



SERO-PREVALENCE OF VIRAL RESPIRATORY DISEASES IN BUFFALOES AT ASSIUT GOVERNORATE

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ABSTRACT:

Respiratory disorders are major concern for Bovidae. Viruses and bacteria in combination with stress play a key role in triggering acute respiratory infections. It is generally accepted that viruses are the first pathogens to intervene, whereas bacteria act as the second invaders to worsen the ill-animal's condition. The most important viral agents are bovine herpes virus type 1 (BoHV-1), bovine respiratory syncytial virus (BRSV), parainfluenza virus type 3 (BPIV-3), bovine viral diarrhoea virus (BVDV) and bovine adenoviruses (BAdVs). This study was conducted to evaluate the serological status of BoHV-1, BVDV, BRSV, BPIV-3 and BAdV-3 in buffaloes, Assiut governorate, Egypt. The samples were tested by commercial indirect ELISA kits. All samples were negative for BoHV-1, BVDV and BRSV; antibodies were detected against BPIV-3 and BAdV-3 in 0.05 and 10.55% of serum samples, respectively. According to the present study, presence of antibody against BAdV-3 were higher than BPIV-3 in buffaloes at Assiut Governorate, Egypt.

INTRODUCTION:

Water buffaloes in different regions have an economic value that is specific to their own regions. Especially, the unique quality of their milk and dairy products increase their value. Italian Mozzarella cheese, which is famous throughout the world, is made from water buffalo milk. Also, water buffalo farming has the advantages related to their resistance for various environmental conditions and diseases, their ability to benefit feed and to turn poor feed into meat and milk, as well as, their low cost compared with cows (Canbolat, 2012).

Respiratory disorders are major concern for Bovidae. They occur in all countries that practice intensive livestock farming. Viruses and bacteria in combination

with stress play a key role in triggering acute respiratory infections. It is generally accepted that viruses were the first pathogens to intervene, whereas bacteria act as the second invaders to worsen the ill-animal's condition (Valarcher and Hägglund, 2006 and Solis-Calderón et al., 2007). The most important viral agents were bovine herpes virus type 1 (BoHV-1), bovine viral diarrhoea virus (BVDV), bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus type 3 (BPIV-3) and bovine adenoviruses (BAdVs) (Hägglund et al., 2007).

BoHV-1 was a member of the genus Varicellovirus in the subfamily Alpha herpesvirinae, which belongs to the Herpesviridae family (Nandi et al., 2009).

These agents (BoHV-1) might represent risks to livestock and even human population (Teshome et al., 2003). Infectious bovine rhinotracheitis (IBR) is one of the most economically important emerging contagious diseases of cattle and buffalo caused by Bovine herpes virus 1 (BoHV-1) (Saravanajayam et al., 2017).

BVDV belongs to genus Pestivirus within the Flaviviridae family (Radostits et al., 2007). It was previously reported that BVDV causing persistent infection in bovines and other ruminant species (Carman et al., 2005 and Uttenthal et al., 2005). Infection of water buffaloes with BVDVs has been confirmed in several studies by serological and molecular techniques (Craig et al., 2015).

BRSV is a pneumovirus in the family Paramyxoviridae. It is a major cause of respiratory disease in young calves. BRSV genomes consisted of a single stranded, negative-sense RNA containing ten genes encoding eleven proteins. Clinical signs of BRSV vary from none to severe, with most outbreaks occurring during the winter season (Valarcher and Taylor, 2007).

BPIV-3 is a member of respirovirus genus in the family Paramyxoviridae (Adams et al., 2016). BPIV-3 is one of most important viral respiratory pathogens of young and adult cattle associated with BRDC (Dong et al., 2012). BPIV-3 could be present and cause economic losses in both cattle and buffalo population. Little was known about epizootic character of BPIV-3 in buffalo population (Akca et al., 2004).

BAdVs are DNA non-enveloped viruses, members of Adenoviridae family (Shirvani et al., 2012). BAdV-3 is one of the most important causes of respiratory

manifestation in cattle especially newborn calves (Zhu et al., 2011). The occurrence and seroprevalence of BAdV-3 infections were detected in buffalo's in Turkey (Akca et al., 2004).

MATERIAL AND METHODS:

1-Animals:

199 randomly selected Egyptian buffaloes with varying sexes and ages including 173 females classified according to puberty to 36 calves & heifers and 137 adult females classified according to pregnancy, 20 pregnant females and 117 non pregnant females and 26 males classified according to puberty, 11 calves and 15 adult males, ages varying from 15 days to 12 years old. Also vaccinated and non-vaccinated animals by master cattle vaccine were used in this study.

2-Samples:

Blood samples (5 ml) were collected aseptically from jugular vein of each animal using plain vacutainer tubes without anticoagulant and transported in ice-box to the laboratory.

Serum was separated by centrifugation of blood at 3000 r.p.m. for 10 minutes at room temperature; the aliquots were transferred into 1.5 ml sterile micro tube (Eppendorf).

These samples were stored at -20 °C until tested.

The samples were collected from 25 July 2016 to 15 January 2018 from different districts at Assiut Governorate.

3- Serological test, Enzyme linked immunosorbant assay (ELISA) according to (Saravanajayam et al., 2017).

3.1. MULTISCREEN ELISA (Bovine respiratory): (Yousef et al., 2013 and Al-Hammadi and Hemida, 2014)

Serum samples were divided to 40 pools each pool contains 5 serum samples except one pool had 4 serum samples. Testing of the serum's groups by using 3 plates of ELISA kit for serodiagnosis of BoHV-1, BVDV, BRSV, BPIV-3 and BAdV-3 (indirect test for blood serum), used in accordance with the manufacturer's instructions (Bio-X Diagnostics, Belgium).

3.2. Select the highest three groups in results for each virus and make analysis for each sample in these groups by using MULTISCREEN ELISA (Bovine respiratory) to confirm the results.

3.3. MONOSCREEN ELISA (Bovine adenovirus 3): (Sinbat et al., 2016)

Make analysis for each sample in the positive groups for BAdV-3 by using ELISA kit for serodiagnosis of BAdV-3, indirect test for blood sera to differentiate the positive samples from the negative ones and to detect the degree of positivity for each positive sample, used in accordance with the manufacturer's instructions (Bio-X Diagnostics, Belgium).

ELISA plates were coated with monoclonal antibodies and inactivated viruses. Tested sera were diluted at a ratio of 1:100 in the dilution buffer. Samples were added to corresponding wells, incubated at 37 °C for 1 hr. plates then washed three times with the

washing buffer by using graduated automatic multichannel pipettes. The conjugate was diluted 1:50 in 1X dilution buffer and added to each wells. Plates were then lidded, incubated at room temperature for one hour and washed as stated above. Undiluted chromogen was added to each well. Plates were then incubated in dark at room temperature for 10 minutes. The reaction was stopped by adding 50 µl per well of the undiluted stop solution followed by reading Optical Density (OD) in the microwells using a plate reader and a 450 nm filter.

4. Interpretation of test results:

4.1 . Interpretation of MULTISCREEN ELISA test results:

Subtract from each value recorded in 1, 2, 3, 4, 5, the signal of the corresponding negative control well 6 and write down the result. In performing this calculation, allow for any negative values that may exist. Carry out the same operations for the column corresponding to the positive and negative controls.

The test can be validated only if the positive control serum yields a difference in optical density at 10 minutes that is greater for each valence than: BoHV-1 > 0.700, BVDV > 1.000, BRSV > 0.800, BPIV-3 > 0.800 and BAdV-3 > 0.600 and the negative control yields a difference in optical density at 10 minutes that is lower than 0,300.

Divide the signal read for each sample well by the corresponding positive control serum signal and multiply this result by 100 to express it as a percentage.

$$\text{Val (ue)} = \frac{\text{Delta OD Sample} * 100}{\text{Delta OD positive}}$$

Using the following table, determine each serum's degree of positivity.

	0		+		++		+++		++++		+++++
BoHV-1	<=Val	30%	<=Val<	67%	=<Val<	104%	<=Val<	141%	<=Val<	178%	Val <
BVDV	<=Val	20%	<=Val<	40%	=<Val<	60%	<=Val<	80%	<=Val<	100%	Val <
BRSV	<=Val	20%	<=Val<	40%	=<Val<	60%	<=Val<	80%	<=Val<	100%	Val <
BPIV-3	<=Val	20%	<=Val<	40%	=<Val<	60%	<=Val<	80%	<=Val<	100%	Val <
BAdV-3	<=Val	10%	<=Val<	33%	=<Val<	56%	<=Val<	79%	<=Val<	102%	Val <

A sample must be considered positive if it yields a result is greater than or equal to one plus sign (+).

4.2 . Interpretation of MONOSCREEN ELISA test results:

Subtract from each value recorded for the odd columns the signal of the corresponding negative control well and write down the result. In performing this calculation, allow for any negative values that may exist. Carry out the same operations for the column corresponding to the positive control.

The test can be validated only if the positive serum yields a difference in optical density at 10 minutes that is greater than 0.600 and the negative serum yields a difference in optical density that is lower than 0.200.

Divide the signal read for each sample well by corresponding positive control serum signal and multiply this result by 100 to express it as a percentage.

$$\text{Val (ue)} = \frac{\text{Delta OD Sample} * 100}{\text{Delta OD positive}}$$

Using the following table, determine each serum's degree of positive.

0		+		++		+++		++++		+++++
<=Val	10%	<=Val<	33%	=<Val<	56%	<=Val<	79%	<=Val<	102%	Val <

A sample must be considered positive if it yields a result is greater than or equal to one plus sign (+).

RESULTS

A- According to the clinical examination all samples were divided to two main groups. First group contained 99 samples were collected from apparently health animals and second group contained 100 samples were collected from 30 animals with respiratory signs such as high temperature (39-40.5 °C), high pules (65-85), high respiration rate (35-45), cough, congested eyes with lacrimation and nasal discharge, 40 animals with digestive troubles such as off food, tympany and enteritis, 20 animals

with reproductive disorders such as repeat breeding and abortions with high temperature and 10 animals appear other clinical signs, 5 animals of them appear udder affections (suspects mastitis) and another 5 animals appear urinary system affections

B- The laboratory and epidemiological findings of the present work were arranged in tables: 1-8. Also, the results were supported by photos of Multiscreen and Monoscreen ELISA plates (1-3).

Table 1; Results of Multiscreen ELISA kit for serodiagnosis of BoHV-1, BVDV, BRSV, BPIV-3 and BAdV-3 in buffalo's sera samples collected from Assiut governorate.

Serial number	District	No. of samples	Results									
			BoHV-1		BVDV		BRSV		BPIV-3		BAdV-3	
			+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
1	Sidfa	12	-----	12	-----	12	-----	12	-----	12	-----	12
2	Abu Tig	8	-----	8	-----	8	-----	8	-----	8	-----	8
3	El Ghanayem	88	-----	88	-----	88	-----	88	1	87	17	71
4	Dayrout	9	-----	9	-----	9	-----	9	-----	9	-----	9
5	Al Qusiyyah	3	-----	3	-----	3	-----	3	-----	3	-----	3
6	Manfalut	14	-----	14	-----	14	-----	14	-----	14	1	13
7	Assiut	24	-----	24	-----	24	-----	24	-----	24	1	23
8	El Fath	11	-----	11	-----	11	-----	11	-----	11	-----	11
9	Al Badari	6	-----	6	-----	6	-----	6	-----	6	-----	6
10	Abnub	22	-----	22	-----	22	-----	22	-----	22	2	20
11	Sahil Salim	2	-----	2	-----	2	-----	2	-----	2	-----	2
	Total	199	-----	199	-----	199	-----	199	1	198	21	178

Table 2; Relationship between districts and results of Monoscreen ELISA Kit used for serodiagnosis of BAdV-3 in buffalo's sera samples from Assiut governorate

Serial No.	district	No. of samples	Results of BAdV-3		
			-ve	+ve	++ve
1	Sidfa	12	12	-----	-----
2	Abu Tig	8	8	-----	-----
3	El Ghanayem	88	71	12	5
4	Dayrout	9	9	-----	-----
5	Al Qusiyyah	3	3	-----	-----
6	Manfalut	14	13	1	-----
7	Assiut	24	23	1	-----
8	El Fath	11	11	-----	-----
9	Al Badari	6	6	-----	-----
10	Abnub	22	20	2	-----
11	Sahil Salim	2	2	-----	-----
	Total	199	178	16	5

Table 3; Relationship between seasons and results of Monoscreen ELISA Kit used for serodiagnosis of BAdV-3 in buffalo's sera samples from Assiut governorate.

Serial No.	Seasons	No. of Samples	Results of BAdV 3		
			-ve	+ve	++ve
1	Winter	53	48	3	2
2	Spring	64	50	11	3
3	Summer	78	76	2	-----
4	Autumn	4	4	-----	-----
	Total	199	178	16	5

Table 4; Relationship between ages and results of Monoscreen ELISA Kit used for serodiagnosis of BAdV-3 in buffalo's sera samples from Assiut governorate

Serial No.	Ages	No. of Samples	Results of BAdV-3		
			-ve	+ve	++ve
1	0-12 Months	30	30	-----	-----
2	1-3 Years	60	58	2	-----
3	3-5 Years	27	26	1	-----
4	>5-12 Years	82	64	13	5
	Total	199	178	16	5

Table 5; Occurance of BAdV-3 infection in diseased and clinically healthy animals according to Monoscreen ELISA Kit.

Serial No.	Case History	No. of Samples	Results of BAdV-3		
			-ve	+ve	++ve
1	Apparently health	99	81	13	5
2	Diseased animals	100	97	3	-----
	Total	199	178	16	5

Table 6; Relationship between sexes and results of Monoscreen ELISA Kit used for serodiagnosis of BAdV-3 in buffalo's sera samples from Assiut governorate

Serial No.	Sexes	No. of Samples	Results of BAdV-3		
			-ve	+ve	++ve
1	Male	26	26	-----	-----
2	Female	173	152	16	5
	Total	199	178	16	5

Table 7; Relationship between pregnancy and results of Monoscreen ELISA Kit used for serodiagnosis of BAdV-3 in buffalo’s sera samples from Assiut governorate

Serial No.	Pregnancy	No. of Samples	Results of BAdV-3		
			-ve	+ve	+++ve
1	Pregnant	20	19	1	-----
2	Non-pregnant	117	97	15	5
	Total	137	116	16	5

Table 8; Relationship between vaccination and results of Monoscreen ELISA Kit used for serodiagnosis of BAdV-3 in buffalo’s sera samples from Assiut governorate.

Serial No.	Vaccination	No. of Samples	Results of BAdV-3		
			-ve	+ve	+++ve
1	Vaccinated animals	13	12	1	-----
2	Non-vaccinated animals	186	166	15	5
	Total	199	178	16	5

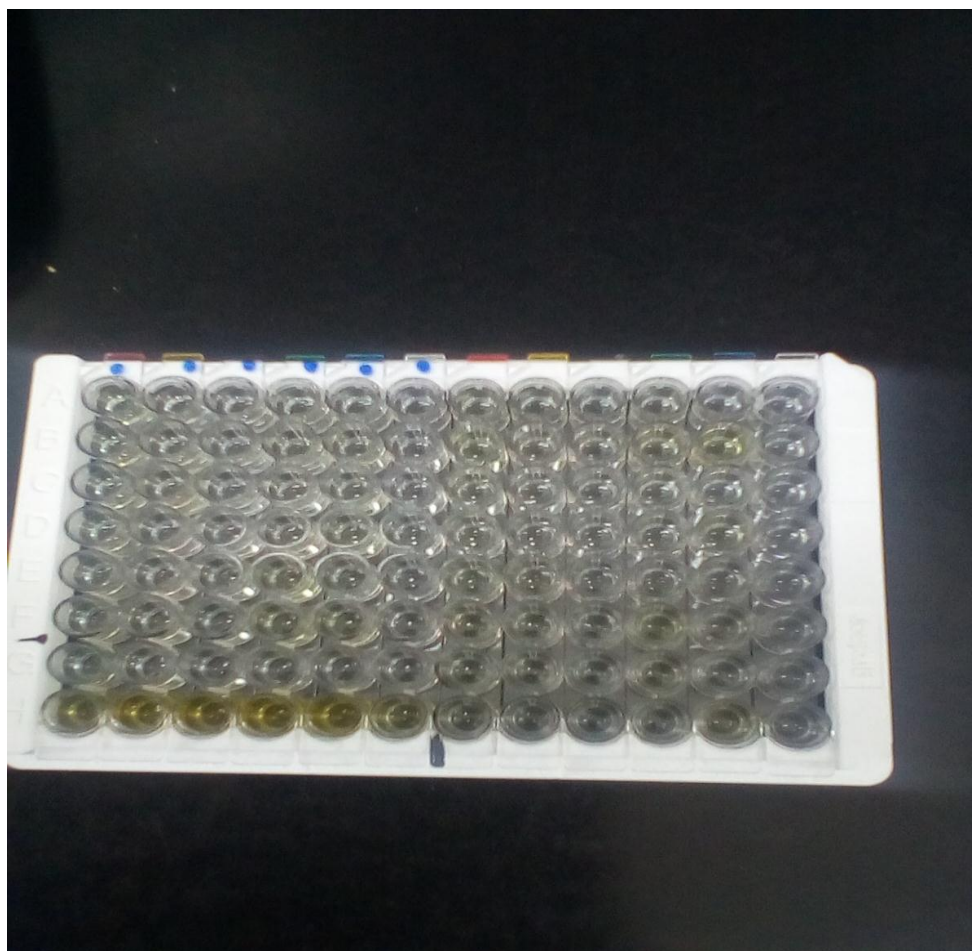


Photo 1; Show the results of plate (1) in MULTISCREEN ELISA (ELISA kit for serodiagnosis of BoHV-1, BVDV, BRSV, BPI3 and Bovine Adenovirus 3 in Buffalo’s serum samples) after dividing of serum samples to 40 groups, each group contains 5 serum samples.

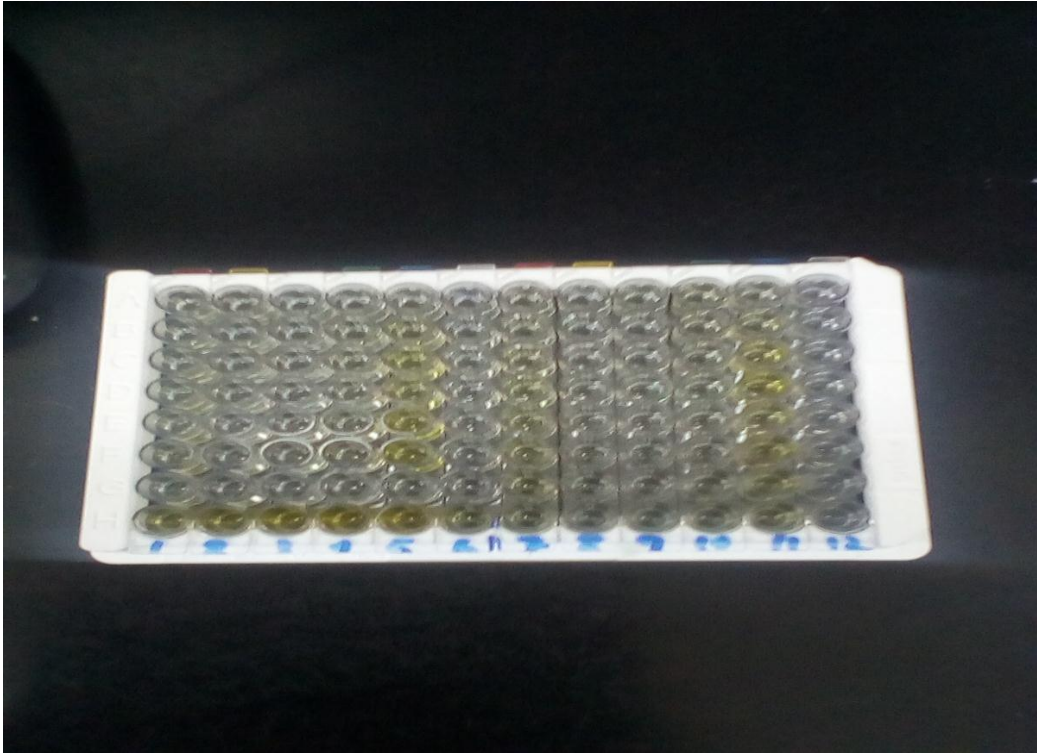


Photo 2; Show the results of plate (2) in MULTISCREEN ELISA (ELISA kit for serodiagnosis of BoHV-1, BVDV, BRSV, BPI3 and Bovine Adenovirus 3 in buffalo's serum samples) after dividing of serum samples to 40 groups, each group contains 5 serum samples.



Photo 3; Show the results of MONOSCREEN ELISA (ELISA kit for serodiagnosis of Bovine Adenovirus 3 in buffalo's serum samples).

DISCUSSION

According to results of Multiscreen and Monoscreen ELISA kits, Table (1) showed that, one positive sample for BPIV-3 1/88 (1.14%) was reported at El Ghanaym and no positive results for BPIV-3 were detected in other districts in this study. The summation of all positive samples for BPIV-3 was 1 sample from totally 199 samples (0.05%). This result was lower than that reported by Akca et al. (2004) who detected that seropositivity rate for BPIV-3 was 11% in buffaloes in Turkey. In this study; also 17 positive samples for BAdV-3 were reported at El Ghanayem 17/88 (19.32%), one positive sample for BAdV-3 was reported at Manfalut 1/14 (7.14%), one positive sample for BAdV-3 was reported at Assiut 1/24 (4.17%) and two positive samples for BAdV-3 were reported at Abnub 2/22 (9.09%). The summations of all positive samples for BAdV-3 were 21 samples from 199 samples (10.55%) these results were lower than that reported by Akca et al. (2004) who detected that seropositivity rates for BAdV-3 were 55% in buffaloes in Turkey. All samples in this study were negative for BoHV-1; this result is similar to that recorded by Anonymous (2000) who reported that Finland was free from IBR and contrast to that reported by Soares et al. (2017) who detected that the occurrence of anti-BoHV-1 antibodies in buffaloes was 56.1% in Pernambuco state - Brazil. All samples were negative for BVDV this result is contrast to that reported by Soares et al. (2017) who detected that the occurrence of anti-BVDV antibodies in buffaloes was 97.9% in Pernambuco state – Brazil and 4.5% of water buffaloes samples were positive for BVDV antibodies in Australia (Evans et al., 2016). These different results of BVDV in different

countries were agreement with Hemmatzadeh et al. (2001) who reported that the prevalence of BVDV infection varies between different countries and even between different provinces within a single country; that may be related to the differences in management, environmental variation, size of herds and existence of Persistent Infection (PI) of animals in these herds. All samples in this study were negative for BRSV, this result was contrast to that reported by Akca et al. (2004) who detected that seropositivity rates for BRSV were 28% in buffaloes in Turkey and Shalaby et al. (2002) who reported that seroprevalence of BRSV infection detected by ELISA was 37.5 % in buffaloes in Giza, Egypt. Finally these total results were similar to that reported by Akca et al. (2004) who detected that the presence of antibody against BAdV-3 was higher than the other viruses and all these low percent may be due to decrease in direct contact with source of infection according to that detected by Soares et al. (2017) who mentioned that the high number of positive animal properties may be related to the absence of biosecurity measures and introduction of infected animals.

According to results of Monoscreen ELISA kit, for serodiagnosis of BAdV-3 Table (2) showed that, out of 21 positive samples, 16 samples (8.04%) with one degree of positivity, 12 samples of them were reported at El Ghanayem 12/88 (13.63), 1 sample was reported at Manfalut 1/14 (7.14%), 1 sample was reported at Assiut 1/24 (4.16%) and 2 samples were reported at Abnub 2/22 (9.09). out of 21 positive samples, 5 samples (2.51%) with two degree of positivity, all these 5 samples recorded at El Ghanayem (5.68%). These results agreed with Hemmatzadeh et al. (2001) who mentioned that the prevalence of

infection differs between different countries and even between different provinces within a single country; this may be related to the differences in management, environmental variation and size of these herds.

According to results of Monoscreen ELISA kit, Table (3) showed that, 3 samples with one degree of positivity (5.66%) and two samples with two degrees of positivity (3.77%) were reported in Winter, 11 samples with one degree of positivity (17.18%) and 3 samples with two degrees of positivity (4.68%) were reported in Spring, 2 samples with one degree of positivity (2.56%) were reported in Summer and no positive samples were reported in Autumn. These results were in contrast to that reported by NADIS (2014) about outbreaks of respiratory disease in UK which occurred within one month of housing in the autumn/early winter and agreed with Henrickson (2003) about high percentage of infected animals with BPIV-3 in cold weather (December-March). According to results of Monoscreen ELISA kits, Table (4) showed that, no positive samples were reported at the age from 0-12 months, 2 samples with one degree of positivity 2/60 (3.33%) were reported at the age from 1-3 years, 1 sample with one degree of positivity 1/27 (3.7%) was reported at the age from 3-5 years and 13 samples with one degree of positivity 13/82 (15.85%) and 5 samples with two degrees of positivity 5/82 (6.11%) were reported at the age >5 years, these results agreed with Also Shirvani et al. (2012) who detected that the infection in adults was usually asymptomatic and recurrent. When signs were present such as respiratory distress, nasal and ocular discharges in addition to hyperthermia may be complicated by secondary bacterial infections.

According to results of Monoscreen ELISA kit, Table (5) showed that, 13 samples with one degree of positivity (13.13%) and 5 samples with two degrees of positivity (5.05%) were reported in apparently health animals. 3 samples with one degree of positivity (3%) were reported in diseased animals (one from animal with high temperature, another one from animal suffering from mastitis and last one from animal suffering from off food for 2 weeks). The result of presence of BAdV-3 in apparently health animals was agreement with Gras et al. (2017) who mentioned that presence of BAdV-3 in cattle related to subclinical infections, in the absence of clinical cases and high levels of their immunity in Brazil.

According to results of Monoscreen ELISA kit, Table (6) showed that, no positive samples were reported in males and 16 samples with one degree of positivity (9.24%) and 5 samples with two degrees of positivity (2.89%) were reported in females animals. This significant difference in this study between males and females may be agreed with Hemmatzadeh et al. (2001) who found significant difference between males and females in infection. The higher susceptibility of female buffaloes may be due to the fact that females are presented for slaughter at older age than males after the end of their breeding and milking period, while, males are fattened for a short period indoors and are fed mainly on dry ration until their slaughter which reduce the chance of contracting the infection (Dorny, 2002).

According to results of Monoscreen ELISA kit, Table (7) showed that, 1 sample with one degree of positivity (5%) was reported in pregnant animals, 15 samples with one degree of positivity (12.82%) and 5

samples with two degrees of positivity (4.27%) were reported in non-pregnant animals. These results may be due to reproductive disorders caused by BAdV-3 (Kahrs, 1981 and Gras et al., 2017).

According to results of Monoscreen ELISA kit, Table (8) showed that, 1 sample with one degree of positivity (7.69%) was reported in vaccinated animals by master cattle vaccine (vaccine against BoHV-1, BVDV, BRSV and BPI3), 15 samples with one degree of positivity (8.06%) and 5 samples with two degrees of positivity (2.68%) were reported in non-vaccinated animals by master cattle vaccine. This high seropositivity in non-vaccinated animals by master cattle vaccine may be due to that co-infection in BAdV-3 was prevalent according to Gras et al. (2017) and absence of biosecurity measures and introduction of infected animals (Soares et al., 2017).

CONCLUSION

In this study all samples were negative for BoHV-1, BVDV and BRSV; antibodies were detected against BPIV-3 and BAdV-3 in 0.05 and 10.55% in serum samples. Antibodies against BAdV-3 were higher than BPIV-3 in buffaloes at Assiut governorate. Also, it could be concluded that the various well known environmental factors plays an important role in spreading of these viral agents.

On the other side, and on the benefit of animal welfare, we surely need to pay attention for the continuous control of the environmental factors. From the obtained results further studies are needed to investigate the prevalence and role of different respiratory viruses especially BAdV-3 in Egyptian buffalo species.

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المسح السيرولوجي للأمراض التنفسية الفيروسيه في الجاموس في محافظة أسيوط

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الملخص العربي :

الاضطرابات التنفسية تمثل خطراً كبيراً علي صحة الجاموس حيث تلعب الفيروسات والبكتيريا تحت تأثير عوامل الضغط والإجهاد دوراً أساسياً في حدوث العدوى التنفسية الحادة. من المقبول بشكل عام أن الفيروسات هي أول مسببات الأمراض ، في حين أن البكتيريا تعمل كمسبب ثانوي للعدوي يؤدي إلي سوء حالة الحيوان. ومن أهم المسببات الفيروسية فيروس الإسهال الفيروسي البقري (BVDV) ، وفيروس الهربس البقري من النوع الأول (BoHV-1) ، والفيروس المخلوي التنفسي البقري (BRSV) ، وفيروس نوع الإنفلونزا البقري ٣ (BPIV-3) ، والفيروس الغدي البقري (BAdVs). أجريت هذه الدراسة لتقييم الحالة المصلية لـ BoHV-1 ، BVDV ، BRSV ، BPIV-3 و BAdV-3 في الجاموس، في محافظة أسيوط ، مصر. لهذا الغرض، تم اختبار العينات من خلال مجموعات الإليزا غير المباشرة التجارية. وكانت جميع العينات سلبية بالنسبة لـ BoHV-1 و BVDV و BRSV ؛ تم الكشف عن الأجسام المضادة ضد BPIV-3 و BAdV-3 في ٠.٠٥ و ١٠.٥٥ ٪ من عينات المصل ، على التوالي. وفقا للدراسة الحالية ، كان وجود الأجسام المضادة ضد BAdV-3 أعلى من BPIV-3 في الجاموس في محافظة أسيوط ، مصر.