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PREVALENCE OF BLUETONGUE VIRUS ANTIBODIESAND ASSOCIATED RISK FACTORS AMONGSHEEP AND GOAT IN EGYPT

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ABSTRACT

Bluetongue is an infectious and non-contagious arthropod borne viral disease of Domestic and wild ruminants namely sheep, goat, cattle, camels, llamas, deer and antelopes. Bluetongue virus is a segmented double-stranded RNA virus and is transmitted by midges belonging to Culicoides spp. This study is to evaluate the current situation of BTV in Egypt particularly no obligatory vaccination, by estimating the prevalence of Bluetongue antibodies in sheep, goats and identify the potential risk factors associated with BTV infection among sheep and goats during 2016-2017. Blood samples were collected from native breed of sheep (237) and goats (370) from twelve provinces, for detection of BTV antibodies and antigen by using ELISA and detection of BTV RNA by using RT-PCR. It was found that the number of positive sera of BTV antibodies were detected in (86/370) 23.2% of sheep and (26/237) 10.9 % of goats indicating higher susceptibility of sheep than goats. The highest prevalence of bluetongue group specific antibodies was detected in Aswan, Elwadi elgadid, Giza and Marsamatrouh governorates while Portsaed governorate was negative. The examined blood (seropositive samples) were negative for the presence of BTV antigen and BTV RNA.This study indicated that costal and border provinces of Egypt, the intensity of the insect vector, seasons of the year, the age and sex of animal are Influential risk factors for BTV infection in sheep and goats. The results showed that ELISA and real-time PCR assay is rapid, sensitive, and equally specific in the diagnosis of BTV.

KEYWORDS Bluetongue virus**,** sheep, goats**,** ELISA, PCR**.**

INTRODUCTION

Bluetongue (BT) is an infectious and non-contagious arthropod borne viral disease of Domestic and wild ruminants namely sheep, goat, cattle, camels, llamas, deer and antelopes. BT primarily affects sheep and deer, but subclinical disease occurs in cattle, goat, and wild Ruminants. Bluetongue is usually considered to be a disease of improved breeds of sheep, particularly the fine-wool and mutton breeds.[1]

The disease is endemic in the tropics and subtropics; it was confined mainly to tropical and temperate areas of America, Africa and parts of Asia.[2] In Africa, several serotypes have been identified in Kenya, and South Africa.[3]

Bluetongue virus (BTV) is a segmented double-stranded RNA virus belonging to the genus Orbivirus of family Reoviridae and is transmitted by midges belonging to Culicoides spp. The spread of Bluetongue virus thus coincides with the distribution of the vector species. There are over 1500 Culicoides species, most significant of which are C. imicola, C. obsoletus, C. variipennis, C. pulicaris, C. sonorensis, C. nubeculosus, C. dewulfi and C. chiopterus. However, only a small number of these have been shown to act as biological vectors.[4]

Twenty-six serotypes of BTV have been reported worldwide.[5] A total of 24 BTV serotypes have been recognized worldwide.[6] Toggenburg orbivirus (TOV) is proposed to be a 25 serotype[7] and complete genome characterization of a 26 BTV serotype from Kuwait.[8] Due to the large number of circulating BTV serotypes, it is generally impossible to predict the serotype for specific season or area.[9] BTV genome is segmented double stranded RNA (10 segments) coded for 7 structural proteins (1-7) and 3 nonstructural proteins (NSI-3). BTV is icosahedral viruses with three layers of capsid, the outer capsid layer comprised of two proteins, VP2 and VP5, where VP2 is the major neutralizing protein and determinant of serotype specificity.[10] The middle layer core particle is made up of two proteins VP7and VP3, the innermost layer made up of three minor proteins, VP1, VP4, VP6.[11]

The manifestation of blue tongue disease range from an in apparent to fatal outcome depending on the serotype of the virus and the species, breed, nutritional, immune status and age of the infected animal ; older animals are generally more susceptible.[12] But can cause fatal disease in a proportion of infected sheep, deer and wild ruminants. Infection of cattle with BTV does not usually result in clinical signs, with the exception of BTV 8 infection in Europe. Cattle are particularly significant in the epidemiology of the disease due to the prolonged viremia in the absence of clinical disease.[13] Clinical signs usually detected in fine wool breeds of sheep and the white tailed deer included fever, facial edema, hemorrhage and ulceration on the oral mucosa and coronitis.[14] Clinical signs of BT are mainly attributable to vascular permeability and include fever, hyperemia and congestion, facial oedema and hemorrhages, and erosion of the mucous membranes. However, in mild cases of the disease, a transitory hyperemia and slight ocular and nasal discharge may be observed.[13] Although infections are observed in domestic and wild ruminants, the clinical disease and mortality are observed only in sheep,. The difference in disease patterns in different parts of the country could be due to varied climatic conditions, sheep population density.[15]

In Egypt, the disease is generally mild in indigenous sheep since the classical symptoms of the disease are not commonly seen, so the detection of the infected animals becomes difficult on the basis of clinical profiles.[16]

The worldwide economic losses due to bluetongue have not been expressed in exact number but the estimate is 3 billion US$ a year. The loses are both direct (death, abortions, weight loss or reduced milk yield and meat efficiency) and, what is important, indirect as result of export restriction for live animals, their semen and products such as foetal bovine serum. the costs of preventive and control measures should also be taken into account. In cases of a wider spread of bluetongue, these measures could have a serious impact on consumer market; therefore, blue tongue is consider a potential biological weapon.[17]

The major control measures include restriction of animal movement, vector control applying insecticides, slaughter of infected animals and vaccination.[6]

MATERIALS AND METHODS

Animals

A total of 370 sheep and 237 goats of both sexes of native breeds, were apparently healthy and unvaccinated belong to twelve provinces in Egypt: Lower Egypt (Marsa Matrouh, Alexandria, Behera, Garbia,Menofia, Dakhlia, Ismalia, Port Said) and upper Egypt (Giza, Sohag, El wadi el gadid, Aswan) representing different geographical regions of the country. All animals samples were screened to BTV antigen in their blood by using sELISA kits and antibodies in their serum by using cELISA kits and detection of BTV RNA by using RT-PCR in blood and culicoides.

Blood samples

were collected from the jugular vein in sterile vacutainers and divided into 2 parts, the first part were mixed with anticoagulant for virus identification. The second part, were allowed to clot without anticoagulant and sera were separated by centrifugation of the blood at 3000 rpm for 10 minutes at room temperature and transferred into 1.5 ml microtube and all serum kept frozen at -20 until used for detection of BTV antibodies.

Mosquitoes samples

Two hundred mosquitoes samples were collected from Aswan during September 2017 with suitable daily temperature; wind speed and relative humidity. Mosquitoes were morphologically identified under a microscope according to the method described by[18] Mosquitoes were submitted for RNA extraction.

Celisa for detection of BTV antibodies

cELISA showed a high sensitivity for examination of immunological response of sheep, goats, cattle and camels to BTV. The test is probably the most widely used and validated method for serogroup specific.

The cELISA was performed on animals' sera using a commercial ELISA kit, the test kit {INGEZIM BTV COMPAC 2.0, 12.BTV. K3}. The kits detect antibodies against all BTV serotypes (non-serotype specific) and is based on the blocking enzymatic immunoassay. They were conducted in accordance with the procedures defined by the manufacturer's instruction[19] The plates coated with VP7 protein of BTV. After adding the sample to well, if it contains specific antibodies against the virus, they will be bind to the antigen absorbed on plate while if the sample does not contain specific antibodies they will not bind to the antigen.

Selisa for detection of BTV Antigen

The kit has been designed for detection and quantification of BTV`s VP7 protein. The test kit {INGEZIM BTV DAS.12. BTV.K2} were supplied by INGEZIM company, Spain. The kit is based on Double antibody sandwich enzymatic immunoassay for detection of BTV, S VP7 protein. The plates are coated with a monoclonal antibodies (MAB) specific of BTV, s VP7 proteins. After adding the sample to the well, if it contains virus, they will bind to MAB absorbed to the plate. It was carried out according to manufacture' s instruction.[20]

Extraction of RNA

Using Patho Gene-Spin™ DNA/RNA Extraction kit (Cat. No.17154). It is specifically designed to isolate high quality nucleic acids from a variety of pathogen such as Virus, bacterium, etc. The sample can be either fresh or frozen plasma, blood, serum or other cell free body fluids. The extraction kit uses advanced silica–gel

membrane technology for rapid and effective purification of DNA or RNA without organic extraction or ethanol precipitation. Chaotropic salt in lysis buffer inactivates immediately DNase /RNase to ensure isolation of intact DNA /RNA.

Rapid and efficient purification of high quality nucleic acid using spin column based centrifugation with no sample cross –contamination. The buffering condition are finally adjusted to provide optimum binding of the DNA /RNA to the column. it was carried out according to manufacturer's instruction.[21]

Real-time Polymerase Chain Reaction (RT-PCR)

BTV real time RT-PCR kit is used for detection of BTV in blood, tissue, sperm or culicoides samples by using the real time PCR system. RT- PCR reaction was done using commercially purchased ready to use Bluetongue virus Real Time RT-PCR Kit supplied by(Shanghai ZJ Bio-Tech Co. Ltd -China) (Cat. No.AR-0117-02). Using apparatus "Stratagene mx 3005 p ". BTV real time RT-PCR kit contains a specific ready to use system for detection of BTV. According to the manufacturer's instructions.[22]

RESULTS AND DISCUSSION

This study was carried out to throw a light on the situation of Bluetongue disease in Egyptian sheep and goat population through evaluating its seroprevalence, that would help decision makers and stakeholders. -BTV antibodies were detected in (86/370) 23.2 % of sheep and (26/237) 10.9% of goats. (Table -1) indicate higher susceptibility of sheep than goats. Sheep is the most susceptible domestic ruminants to BTV and serve as an indicator host for the virus[23] but in Egypt, BT is generally mild in sheep since the classical symptoms of the disease are not commonly seen so it is often difficult to diagnose.[16]

Table 1: Detection of BTV antibodies in the serum samples of sheep and goats.

Province	Total No. of sheep and goat sample	Sheep sample			Goat sample		
		Total No.	Positive	%Positive	Total No.	Positive	%Positive
Marsa Matrouh	100	65	14	21.5	35	5	14.2
Alexandria	59	31	4	12.9	28	2	7.1
Behera	59	30	5	16.6	29	2	6.8
Garbia	46	30	5	16.6	16	3	18.7
Menofia	50	35	6	17.1	15	1	6.6
Dakhlia	41	27	6	22.2	14	2	14.2
Ismalia	28	16	4	25	12	1	8.3
Port saed	43	28	----	0	15	----	0
Giza	41	18	6	33.3	13	2	15.3
Sohag	30	17	3	17.6	13	1	7.6
El wadi el gadid	60	33	16	48.4	27	3	11.1
Aswan	60	40	17	42.5	20	4	20
Total	617	370	86	23.2	237	26	10.9

No= Number %= percent

BTV antibodies were detected in sheep sera in El wadi El gadid 16/33 (48.4), followed by Aswan 17/ 40 (42.5 %), Giza 6/18 (33.3 %), Ismalia 4/16 (25 %), Dakhlia 6/27 (22.2 %), Marsa matrouh 14/65 (21.5%), Sohag 3/17 (17.6 %), Menofia 6/35 (17.1 %), Behera and Garbia 5/30 (16.6 %), Alexandria 4/31 (12. 9%) and Port saed 0%.

Some province showed 0% as in Port saed in their samples, Like this governorate, more epidemiological data is required and further examinations of large number of animals are recommended for complete conclusion of this area.

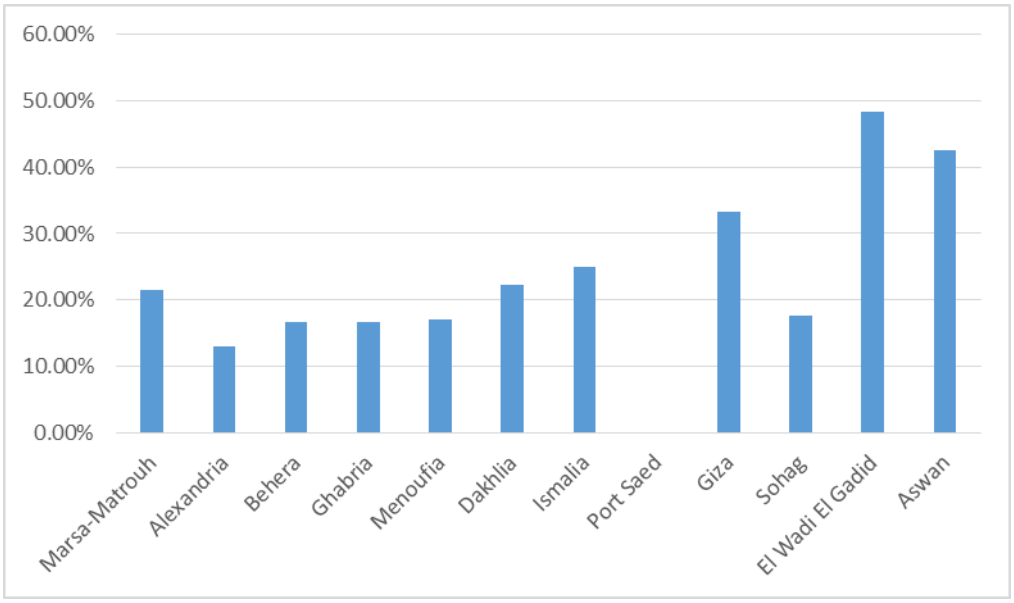

Fig. 1: Positive percentage BTV antibodies in sheep detected by cELISA in different Egyptian provinces.

BTV antibodies were detected in goats sera in (4/ 20) 20% in Aswan, followed by (3/ 16) 18.7 % in Garbia, then (2/13) 15.3 % in Giza, then (2/14) 14.2 % in Dakhlia and (5/ 35) 14.2 % in Marsamatrouh, then (3/ 27) 11.1 % in El wadi el gadid, then (1/12) 8.3 % in Ismalia, then (1/13) 7.6 % in Sohag, (2/ 28)7.1 % in Alexandria, then (2/29) 6.8 % in Behera, then (1/15) 6.6 % in Menofia, then 0% in Port saed. These different results among geographical areas in the country came supported by the results, previously obtained by.[24]

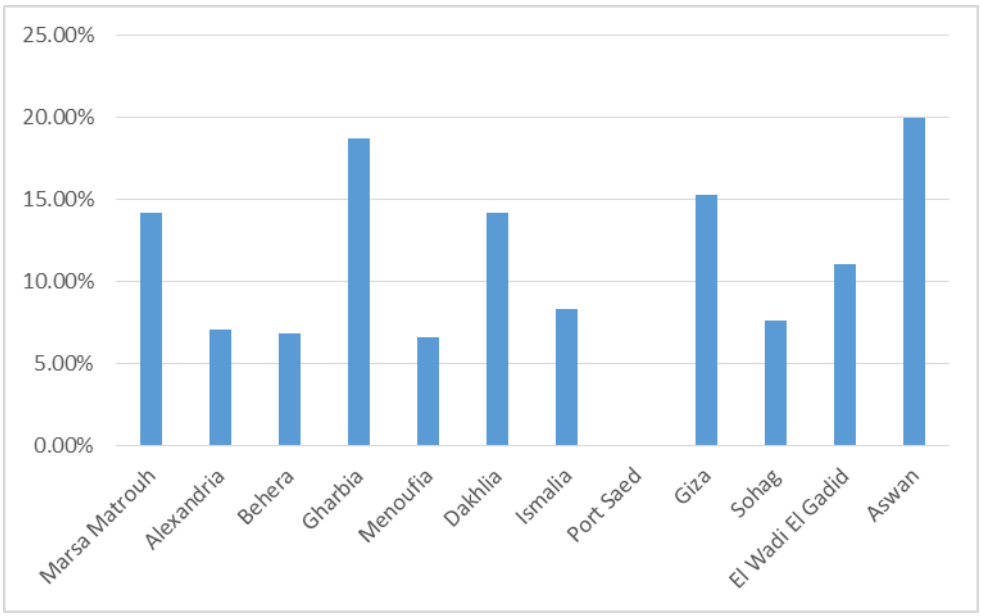

Fig. 2: Positive percentage BTV antibodies in goat detected by cELISA in different Egyptian provinces.

For studying the prevalence of BTV antibodies in coastal and border provinces of Egypt, geographical location considered a risk factor, might be attributed to climatic factors that favor the maintenance and recirculation of the BTV in its vertebrate and non-vertebrate hosts implicated in transmission of BTV. In addition unrestricted movement of animal population between these provinces and the importation of European and African countries where the enzootic nature of BTV.

The environmental changes in the different provinces, high prevalence of mosquitoes and wide area of shallow stagnant water and irrigation projects in delta governorates might be also incorporated in the epidemiology of the virus provide suitable climatic condition for survival of the adults and larvae of Culicoides vectors which considered as a big risk factor. In addition, animal source (contact with other herds) is another factor that affects BTV seropositivity results. It is probable that introducing infected animals into the herd would allow the local midge population to become

infected, with a subsequent increase in BTV infection rates.

Using competitive ELISA kit (are recommended as prescribed tests for international trade in the OIE Manual of Standards for Diagnostic Tests and Vaccines).[25] cELISA showed a high sensitivity for examination of immunological response of sheep, goats, cattle and camels to BTV. The test is probably the most widely used and validated method for serogroup specific.[19] It is used as a spot test and provide a qualitative measurement of positivity. This method is easy to implement, rapid and reliable and it is suited to analyzing a large number of samples.[25]

Positive serum samples means that BTV –specific antibodies that are still circulating in the examined animals with undetectable signs is due to subclinical infection where Bluetongue is endemic disease, but the virus may be of low virulence as well as the local breeds, resistant species have an enough immunity level.

Fig.3 demonstrated that examined sera all over the four seasons of the year 2016-2017 indicates the highest percentage of prevalence of BTV antibodies were detected by 22.1 % during autumn, 19.5% during summer, 12.4 % during spring and 8 % during winter. Our result agreed with those of[26] who concluded that autumn in Egypt characterized by high humidity and moderate temperature, both of which favors the rapid breeding and multiplication of the vector where culicoides were found to build up a peak in late summer and early Autumn. The central role of the insect in BTV epidemiology ensures that the prevalence of disease is governed by ecological factors such as high rainfall, temperature, high humidity and high organic matter content of the soil, which favor insect survival therefore in many parts of the world the disease, has a seasonal occurrence[27] Which considered as a risk factor.

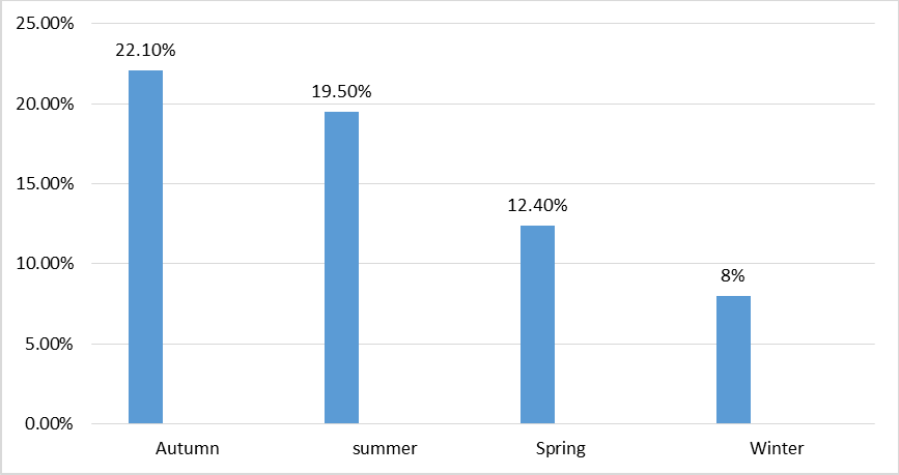

Fig. 3: Positive percentage BTV antibodies detected by cELISA in different seasons.

Our result reported higher risks of older animals for BTV infections, (Table. 2) showing the rate was 12.8% in sheep and 7.6% in goat (less than one year old), 24% in sheep and 11.2% in goat, (1-2 years old), 30.6% in sheep and12.8% in goats, (2-4 years old).

Table 2: Prevalence of BTV antibodies in sheep and goats in relation to different age as detected by cELISA.

Age	Tested sheep sera			Tested goat sera		
	No.	No. of +ve	%	No.	No. of +ve	%
Less than 1 year	70	9	12.8	52	4	7.6
≥ 1-2 year	225	54	24	107	12	11.2
≥ 2-4 year	75	23	30.6	78	10	12.8
Total	370	86	23.2	237	26	10.9

No. = Number +ve = positive % = percent

Regarding the effect of age on the susceptibility of animals to BTV, The present findings were parallel to the results obtained by[28]) who reported significant increase in seroprevalence of BTV antibodies by age as maternal antibodies wane, followed by an increase with age and following exposure and infection as The rates of infection were increased with age of animals (sheep and goats). At this age, the animals are usually released into the pasture for grazing, where they are likely to be exposed to infected vectors and subsequent BTV infection. Young animals are usually kept indoors and are well taken care of by the animal owners from contracting infectious diseases, particularly the insect and tick-borne infections.[29]

Female animals of all tested species (sheep and goats) were more liable to infection with a higher percentage (24.7 % and 12.9 %) respectively, than male (20.3 % and 8.4 %) respectively (Table. 3), The female hormones may play an important role in such phenomenon.

Table 3: Prevalence of BTV antibodies in sheep and goats in relation to sex as detected by cELISA.

Species		Male			female	
	No.	No. of +ve	%	No.	No. of +ve	%
Sheep(370)	128	26	20.3	242	60	24.7
Goat (237)	106	9	8.4	131	17	12.9
Total(607)	234	35	14.9	373	77	20.6

No=Number +ve = positive %= percent

A total of 112 whole blood samples from native breed of sheep and goats from seropositive animals to BTV antibodies were examined for BTV antigen by sELISA and detection of BTV RNA by using RT-PCR. All the examined blood samples were negative for the presence of BTV antigen or BTV RNA.

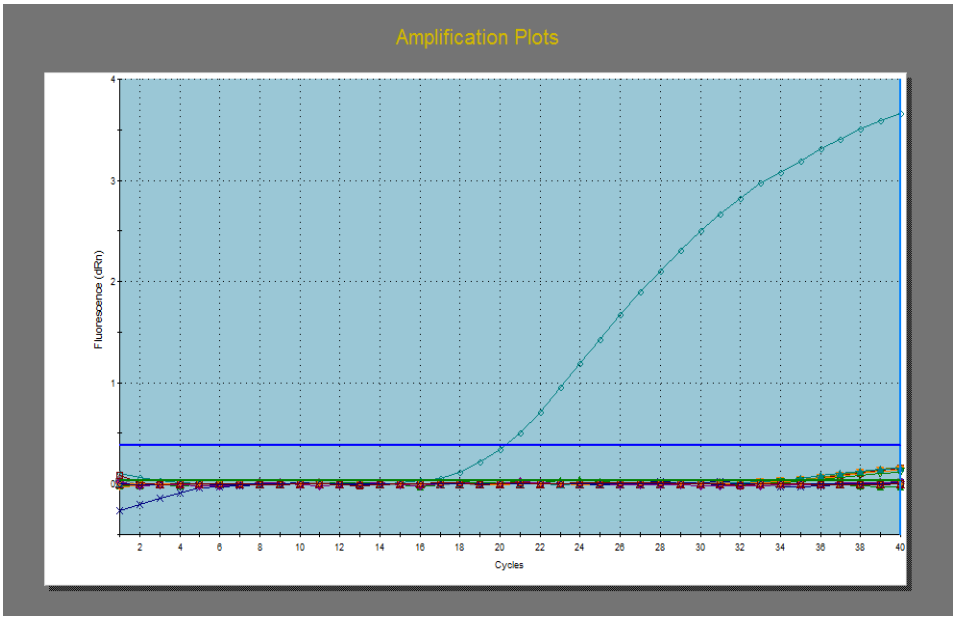

Fig. 4: Negative result of examined blood from different animals to RT-PCR.

A total of 200 culicoides and 100 larvae as a pooled group (5 pools) trapped from Aswan province were examined and screened for BTV RNA using RT-PCR. All samples showed negative results compare with the positive control (included in kits).Our results do not agree with the result mentioned by[30] who detected positive mosquitoes pool to BTV in Ismailia. This result may be due to different climatic condition according to season of the year, organic matter in the soil, which favor insect survival and presence of virulent strain of BTV in that time in this province which come over the immune status of animals and large vector population without control of insects.

The positive seroprevalence result is due to old infection where Bluetongue disease is an endemic disease in Egypt, the result refers that the virus may be of low virulence as well as the local breeds have an enough immunity level and no obligatory vaccination program for our livestock, No official records in Egypt till now for BTV outbreak and no announce from General Organization for Veterinary Services or OIE for any outbreak in Egypt and the negative result of samples from blood for antigen detection by using sELISA and negative result of samples from blood and mosquitoses for detection of BTV RNA by using RT-PCR proved that.

CONCLUSION

From these results, we can conclude that ELISA Test may be sufficient for monitoring the disease condition in endemic area and with the use of sensitive technique RT-PCR for the detection of virus RNA.

This study reflected high seroprevalence of bluetongue infection in sheep than goats. The present study indicated that costal and border provinces of Egypt, where the legal and illegal importation of animals to Egypt, the intensity of the insect vector, seasons of the year, the age and sex of animal are Influential risk factors for BTV infection in sheep and goats. The results showed that ELISA and real-time PCR assay is rapid, sensitive, and equally specific in the diagnosis of BTV. Surveillance for BTV infection should be extended to include other susceptible ruminants.

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