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ALTERNATIVE METHODS FOR EVALUATION OF ATTENUATED BOVINE RESPIRATORY SYNCYTIAL VIRUS VACCINES.

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A B S T R A C T

Different batches of attenuated BRSV vaccine was examined for Safety and sterility. Identity of the BRSV vaccine was ensured using RT-PCR and its titration on MDBK cell line was done using IFAT. The six evaluated vaccine batches gave $10^{5.3}$, $10^{5.2}$,10^{5}, 10^{4},$10^{4.3}$ and $10^{4.6}$ $TCID_{50}$/ml titers respectively. Potency of the attenuated BRSV vaccines was evaluated in vaccinated calves using SNT and ELISA. Protective serum neutralizing antibody titer started at 3 weeks post vaccination for 1st, 2nd, and 3rd batches, and persisted till 18 weeks post vaccination, while the 4th, 5th and 6th batches did not give protective serum neutralizing antibody titer till the 4th week post vaccination. All the results of SNT were confirmed by using ELISA. It was concluded that evaluation of BRSV vaccines via RT-PCR and its titration on MDBK cell line using IFAT beside identification and safety tests, were more economic, rapid and safe giving a good guide for the effectiveness of the vaccine used.

KEY WORDS: BRSV, PCR, SNT, ELISA

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1. I N T R O D U C T I O N

Bovine respiratory syncytial virus (BRSV), being a pneumovirus of the family Paramyxoviridae, is a widespread cause of lower respiratory tract disease in cattle and responsible for significant economic losses to the livestock industry [4], [19]. BRSV has been established as an important viral component in the bovine respiratory disease complex (BRDC) [3]. BRSV was first diagnosed in 1967 in Switzerland [12]. The virus was detected in Japan, Belgium, Switzerland, England and USA [11]. BRSV appears to be spread worldwide [2]. In Egypt, the characteristic pathological picture of BRSV infection was observed firstly [20] then the first isolation was done [9] followed by detection of BRSV antigen in lung tissue [14]. Control of BRSV infection as part of a herd health program is therefore important so different approaches have been followed for prevention through developing safe and efficacious BRSV vaccines including inactivated vaccines [8], (genetically) modified live vaccines [16], subunit vaccines, [18], DNA vaccines [21] and vector vaccines [22]. Today inactivated and modified live BRSV vaccines are commercially available, most of them as combination vaccine with other antigens related to the BRDC. The present work aimed to evaluate different batches of live attenuated BRSV vaccine in vitro using titration of batches on MDBK using IFAT and in vaccinated calves using SNT and ELISA.

2. MATERIAL AND METHODS

2.1. *BRSV vaccines*:

Six batches of commercial polyvalent vaccine contain live attenuated BRSV (strain 375) $10^{5.3}$ $TCID_{50}$ /ml.

2.2. *Molecular detection of viral vaccine identity using RT-PCR*:
It was performed to detect identity of viral vaccines with BRSV [1]. Specific primers were used for detection of P gene sequence of BRSV with specific primer sequences:
Primer1:
5'GAAATTTCCATGGAAAAATTTGCACCTG3'
Primers 2:
5'GAAATCTTCAAGTGATAGATCATT G3'.

2.3. *Titration of BRSV vaccines in cell culture using indirect fluorescent antibody technique (IFAT)*:
2.3.1. Titration of BRSV vaccine using infectivity test (CPE method) [10].
2.3.2. Indirect fluorescent antibody technique [13].

2.4. *Calves and experimental design*:
Forty two susceptible Friesian calves (6 months old), clinically normal, healthy and free from antibodies for BRSV were used (24 calves for potency, 12 calves for safety and 6 calves were kept as a control for the experiment). Calves used for evaluation of vaccine potency were inoculated with 2ml intramuscularly (I/M) from each vaccine batch and the inoculation was repeated after 2 weeks (booster dose), were kept under close observation during the whole time of experiment and subjected for serum samples collection.

2.5. *Serum samples*:
Serum samples were collected from vaccinated calves weekly. The sera were collected and stored at -20°C and inactivated at 56°C for 30 minutes before being used in the test.

2.6. *Serum neutralization test (SNT)*:
The humeral immune response of vaccinated calves against live attenuated BRSV vaccine using SNT. SNT was carried out in 96-well micro titer plate using BRSV strain as an antigen at its 8th passage level in MDBK cell culture [7].

2.8. *Indirect Enzyme linked immunosorbent assay* (ELISA):
The humoral immune response against BRS antigens in the vaccines was measured by ELISA kit for diagnosis of bovine respiratory syncytial virus in cattle (Bio-X BRS ELISA kit).

3. RESULTS AND DISCUSSION

Bovine respiratory syncytial virus (BRSV), being a pneumovirus of the Paramyxoviridae family, is a widespread cause of lower respiratory tract disease in cattle and is responsible for significant economic losses to the livestock industry [19]. The virus can cause severe disease in animals of all ages, but primarily affects young native calves in recurrent seasonal outbreaks [4]. Field studies have shown a variable effect of BRSV vaccination on clinical disease, productivity of young calves, weaned animals and cows, perhaps related to variations in virus prevalence or prior exposure [6]. In the present study we have attempted to apply alternative methods for evaluation of live attenuated vaccine of BRSV to be more effective, cheaper, faster and easily applied method for evaluation the efficacy of live attenuated BRSV vaccine. From the results we can observe that the evaluated vaccine batches were proven sterile and free from anaerobic bacteria, aerobic bacteria, fungal contamination and mycoplasma. These results agreed with those the Egyptian veterinary codex – CLEVB (2009), and the Code of Federal Regulations (2005), who reported that the final product should be free from anaerobic bacteria, aerobic bacteria, fungi and mycoplasma.
Identity test was carried out on the live attenuated virus of the vaccine batches using RT-PCR which give positive amplification of 700 pb fragment indicating presence of antigens as show in photo (1), which coincides with the results obtained by previous authors [1].

Photo 1 The PCR amplification of the conserved region of P gene of Bovine RSV. Note the amplification of 700 pb fragment.

Safety of the evaluated vaccine batches were detected in mice, guinea pigs and also detected in calves (as 10X of vaccine dose), all the evaluated vaccine batches were safe, where there were not any clinical abnormalities observed in inoculated animals, and with no rise in body temperature that agreed with the Egyptian veterinary codex – CLEVB (2009), and the Code of Federal Regulations (2005).

The results of titration of BRSV vaccines in cell culture using IFAT, shows the titer of vaccine batches, where the 1st batch titer was $10^{5.3}$ $TCID_{50}$/ml, the 2nd batch titer was $10^{5.2}$ $TCID_{50}$/ml, and the 3rd batch was 10^{5} $TCID_{50}$/ml as shown in photo (2), and confirmed by IFAT which gave satisfactory positive results indicating presence of antigens as show in photo (3), which prove that the CPE of titration is belonged to BRSV. The results of virus titration and IFAT were agreed with earier works [17, 24]. The humeral immune response to of live attenuated BRSV vaccines in vaccinated calves using SNT showed that protective neutralizing serum antibody titer (0.6) started from 2nd week post vaccination and persisted in protective level until 18 weeks, the highest level of antibody was recorded in 8th week post vaccination for batches 1, 2 and 3 of the vaccine as shown in table (1) and figure (1), while ELISA positive antibody titer against BRSV started from the first week post vaccination and persisted for 24 weeks post vaccination as These results agreed with that of former studies [5, 7, 15, 23], that demonstrated the efficacy and duration of a quadrivalent vaccine containing BRSV. shown in table (2) and figure (2), that confirmed the results of SNT.

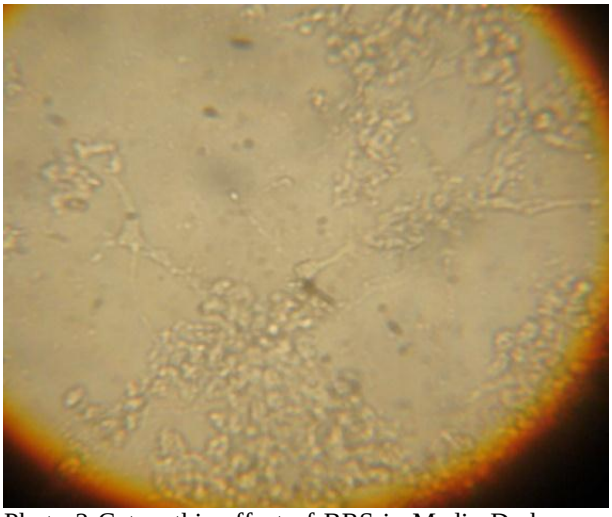

Photo 2 Cytopathic effect of BRS in Madin Darby Bovine Kidney cell shows: (syncytium formation).

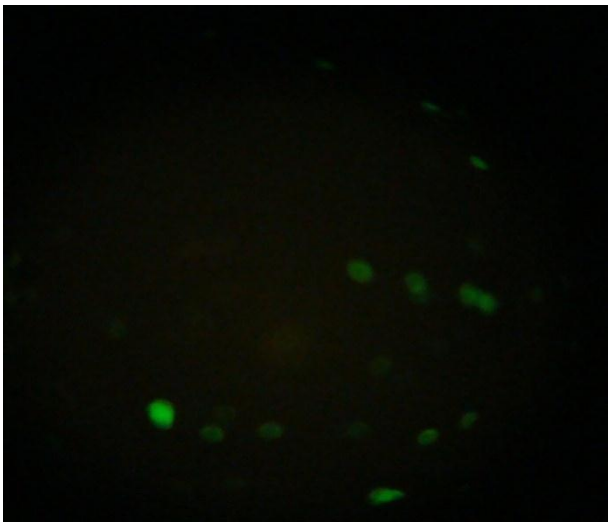

Photo 3 Positive IFAT in MDBK cell inoculated with BRSV showing apple green fluorescence inside the cells under fluorescent microscope (×400).

The humeral immune response to 4th, 5th and 6th batches of live attenuated BRSV vaccine in vaccinated calves using SNT showed that un protective neutralizing

serum antibody titer less than (0.6) till the 4th week as shown in table (3), also ELISA showed that negative titer less than (1000) till the 4th week as shown in table (4). The result above indicated with those of the titration of the three evaluated vaccine batches (1, 2 and 3) gave $10^{5.3}$, $10^{5.2}$ and 104^{5} in corresponding to SNT and ELISA for the sera of vaccinated calves gave in 4th week (1.275, 1.2875, 1.225) and (2112.25, 1736.25, 1945.75) respectively. in the other hand we observed that the titration of the three evaluated vaccine batches (4, 5 and 6) gave 10^{4} , $10^{4.3}$ and $10^{4.6}$ in corresponding to SNT and ELISA for the sera of vaccinated calves gave in 4th week (0.15, 0.30, 0.45) and (779, 883.5, 988), respectively.

Table 1 Mean serum neutralizing antibody titers of calves vaccinated with attenuated BRSV vaccine with batches 1, 2 and 3 using SNT:

	*SNT titers weeks post vaccination													
Type of Batch	-	1st dose			2nd dose (Booster dose)									
	0	1	2	3	4	6	8	10	12	14	16	18	20	24
Batch 1	0.08	0.3	0.75	0.68	1.28	1.80	2.03	1.95	1.73	1.34	1.09	0.83	0.56	0.41
Batch 2	0.23	0.38	0.98	0.94	1.29	1.61	1.91	1.95	1.79	1.36	1.04	0.71	0.41	0.26
Batch 3	0.15	0.60	0.83	0.73	1.23	1.49	1.78	1.99	1.74	1.56	1.14	0.83	0.50	0.30
Control	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30

* Log_{10} serum neutralizing antibody titer, Protective serum neutralizing antibody titer = 0.6

Table 2 Mean serum antibody response of calves vaccinated with attenuated BRSV vaccine with batches 1, 2 and 3 using ELISA:

	* ELISA Optical Densities weeks post vaccination													
Type of batch	-	1st dose			2nd dose (Booster dose)									
	0	1	2	3	4	6	8	10	12	14	16	18	20	24
Batch 1	554.25	1071.75	1523	1487.75	2112.25	2455	2407.25	2215.25	1980.25	1784.5	1672.5	1450	1318.5	1212.75
Batch 2	757.5	1091.5	1508.75	1476.5	1736.25	2116.75	2421.25	2379	1940.5	1778	1590	1370.5	1296.5	1203.5
Batch 3	705	1360.5	1771.25	1711.5	1945.75	2310.5	2535.5	2338.25	1920.75	1728.5	1570.5	1400	1320.5	1212.75
control	684	714	722.33	747	753.33	771.66	739.166	681.33	683	676.83	689.83	676.66	685.33	718.66

*(+ve) value 1000 or more according to kit. and (-ve) value less than 1000 according to kit

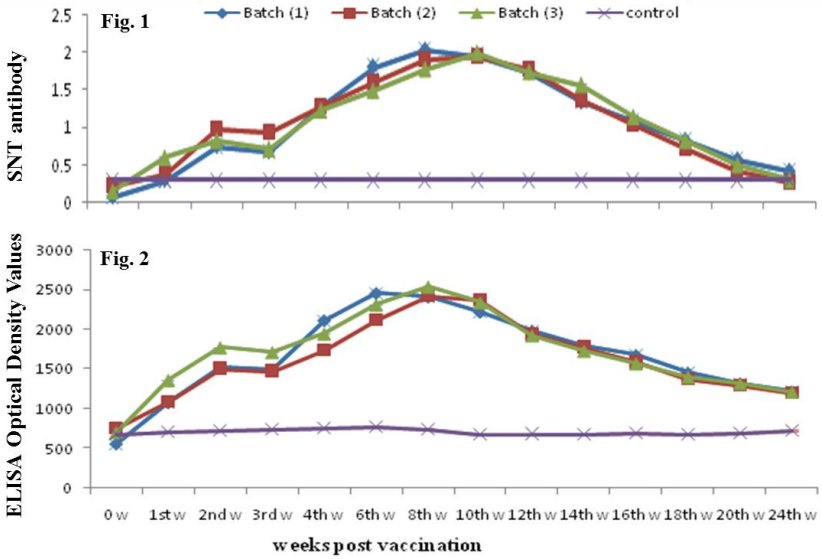

Fig. 1 Comparative SNT results of different batches 1,2 and 3 of attenuated BRSV, and fig. 2 Comparative ELISA results of different batches 1,2 and 3 of attenuated BRSV.

In regarding to titration results we can determine that the titer 5 log10 $TCID_{50}$ gave satisfactory response more than the protective SNT (0.6 log TC ID_{50}), while the titer 4.6 log10 $TCID_{50}$ gave unsatisfactory response, So we can conclude that the evaluation of attenuated BRSV vaccines can be carried out via vitro techniques beside identification test and safety test, which are more economic, rapid and safe giving a good guide for the effectiveness of the vaccine used.

Table 3 Mean serum antibody response of calves vaccinated with attenuated BRSV vaccine with batches 1, 2 and 3 using SNT:

Type of batch	*SNT titers weeks post vaccination				
	-	1st dose		2nd dose (Booster dose)	
	0	1	2	3	4
Batch 4	0.15	0.3	0.3	0.15	0.15
Batch 5	0.0	0.15	0.3	0.3	0.3
Batch 6	0.0	0.3	0.45	0.3	0.45
Control	0.3	0.3	0.3	0.3	0.3

* Log_{10} serum neutralizing antibody titer. Protective serum neutralizing antibody titer = 0.6.

Table 4 Mean serum antibody response of calves vaccinated with attenuated BRSV vaccine with batches 1, 2 and 3 using ELISA:

Type of Batch	** ELISA Optical Densities weeks post vaccination				
	-	1st dose		2nd dose (Booster dose)	
	0	1	2	3	4
Batch 4	650.34	799.89	856.33	720.356	779
Batch 5	489.16	667.75	897	857.33	883.5
Batch 6	515.78	880.75	968.891	878.29	988
Control	739.12	684.33	645	675	690

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طرق بديلة لتقييم لقاحات مستضعفة للفيروس التنفسى البقرى المتضخم.

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الملخص العربى

تم تقييم نقاوة وأمان تشغيلات مختلفة للقاحات مستضعفة للفيروس التنفسى البقرى المتضخم. تم التأكد من مطابقة اللقاحات باستخدام اختبار البلمرة المتسلسل ذو النسخ العكسى وبعد ذلك تم تقييم اللقاحات عن طريق معايرة اللقاحات على خط خلايا مادين وداربى وباستخدام اختبار الوميض الفلورسينتى الغير المباشر. أعطت اللقاحات الستة عيارية تبلغ $10^{5.3}$، $10^{5.2}$، 10^{5}، 10^{4}، $10^{4.3}$، و $10^{4.6}$ $TCID_{50}/ml$ على الترتيب. تم تقييم فعالية اللقاحات بعد حقنها فى العجول ثم اجراء اختبارات المصل التعادلى والاليزا. أظهر اختبار المصل التعادلى بداية المستوى الواقى لعيارية الاجسام التعادلية المضادة عند الاسبوع الثالث بعد التحصين للقاحات الاول والثانى والثالث واستمرت حتى الاسبوع الثامن عشر بعد التحصين بينما لم تعطى اللقاحات الرابع والخامس والسادس المستوى الواقى لعيارية الاجسام التعادلية المضادة حتى الاسبوع الرابع بعد التحصين. أظهر اختبار الاليزا نتائج متوافقة مع نتائج اختبار المصل التعادلى. تم استنتاج ان تقييم اللقاحات المستضعفة للفيروس التنفسى المتضخم باستخدام اختبار البلمرة المتسلسل ذو النسخ العكسى ومعايرتها فى خط خلايا مادين وداربى وباستخدام اختبار الوميض الفلورسينتى الغير المباشر بالاضافة الى اختبارات الامان حيث كانت اوفراقتصاديا واكثر سرعة وامان وكانت مؤشر جيد على فعالية اللقاح المستخدم.

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