $TCID_{50}$ of the used virus.

Table (2): Homologous and heterogonous serum neutralizing antibody titers in the prepared rabies and BEF antisera.

Used Virus	Serum neutralizing antibody titer*			
	BEF antiserum		Rabies antiserum	
	Prepared in			
	Cattle	Rabbits	Cattle	Rabbits
Rabies	8	16	512	1024
Cattle	2048	1024	4	8

*Antibody titer= the reciprocal of the final serum dilution which neutralized and inhibited the cytopathic effect of 100-200 $TCID_{50}$ of the used virus.

Table (3): Application of AGPT in homologous and heterogonous manners on rabies and BEF prepared anti-sera

Used Virus	Precipitation antibody titer			
	BEF antiserum		Rabies antiserum	
	Prepared in			
	Cattle	Rabbits	Cattle	Rabbits
Rabies	16	0	128	512
Cattle	128	256	4	8

Table (4): Homologous and heterogonous fluorescent antibody reactions between rabies and BEF viruses and their prepared anti-sera

Used Virus	FA reactions between rabies and BEF viruses and their anti-sera			
	BEF antiserum		Rabies antiserum	
	Prepared in			
	Cattle	Rabbits	Cattle	Rabbits
Rabies	2+	+	4+	4+
Cattle	4+	4+	±	+

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الملخص العربى

تقييم مقارن لعلاقة التجانس والاختلاف بين أمصال السعار والحمى العابرة عالية العيارية المناعية عند إستخدامها سيرولوجياً

زينب طه سالم سلامه

معهد بحوث الأمصال واللقاحات البيطرية - العباسية - القاهرة

لقد إستهدفت الدراسة الحالية التوصل إلى معرفة إلى أى مدى يمكن إستخدام المصل عالى العيارية المناعية للسعار والحمى العابرة فى الاختبارات السيرولوجية المختلفة المستخدمة فى تشخيص كلا المرضين، وعلى ذلك فقد تم تحضير أمصال عالية العيارية المناعية ضد كل من الڤيروس المسببين فى الأرانب والأبقار ثم إقران أجزاء من هذه الأمصال بالفلويسين إيزوثيوسينات ثم تمت معايرتها وإجراء إختبارات المصل المتعادل الترسيب فى الآجار والوميض الفلوريسنتى المناعى المباشر تجانسياً وتبادلياً، وقد أوضحت نتائج هذه الاختبارات أن معيار المصل المضاد للحمى العابرة يكون أعلى عند تحضيره فى الأبقار عنه عند تحضيره فى الأرانب، بينما يكون المصل المحضر ضد السعار ذو معيار أعلى فى الأرانب عنه فى الأبقار، كذلك تبين أن تفاعلات الاختبارات السيرولوجية المطبقة تكون أعلى وأوضح عند إستخدام أمصال وڤيروسات متجانسة عنه عند إستخدام أمصال مخالفة للڤيروسات المختبرة، وعلى ذلك يمكن القول بوجود علاقة أنتيجينية ضئيلة بين الڤيروسين يمكن معها إستخدام أى المصلين فى تشخيص المرض الآخر مع الأخذ فى الإعتبار تاريخ الحياة المرضية من تعرض للعقر من حيوانات ضالة أو وجود أوبئة من الحمى العابرة إضافة إلى الأعراض الإكلينيكية التى تظهر على الحيوانات.