

EPIDEMIOLOGICAL STUDY ON BOVINE EPHEMERAL FEVER VIRUS (BEFV) INFECTION IN CATTLE AND BUFFALOES IN EGYPT

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ABSTRACT

The present study was conducted on 156 cattle and buffaloes. The age of these animals between 6 months to 4 years, they examined during the period from January 2018 to September 2019. These animals from different localities in Assiut, Sohag and El-Menia governorates. The investigated animals characterized by sudden onset of fever in 25.64% (40/156) ranged from 40° to 42°C associated with severe congested mucous membranes, dry muzzle, foamy salivation, dullness, sickness, shivering and trembling, rapid shallow respiration in 19.23% (30/156) and ruminal stasis in 25% (39/156), enlargement of superficial lymph nodes. 7.05% (11/156) and 6.41% (10/156) of the examined cases showed drop in milk production and abortion, respectively. Little number of cases showed recumbency, subcutaneous emphysema and lameness respectively 1.28% (2/156), 0.64% (1/156) and 3.21% (5/156). The serological detection for ephemeral fever virus antigen was 9.52% (8/84), and 4.76% (2/42) were positive for virus antibodies. 40/50 (80%) were positive for RT-PCR. The prevalence of infection was 17.95% (28/156) in Assiut governorate, 5.77% (9/156) in Sohag, 1.92% (3/156) in El-Menia. Frezian breeds had higher rate of infection 13.46% (21/156) than native breeds 6.41% (10/156) followed by buffaloes was 5.77% (9/156). The infection in females was 17.31% (27/156) but in males was 8.33% (13/156). The age group >2-4 years (13.45%) was more susceptible to virus infection than age group 6 months-2 years (12.5%). The infection rate was higher in hot months 19.23% (30/156) than non-hot months 6.41% (10/156).

Key words: BEFV infection, Assiut Governorate, Epidemiology

INTRODUCTION

Bovine ephemeral fever (BEF) is a non-contagious viral disease of cattle and water buffaloes (Zaghawa *et al.*, 2017; Lee, 2019 and Huihui *et al.*, 2020). It is a vector born acute febrile disease in tropical and

subtropical regions of Africa, Asia, Australia and the Middle East (Aziz-Boaron *et al.*, 2012; Kun *et al.*, 2020). BEF is characterized by high morbidity rates may be up to 100%, however the mortality rate is generally low (rarely exceeds 1%), but cattle in good condition are usually affected more severely and the mortality rate can be as high as 30% in very fat cattle and in outbreaks of bovine ephemeral fever the morbidity rate may be as high as 80% (Momtaz *et al.*, 2012 and Zaghawa *et al.*, 2017). BEF is considered one of the diseases

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which have economic impact as loss of milk production in quality and quantity, loss of condition in beef herds, infertility and abortion (Bakhshesh and Abdollahi, 2015 and Jiang *et al.*, 2019). The disease is characterized clinically by a sudden onset of fever as high as 41°C, sudden and severe drop in milk production, inappetence, lethargy, salivation, nasal discharge and depression (Tonbank *et al.*, 2012 and Pasandideh *et al.*, 2019). Diagnosis of BEF begins with the history of the outbreak and clinical examination of the affected animals. Serological tests as blocking Enzyme linked immunosorbent assay (ELISA) and reverse transcriptase polymerase chain reaction (RT-PCR) are the more accurate methods used for diagnosis of the disease (Zaher and Ahmed, 2011; Kasem *et al.*, 2014 and Alkan *et al.*, 2017). Because of lack of information about this virus in Assiut governorate, the aim of our study is to determine the virus prevalence by using ELISA test and RT-PCR test.

MATERIALS AND METHODS

Animals:

During the period of investigation from January 2018 to September 2019, a total number of 156 cattle and buffaloes (72 Frezian, 33 native breed cattle and 51 buffaloes) of different ages (6 months to 4 years divided into different groups) and sexes, were clinically examined, serologically and molecularly tested for the presence of BEFV. These animals were admitted to Veterinary teaching hospital in Faculty of Veterinary medicine, Assiut University from different localities in Assiut Governorate, the farm of El-Dyabat in Sohag and El-Menia governorate. Depending on history taking, the clinically examined animals were not vaccinated against BEFV. These animals examined clinically according to (Rosenberger, 1979 and Radiostits *et al.*, 2007) any deviation from normal was recorded.

Samples:

Whole blood and serum samples were collected from jugular vein of 156 cattle and buffaloes in vacutainer tubes with or without anticoagulant on which the date, number, age and sex of the animal in addition to the address of the owner were registered. The whole blood samples were preserved at -80°C deep freeze until used for molecular examination. The serum samples were collected by taking blood samples in vacutainer tube without anticoagulant, then preserved in refrigerator for 1 hour, and centrifuged at 3000 r.p.m for 15 minutes. Serum samples were preserved at -20°C deep freeze until used for serological examination (ELISA antibody and antigen).

Serological Diagnosis:

- **Bovine ephemeral fever virus antigen (BEFV- Ag) ELISA kit:** (Sino Gene Clone Biotech Co., Ltd), (Catalog No.: SG- 60602).
- **Bovine ephemeral fever virus antibody (BEFV- Ab) ELISA kit:** (Sino Gene Clone Biotech Co., Ltd), (Catalog No.: SG- 60601)

Molecular diagnosis:

- **Kits for RNA extraction according to (Momtaz *et al.*, 2012) was Qiagen® QIAamp Viral RNA Mini Kit,** (QIAGEN, GmbH, Germany). LOT (151034653), CAT. No. 5290, No. of Preps 50.
- **Primers:**
The presence of BEFV was confirmed by RT-PCR as described by (Kasem *et al.*, 2014) by using the sequences of the primers as follows:
380Forward (F) (5' AGA GCT TGG TGT GAA TAC 3')
380Reverse (R) (5' CCA ACC TAC AAC AGC AGA TA 3') (Zheng and Qui, 2012).
- **Master Mix: Qiagen® OneStep RT-PCR Kit** (Qiagen, Hilden, Germany).
- **Identification of PCR products**

After amplification, 1.5% agarose was prepared for the step of Gel electrophoresis and the bands of PCR products at 380 bp

Statistical analysis

- All data were analyzed by Chi-square of independence formula that used by Statistical package for the social sciences (SPSS) version 16 software program (2007).

RESULTS

During the period of investigation from January 2018 to September 2019, one hundred and fifty-six (156) cattle and buffaloes were inspected and clinically, serologically and molecularly examined for BEFV infection.

Results of clinical examination:

The clinical signs of BEFV infection noticed were sudden onset of fever in 25.64% of cases (40 out of 156) ranged from (40° to 42°C) associated with severe congested mucous membranes as in photo (1), dry muzzle, foamy salivation as in photo (2), dullness, sickness, shivering and trembling, 19.23% (30/156) from the examined cases showed rapid shallow respiration and 25% (39/156) of the investigated animals suffered from rumenal stasis. Most of the observed animals showed enlargement of superficial lymph nodes as in photo (3). As a result of fever, 7.05% (11/156) and 6.41% (10/156) of the examined cases showed drop in milk production and abortion, respectively. Little number of cases showed recumbency as, subcutaneous emphysema and lameness respectively 1.28% (2/156), 0.64% (1/156) and 3.21% (5/156).



Photo (1): Congested mucus membrane of the eye (Fever)

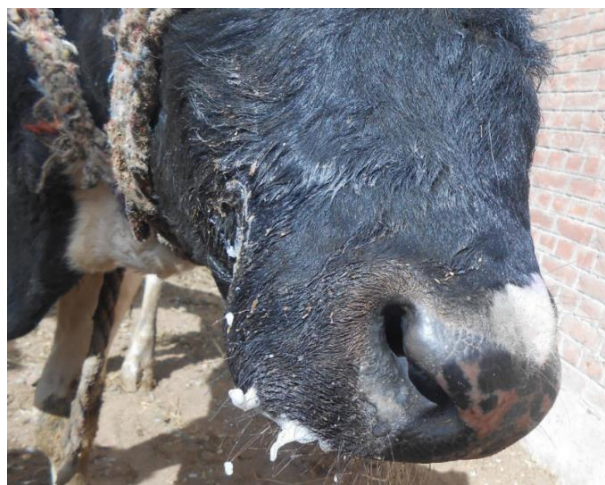


Photo (2): Dry muzzle (fever)



Photo (3): Enlargement of prescapular lymph node.

Table 1: Results of clinical examination:

Clinical finding	No. of +ve cases	%
Fever (congested mucus membranes, dry muzzle, foamy salivation, dullness, sickness, shivering and trembling)	40/156	25.64
Respiratory signs (cough and shallow, rapid respiration)	30/156	19.23
Ruminal stasis	39/156	25
Enlarged superficial lymph nodes	35/156	22.44
Drop in milk production	11/156	7.05
Abortion	10/156	6.41
Recumbency	2/156	1.28
Subcutaneous emphysema	1/156	0.64
Lameness	5/156	3.21

Results of serological diagnosis:

ELISA test used for detection of BEFV Antigen revealed that 9.52% (8 out of 84) of serum samples were positive, while ELISA

test used for detection of BEFV Antibodies revealed that 4.76% (2 out of 42) of serum samples were positive as in Table (2).

Table 2: Prevalence of BEFV infection in the examined animals by ELISA test.

ELISA test	Number of the examined cases	Number of positive cases	percentage
Ag	84	8	9.52
Ab	42	2	4.76

Results of molecular diagnosis:

40 out of 50 (80%) examined samples showed the specific band at 380 bp after

PCR amplification of G gene of BEFV by using RT-PCR as shown in Photo (4) and Table (3).

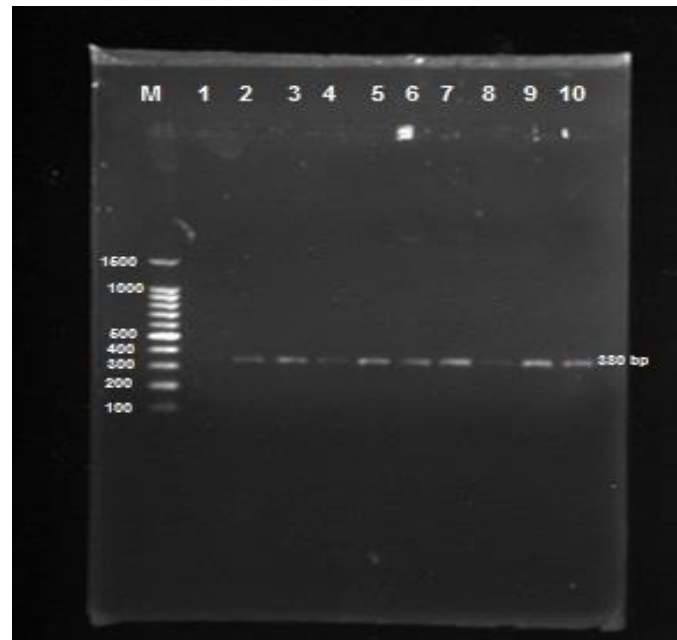


Photo (4): Agarose gel electrophoresis of RT-PCR amplification of G gene of BEFV
Lane M: DNA Marker of 100bp.
Lanes 2, 3, 4, 5, 6, 7, 8, 9 & 10: Positive samples with amplified product at 380 bp.

Table 3: The positive results of RT-PCR

Examined samples	RT-PCR	
	No.	%
50	40	80

Comparison between ELISA and RT-PCR in diagnosis of BEFV:

The positive BEFV examined animals by ELISA and RT-PCR was higher significant than investigated animal by ELISA only or RT-PCR only. Total numbers of examined cases in our study were 156 animals, 106

cases out of 156 animals were tested by ELISA only and 10 cases from them were positive for BEFV infection. 50 out of 156 cases were tested by PCR only and 40 cases were positive for BEFV infection as shown in table (4).

Table 4: Prevalence of BEFV infection in the examined cases by ELISA and RT-PCR

Total number of examined animals	ELISA			RT-PCR		
	No.	Positive	%	No.	Positive	%
156	106	10	9.43	50	40 ***	80%

*** Highly significant increase at $p < 0.001$

Table 5: Difference between serological and molecular techniques for diagnosis of BEFV
The 20 cases in this table were out of 156 animals (the total number of the examined cases) were tested by both ELISA and PCR and 4 cases from them were positive by ELISA and the other 16 cases were positive by PCR.

Examined cases	Serological test (ELISA)		Molecular technique RT-PCR	
	No.	%	No.	%
20	4	20	16	80***

***Highly significant increase at $p < 0.001$.

Table (6): Results of molecular examination:

These 20 cases out of 156 animals were examined by both ELISA and PCR and resulted those 4 cases being positive for both ELISA and PCR, 13 cases were positive for PCR and negative for ELISA and 3 cases were negative for both PCR and ELISA.

Total No. of the examined cases	+ve RT-PCR and +ve ELISA		+ve RT-PCR and -ve ELISA		-ve PCR and -ve ELISA	
	No.	%	No.	%	No.	%
20	4	20	13	65***	3	15

***Highly significant increase at $p < 0.001$.

Epidemiological findings:

A- Prevalence of BEFV infection:

Prevalence of BEFV infection in the examined cases was 25.64% (40/156). The prevalence of BEFV infection in different localities in Upper Egypt indicated that

Assiut had the highest rate of infection 17.95% (28/156). The prevalence of BEFV infection in Sohag and El- Menia were 5.77% (9/156) and 1.92% (3/156), respectively as observed in Table (7).

Table 7: Percentage of infection with BEFV in different governorates in Upper Egypt.

Locality	Examined cases	Positive cases	
		No.	%
Assiut	80	28	17.95***
Sohag	36	9	5.77
El-Menia	40	3	1.92
Total	156	40	25.64

B- Breed susceptibility:

The relationship between BEFV infection and breed susceptibility revealed that frezian breeds had higher rate of infection 13.46%

(21/156) than native breeds 6.41% (10/156) followed by buffaloes was 5.77% (9/156) as observed in Table 8.

Table 8: Prevalence of BEFV infection in different breeds of cattle and buffaloes.

Breed	Examined cases	Positive cases	
		No.	%
Frezian	72	21	13.46
Native	33	10	6.41
Buffaloes	51	9	5.77
Total	156	40	25.64

Non-significant difference at $p < 0.05$.

C-Sex susceptibility:

Female animals had higher infection rate than males to BEFV. The prevalence of

infection in females was 17.31% (27 out of 156) but in males was 8.33% (13 out of 156) as shown in Table 9.

Table 9: Prevalence of BEFV infection in both sexes of cattle and buffaloes.

Sex	Examined cases	Positive cases	
		No.	%
Male	58	13	8.33
Female	98	27	17.31
Total	156	40	25.64

Non- significant at $p < 0.05$.

D-Age susceptibility:

In our study, the observed animals ranged from 6 months to 4 years. In the age group

(>2 to 4 years) had higher infection rate 13.45% (21/156) than age group (6 months to 2 years) 12.18% (19/156) as in Table 10.

Table 10: Prevalence of BEFV infection in cattle and buffaloes at different age groups.

Age	Examined cases	Positive cases	
		No.	%
6 months-2 years	40	19	12.18
>2-4 years	116	21	13.45***
total	156	40	25.64

***Highly significant increase at $p < 0.001$.

E-Seasonal variation:

In the present study, the prevalence of BEFV infection in the examined cases was higher

in hot months 32.61% (30/156) than non-hot months 15.62% (10/156) as in Table 11.

Table 11: Influence of seasonal variation on the prevalence of BEFV infection.

Months	Examined cases	Positive cases	
		No.	%
Hot months	92	30	19.23*
Non-hot months	64	10	6.41
Total	156	40	25.64

* Significant difference at $p < 0.05$.

DISCUSSION

BEF is an arthropod- borne disease of cattle and water buffaloes. BEFV infection occurs seasonally in tropical, subtropical and high temperate regions of Africa, Asia and Australia, BEF is considered as a member of the genus *Ephemerovirus* in the family *Rhabdoviridae*, the characteristic clinical signs of BEF are sudden onset of a

high fever, anorexia, depression, ocular and nasal discharges, salivation, muscle stiffness, lameness, rumenal stasis, sternal recumbency and other inflammatory responses. The disease can cause economic impact through the sudden drop of milk production in dairy cattle and loss of condition in beef cattle, these detected results agreed with (Zaher and Ahmed, 2011; Bakhshesh and Abdollahi, 2015;

Hayama *et al.*, 2016; Mirazaie *et al.*, 2017; Lapira *et al.*, 2018 and Lee, 2019).

The investigated animals showed a very high temperature ranged from (40 to 42°C) or more, severe congested mucous membranes, rapid and shallow respiration, body shivering and muscle trembling. The examined cases showed dullness, sickness, foamy salivation, sudden drop in milk production, enlargement of superficial lymph nodes, cessation of rumination, some cases had constipation, and some had diarrhea. A small number from the examined cases showed lameness and recumbency, the pregnant cows aborted during the stage of fever. A very small number of cases showed subcutaneous emphysema at the region of the hind quarters and in the gluteal muscles and recumbency, these results were in agreement with (El-Nesr *et al.*, 2010 and Zaher and Ahmed, 2011). The clinical signs related to BEFV infection which detected in our study mainly may be attributed to increasing the vascular permeability and release of cytokines and Interleukins (IL-2 and IL-6) and inflammatory biomarkers as (cortisone, CRP) and decrease in calcium concentration that resulted from the inflammatory response associated with the disease, pulmonary and subcutaneous emphysema may be indicated to the nutritional Selenium deficiency and replication of BEFV in the reticuloendothelial tissues as lung, spleen and lymph nodes. The short incubation period, sudden onset and sudden recovery of BEF may be interpreted by the key role of the released neutralizing antibodies in protection against the disease and these interpretations were in agreement with (El-Nesr *et al.*, 2010; Al-Behwar *et al.*, 2018 and Abo-Sakaya and Bazan, 2020).

The prevalence of BEF infection in the current study revealed that disease could

be affected by several factors including age, locality and seasonal variation. Our study revealed that the infection rate of BEFV infection in our examined cases was 25.64% (40/156) in Assiut governorate, these results were nearly similar to (Momtaz *et al.*, 2012) who detected 29% of the examined cases were positive for BEFV infection in Iran, (Bakhshesh and Abdullahi, 2015) in Iran that detected 27% of cases were positive in Turkey, (Degheidy *et al.*, 2011) also detected 23.1% of the examined cases were positive in el-Giza, Egypt; in addition to (Alkan *et al.*, 2017) who detected 25 % of cases were positive for BEFV infection in Turkey-Ankara for BEFV G gene, although (Zaghawa *et al.*, 2017) detected 25% of the examined cases were positive in Saudia Arabia and (Al-Sultany and Hassan, 2013) who reported 24.44% from the examined cases were positive for BEFV infection in Iraq. The higher intensity of infection in cattle and buffaloes may be attributed to the bad hygienic condition which helps on the spreading of vector (*Culicoides* midges and mosquitoes) of the disease, in addition to the climatic condition that occurs in summer season, as due to lack of rains in summer season together with presence of stagnant water may create a favorable habitat for the vector reproduction leading to BEFV expansion.

In the present study, the serological technique used for detection of BEFV infection was blocking ELISA-Ag and ELISA-Ab. Using of ELISA for detection of antigen of BEFV revealed that 9.30% (8 out of 86) of serum samples were positive, these results were lower than that recorded by (Zheng *et al.*, 2010) who detected that ELISA test had 100% sensitivity and 96.7% specificity and gave 336 positive samples in Japan. Although our detected results were nearly similar to that detected by (Momtaz *et al.*, 2011) in Iran, the

variation between our results and those of other studies may be attributed to geographical variation and differences in timing of samples collection and also may be due to the small number of the collected samples in addition to the high proportion of non-immunized cattle in the examined serum samples and this allowed the BEFV to be transmitted and caused the disease among cattle herds and this in agreement with (Li *et al.*, 2015).

Currently, RT-PCR was more efficient and reliable than the used serological method (Blocking ELISA Ag and Ab) for detection of BEFV infection in cattle and buffaloes (RT-PCR 80% (40/50) – ELISA-Ag 9.30% (8 out of 86)), the serological technique was lower than that recorded by the molecular techniques (RT-PCR) and these results were similar to previously recorded by (Kasem *et al.*, 2014; Alkan *et al.*, 2017; Zaghawa *et al.*, 2017 and Lapira *et al.*, 2018) who observed that RT-PCR was the most sensitive test used for diagnosis of BEF compared to other tests especially ELISA because RT-PCR has many advantages such as possibility to detect as little as 2 fragments of viral RNA and confirmation of diagnosis of BEFV, sensitive, specific and rapid diagnosis of the disease in Egypt, moreover we found that RT-PCR assay was useful for testing RNA samples extracted from whole blood.

Our results were also in agreement with (Bakhshesh and Abdollahi, 2015) who found that an accurate and reliable diagnosis by using molecular approach for detection of widely spread of viral agent and the application of RT-PCR in the future will definitely increase the sensitivity of BEFV diagnosis and will help to reveal the real extent of virus, our results were in agreement with (Tonbank *et al.*, 2012) who detected 80% of cases were positive for BEFV infection by RT-PCR in Turkey, but in contrast with

(Degheidy *et al.*, 2011) in which his results were less than our results who detected 45.4% positive cases by RT-PCR and this may be contributed to there was no vaccination program against BEFV in Egypt.

Additionally, our observations in the current study were in agreement with (Zheng *et al.*, 2010 and Mirazaie *et al.*, 2017) who revealed that rapid spread of BEF epidemics from the primary foci was most probably supported by dense populations of susceptible cattle, breeding places of vectors, climatic and ecological conditions that susceptible for propagation and dispersal of massive numbers of vectors and so contributed considerably to the fast spread of the disease, in addition to virus strain and high sensitivity of cattle population, low level of awareness among practitioners, owners, and farm managers about the disease, inappropriate management of collecting manure and communication between farm workers were identified as a risk factors for disease occurrence. The results reported by (Tamam and Abdel-Moneim, 2005) in Egypt were in agreement with our study and this may be due to the majority of domestic animals populations were not subjected to vaccination for BEFV, although high prevalence rate may be due to the ability of the virus to reactivate from latency that it is responsible for recurrence of the disease and increased rate of virus transmission.

According to Locality in the current study, there was a relationship between the rate of BEFV infection and localities, in which the percentage of infection in Assiut 17.95% was higher than in Sohag and El-Menia, 5.77% and 1.92%, respectively, this may be attributed to number of collected samples in Assiut governorate were more than those collected from Sohag and El-Menia governorates because most of our

samples were collected from the incoming cases to the Veterinary teaching hospital, Faculty of Veterinary medicine, Assiut governorate and the high percentage of infection in Assiut may be due to lack of immunization and the owners didn't use the vaccination program against BEF.

In relation to the breed of the animals in our study, the infection rate of BEFV was 13.46% in Frisian breeds of cattle and 6.41% in native breeds of cattle and 5.77% in buffaloes, in which, the infection rate in Frisian breeds of cattle was higher than in native breeds of cattle and buffaloes that similar to the results showed previously by (Momtaz *et al.*, 2012 in Iran; Zaher and Ahmed, 2012 in Egypt; Niwa *et al.*, 2015 in Japan and Zaghawa *et al.*, 2017 in Saudia Arabia) and this may be due to buffaloes less harassed by mosquitoes than cattle, variation in number of the investigated cases, stress factors to which the animal exposed, sanitary conditions and also immune status of animals which is lower in Frisian breeds than native breeds and buffaloes, but (Walker and Klement, 2015) showed that clinical BEF occurred in cattle and water buffaloes as the same.

Regarding to sex, our study revealed that rate of infection in female animals (17.31%) was higher than male animals (8.33%), this result was agreement with that reported by (Momtaz *et al.*, 2012; Al-Sultany and Hassan, 2013 and Akakpo, 2015) and this may be attributed to the higher number of female animals than male ones and inclination of carrier insects to sting females more than males.

Animal age plays a great role in animal susceptibility to BEFV infection as it has been observed that animals aged group >2-4 years (13.45%) was more susceptible to infection than animals aged group 6 months-2 years (12.5%). Our result similar

to that previously obtained by (Momtaz *et al.*, 2012; Al-Sultany and Hassan, 2013 and Akakpo, 2015) who concluded that may be due to the frequency of stinging by insects. Contrariwise, (Zaher and Ahmed, 2011) found that young cattle and buffaloes were more susceptible to infection with BEFV than older ages and this may be attributed to lack of immunity in young ages. (Mirazaie *et al.* 2017) reported that all age groups of cattle were susceptible to BEF, but it has been usually showed more frequently in calves less than 2 years old compared to the other age groups which may be due to delayed methods for diagnosis and treatment.

Dealing with the seasonal variation and BEF infection among infected animals, the present study showed that there was a relationship between the rate of BEFV infection and the seasonal changes and there was higher rate of infection in hot months (19.23%) than non-hot months (6.41%), this might be attributed to wide spread of flying insect vectors (mosquito species) that are responsible for transmission of the disease mainly during summer season, although due to lack of rains during summer season, the high temperature together with presence of stagnant water, may create a favorable habitat for vector reproduction, this finding was in agreement with (Yeruham *et al.*, 2010; Al-Sultany and Hassan, 2013; Kasem *et al.*, 2014; Mirazaie *et al.*, 2017 and Zaghawa *et al.*, 2017), but our result was disagreement with that recorded by (Hayama *et al.*, 2016) who mentioned that maintenance of BEFV would be possible in winter season in addition to summer.

CONCLUSION

BEF is one of the most important viral disease which infect cattle and buffalo. This disease can be affected by some risk factors such as age, and seasonal variation,

breed and sex had no effect upon the occurrence of the disease. Serological test (ELISA) is an important tool for the detection of this infection. Molecular technique (RT-PCR) is an important confirmatory and reliable technique for diagnosis of BEFV infection.

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دراسة وبائية لعدوي فيروس حمى الأبقار سريعة الزوال في الماشية و الجاموس في مصر

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أجريت الدراسة الحالية على ١٥٦ بقرة وجاموس. وتتراوح أعمار هذه الحيوانات ما بين ٦ أشهر إلى ٤ سنوات ، تم فحصها خلال الفترة من يناير ٢٠١٨ إلى سبتمبر ٢٠١٩. هذه الحيوانات من محليات مختلفة بمحافظة أسيوط وسوهاج والمنيا. تميزت الحيوانات التي تم فحصها بظهور مفاجئ للحمى في ٢٥,٦٤% (١٥٦/٤٠) تتراوح من ٤٠ درجة مئوية إلى ٤٢ درجة مئوية مصحوبة باحتقان شديد في الأغشية المخاطية ، وجفاف الكمامة ، وسيلان اللعاب الرغوي ، والبلادة ، والمرض ، والارتعاش والارتجاف ، والتنفس الضحل السريع في ١٩,٢٣% (١٥٦/٣٠) وركود الكرش في ٢٥% (١٥٦/٣٩) ، تضخم الغدد الليمفاوية السطحية ، ٧,٠٥% (١٥٦/١١) و ٦,٤١% (١٥٦/١٠) من الحالات التي تم فحصها أظهر انخفاضاً في إنتاج الحليب والإجهاض ، على التوالي. أظهر عدد قليل من الحالات الاستلقاء وانتفاخ الرئة تحت الجلد والعرج على التوالي ١,٢٨% (١٥٦/٢) ، ٠,٦٤% (١٥٦/١) و ٣,٢١% (١٥٦/٥). كان الاكتشاف المصلي لمستضد فيروس الحمى سريعة الزوال ٩,٥٢% (٨٤/٨) ، و ٤,٧٦% (٤٢/٢) كان موجياً للأجسام المضادة للفيروسات. ٥٠/٤٠% (٨٠%) كانت موجبة لـ RT-PCR. بلغ معدل انتشار الإصابة ١٧,٩٥% (١٥٦/٢٨) في محافظة أسيوط ، ٥,٧٧% (١٥٦/٩) في سوهاج ، ١,٩٢% (١٥٦/٣) في المنيا. كانت السلالات الفرزبان أعلى معدل إصابة ١٣,٤٦% (١٥٦/٢١) من السلالات المحلية ٦,٤١% (١٥٦/١٠) تليها الجاموس بنسبة ٥,٧٧% (١٥٦/٩). وبلغت نسبة الإصابة عند الإناث ١٧,٣١% (١٥٦/٢٧) أما الذكور فكانت ٨,٣٣% (١٥٦/١٣). كانت الفئة العمرية < ٢-٤ سنوات (١٣,٤٥%) أكثر عرضة للإصابة بالفيروس من الفئة العمرية ٦ أشهر إلى سنتين (١٢,٥%). كان معدل الإصابة أعلى في الأشهر الحارة ١٩,٢٣% (١٥٦/٣٠) من الأشهر غير الحارة ٦,٤١% (١٥٦/١٠).