COMPARATIVE STUDIES ON BOVINE EPHEMERAL FEVER (BEF) VACCINES PREPARED FROM THE EGYPTIAN STRAIN "BEF-AVS/2001" AND THE AUSTRALLIAN STRAIN "WEBSTER-919V10"

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Keywords: Bovine ephemeral fever strains — inactivated viral vaccine,

ABSTRACT

In the present study, two cell culture inactivated vaccines were prepared from the local and Australian strains of BEF virus. Each vaccine was inoculated in a group of susceptible cattle using the same dose and giving a booster dose two weeks after the initial dose. It was found that cattle responded better to the local strain vaccine than to the Australian one recorded higher levels of serum neutralizing antibodies suggesting a longer period of protection. The prepared hyperimmune sera; in rabbits and conjugated with Fluorescein Isothiocyanate "FITC" showed antibodies similar in their titer behavior to those in vaccinated cattle i.e. the local strain induced higher antibody titer than the Australian one. It was clear that the Fluresent antibody technique "FAT" is a rapid and sensitive technique to diagnosis BEF using either conjugated antiserum.

INTRODUCTION

During summer 2000, a sever outbreak of Bovine ephemeral fever (BEF) disease was recorded in Egypt **(Soad, et al. 2001)**, resulting in a great eeonomic losses in cattle herds **(Zaghawa, et al. 2000)**. This disease eaused by a virus belongs to family Rabdoviridae, genus ephemorovirus. It is known as an arthropod-born viral disease **(Murphy, et al. 1999)**.

The disease represents an economie problem in tropical and subtropieal regions of Africa, Asia and Australia **(Nandi and Nega, 1999)**, so it should be kept under control.

Control of BEF viruses based on the eradication of insects and vaccination of susceptible hosts "cattle and buffaloes" **(Sewell and Brocklesby, 1990)**. The first trial to produce a BEF vaccine was developed in South Africa by Van-der **Westhuizen (1967)** by attenuating the virus

through several passages in suckling mice, the brain suspention was combined with Freund's incomplete adjuvant and given as multiple injections. **Heuschele and Johnson (1969)** produced an effective experimental vaccine by combining BEF virus passage in BHK cells with Freund's incomplete adjuvant and given also as multiple injections. **Tzipori and Spradbrow (1973)** produced a vaccine from the third generation of mouse brain passage virus combined with the adjuvant aluminium hydroxide gel and given as a course of two injections. **Webster, (1988)** produced a vaccine from the strain $919V_{10}$ with Quil A as an adjuvant and given as a multiple injection.

Inaba; et al. (1969) observed that the attenuated BEF viruses had largely lost their antigenicity. So al 1973; **Inaba et al.; Theodoridis et al.** and Tziport and Spradbrow developed a formalin inactivated aluminum phosphate gel adsorbed vaccine which was given as a course of two injections with annual booster inoculation. **Della-Porta and Snowdon (1979)** vaccinated cattle with an inactivated BEF vaccine with D- propiolactone or formalin and then combined with aluminum phosphate gel or Freund's incomplete adjuvant. They found that such vaccines resulted in a poor resistance to experimental challenge with the virulent virus. **Vansclow, et al. (1985)** used strain $919V_{10}$ of BEF virus with various adjuvant and vaccine regimes, these tests resulted to give the Quil A as an adjuvant and the vaccines given in two successive doses, these vaccines shown to provide a good protection level. **Daoud et al., (2001)** prepared a potent inactivated BEF vaccine from the local Egyptian isolate. Such vaccine was prepared on VERO cells and inactivated with Binary ethelemine, using alhydragel as an adjuvant. It was found that the vaccinated cattle exhibited a neutralizing antibody level that was able to withstand the experimental challenge with the virulent strain.

Many workers suggested that there are different strains of BEF and they have some antigenic variations where there are certain isolates fail to induce antibody to heterologous strains but do so to the homologues strains **(Losos, 1986; Tian et al.,1987 and Cybinski et al., 1990 & 1992)**.

Finally, it was of interest to study the immunogenic relationship between the Egyptian and Australian strain used in the vaccine production.

MATERIAL & METHODS

I- VIRUS STRAINS:

1.1-THE LOCAL STRAIN:

BEF-AVS was used in the present work as a local strain of bovine ephemeral fever **(Soad, et al. 2001)**. It was propagated three times in BHK_{21} cell line **(Azab, et al, 2002)**. It has a titer of

10^7TCID$_{50}$/ML .This virus was used to prepare the local inactivated vaccine according to **(Daoud et al., 2001)** and in the serological tests.

1.2- THE AUSTRALIAN STRAIN:

BEF stain 919V$_{10}$ was used to prepare an inactivated vaccine on BHK$_{21}$ cell line, following up the same procedure adapted to the local one. It was supplied kindly by the Faculty of Veterinary Medicine, Cairo University.

It was propagated on VERO cell culture twelve times **(Vanselow, 1985)**. This virus was also passaged three times in BHK21 cell culture and had a titer of 10^6TCID$_{50}$/ml. It was also used in comparative serological tests.

On the time of vaccine preparation, the titer of the two strains was adjusted to be 10^6TCID$_{50}$/ml. BEI was used as an in activator at a final concentration of 0.01M for 3.5 hours at 37°c and the Alhydragel was added as adjuvant at a ratio of 20% **(Daoud et al., 2001)**.

2-ANIMALS:

2.1- CATTLE:

Nine cross-breed Friesian calves of about 1-1.5 year old were screened using SNT and found to be free from BEF neutralizing antibodies. They were classified into three groups (3 calves/ groups). Group one was vaccinated with the local vaccine and group two was vaccinated with the vaccine prepared from the Australian strain. The used dose was the same of each vaccine (2ml for each contain 2 X 10^6TCID$_{50}$) animal inoculated s/c and followed by a booster one after 2 weeks from the first dose. The 3rd group was kept as unvaccinated animal control.

All animals were kept under hygiene measures receiving balanced ration and adequate water; and subjected to daily clinical examinations.

2.2- RABBIT:

Ten adult healthy boscat rabbits were used to prepare hyperimmune sera against the local and Australian BEF viruses where five rabbits were used to each one.

3-SAMPLING:

Serum samples were obtained from all animal groups weekly post vaccination up to 3 weeks

post the booster dose. These samples were subjected to serological examination to estimate; in a comparison; the induced antibodies by each vaccine.

4- SERUM NEUTRALIZATION TEST (SNT):

Quantitative SNT was carried out using the micro titer technique according to **Young and Spradbrow, (1990)** and the antibody titer was calculated as the reciprocal of the serum dilution which neutralizes and inhibits the CEP of 100-200 $TCID_{50}$ of the used virus aecording to **Singh et al., (1967)**.

5- PREPARATION OF BEF HYPERIMMUNE SERA CONJUGATED WITH FLUORESCEIN ISOTHIOCYANATE:

The hyperimmune sera were prepared in rabbits according to **Edries et al., (1999)**.

The immunoglobulin were precipitated and then conjugated with FITC according to **(Narin and Marrack, 1964)**.

6- DIRECT FLOUREST ANTIBODY TECHNIQUES (FAT):

It was carried out against the local and Australline strains of BEF virus in infeeted BHK cell cultures.

FAT was carried out according to **(Peter,K. 1969)**.

* Both of SNT and FAT were carried out homogonously and heterogencously and in a quantitative manner.

RESULTS AND DISCUSION

BEF was described in several African countries and several outbreaks were reported in Egypt especially during the last few years **(Hassan et al., 1991; St. George, 1994; Abdel Maneiam et al., 1995; Soad et al., 2000 and El Naggar, 2003)**.

In Egypt as in many developing countries, it is necessary to protect animal's wealth against dangerous diseases which usually affect dramatically this wealth due to the great economic losses in both meat and milk production.

BEF appears to exist as a single serotype worldwide. An analysis of a number of different iso-

lates has revealed some antigenic variation and to put such disease under control, it is necessarily to provide a highly immunogenic specific vaccine which has the ability to induce high immune response in vaccinated cattle to be able to withstand natural infection (St. George, 1994).

In the present study it was of interest to compare between the efficiency in the immune response of local breed cattle to vaccines prepared from the local strain of BEF and the Australian one. In addition to compare between the antigenic relationships between the two strains using hyperimmune sera prepared in rabbit and conjugated with FITC.

The experimental result show that the serum neutralizing antibody induced in cattle vaccinated with the vaccine prepared from the local strain recorded a higher titer than those induced by the Australian strain vaccine.

Also carrying out SNT using heterologous viruses revealed that homologous viruses showed higher antibody titer than heterologous one as shown in table (1) & (2). This result showed that the local strain is more immunogenic than the Australian one, suggesting that it could be of longer protection period where it was suggested that there was an apparent relationship between neutralizing antibody response and the level of protection. Such immunogenic difference could be attributed to the low passage level in T.C. of the local strain (which was not more than three passages) than the Australian strain (11-12 passage in T.C.) as described by **Inaba, et al.(1969) and Theodoridis et al., (1973)** who mention that the virus losses its immunogenic for cattle by the repeated passages in BHK or VERO cell lines .Or to the variable mutant in the surface glycoprotein G which is the major neutralizing and protective antigen of BEF virus **(Kongsuwan,et al. 1998)**.

The obtained serum neutralizing antibody titers at their peak (128) for the local strain and (32) for the Australline one were recorded by the 4^{th} week post the second dose similar result were reported by **Chiu and Lu (1987)** who suggested that such titers (128) could protect cattle against infection up to 12 month.

On the other side preparation of hyperimmune sera in rabbit showed that rabbit responded immunogically nearly in the same manner to the two strains as indicated by SNT (table 3), where it was also noticed that the detected antibody titer were higher in the use of homologous virus than in the use of heterologous one. These findings appear to be come in a parallel manner with those obtained from the SNT carried out in the sera of vaccinated cattle, the FAT showed that both conjugated hyper immune serum reacted positively with the two viruses spotting the light on the possibility of the use of any antiserum to diagnosis of BEF virus as stated by **(Mellor, 2001 and Zaghawa, et al.2002)**.

However FAT reaction power was also depending on the titer of antibody in the prepared

hyper immune serum (conjugated globulins).

The use of FAT to protect BEF virus was recorded by many authors as **Young and Spradbrow, (1981); St Georgwe, (1988); Hassan, et al., (1991) ; Zaghawa, et al.(2002) and Azab, et al (2003).**

It could be concluded that to obtain a good level of immunity against BEF disease among local breed of cattle it is preferable to use the local strain from vaccine preparation. Conjugated hyper immune serum used in FAT is useful to obtain rapid sensitive and accurate diagnosis of BEF using either strains, this fact is true since **Azab, et al (2003).**

Table (1): BEF neutralizing antibody titers in vaccinated calves as estimated by SNT using the local viral strain.

Animal group	Used vaccine	*Serum neutralizing antibody titer					
		**1WPV	2WPV	"1WPB	2WPB	3WPB	4WPB
1	Local strain	4	8	32	64	128	128
2	Australian strain	2	8	16	32	32	32
3	control	0	0	0	0	0	0

Table (2): BEF neutralizing antibody titers in vaccinated calves as estimated by SNT using the Australian viral strain.

Animal group	Used vaccine	*Serum neutralizing antibody titer					
		**1wpv	2wpv	"1wpb	2wpb	3wpb	4wpb
1	Local strain	2	4	16	32	64	64
2	Australian strain	4	8	16	32	32	32
3	control	0	0	0	0	0	0

*Antibody titer= the reciprocal of serum dilution which neutralizing and inhibit the cytopathic effect (CPE) of 100-200 $TCID_{50}$ of used virus.

**WPV= Weck post vaccination. "WPB= Weck post booster vaccination

Table (3): BEF neutralizing antibody titers in rabbit hyperimmune sera.

Type of hyperimmune sera	*Neutralizing antibody titers using	
	Local strain	Australian strain
Against local strain	2^{10}	2^{8}
Against Australian strain	2^{7}	2^{9}

*Antibody titer= the reciprocal of serum dilution which neutralizing and inhibit the cytopathic effect (CPE) of 100-200 $TCID_{50}$ of used virus.

Table (4): Direct FAT on the prepared rabbit BEF hyperimmune sera conjugated with FITC.

Type of conjugated hyperimmune sera	FAT reacting with	
	Local virus strain	Australian virus strain
Against local strain	10^{4}	10^{3}
Against Australian strain	10^{2}	10^{3}

N.B.: The technique was carried out using the same virus titer of both ($100TCID_{50}$)

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الملخص العربى

دراسات مقارنة على لقاحات حمى الثلاث أيام المحضرة من العترة المصرية (DEFAVS/2001) والعتره الاسترالية (Webester 919V$_{10}$)

المشتركون فى البحث

نجلاء إبراهيم على زينب طه سالم سلامه أحمد محمود داود

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فى هذه الدراسة تم تحضير لقاحى نسيجين مثبطين ضد مرض حمى الثلاث أيام إحداهما من العترة المحلية والآخر من العترة الاسترالية، وقد تم تحصين مجموعة من الأبقار باللقاح الأول وأخرى باللقاح الثانى مع ترك مجموعة ثالثة كضابط للتجربة باستخدام نفس الجرعة من اللقاحين وبإعطاء جرعة منشطة بعد إسبوعين من الجرعة الأولى. وهذا وقد أظهرت نتائج إختبار المصل المتعادل أن إستجابة الأبقار تكون أفضل وأعلى للقاح المحضر من العترة المحلية عنها للقاح المحضر من العترة الاسترالية مما يتيح فرصة لمدة حماية أطول.

كما تم تحضير مصلين مناعين عالية العيارية ضد كلا العترتين وتم أقرانهما بمادة الفلورسين أيسو سيوسيانيت حيث كانت إستجابة الأرانب لكل منهما متماثلة لما حدث فى الأبقار، وقد أوضح إختبار الوميض الفلورسنتى المناعى المباشر لمكانية إستخدام هذه التقنية فى تشخيص المرض باستخدام أى من المصلين.