PREVALENCE OF ANTIBODIES TO BLUETONGUE VIRUS IN SMALL AND LARGE RUMINANTS AT DIFFERENT PROVINCES OF EGYPT

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A B S T R A C T

A total of 618 serum samples of sheep, lambs (< 3 months), goats and cattle at five provinces in Egypt were screened for qualitative analysis of the BTV antibodies using a commercial competitive ELISA (cELISA) kit. The results showed an overall percentage of BTV positive sheep, lambs (< 3 months), goats and cattle serum samples were 23.5%, 15%, 10.9% and 10.7% respectively. The results based upon the serum samples showing optical density values more than 50 percent of the mean of negative control were taken as positive for presence of BTV antibodies. A highest percentage of seropositivity was found in Gharbia (20.5%), Alexandria (17.9%), Kafer-El sheikh (16.8%), Mounofia (9%) and Bahera (9.6%) provinces. From examined sera all over the four seasons of the years 2011 and 2012 indicated the prevalence of BTV antibodies by 21.1% during autumn, 15.6% during winter, 11.1% during summer and 10.2% during spring. In conclusion, BTV antibodies were widely prevalent in sheep, lambs (< 3 months), goats and cattle in these provinces of Egypt and cELISA were found to be sensitive and effective for screening of BTV group specific antibodies.

Key Words: Bluetongue, cELISA, Small and large ruminants, Seroprevalence

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1. INTRODUCTION

Bluetongue virus (BTV) is the causative agent of bluetongue disease in sheep and cattle, an insect transmitted disease of ruminants [2]. BTV belongs to Orbivirus genus of family Reoviridae it has double stranded segmented RNA genome having ten discrete segments The seven of these segments encode structural protein (VP1 to VP7) and the remaining three encode for non structural proteins (NS1, NS2, NS3 and NS3A), NS3 and NS3A are encoded by tenth segment [3].

There are 24 distinct BTV serotypes and recently Toggenburg orbivirus (TOV) is proposed to be a 25th serotype [4] and, complete genome characterization of a 26th BTV serotype from Kuwait [5]. It is a notifiable disease of the World Organization for Animal Health (Office of international epizooties: OIE) due to its economic impact [6].

Virus is transmitted within its vertebrate hosts via bites of culicoides species. The severity of infection depends on various factors, such as species, breed, age, nutritional and immune status of animals and environmental stresses as well as the virulence of BTV strain involved. Although clear differences in virulence of BTV isolates are known, the virulence determinants are still poorly defined. [7].

Bluetongue is generally mild in indigenous sheep of Egypt since the classical symptoms of the disease are not commonly seen [8] except for abortion syndrome [9] so the detection of infected animals

becomes difficult on the basis of clinical profiles or isolation of the virus. However, presence of BTV antibodies in a herd indicates the presence of viral infection [10]

In order to overcome serological cross-reactions among orbivirus serogroups, which can hinder the accurate diagnosis of bluetongue virus (BTV) infection of livestock, a blocking ELISA (B-ELISA) incorporating a monoclonal antibody (20E9B7G2) with specificity for the BTV serogroup was developed [11]

The competitive enzyme linked immunosorbent assay (cELISA) is a superior test for serologic diagnosis of BTV infection of ruminants because it requires significantly less time to run, and provide objective results [12]. It is also approved by OIE for testing BTV infection in international trade of livestock. In the present study, we have assayed seroprevalence in sheep, lambs (< 3 months), goats and cattle at five provinces in Egypt using commercially available competitive ELISA kit.

2. MATERIALS AND METHODS

2.1. Serum samples:

A total of 618 field sera were randomly collected from sheep, lambs (< 3 months), goats and cattle from 5 provinces in Egypt all over the four seasons of the years 2011 and 2012 (table1). No record of bluetongue activity or any serologic evidence of BTV infection has ever been reported in these Egyptian provinces. These sera were stored at -20ºC in small aliquots till used for the detection of BTV- group specific antibodies using competitive ELISA (cELISA).

2.2. BTV group specific antigen:

it was supplied with bluetongue antibody test kit by Veterinary Diagnostic Technology, (VDT) Inc. USA was used in the cELISA test.

2.3. *Competitive Enzyme-linked Immunosorbent Assay (cELISA):*

The Bluetongue Competitive ELISA Kit: (BDSL; Biological Diagnostic Supplies Ltd., Surrey, UK) were used. The test is based on the detection of antibodies specific to the highly conserved segment 7 (VP7) of BTV. It is therefore designed to detect infection by any type of BTV and/or vaccination by any vaccine presenting the VP7 antigen. The test was carried out as described in the protocol supplied by the manufacturers, and the percentage supplied by the manufacturers, and the percentage inhibition (PI) values were calculated as described by Afshar et.al. [**1**]. Samples with PIs equal to or greater than 50% were considered to be positive, and those with PIs of less than 50% were taken as negative.

Table 1. Number of serum samples of sheep, lambs (< 3 months), goats and cattle in relation to Egyptian provinces.

province	sheep	goats	Lambs	cattle	Total
Gharbia	85	33	21	61	200
Kafer-El sheikh	71	29	17	55	172
Alexandria	40	18	8	40	106
Mounofia	35	11	10	32	88
Bahera	29	10	4	9	52
Total	260	101	60	197	618

3. RESULTS

A serological survey involving 618 serum samples (260 sheep, 60 lambs,101goats and 197 cattle) from different Egyptian provinces was carried out employing cELISA for qualitative analysis of the BT antibodies. The serum samples showing optical density values > 50 % of the mean of negative control were taken as positive for presence of BTV antibodies.

As shown in Table (2) the number of total positive samples were 102 samples out of 618 samples (16.5%). From examined sera for each species 23.5% (61/260) sheep,

15% (9/60) lambs, 10.9% (11/101) goats and 10.7% (21/197) cattle were positive for BTV –antibodies.

From examined sera at 5 Egyptian provinces 20.5% (41/200) at Gharbia, 16.8% (29/172) at Kafer-El sheikh, 17.9% (19/106) at Alexandria, 9% (8/88) at Mounofia and 9.6% (5/52) at Bahera were positive revealed circulation of BTV – group specific antibodies as shown in Table (3).

A critical observation of (Table-4) indicates that from a total positive sera examined at 5 Egyptian provinces a high percentage BT antibodies were detected in sheep (56.1% at Gharbia, 58.8% at Kafer-El sheikh, 52.6% at Alexandria, 87.5% at Mounofia and 80% at Bahera), then cattle (21.9% at Gharbia, 24.1% at Kafer-El sheikh, 21.1% at Alexandria, 12.5% at Mounofia), then goats (12.2% at Gharbia, 6.9% at Kafer-El sheikh, 15.8% at Alexandria, 20% at Bahera) and finally lambs (9.7% at Gharbia, 10.3% at Kafer-El sheikh, 10.5% at Alexandria). Also both lambs and goats sera were free from BTV –group specific antibodies at Mounofia province while lambs and cattle sera were free from BTV – group specific antibodies at Bahera.

As shown in Table (5) From examined sera all over the four seasons of the year 2011 and 2012 indicates prevalence of BTV antibodies by 21.1% (51/242) during autumn, 15.6% (34/218) during winter, 11.1% (11/99) during summer and 10.2% (6/59) during spring.

Table (2): Prevalence of BTV antibodies in sheep, lambs (< 3 months), goats and cattle as determined by cELISA

Animal Species	No of serum samples	No. of Positive	Percent Positive
Adult sheep	260	61	23.5
Lambs (< 3month)	60	9	15
goats	101	11	10.9
cattle	197	21	10.7
total	618	102	16.5

Table (3): Prevalence of BT Antibodies in different Egyptian provinces as determined by cELISA

province	Total Examined sera	Total positive sera	Percent Positive
Gharbia	200	41	20.5
Kafer-El sheikh	172	29	16.8
Alexandria	106	19	17.9
Mounofia	88	8	9.0
Bahera	52	5	9.6

Table (4): Prevalence of BT Antibodies in sheep, lambs (< 3 months), goats and cattle at different Egyptian provinces as determined by cELISA.

province	Total	No. of Positive				Percent Positive			
	positive sera	sheep	Lambs	goats	cattle	sheep	Lambs	goats	cattle
Gharbia	41	23	4	5	9	56.1	9.7	12.2	21.9
Kafer-El sheikh	29	17	3	2	7	58.6	10.3	6.9	24.1
Alexandria	19	10	2	3	4	52.6	10.5	15.8	21.1
Mounofia	8	7	0	0	1	87.5	0	0	12.5
Bahera	5	4	0	1	0	80	0	20	0

Table (5): Seasonal distribution of BTV antibodies all over the year 2011 and 2012.

Season	No of serum samples	No. of Positive	Percent Positive
Summer	99	11	11.1
Autumn	242	51	21.1
Winter	218	34	15.6
Spring	59	6	10.2

4. DISCUSSION

Bluetongue affects both domestic and wild ruminants, and its origin is probably African ruminants. It was first identified in South Africain Merino sheep in the late 18th century [13]. Various techniques have been used to detect antibodies against BTV. Only AGID and competitive-ELISA are recommended as prescribed tests for international trade in the OIE Manual of Standards for Diagnostic Tests and Vaccines [6].

In Egypt, there is no vaccination program is running or used, so positive serum samples means that BTV-specific antibodies which are still circulating in the tested animals without any detectable signs is due to subclinical infection . Sheep is the most susceptible of the domestic ruminants to BTV and serve as an indicator host for the virus [7].

This study estimates the prevalence and distribution of antibodies to BTV in different domesticated animals in 5 provinces of Egypt. Our results revealed low seroprevalence (16.5%) of BTV infection which was comparable to that has been described amongst ruminants in regions of Saudi Arabia (47.3% seroprevalence) [14], Turkey (29.5%) [15], India (up to 45.7%) [16] and Pakistan (48.8%) [17].

Due to the large number of circulating BTV serotypes, it is generally impossible to predict the serotype for a specific season or area. Furthermore, several serotypes tend to circulate simultaneously [18].

The highest proportion of seropositives in different livestock in Gharbia, , Alexandria, Kafer-El sheikh, Mounofia and Bahera Province could be attributed to climatic factors that favour the maintenance and recirculation of the BTV in its vertebrate and non-vertebrate hosts in addition to unrestricted movement of animal population between these provinces and the importation from Asia and the Horn of Africa (Ethiopia, Somalia, Eritrea and Djibouti) where the enzootic nature of BTV in large regions of the African continent is reported [19] and also there were possibility of windborne carriage of infected Culicoides from distant endemic areas [20].

Our findings not only detected BTV antibodies in serum samples of adult sheep but also in serum samples of lambs up to three months and at considerable level. This results denotes that these lambs were of infected dams .this agreed with results obtained by Livingston and Hardy [21] who found that antibodies passively transferred in the colostrums of BT-immune dams persisted in lamb sera for as long as three months.

The BTV antibodies were detected in the four seasons of the year although the disease in sheep has a seasonal variation in incidence [22]. unfortunately, Autumn in Egypt characterized by high humidity and moderate temperature (Temperature is about 29oc and relative humidity is about 72%-Meteorological office, 1988), 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 [9] .therefore the highest percent of BTV antibody were detected in autumn.

5. REFERENCES

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انتشار الاجسام المضادة لفيروس اللسان الازرق فى المجترات الصغيرة والكبيرة بمختلف محافظات مصر.

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1 قسم الفيرولوجي كلية الطب البيطري بمشتهر جامعة بنها –القليوبية –مصر 2 معهد بحوث صحة الحيوان -الدقي-الجيزة –مصر

الملخص العربي

تم عمل مسح سيرولوجى لإجمالي 618 عينة مصلية من الاغنام والحملان الصغيرة (اقل من 3 شهور) والماعز والابقار فى خمس محافظات مصرية للتحليل الكيفي للأجسام المضادة لفيروس اللسان الازرق باستخدام اختبار الإليزا التنافسي التجاري. اوضحت النتائج ان النسبة الايجابية الكلية لفيروس اللسان الازرق فى كل من العينات المصلية للأغنام والحملان الصغيرة (اقل من 3 شهور) والماعز والابقار كانت 23.5%، 15%، 10.9%و10.7% على التوالي. اعتمدت النتائج على اعتبار العينات المصلية التي تظهر قيم كثافة بصرية أكثر ب50% من متوسط العينات السلبية الضابطة عينات ايجابية تحوى اجسام مضادة لفيروس اللسان الازرق. كما وجدت اعلى نسب مصلية ايجابية للفيروس فى محافظات كلا من الغربية (20.5%)، الاسكندرية (17.9%)، كفر الشيخ (16.8%)، المنوفية (9%) والبحيرة(9.6%). وقد اشارت العينات المصلية التي فحصت خلال الاربع فصول لأعوام 2011 و2012 انتشار الاجسام المضادة لفيروس اللسان الازرق ب 21.1% اثناء الخريف و15.6% اثناء الشتاء و11.1% اثناء الصيف و10.2% اثناء الربيع. واستنتاجا لذلك فان الاجسام المضادة لفيروس اللسان الازرق كانت واسعة الانتشار فى كلا من الاغنام والحملان الصغيرة (اقل من 3 شهور) والماعز والابقار فى تلك المحافظات المصرية وان اختبار الإليزا التنافسي كان ذو حساسية وفاعلية فى المسح السيرولوجى الاجسام المضادة للجمع الخاص بفيروس اللسان الازرق.

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