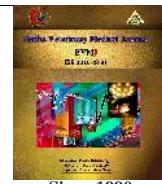




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### Original Paper

## Field, serological and biochemical evaluation of Bovine Ephemeral Fever and Rabies vaccines in cattle and buffaloes

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### ABSTRACT

Family *Rhabdoviridae* contains 2 genera *Ephemerovirus* and *Lyssavirus* which contain viruses responsible for two destructive diseases Bovine Ephemeral Fever (BEF) and rabies, respectively. Both diseases causes direct and indirect economic losses related to deaths, abortions, cost of treatment and prevention, zoonotic impact and restriction of animal movement. The main objective of this study is to evaluate the immune responses of cattle and buffalo vaccinated with BEF and rabies vaccines when administered separately in comparison with co vaccination. Serum samples were collected from 16 cattle and buffaloes (eight of each) then subjected to serum neutralization test SNT, serum biochemical, liver and kidney function for comparison according to the vaccinated groups. Each animal species divided into 4 groups (2 animals/group) group 1 was vaccinated by 2 doses of attenuated BEF vaccine inactivated at time of use with 2 weeks in between, group 2 was vaccinated by 1 dose of inactivated rabies vaccine, group 3 was vaccinated by rabies vaccine simultaneous with 1<sup>st</sup> dose of BEF vaccine and booster dose of BEF vaccine after 2 weeks, group 4 not vaccinated and let to be control group. The results showed that both live attenuated BEF and inactivated cell culture rabies vaccines are safe because they didn't harmful effect on liver and kidney functions, they are immunogenic as they lead to significant increase in total serum protein due to increase in globulin. Co-vaccination of both vaccines together has higher levels of specific antibodies against both BEF and rabies in all vaccinated animals

## 1. INTRODUCTION

Cattle and buffalo industry is one of the most important industries that provide human with their main source of animal proteins, minerals and vitamins through their meat and milk production such elements are essential requirements for good health and normal body building (Zaghawa et al., 2002). These animal species may be threatened by many infectious diseases resulting in huge economic losses. Viral diseases represent the most dangerous diseases facing cattle industry where trials of treatment are difficult and of high cost, but it is likely that most of them are preventable through following up accurate control measures and vaccination programs (Daoud et al., 2005). Bovine ephemeral fever and rabies are important and antigenically related viral diseases that affecting cattle and buffalo (Tsuyoshi et al., 2014; CDC, 2019).

Bovine ephemeral fever or three-day sickness is an acute febrile noncontiguous epizootic arthropod born viral disease affecting mainly cattle and water buffaloes (Nabila et al., 2006). The disease is caused by bovine ephemeral fever virus (BEFV) that belongs to family Rhabdoviridae under Ephemerovirus genus (Tsuyoshi et al., 2014). Moreover, it is considered an arbovirus that can be transmitted by insect biting of different vectors as: culicoides biting midges or mosquitoes (Tsuyoshi Niwa et al., 2014; Yang et al., 2018).

Clinically, the diseased animal by BEF exhibits drastic drop in milk production, high fever, anorexia, nasal and ocular discharge, excessive salivation, and muscle stiffness then inability to stand, reluctance to move (Lee, 2019). High morbidity and low mortality and rapid recovery within 3-4 days from the onset of clinical signs are characteristic to the disease in its mild form whereas, some animals may show sever signs and complications with more extended course (Robinson and Robinson, 2016). Although BEF is an infectious, it is a preventable disease as primary vaccination in calves that followed by regular booster doses with a good quality vaccine usually achieves satisfactory protection with efficient vector control strategy (Lee, 2019).

On the other hand, rabies is a vaccine preventable viral disease that presents in more than 150 countries and territories (WHO, 2020). It is a widely feared disease for thousands of years, with records of rabid dogs from ancient Egyptian and Mesopotamian texts (Gold et al., 2020). Unlike BEF, Rabies is a zoonotic disease that kills an estimated 35.000 per year, mostly in Africa, Asia, and Latin America (Bread, 2001). It affects all warm-blooded animals and humans, but bats, dogs and cats are the main reservoir to the disease transmitting it to other animals or human mainly by biting them (Takayama, 2005). Rabies virus is the main cause of rabies disease and antigenically related to BEF as both of them are belong to the same family

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(*Rhabdoviridae*), but rabies virus belongs to genus *Lyssavirus*. It has distinct bullet shape and non-segmented, negative single-stranded RNA genome (Smith, 1996; CDC, 2019). The disease affects the CNS of infected host leading to nervous manifestations as signs of mania, difficult in swallowing, paralysis that started from the hind limbs then directed to forward (trunk and fore limbs), recumbence and ends usually with death (Adedeji *et al.*, 2010). There is no known treatment for rabies, but it mainly replaced by post exposure prophylaxis (PEP). This involves administration of rabies immunoglobulin and vaccine soon after exposure to the virus, followed by a series of injections over 30 days (Smith and Pharm, 2012).

Depending on the antigenic relationship between BEF and Rabies viruses, some trials have been carried out to use rabies vaccines to protect cattle against BEF and reverse (Kongsuwan *et al.*, 1998). Therefore, the aim of this research is to investigation of the immune response of cattle and buffalo to the local inactivated cell culture bovine ephemeral fever vaccine and to evaluate the effect of mutual co-vaccination with both inactivated bovine ephemeral fever vaccine and rabies vaccine on the immune response of cattle and buffalo and also to estimate the effect of such vaccines on the level of serum proteins and liver and kidney functions

## 2. MATERIAL AND METHODS

### 2.1. Experimental animals

A total number of 16 cross breed cattle and buffaloes (eight of each), 1.5 - 2 years old in a private farm were used in this study. Each animal species (cattle or buffaloes) was divided into 4 groups group 1 was vaccinated by 2 doses of attenuated BEF vaccine inactivated at time of use with 2 weeks in between, group 2 was vaccinated by 1 dose of inactivated rabies vaccine, group 3 was vaccinated by rabies vaccine simultaneous with 1st dose of BEF vaccine and boosting dose of BEF vaccine after 2 weeks, group 4 not vaccinated and let to be control group. All vaccines were locally produced at the veterinary serum and vaccine research institute (VSVRI), Abbassia, Cairo, Egypt.

### 2.2. Blood samples

Blood samples from the experimental cattle and buffalos were collected under complete aseptic conditions and allowed for clotting then serum was separated and centrifuged at 2000 rpm for 15 minutes according to (Lannette, 1964) then kept in sterile screw capped vials at -20 °C till used for SNT and serum biochemical analysis.

### 2.3. Serum neutralization test (SNT)

Table 1 Cumulative table showing BEF neutralizing antibody titers in different vaccinated cattle and buffalo groups.

Period of vaccination	BEF SNA titer* in cattle and buffalo					
	SNA titer* in cattle			SNA titer* in buffalo		
	Group 1	Group 3	Control	Group 1	Group 3	Control
Pre vaccination	0	0	0	0	0	0
<u>First dose of vaccination</u>						
2WP 1 <sup>ST</sup> V**	8	8	0	8.00	8	0
<u>Second dose of vaccination</u>						
2WP 2 <sup>ND</sup> V***	32.00	32.00	0	32.00	64.00	0
4WP 2 <sup>ND</sup> V***	64.00	128.00	0	32.00	128.00	0
2MP 2 <sup>ND</sup> V****	64.00	128.00	0	64.00	128.00	0
3MP 2 <sup>ND</sup> V****	128.00	128.00	0	128.00	128.00	0
4MP 2 <sup>ND</sup> V****	128.00	128.00	0	128.00	128.00	0
5MP 2 <sup>ND</sup> V****	128.00	128.00	0	128.00	128.00	0
6MP 2 <sup>ND</sup> V****	128.00	128.00	0	128.00	128.00	0

(Group 1: vaccinated by BEF vaccine only, Group 3: vaccinated by BEF and rabies vaccines, Group 4: Control group). SNA titer\* = Serum Neutralizing Antibody titer= the reciprocal of the final serum dilution which neutralized 100-200 TCID50 of BEF virus. WP 1<sup>ST</sup> V\*\* = Week Post first Vaccination, WP 2<sup>ND</sup> V\*\*\* = Week Post second Vaccination. MP 2<sup>ND</sup> V\*\*\*\* = Month Post second Vaccination

SNT was carried out by using microtiter technique according to (Nurtop *et al.*, 2018). The neutralizing antibodies titer against the challenged viral vaccines was expressed as the reciprocal of the final serum dilution which neutralized the CPE of 100TCID50 of the used virus according to (Singh *et al.*, 1967)

### 2.4. Estimation of serum proteins and other biochemical assays

Serum samples were evaluated for estimation of total serum protein and albumin and for the enzymatic activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in addition to serum concentrations of creatinine. These parameters were determined spectrophotometric method using commercially available test kits supplied by Centronic GmbH Am Aleinfeld 11, 85456 Wartenberg (Germany) and also serum concentrations of Urea was determined spectrophotometric method using commercially available test kits supplied by MDSS GmbH (Germany) according the manufacturer's instructions

### 2.5. Statistical analysis

First, all data were tested for normality and homogeneity. Then, one-way analysis of variance used to determine the statistical significance of differences among groups followed by Duncan's test as post hoc for making a multiple comparisons using the Statistical Package for Social science Software (Version 25, SPSS Inc., and Chicago, IL, USA). The values were expressed as the mean ± standard error of the mean. A significant difference was used at the 0.05 probability level.

## 3. RESULTS

### 3.1. Results of SNT of serum collected from cattle and buffalo vaccinated with BEF vaccine.

Cattle and buffalo receiving two doses attenuated cell culture bovine ephemeral fever vaccine inactivated on the time of use with two weeks in between began to record a detectable level of specific BEF neutralizing antibodies by the 2nd week after the first dose of vaccination with a mean titer 8 in cattle and 6 in buffalo as measured by SNT. The antibody titer was increased gradually after the 2nd dose to reach a peak titer at the 2nd month to be 128 in cattle and buffalo and remain constant till 6 months post 1<sup>st</sup> vaccination (the experiment period) as demonstrated in table (1)

**3.2. Results of SNT of serum collected from cattle and buffalo vaccinated with inactivated cell culture rabies vaccine.**

Vaccination of cattle and buffalo with a single dose of the inactivated cell culture rabies vaccine resulted in induction of specific rabies neutralizing antibodies from second week post vaccination with a mean titer of 32 in cattle and 16 in buffalo and increased gradually to reach its peak (128) by the 3rd month post vaccination and by the 6th week post vaccination in buffalo and still within such titer up to 6 months as shown in table (2)

**3.3. Serum neutralizing antibody titer of serum collected from cattle and buffalo vaccinated simultaneously with 2 doses of live attenuated cell culture BEF vaccine and one dose of inactivated cell culture rabies vaccine.**

Simultaneous vaccination of cattle and buffalo with 1st dose of inactivated cell culture BEF vaccine and inactivated cell culture rabies vaccine then booster dose with BEF vaccine after inactivation 2 weeks post the 1st dose resulted in better immune levels against each of BEF and rabies specially in buffalo even from the second week post vaccination. It was found that serum BEF neutralizing antibodies had a mean titer of 8 by second week post vaccination in cattle and 8 in buffalo at the same time and both titers increased gradually to reach to their peak by the 4th week post 2nd vaccination by a mean titer of 128 in both cattle and buffalo and still unchanged till 6 months post vaccination as illustrated in table (1)

Rabies serum neutralizing antibody titers nearly follows the same behavior as it had also a mean titer of 16 in cattle and 32 in buffalo by the second week post vaccination and

increased gradually to reach to its peak by a mean titer of 128 in both cattle and buffalo by 6th week post vaccination and not declined up to 6months as demonstrated in table (2)

**3.4. Estimation of serum protein in sera of vaccinated cattle.**

Total protein concentration in group 1 showed significant increase when compared to control group 2, 4, 6- and 8-weeks post vaccination. Contrary, group 2 showed non-significant change 2, 4, 6- and 8-weeks post vaccination when compared to control group. in addition, group 3 showed non-significant change 2 weeks post vaccination followed by significant increase 4, 6- and 8-weeks post vaccination when compared to control group table (3).

Albumin concentration in group 1 showed non-significant increase when compared to control group 2 weeks post vaccination followed by significant increase 4, 6- and 8-weeks post vaccination. Moreover, group 2 showed non-significant change 2, 4, 6- and 8-weeks post vaccination when compared to control group while group 3 showed significant decrease 2- and 4-weeks post vaccination followed by non-significant change 6- and 8- weeks post vaccination when compared to control group table (3).

Globulin concentration in group 1 and 3 showed significant increase when compared to control group 2, 4, 6- and 8-weeks post vaccination. Moreover, group 2 showed non-significant change 2 weeks post vaccination followed by significant increase 4, 6- and 8-weeks post vaccination when compared to control group table (3). A/G ratio in group 1, 2 and 3 showed significant decreases 2, 4, 6- and 8-weeks post vaccination when compared to control group as showed in table (3).

Table 2 Cumulative table showing rabies neutralizing antibody titers in different vaccinated cattle and buffalo groups

Period of vaccination	Rabies SNA titer* in cattle and buffalo					
	SNA titer* in cattle		SNA titer* in buffalo			
	Group 2	Group 3	Control	Group 2	Group 3	Control
Pre vaccination	0.00	0.00	0	0.00	0.00	0
2WP v**	32.00	16.00	0	32.00	32.00	0
4WP v**	64.00	32.00	0	64.00	64.00	0
6WP v**	64.00	128.00	0	128.00	128.00	0
2MP v***	64.00	128.00	0	128.00	128.00	0
3MP v***	128.00	128.00	0	128.00	128.00	0
4MP v***	128.00	128.00	0	128.00	128.00	0
5MP v***	128.00	128.00	0	128.00	128.00	0
6MP v***	128.00	128.00	0	128.00	128.00	0

(Group 2: vaccinated by rabies vaccine only, Group 3: vaccinated by BEF and rabies vaccines, Group 4: Control group). SNA titer\* = Serum Neutralizing Antibody titer= The reciprocal of the final serum dilution which neutralized 100-200 TCID50 of BEF virus. WP V\*\* = Week Post Vaccination. MP V\*\*\* = Month Post Vaccination

Table 3 Serum protein values in the sera of different vaccinated cattle groups:

Parameter	Cattle Groups	Duration of Experiment			
		2 WPV	4 WPV	6 WPV	8 WPV
Total Protein (g/dl)	Group 1:	10.42 ± 0.56 <sup>a</sup>	10.74 ± 0.57 <sup>a</sup>	10.83 ± 0.58 <sup>a</sup>	10.91 ± 0.58 <sup>a</sup>
	Group 2:	8.09 ± 0.43 <sup>b</sup>	8.21 ± 0.44 <sup>bc</sup>	8.56 ± 0.46 <sup>bc</sup>	8.90 ± 0.47 <sup>bc</sup>
	Group 3:	9.23 ± 0.49 <sup>ab</sup>	9.54 ± 0.51 <sup>ab</sup>	9.57 ± 0.51 <sup>ab</sup>	9.90 ± 0.53 <sup>ab</sup>
	Group 4:	7.68 ± 0.41 <sup>b</sup>	7.11 ± 0.38 <sup>c</sup>	7.42 ± 0.40 <sup>c</sup>	7.56 ± 0.40 <sup>c</sup>
Albumin (g/dl)	Group 1:	4.10 ± 0.22 <sup>a</sup>	4.40 ± 0.23 <sup>a</sup>	4.61 ± 0.25 <sup>a</sup>	4.87 ± 0.26 <sup>a</sup>
	Group 2:	3.17 ± 0.17 <sup>b</sup>	3.20 ± 0.17 <sup>bc</sup>	3.21 ± 0.17 <sup>b</sup>	3.69 ± 0.20 <sup>b</sup>
	Group 3:	2.43 ± 0.13 <sup>c</sup>	2.87 ± 0.15 <sup>c</sup>	3.53 ± 0.19 <sup>b</sup>	3.55 ± 0.19 <sup>b</sup>
	Group 4:	3.60 ± 0.19 <sup>ab</sup>	3.64 ± 0.19 <sup>b</sup>	3.46 ± 0.18 <sup>b</sup>	3.77 ± 0.20 <sup>b</sup>
Globulin (g/dl)	Group 1:	6.32 ± 0.34 <sup>a</sup>	6.34 ± 0.34 <sup>a</sup>	6.22 ± 0.33 <sup>a</sup>	5.86 ± 0.31 <sup>ab</sup>
	Group 2:	4.92 ± 0.26 <sup>b</sup>	5.01 ± 0.27 <sup>b</sup>	5.35 ± 0.29 <sup>a</sup>	5.21 ± 0.28 <sup>b</sup>
	Group 3:	6.80 ± 0.36 <sup>a</sup>	6.66 ± 0.36 <sup>a</sup>	6.04 ± 0.32 <sup>a</sup>	6.35 ± 0.34 <sup>a</sup>
	Group 4:	4.08 ± 0.22 <sup>b</sup>	3.67 ± 0.20 <sup>c</sup>	3.96 ± 0.21 <sup>b</sup>	3.79 ± 0.20 <sup>c</sup>
A/G Ratio (g/dl)	Group 1:	0.65 ± 0.03 <sup>b</sup>	0.69 ± 0.04 <sup>b</sup>	0.74 ± 0.04 <sup>b</sup>	0.83 ± 0.04 <sup>b</sup>
	Group 2:	0.64 ± 0.03 <sup>b</sup>	0.64 ± 0.03 <sup>b</sup>	0.60 ± 0.03 <sup>c</sup>	0.70 ± 0.04 <sup>b</sup>
	Group 3:	0.36 ± 0.02 <sup>c</sup>	0.43 ± 0.02 <sup>c</sup>	0.58 ± 0.03 <sup>c</sup>	0.56 ± 0.03 <sup>c</sup>
	Group 4:	0.88 ± 0.05 <sup>a</sup>	0.98 ± 0.05 <sup>a</sup>	0.87 ± 0.05 <sup>a</sup>	0.98 ± 0.05 <sup>a</sup>

(Group 1: Cattle vaccinated by BEF vaccine only, Group 2: Cattle vaccinated by rabies vaccine only, Group 3: Cattle vaccinated by BEF and rabies vaccines, Group 4: Control cattle group). WPV: Weeks Post Vaccination. Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P<0.05).

### 3.5. Estimation of serum proteins in the sera of vaccinated buffalo groups.

It was found that the total serum protein concentration in buffalo in group 1, 2 and 3 showed significant increase when compared to control group in the 2, 4, 6- and 8-weeks post vaccination table (4).

Serum protein fractionation revealed that Albumin concentration in group 1 showed significant increase when compared to control group in the 2nd, 4th, 6th and 8th weeks post vaccination. Moreover, group 2 showed non-significant change 2 weeks post vaccination and significant increase 4, 6- and 8-weeks post vaccination when compared to control group. Group 3 showed non-significant change in the 2nd and 6th weeks post vaccination followed by significant increase on the 4th and 8th weeks post vaccination when compared to control group table (4).

Globulin concentration in group 1 showed significant increase when compared to control group 2- and 8-weeks post vaccination and non-significant change 4- and 6-weeks post vaccination. Moreover, group 2 showed significant increase 2, 4, 6- and 8-weeks post vaccination when compared to control group. Group 3 showed significant increase 2, 4- and 6-weeks post vaccination and non-significant change 8 weeks post vaccination when compared to control group table (4).

A/G ratio in group 1 and 2 showed significant decrease 2, and 8 weeks post vaccination, significant increase 4 weeks post vaccination and non-significant change 6 weeks post vaccination when compared to control group. Moreover, group 3 showed significant decreases 2, 4- and 6-weeks post vaccination and significant increase 8 weeks post vaccination when compared to control group as demonstrated in table (4).

### 3.6. Estimation of liver function (AST, ALT) in the sera of vaccinated cattle groups.

AST activity in group 1 showed non-significant change 2- and 8-weeks post vaccination and significant decrease 4- and 6-weeks post vaccination when compared to control group. Group 2 exhibited non-significant change in AST activity 2, 4- and 8-weeks post vaccination and significant decrease 6 weeks post vaccination when compared to control group.

Moreover, group 3 exhibited significant decrease 2- and 6-weeks post vaccination and non-significant change 4- and 8-weeks post vaccination when compared to control group. ALT activity in group 1 and 2 showed significant increase 2, 4, 6- and 8-weeks post vaccination when compared to control group. Group 3 exhibited significant increase in ALT activity 2- and 4-weeks post vaccination followed by non-significant change 6 weeks post vaccination and significant decrease 8 weeks post vaccination when compared to control group as shown in table (5).

### 3.7. Estimation of kidney function (Urea, Creatinine) in the sera of vaccinated cattle groups.

Urea concentration in group 1, 2 and 3 showed significant increase 2, 4, 6- and 8-weeks post vaccination when compared to control group.

Creatinine concentration in group 1, 2 and 3 showed significant increases 2, 4, 6- and 8-weeks post vaccination when compared to control group as shown in table (5).

### 3.8. Estimation of liver function (AST, ALT) in the sera of vaccinated buffalo groups.

AST activity in group 1 showed non-significant change 2, 4, 6- and 8-weeks post vaccination and significant decrease 4- and 6-weeks post vaccination when compared to control group. Group 2 exhibited non-significant change in AST activity 2, 6- and 8-weeks post vaccination and significant decrease 4 weeks post vaccination when compared to control group. Moreover, group 3 exhibited non-significant decrease 2, 4- and 8-weeks post vaccination and significant decrease 6 weeks post vaccination when compared to control group. ALT activity in group 1 showed significant decrease 2, 6- and 8-weeks post vaccination and significant increase 4 weeks post vaccination when compared to control group. Group 2 exhibited non-significant changes in ALT activity 2- and 6-weeks post vaccination, significant increase 4 weeks post vaccination and significant decrease 8 weeks post vaccination when compared to control group. Group 3 exhibited significant decrease in ALT activity 2, 4, 6- and 8-weeks post vaccination when compared to control group as in table (6).

Table 4 Serum protein values in the sera of different vaccinated buffalo groups

Parameter	Buffalo Groups	Duration of Experiment			
		2 WPV	4 WPV	6 WPV	8 WPV
Total Protein (g/dl)	Group 1:	5.49 ± 0.10 <sup>a</sup>	5.84 ± 0.18 <sup>b</sup>	5.85 ± 0.18 <sup>b</sup>	5.57 ± 0.17 <sup>b</sup>
	Group 2:	5.19 ± 0.16 <sup>a</sup>	7.73 ± 0.24 <sup>a</sup>	7.45 ± 0.10 <sup>a</sup>	8.09 ± 0.03 <sup>a</sup>
	Group 3:	5.33 ± 0.07 <sup>a</sup>	7.65 ± 0.24 <sup>a</sup>	5.24 ± 0.16 <sup>c</sup>	5.69 ± 0.15 <sup>b</sup>
	Group 4:	4.33 ± 0.10 <sup>b</sup>	4.63 ± 0.11 <sup>c</sup>	4.62 ± 0.21 <sup>d</sup>	4.44 ± 0.22 <sup>c</sup>
	Group 1:	2.47 ± 0.11 <sup>a</sup>	3.45 ± 0.11 <sup>b</sup>	3.25 ± 0.10 <sup>b</sup>	2.42 ± 0.07 <sup>c</sup>
Albumin (g/dl)	Group 2:	2.31 ± 0.06 <sup>ab</sup>	4.18 ± 0.04 <sup>a</sup>	3.87 ± 0.30 <sup>a</sup>	3.61 ± 0.11 <sup>a</sup>
	Group 3:	2.27 ± 0.07 <sup>ab</sup>	2.68 ± 0.08 <sup>c</sup>	2.36 ± 0.09 <sup>c</sup>	3.09 ± 0.10 <sup>b</sup>
	Group 4:	2.10 ± 0.07 <sup>b</sup>	2.23 ± 0.07 <sup>d</sup>	2.47 ± 0.09 <sup>c</sup>	2.17 ± 0.05 <sup>c</sup>
	Group 1:	3.02 ± 0.02 <sup>a</sup>	2.39 ± 0.07 <sup>c</sup>	2.60 ± 0.08 <sup>bc</sup>	3.15 ± 0.10 <sup>b</sup>
	Group 2:	2.88 ± 0.10 <sup>a</sup>	3.55 ± 0.28 <sup>b</sup>	3.58 ± 0.29 <sup>a</sup>	4.49 ± 0.09 <sup>a</sup>
Globulin (g/dl)	Group 3:	3.06 ± 0.14 <sup>a</sup>	4.96 ± 0.15 <sup>a</sup>	2.98 ± 0.09 <sup>b</sup>	2.61 ± 0.05 <sup>c</sup>
	Group 4:	2.22 ± 0.04 <sup>b</sup>	2.40 ± 0.06 <sup>c</sup>	2.16 ± 0.13 <sup>c</sup>	2.27 ± 0.19 <sup>c</sup>
	Group 1:	0.82 ± 0.04 <sup>b</sup>	1.49 ± 0.05 <sup>a</sup>	1.30 ± 0.04 <sup>a</sup>	0.79 ± 0.02 <sup>c</sup>
	Group 2:	0.80 ± 0.01 <sup>b</sup>	1.19 ± 0.11 <sup>b</sup>	1.11 ± 0.19 <sup>a</sup>	0.80 ± 0.04 <sup>c</sup>
	Group 3:	0.75 ± 0.06 <sup>b</sup>	0.56 ± 0.02 <sup>d</sup>	0.78 ± 0.02 <sup>b</sup>	1.99 ± 0.06 <sup>a</sup>
A/G Ratio (g/dl)	Group 4:	0.95 ± 0.01 <sup>a</sup>	0.93 ± 0.02 <sup>c</sup>	1.14 ± 0.03 <sup>a</sup>	0.97 ± 0.07 <sup>b</sup>

(Group 1: Buffalo vaccinated by BEF vaccine only, Group 2: Buffalo vaccinated by rabies vaccine only, Group 3: Buffalo vaccinated by BEF and rabies vaccines, Group 4: Control buffalo group). WPV: Weeks Post Vaccination. Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P<0.05).

**3.9. Estimation of kidney function (Urea, Creatinine) in the sera of vaccinated buffalo groups:**

Urea concentration in group 1 showed significant increase 2- and 4-weeks post vaccination followed by significant decrease 6 weeks post vaccination and non-significant change 8 weeks post vaccination when compared to control group. Group 2 showed significant increases in urea concentration 2, 4- and 6-weeks post vaccination followed by significant decrease 8 weeks post vaccination when compared to control group. Group 3 showed significant

increase in urea concentration 2- and 4-weeks post vaccination followed by significant decrease 6- and 8-weeks post vaccination when compared to control group. Creatinine concentration in group 1 showed significant increase 2, 4, 6- and 8-weeks post vaccination when compared to control group. Group 2 and 3 showed significant decrease in urea concentration 2- and 4-weeks post vaccination followed by significant increase 6- and 8-weeks post vaccination when compared to control group as in table (6).

Table 5 AST, ALT, Urea and creatinine values in the sera of different vaccinated cattle groups

Parameter	Cattle Groups	Duration of Experiment			
		2 WPV	4 WPV	6 WPV	8 WPV
AST (U/L)	Group 1:	34.37 ± 1.06 <sup>bc</sup>	36.18 ± 1.12 <sup>b</sup>	28.95 ± 0.90 <sup>b</sup>	39.80 ± 1.23 <sup>a</sup>
	Group 2:	40.45 ± 2.80 <sup>a</sup>	39.80 ± 1.23 <sup>a</sup>	21.71 ± 0.67 <sup>c</sup>	41.61 ± 1.29 <sup>a</sup>
	Group 3:	30.75 ± 0.95 <sup>c</sup>	40.86 ± 0.34 <sup>a</sup>	31.91 ± 0.99 <sup>b</sup>	39.58 ± 1.22 <sup>a</sup>
	Group 4:	39.29 ± 1.21 <sup>ab</sup>	38.54 ± 1.19 <sup>a</sup>	37.67 ± 1.16 <sup>a</sup>	39.17 ± 1.21 <sup>a</sup>
ALT (U/L)	Group 1:	34.37 ± 1.06 <sup>b</sup>	14.47 ± 0.45 <sup>c</sup>	28.95 ± 0.90 <sup>b</sup>	25.32 ± 0.78 <sup>b</sup>
	Group 2:	63.32 ± 1.96 <sup>a</sup>	46.75 ± 1.45 <sup>a</sup>	48.84 ± 1.51 <sup>a</sup>	36.18 ± 1.12 <sup>a</sup>
	Group 3:	30.75 ± 0.95 <sup>b</sup>	48.84 ± 1.51 <sup>a</sup>	23.52 ± 0.73 <sup>c</sup>	13.98 ± 0.43 <sup>d</sup>
	Group 4:	23.52 ± 0.73 <sup>c</sup>	21.65 ± 0.67 <sup>b</sup>	24.45 ± 0.76 <sup>c</sup>	21.24 ± 0.66 <sup>c</sup>
Urea (mg/dl)	Group 1:	14.62 ± 0.45 <sup>b</sup>	11.00 ± 0.34 <sup>c</sup>	9.96 ± 0.31 <sup>b</sup>	9.78 ± 0.30 <sup>a</sup>
	Group 2:	18.25 ± 0.56 <sup>a</sup>	17.37 ± 0.54 <sup>a</sup>	9.89 ± 0.31 <sup>b</sup>	10.28 ± 0.32 <sup>a</sup>
	Group 3:	13.81 ± 0.43 <sup>b</sup>	13.93 ± 0.43 <sup>b</sup>	12.46 ± 0.39 <sup>a</sup>	8.25 ± 0.25 <sup>b</sup>
	Group 4:	7.65 ± 0.24 <sup>c</sup>	8.93 ± 0.28 <sup>d</sup>	7.81 ± 0.24 <sup>c</sup>	7.27 ± 0.22 <sup>c</sup>
Creatinine (mg/dl)	Group 1:	0.96 ± 0.03 <sup>c</sup>	0.97 ± 0.03 <sup>b</sup>	1.33 ± 0.04 <sup>b</sup>	1.26 ± 0.04 <sup>b</sup>
	Group 2:	1.69 ± 0.05 <sup>a</sup>	1.41 ± 0.04 <sup>a</sup>	1.94 ± 0.06 <sup>a</sup>	1.04 ± 0.03 <sup>c</sup>
	Group 3:	1.12 ± 0.03 <sup>b</sup>	1.52 ± 0.05 <sup>a</sup>	0.80 ± 0.02 <sup>c</sup>	1.38 ± 0.04 <sup>a</sup>
	Group 4:	0.54 ± 0.02 <sup>d</sup>	0.48 ± 0.01 <sup>c</sup>	0.60 ± 0.02 <sup>d</sup>	0.44 ± 0.01 <sup>d</sup>

(Group 1: Cattle vaccinated by BEF vaccine only, Group 2: Cattle vaccinated by rabies vaccine only, Group 3: Cattle vaccinated by BEF and rabies vaccines, Group 4: Control cattle group). WPV: Weeks Post Vaccination. Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P<0.05)

Table 6 AST, ALT, Urea and creatinine values in the sera of different vaccinated buffalo groups

Parameter	buffalo Groups	Duration of Experiment			
		2 WPV	4 WPV	6 WPV	8 WPV
AST (U/L)	Group 1:	69.36 ± 3.69 <sup>a</sup>	69.50 ± 3.08 <sup>a</sup>	61.61 ± 1.29 <sup>ab</sup>	64.47 ± 0.45 <sup>a</sup>
	Group 2:	71.23 ± 3.75 <sup>a</sup>	54.27 ± 1.68 <sup>b</sup>	65.23 ± 1.40 <sup>a</sup>	65.88 ± 2.96 <sup>a</sup>
	Group 3:	60.35 ± 3.41 <sup>a</sup>	72.99 ± 3.80 <sup>a</sup>	56.18 ± 1.12 <sup>b</sup>	64.18 ± 2.29 <sup>a</sup>
	Group 4:	64.35 ± 0.39 <sup>a</sup>	69.33 ± 2.76 <sup>a</sup>	63.52 ± 2.89 <sup>a</sup>	66.17 ± 2.97 <sup>a</sup>
ALT (U/L)	Group 1:	43.41 ± 1.34 <sup>c</sup>	72.36 ± 2.24 <sup>b</sup>	41.61 ± 1.29 <sup>b</sup>	18.09 ± 0.56 <sup>c</sup>
	Group 2:	72.36 ± 2.24 <sup>a</sup>	79.59 ± 2.46 <sup>a</sup>	72.36 ± 2.24 <sup>a</sup>	30.75 ± 0.95 <sup>b</sup>
	Group 3:	50.66 ± 1.57 <sup>b</sup>	36.18 ± 1.12 <sup>d</sup>	19.90 ± 0.62 <sup>c</sup>	21.71 ± 0.67 <sup>c</sup>
	Group 4:	70.55 ± 2.18 <sup>a</sup>	64.30 ± 1.99 <sup>c</sup>	68.94 ± 2.13 <sup>a</sup>	63.53 ± 1.96 <sup>a</sup>
Urea (mg/dl)	Group 1:	22.31 ± 0.69 <sup>b</sup>	29.60 ± 0.92 <sup>a</sup>	3.86 ± 0.12 <sup>d</sup>	9.05 ± 0.28 <sup>a</sup>
	Group 2:	27.78 ± 0.86 <sup>a</sup>	31.19 ± 0.96 <sup>a</sup>	8.74 ± 0.27 <sup>a</sup>	7.67 ± 0.24 <sup>b</sup>
	Group 3:	19.54 ± 0.60 <sup>c</sup>	18.59 ± 0.57 <sup>b</sup>	5.65 ± 0.17 <sup>c</sup>	6.04 ± 0.19 <sup>c</sup>
	Group 4:	8.31 ± 0.26 <sup>d</sup>	8.16 ± 0.25 <sup>c</sup>	7.93 ± 0.25 <sup>b</sup>	8.91 ± 0.28 <sup>a</sup>
Creatinine (mg/dl)	Group 1:	1.71 ± 0.05 <sup>a</sup>	1.20 ± 0.04 <sup>a</sup>	1.34 ± 0.04 <sup>b</sup>	1.31 ± 0.04 <sup>b</sup>
	Group 2:	0.12 ± 0.00 <sup>d</sup>	1.04 ± 0.03 <sup>b</sup>	1.44 ± 0.04 <sup>b</sup>	1.50 ± 0.05 <sup>a</sup>
	Group 3:	0.91 ± 0.03 <sup>c</sup>	0.84 ± 0.03 <sup>c</sup>	1.63 ± 0.05 <sup>a</sup>	1.45 ± 0.04 <sup>a</sup>
	Group 4:	1.38 ± 0.04 <sup>b</sup>	1.24 ± 0.04 <sup>a</sup>	1.04 ± 0.03 <sup>c</sup>	1.16 ± 0.04 <sup>c</sup>

(Group 1: Buffalo vaccinated by BEF vaccine only, Group 2: Buffalo vaccinated by rabies vaccine only, Group 3: Buffalo vaccinated by BEF and rabies vaccines, Group 4: Control buffalo group). WPV: Weeks Post Vaccination. Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P<0.05).

#### 4. DISCUSSION

Bovine ephemeral fever (BEF) is an infectious and preventable disease (Fan, 2019) that negatively affecting on productive and reproductive activities of cattle and water buffalo and characterized by sudden onset of fever, stiffness, lameness, nasal and ocular discharges, depression, cessation of rumination and constipation (Kawther and Wahid, 2011). Several outbreaks of BEF were reported in Egypt through 2000, 2004, 2014 and 2018 (Hassan, 2000; El.Bagoury et al., 2014; Albhwar et al., 2018). It is an acute vector-born viral disease caused by BEF virus as a member of family Rhabdoviridae. Subsequently, eradication of the arthropod

vectors and vaccination are necessary to control such disease. There are 2 types of vaccines (live and inactivated) but the live virus vaccine which inactivated on the time of use is preferable as it has prolonged immunity due to saponin action as virus inactivator and immune stimulating agent (Chenq et al., 2006; Albhwar et al., 2010; Orly et al., 2012). Rabies disease is a fatal disease that has been recorded for thousands of years having the ability to affect all mammalian species (Gold et al., 2020). It is caused by rabies virus that belongs to family Rhabdoviridae and transmitted mainly biting of rabid animal to susceptible host (CDC, 2019). Depending on the antigenic relationship between rabies virus and BEF virus, several studies were applied to know

possibility of cross protection induced by one of them against the other where there was that a suggested possible protection against BEF virus in cattle vaccinated with killed rabies vaccine (Zaghawa et al., 2001). In addition, another study was applied for rapid diagnosis of BEF virus infection among cattle in Egypt during summer 2001 using anti-serum specific to rabies virus for rapid diagnosis by immunoperoxidase (IP) and immunofluorescence (IF) techniques in leukocyte and blood films of naturally infected cattle also impression smears of brain of inoculated mice (Zaghawa et al., 2002) and it was concluded that the anti-serum specific to rabies virus can be used for diagnosis of BEF using IF and IP and this is a good idea for diagnosis of BEF when a sudden outbreak occurs and there is no available BEF virus anti-serum (Gehan et al., 2004). It was stated that cattle could be protected successfully against rabies and BEF at the same time with regard to the benefit of the antigenic relationship between both rabies and BEF virus (El-Shamy, 2006).

The present work was planned to investigate the cross-protection efficacy and safety of vaccination of cattle and buffalo with BEF and rabies vaccines when given separately and simultaneously. To fulfillment the planned work, sero-negative BEF and rabies cattle and buffaloes in groups were used. Each of cattle and buffalo groups was classified into 4 groups in which group-1 vaccinated by 2 doses of the locally produced cell culture attenuated BEF, group-2 vaccinated one dose of the inactivated cell culture rabies vaccine, group-3 vaccinated by the first dose of BEF vaccine simultaneously with a dose of inactivated rabies then a second dose of BEF vaccine after 2 weeks; while group-4 includes non-vaccinated cattle kept as test control.

It was found that all vaccinated cattle and buffalo did not show any local or systemic abnormal post vaccination reactions confirmed the safety of BEF and rabies vaccines (Daoud et al., 2001; Khalid, 2004; Daoud et al., 2005; Younis et al., 2005; El-Shamy, 2006).

The results in table 1 showed that double dose of BEF vaccination success to produce a protective immune response for 6 months post 1<sup>st</sup> vaccination this finding in agreement with (Daoud et al., 2001; Moustafa 2004; Khalid, 2004; Saber 2004; Daoud et al., 2005; Younis et al., 2005; El-Shamy, 2006). On the other hand, vaccination of cattle and buffalo with a single dose of the inactivated cell culture rabies vaccine resulted in induction of specific rabies neutralizing antibodies for 6 months post vaccination as shown in table (2) (El-Karamany 1986; Khodier et al., 1998; Khodier 1999; El-Shamy, 2006). Meanwhile, simultaneous vaccination of cattle and buffalo with two doses of BEF vaccine and one dose of inactivated cell culture rabies vaccine resulted in better immune response and plenty protective levels of specific antibodies against both BEF and rabies especially in buffalo for 6 months post vaccination as illustrated in table (1). Similarly, rabies serum neutralizing antibody titers nearly has the same behavior of BEF vaccine in both vaccinated cattle and buffalo groups with high levels of specific rabies antibodies for 6 months as demonstrated in table (2). These findings in this study almost the even in local level but there were previous trial by El-Shamy (2006), who vaccinated cattle only by inactivated cell culture BEF and rabies simultaneously but with a single dose for each vaccine and recorded better immune response for each vaccine than when vaccinated each one separately.

The findings regarding the total protein concentration showed significant increase in all vaccinated cattle and buffalo groups when compared to control group at 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> weeks post vaccination due to increase in globulin

levels. Also, albumin and Globulin concentration showed significant increase when compared to control group at 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> weeks post vaccination, but A/G ratio in group 1, 2 and 3 showed significant decrease at 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> weeks post vaccination when compared to control group as showed in table (3) in cattle and in table (4) in buffalo.

These findings appear to be the same as those obtained formerly (Safaa 2004; Sayed Ahmed 2005; El-Shamy, 2006) in both cattle and buffalo groups and also when cattle vaccinated by BEF vaccine leading to increase in total serum protein and decrease in serum albumin to the increase in the globulins to form the humeral antibodies after vaccination that recorded by (Taha et al., 1984; Lila 1993).

Estimation of liver function (AST, ALT) in the sera of vaccinated cattle groups revealed that AST and ALT activity is increased in all vaccinated cattle groups except in some groups at certain time when compared to control non-vaccinated cattle group as shown in table (5). While in buffalo groups the AST and ALT activity are in opposite to those in cattle as both levels showed decrease in their concentration in all vaccinated buffalo groups that may be significant or non-significant when compared to control non-vaccinated buffalo group as in table (6).

Estimation of kidney function (Urea, Creatinine) in the sera of vaccinated cattle groups showed that both parameters concentration in group 1, 2 and 3 showed significant increase at 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> weeks post vaccination when compared to control non-vaccinated group as shown in table (5). While in buffalo groups urea and creatinine concentrations may showed significant increase or decrease in all vaccinated buffalo groups when compared to control non-vaccinated buffalo group as in table (6). Regarding the obtained liver and kidney function parameters investigated through the present work, there were no available data discuss the effect of BEF and rabies vaccines on them in vaccinated cattle and buffalo although (Taha et al., 1984; Lila 1993) showed that cattle and buffalo vaccination with one of viral vaccines (Rinderpest vaccine) did not affect liver and kidney functions in such animals. Also, in monitoring the serum values of some biochemical parameters (AST) in cattle vaccinated by a combination of hexavalent and trivalent FMD vaccine revealed that a significant increase in serum activity of AST that may suggest some degree of hepatic dysfunction (Shawky et al., 2015)

## 5. CONCLUSIONS

From the obtained results in the present study, it could be concluded that 1) All used vaccines cell culture BEF and inactivated cell culture rabies vaccines are safe and immunogenic for cattle and buffalo, 2) Simultaneous vaccination of cattle and buffalo with inactivated cell culture BEF and rabies vaccine provide them with good levels of specific BEF and rabies antibodies suggesting successful protection against rabies and BEF at the same time with regard to the benefit of the antigenic relationship between the two viruses.

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