EVALUATION OF HEAMAGGLUTINATION-INHIBITION ASSAY AND SERUM NEUTRALIZATION TEST FOR DETECTION OF BOVINE EPHEMERAL FEVER VIRUS ANTIBODIES IN CATTLE

By

Sharawi,* S.S.A and Khadr**A.M.
*Dept. Virology, Fac, Vet. Med. Banha Univ.
** Dept. Medicine, Fac. Vet. Med. Alex. Univ.

SUMMARY

Thirty seven serum samples of naturally infected cattle of different ages in Kalubia Governorat during summer season of 2005, were submitted for detection and titration of antibodies to bovine ephemeral fever virus (BEFV) by heamagglutination-inhibition (HI) test comparing with those obtained by the standard serum neutralization test (SNT).

The result of detection of BEFV antibodies in all cattle sera by SNT indicated that; 19 out of 37 (51.4%) were positive. The serum neutralizing antibodies not detected in calves less than 6 months old while 6 out of 13 (46.7%), 13 out of 15 (86.7%) were positive for 1-2 years and < 3 years old cattle respectively.

Concerning the use of HI test for detection of heamagglutination-inhibition antibodies to BEFV, 29 out of 37 (78.9%) were positive, on the other hand 3 out of 9 (33.3%), 11 out of 13 (84.6%), 15 out of 15 (100%) of calves less than 6 months, 1-2 yeas and < 3 years old cattle were positive respectively. HI test gave a higher antibody titer in comparison to SNT. The percentage of agreement between HI test and SNT for detection of antibody to BEFV was 72.9%. The sensitivity and specificity of HI test in comparison to SNT for detection and titration of antibodies against BEFV were 100% and 44.4% respectively. It could be concluded that HI test can be established for detection and titration of antibodies to BEFV. The inherent advantage of HI test makes it an attractive assay for the seroepidemiology of bovine ephemeral fever disease.

INTRODUCTION

Bovine ephemeral fever (BEF) is a disabling viral disease of cattle and water buffaloes *(Walker 2005)*. Bovine ephemeral fever virus (BEFV), like other rhabdovirus, has a (-ve) ss RNA genome and five structural proteins including a nucleoprotein (N), a polymerase-associated protein (P), a matrix protein (M_1 & M_2) a large RNA dependent RNA polymerase (L) and a surface

glycoprotein (G) *(Walker et al, 1991)*. The G protein contains specific and neutralizing antigenic sites as well as heamagglutinating site (heamagglutinin) *(Cybinski et al., 1990)* that induce protective immunity in cattle *(Hertig et al., 1996)*. Variation and mutation of BEFV were recorded *(Davis et al., 1993 and Wang et al., 2001)*.

Diagnosis of BEFV depends on isolation and serological identification of viral antigen as well as detection of specific antibody by serum neutralization test (SNT) *(Tzipori, 1975)*.

SNT has many disadvantages as time consuming, bacterial contamination and toxic effect of samples on tissue culture *(Burleson et al 1997)*, on the other hand, the occurrence of autointerference due to the presence of defective interfering BEFV particles in cell culture had been noted *(Tzipori, 1975)*, so abortive infection of infected cell could be suspected.

Although the heamagglutination of Adelaide River virus, (a member of the bovine ephemeral fever viruses) was reported *(Kaneko et al., 1986 and Shorthose et al., 1986)*, only one document by *Khalil et al., 2005* was available for detection heamagglutination of BEFV and application of HI test for titration of heamagglutination inhibition antibodies in bovine sera.

The objective of this work was aimed to study the relationship between haemagglutination inhibition and neutralizing antibodies to compare between HI test and SNT as a diagnostic tools for detection of BEFV antibodies in sera of naturally infected cattle with BEFV.

MATERIALS AND METHODS

1. Blood serum samples:

Thirty seven serum samples were collected from native cattle suspected to be infected with BEFV in Kalubia Governorate during summer 2005.

2. Bovine ephemeral fever virus (BEFV):

BEFV was isolated from cattle in Menoufia Governorate during summer 2000 and it designated as Menoufia 2000 strain *(Khaliel et al., 2001)*. The virus was propagated in brain of 2-day-old suckling mice and Vero cell line for one passage. It was used for SNT and HI test.

1. Serum neutralization test (SNT):

The β- procedure was applied in microtitier plate using 100 TCID$_{50}$ of tissue culture- adapted BEFV *(Young and Spradbrow 1990)*.

2. Hamagglutination (HA) test and Haemagglutination inhibition (HI) test:

HA test was carried out in microtiter plates at 37°C using 0.5% chicken RBCs, to test the heamagglutination activity of BEFV after its passage in vero cells while the HI test was done to estimate the haemagglutination inhibition antibodies in cattle sera using 4HA units. The reciprocal of the highest serum dilution showing complete inhibition of heamagglutination was considered as the HI titer *(Burleson et al., 1997)*.

3. Statistical analysis:

It was carried out using Epi-info computer program designed by *Dean et al., (1994)* and produced by world health organization (WHO). The percentage of agreement, sensitivity and specificity were calculated according to *Knapp and Miller (1991)*.

RESULTS

The results presented in table (1) illustrated the prevalence of antibodies to BEFV in cattle sera using SNT, and HI test. Table (2) indicated the frequency distribution of BEFV antibody titer using SNT, and HI test. Table (3) revealed the percentage of agreement between SNT and HI test for detection of antibodies to BEFV in cattle sera, while table (4) presented, the results of sensitivity and specificity of HI test in comparision to SNT for detection of antibodies to BEFV.

DISCUSSION

The neutralizing antibodies of infected cattle in response to BEFV are considered of diagnostic value *(Cybinski 1987)* in conjunction with clinical signs of the disease *(Bi et al 1993)*. Detection of BEFV antibodies by SNT (table 1) revealed that, 19 out of 37 (51.4%) examined sera were positive. This result came in agreement with the findings of *Chiu and Lu 1984, Wongwatcharadumrong et al 1985 and Jung 2001*. On the other hand no neutralizing antibodies were detected among calves less than 6 months old. This result confirms the previous results of *Abu-El Zein et al., 1997* who recorded that the BEFV was not isolated from calves less than 5 months old. The detection of neutralizing antibodies of cattle aged 1-2 years and over 3 years old were 6 out of 13 (46.7%) and 13 out of 15 (86.7%) respectively, these result simulated the data obtained by *Uren et al 1987,* who stated that the disease was most frequently recorded in cattle under 3 years of age but also occurred in older cattle.

Concerning the use of HI test for detection of heamagglutination inhibition antibodies to BEFV, 29 out of 37 (78.9%) examined cattle sera were positive. On the other hand 3 out of 9 (33.3%), 11 out of 13 (84.6%), 15 out of 15 (100%) of 6 months old calves, 1-2 years and over 3 years old cattle sera respectively were positive. The results presented in table (2) showed that the HI test had been given high antibody titer to BEFV in comparison to SNT. The examined sera had a titer between (2-8), which is in agreement with *Khalil et al., 2005*. The high percentage and titer of HI test compared with SNT (table 1 and 2) may be attributed to the predominance of the heamagglutinin epitopes allover the viral envelope, which increases the chance of the formation of more heamagglutinating inhibition antibodies that can be detected even in young calves less than 6 months old.

The percentage of agreement between HI test and SNT for detection of antibodies to BEFV was 72.9% (table 3).

Morover sensitivity and specificity of HI test in comparison to SNT for detection and titration of antibodies against BEFV were 100% and 44.4% respectively (table 4). However earlier studies presented HI test as a sensitive and specific test in comparison to the standard SNT for detection of antibody to para influenza-3 (PI3) *(Abinanti et al., 1961)* and for dengue or yellow fever in comparison to ELISA *(De-araujo et al., 2002)*. The correlation between HI test and SNT was very high and heamagglutination inhibition antibody persisted for long time for many viruses as pseudorabies *(Testue et al., 1989)*, chuzan virus *(Goto et al. 1991)* and equine arteritis *(Kubota et al., 1997)*.

It was concluded that, HI test can be established for detection and titration of antibodies against BEFV, as it is simple does not require sophisticated equipment, cheap, sensitive and was also found to be the method of choice when early detection of BEFV antibodies are required, so the inherent advantages of HI test make it an attractive assay for the seroepidemiology of BEFV.

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Table (1): Prevalence of antibodies to BEFV in cattle sera using serum neutralization test (SNT) and haemagglutination inhibition (HI) test.

Age	No. of examined samples	SNT			HI test		
		Positive	Negative	%	Positive	Negative	%
> 6 months	9	0	9	0.0	3	6	33.3
1-2 year	13	6	7	46.7	11	2	84.6
< 3 year	15	13	2	86.7	15	0	100
Total	37	19	18	51.4	29	8	78.9

Table (2): Frequency distribution of antibody titers to BEFV in cattle sera using SNT and HI test.

Antibody titers	SNT		HI test	
	Frequency	**%**	**Frequency**	**%**
2	7	36.9	8	27.6
4	5	26.2	8	27.6
8	7	36.9	8	27.6
16	0	0.0	3	10.4
32	0	0.0	2	6.8
Total	**19**		**29**	

Table (3): Percentage of agreement between SNT and HI test for detection of antibodies to BEFV in cattle sera.

No. of examined sera	SNT	HI test	Results	
			Agreement	**Disagreement**
19	Positive	Positive	19	0
8	Negative	Negative	8	0
10	Negative	Positive	0	10
37			27	10

*Percentage of agreement = $\dfrac{total\ agreement}{total\ No.of\ exa\min ed\ sample} x100$

** Percentage of agreement = $\dfrac{27}{37} x\ 100 = 72.9$

Table (4): Results of sensitivity of HI test in comparsion to SNT for detection of antibodies to BEFV in cattle sera.

HI results	SNT results		
	Positive	**Negative**	**Total**
Positive 29	19 (A)	10(B)	29
Negative 0	0 (C)	8 (D)	8
Total	19 (A+C)	18 (B+D)	37

*Sensitivity = $\dfrac{A}{A+C} X100 = \dfrac{19}{19} X100 = 100\%$

** Specificity = $\dfrac{D}{B+D} x\ 100 = \dfrac{8}{18} x100 = 44.4$

<u>الملخص العربى</u>

تقييم اختبار تثبيط التلزن الدموى واختبار التعادل المصلى لاستكشاف

الأجسام المضادة لفيروس الحمى العابرة فى مصل الماشية

سعد شعراوى على شعراوى* وعادل محمد خضر**

*قسم الفيرولوجيا – كلية الطب البيطرى – جامعة بنها

** قسم الأمراض الباطنة– كلية الطب البيطرى – جامعة الإسكندرية

صيف عام 2005 وبمحافظة القليوبية تم جمع عدد 37 عينة من مصـــل الماشـــية ذات الأعمـــار المختلفة والمصابة طبيعياً بالفيروس المسبب لمرض الحمى العابرة (BEFV)حيث أخضعت هـــذه العينـــات لاستكشاف وقياس عيارية الأجسام المضادة بها لهذا الفيروس وذلك باستخدام اختبار تثبيط التلزن الدموى (HI) مقارنة مع اختبار التعادل المصلى(SNT)0 باستخدام اختبار التعادل المصلى أشارت النتائج إلـــى أن 19 عينة من الـــ 37 عينة (بنسبة 51.4%) إيجابية وبها أجسام مناعية تعادلية ضد الفيروس وقد اختلفت نسبة توزيع الأجسام المناعية التعادلية حسب سن الماشية، حيث لم توجد هذه الأجسام المناعية التعادلية فى العجول التى عمرها أقل من ستة أشهر فى حين كانت 6 من 13 (بنسبة 46.7%) للماشية التى تتراوح أعمارها بين عام وعامان فى حين كانت 13 من 15 (بنسبة 86.7%) للماشية التى عمرها أكبر من ثلاث سنوات.

فيما يخص استخدام تثبيط التلزن الدموى لاستكشاف وعيارية الأجسام المثبطة للتلزن الـــدموى فـــى مصل الماشية ضد فيروس الحمى العابرة (BEFV) أشارت النتائج إلى أن 29عينة من جملة الـــ 37 عينة كانت إيجابية (بنسبة 78.47%) وقد اختلفت نسبة توزيع الأجسام المثبطة للتلزن الدموى حسب سن الماشية كالآتى: 3 من 9 (بنسبة 33.3%) كانت فى العجول التى عمرها أقل من ستة أشهر فى حين كانت 11 مـــن 13 (بنسبة 84.6%) فى الماشية التى يتراوح أعمارها بين عام وعامين وكانت 15 من 15 (بنسبة 100%) فى الماشية التى عمرها أكبر من ثلاث سنوات0 هذا وكانت عيارية الأجسام المضادة للفيـــروس المســـبب لمرض الحمى العابرة فى الماشية أعلى عيارية باستخدام اختبار تثبيط التلزن الدموى مقارنة باختبار التعـــادل المصلى.

كانت نسبة الاتفاق بين اختبار تثبيط التلزن الدموى واختبار التعادل المصلى 72.9% فى حين كانت حساسية واختصاص اختبار تثبيط التلزن الدموى مقارنة باختبار التعادل المصلى 100% و 44.4% علـــى الترتيب.

من هذه النتائج يمكن الاستنتاج إلى أنه يمكن اعتماد اختبار تثبيط التلزن الدموى لاستكشــاف وعياريـــة الأجسام المضادة للفيروس المسبب للحمى العابرة فى الماشية كما وأن مميزات هذا الاختبار تجعله مفضل فى دراسة وبائية هذا المرض.

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