COMPETITIVE ELISA TEST FOR DIAGNOSIS OF BLUETONGUE IN EGYPT

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SUMMARY

Bluetongue (BT) is an infectious non contagious, insect born viral disease of sheep and other ruminants. The clinical diagnosis of bluetongue is often difficult due to the marked variations in the severity of the symptoms and lesions. The primary problem caused by BTV in enzootic areas was the decrease in lamb and calf production. competitive ELISA test kit was used to detect bluetongue group specific antibodies in 184 serum samples collected from apparently healthy native breeds of sheep, goats and cattle of both sexes during the four seasons of the year. The tested animals were adult except 49 lambs which were up to three months. 54.5% of the tested animals were positive for BTV group specific antibodies. The highest percent of seropositive samples were in autumn (68.3%) while in summer; winter and spring the percent were 48%, 66.7% and 44.1% respectively. BTV antibody values were classified into four group, the first found to be high in 20.7% of sheep, 12.5% of goats and 57.1% of cattle; the second found to be moderate in 33.1% of sheep, 37.5% of goats and 42.9% of cattle and the third found to be low in 7.8% of sheep and 50% of goats. 63.3% of the tested lambs were positive for BTV antibodies. As no vaccination programme is adopted in Egypt since a considerable time and competitive ELISA (C-ELISA) is a prescribed test for international trade due to its sensitivity, specificity and accuracy, seropositive samples reflect subclinical infection of adult tested animals and the positive resultes of testd lambs resulted from the passively transferred of BTV antibodies from their mother's colostrum which persisted in lamb sera for long period as three months.

INTRODUCTION

Bluetongue (BT) is an infectious non contagious insect born viral disease of sheep and other domestic and wild ruminants *(Anthony and Werner, 1992).*

Bluetongue virus (BTV) infection is endemic through out much of the world *(Gibbs and Greiner, 1994)* where its global distribution is currently between latitudes of 50°N and 35°S *(OIE, 2005).* Numerous countries in the

tropics and subtropics have blue tongue virus unknowingly circulating subclinically in cattle and other ruminants *(Gibbs and Greiner, 1988).*

BTV is a member of the Orbivirus genus, one of nine genera classified in the family Reoviridae *(OIE, 2004).* Midges of the genus cullicoides act as biological vectors of blue tongue virus. Of the approximately 1400 species of cullicoides world-wide, less than 20 are considered actual or possible vectors *(Mellor et al., 2000)*

Clinical signs of disease in domestic and wild ruminants range from subclinical in the vast majority of cases to an acute febrile response characterized by panting, fever, salivation and frothing at the mouth, oedema (of lips, nose, face, submandibulum, eyelids and sometimes ears), congestion (of mouth, nose, nasal cavity, conjunctiva, skin and coronary band). The oedema of lips and nose can give the sheep a "monkey –face" appearance.The congestion of nose and nasal cavity produces a "sore muzzle" effect with ulcerations in and around the mouth. A "blue" tongue may develop if the blood is impaired. Lameness due to coronary band congestion, may occur early in the disease and lameness or torticollis, as a result of skeletal muscle damage, may occur later. *(Anthony and Werner, 1992 and OIE, 1998).*

The primary problem caused by BTV in enzootic areas was the decrease in lamb and calf production due to early fetal deaths and congenital defects *(Jones et al., 1981 and Anthony and werner, 1992).*

There are many serologic tests for the diagnosis of BT antibodies such as modified direct complement fixation test (MDCFT) which has lost favor because of the variability of test results among laboratories, anticomplementary reaction and the short lived antibodies. Immuno fluorescent and indirect ELISA tests are complicated by cross reactions between BT serogroup viruses and the growing list of related Orbiviruses. Virus neutralization (VN) and haemagglutination inhibition (HI) are serotype specific antibody tests and more useful in research than for diagnostic purposes *(Anthony and Werner, 1992).* Agar gel immunodiffusion (AGID) test was the standard method for the detection of group specific anti-BTV antibodies in animal sera for many years although the test lacks sensitivity and the BTV antigen used in the test may cross react with other Orbiviruses, such as epizootic hemorrhagic disease (EHD) viruses *(Della-Porta et al., 1985).* Monoclonal antibody – based competitive ELISA (cELISA) has solved this problem *(OIE, 2004).* The cELISA is highly specific and sensitive in detecting bluetongue group specific antibodies and should be routinely used for detection of antibodies against BTV in small ruminants *(Smirti and Shringi, 2005)*

The aim of the present study is the detection of BTV antibodies in sheep, goats and cattle sera using competitive ELISA commercial kit to know the situation of the disease in Egypt.

MATERIAL AND METHODS

1) Animals

A total number of 184 apparently healthly animals were used, out of them 145 sheep, 21 goats and 18 cattle of both sexes. All animals were of native breeds and adult except 49 lambs which were up to 3 months. they belongs to El-Behera, Alexandria and Matrouh governorates.

2) Samples

Blood samples of the tested animals were collected from March, 2005 to February, 2006. The separated sera were stored properly until used.

3) Serological detection for BTV antibodies

The collected sera were tested for the presence of antibodies for group specific BTV antibodies by competitive ELISA commercial kit (VMRD, Inc. USA).

Test description

Competitive ELISA detects BTV antibodies in ruminant sera. serum Sample of BTV antibody inhibits binding of horse radish peroxidase (HRP) - labeled BTV specific monoclonal antibody to BT viral antigen coated on the plastic wells. Binding of the HRP- labeled monoclonal antibody conjugate is detected by the addition of enzyme substrate and quantified by subsequent colour product development. Strong colour development indicates little or no blockage of HRP-labeled monoclonal antibody binding and therefore the absence of BTV antibody in sera sample. Weak color development due to inhibition of the monoclonal antibody binding to the antigen on the solid phase indicates the presence of BTV antibodies in sample sera.

Test validation

The mean optical density of the negative controls must be greater than 0.300 and less than 2.00. The mean optical density of the positive controls must be less than or equal to 50% of the mean optical density of the negative controls.

Interpreting the results

Test sera are positive if they produce an optical density less than 50% of the mean of the negative controls. Test sera that produce an optical density greater than or equal to 50% of the mean of the negative controls are negative.

RESULTS

The present study summarizes the results obtained with the BTV antibody detection by C-ELISA test kit as follows:

I) One hundred serum samples (54.3%) out of 184 tested sera were positive for BTV-antibodies which were 85 (58.6%) out of 145 sheep sera, 8 (38.1%) out of 21 goats sera and 7 (38.9%) out of 18 cattle sera (Table 1 and Fig. 1).

II) Twenty four serum samples (48.0%) out of 50 sera collected in Summer, 41 serum samples (68.3%) out of 60 sere collected in Autumn, 20 serum samples (66.7%) out of 30 sera collected in winter

and 15 serum samples (14.3%) out of 31 sera collected in Spring were positive for BTV antibodies (Table 2 and Fig.2).

III) BTV antibody values were found to be high in 30 (2.07%) sheep, I (12.5%) goat and 4 (57.1%) cattle, moderate in 48 (33.1%) sheep, 3(37.5%) goats and 3(42.9%) cattle and low in 7(7.8%) sheep and 4 (50.0%) goats (Table 3 and Fig.3)..

IV) Thurty one lambs (up to three months) serum samples (63.3%) out of 49 and 54 adult sheep serum samples (56.3%) out of 96 were positive for BTV antibodies (Table 4 and Fig.4).

DISCUSSION

The BTV has been shown by serology to be present in regions where the culicoides vector is present *(OIE, 2002)*.

Current procedures to determine the serotype of antibodies in sera (for specific diagnosis) are cumbersome because they require determination of the capacity of test sera and infectivity of panels of known virus serotypes in time-consuming neutralization test. AGID (a prescribed test for international trade) test to detect anti-BTV antibodies is simple to perform and the antigen used easy to generate but the test lacks specificity and the subjectivity exercised in reading the results have encouraged the development of ELISA – based procedures for the specific detection of antibodies *(OIE, 2004)*. C-ELISA which is also a prescribed test for international trade was developed to measure BTV – specific antibody without detecting cross-reacting antibody to other Orbiviruses (*Afshar et al., 1989 and Reddington et al., 1991*). The specificity is the result of using BT serogroup – reactive monoclonal antibody which bind to the amino- terminal region of the major core protein *VP7 (OIE, 2004)*. C-ELISA antibodies appear earlier than AGID antibodies *(Koumbati et al., 1999)*.

VMRD, s C-ELISA detects antibody to BTV in ruminant sera. It detects all 24 known serotypes of BTV and does not detect antibody to serotypes 1 or 2 EHD virus. It saves in technician time since sample dilution is unnecessary and this agreed with results obtained by *Sorensen and Lei (1986)* who found that screening of serum samples undiluted reduce the time for completion of the test. Furthermore, incubation time in this kit is 40 minutes (another cause for saving technician time). Undulation of serum samples may increase the sensitivity of the C-ELISA for samples with low titers. The automation of the test and the application of target timing allow the test results to be interpreted objectively, with a built- in quality assurance system *(Afshar et al., 1989)*. There is no vaccination program is running or used in Egypt since a considerable time, 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 *(Anthony and Werner, 1992)* In Egypt, BT is generally mild in indigenous sheep since the classical symptoms of the disease are not commonly seen so

it is often difficult to diagnose *(Ayoub and Singh, 1970)*. Cattle and goats can be also infected but usually with mild or no clinical signs *(Mellor, 1996)*. These previously obtained data explain the subclinical infections demonstrated serologically in sheep, goats and cattle in this study.

Viraemia is essential for transmission of BTV by culicoides which when become infected it remains infected for life and can harbor up to million BTV particles. Also viraemia is cell associated *(Anthony and Werner, 1992)* and virus particles appear to be sequestered in invaginations of the erythrocyte memberane, allowing prolonged viraemia in the presence of neuteralized antibodies and could account for prolonged survival of infective particles during convalescence *(Bowne and Ritchie, 1970 and OIE, 1998)*. These factors have great significance in areas with a temperate climate, where over wintering of the virus is possible only in the presence of animal hosts with long viraemia bridging the insect free season *(Koumbati et al., 1999)*. This agreed with our results where BTV antibodies were detected in the four seasons although the disease in sheep has a seasonal variation in incidence *(Howell, 1963)*. Unfortunately, Autumn in Egypt characterized by high humidity and moderate temperature (Temperature is about 29°C and relative humidity is about 72% - Meteorological office, 1988), both of which favours the rapid breeding and multiplication of the vector , where culicoides were found to build up a peak in late Summer and early Autumn *(Iman, 1990&Walker and Davies, 1971)*. Therefore the highest percent of BTV antibody were detected in autumn.

Cattle have been regarded as being much more important in the epidemiology of BT than sheep and goats due to firstly, cattle are considered to be more attractive to the vector, secondly, they rarely exhibit clinical signs of the disease and thirdly, they are presumed to support a viraemia of considerably longer duration than sheep and goats. Therefore in enzootic areas cattle may be a reservoir of BTV and has up to 100 days viraemia *(MacLachlan et al., 1991)* enabling the virus to "bridge" vector free periods *(Koumbati et al., 1999)*. Due to the previous factors, it was essential to examine cattle for having BTV antibody which was of considerable level due to infected cattle develops a viraemia of high titer *(Anthony and Werner, 1992)*. Also it was important to examine goats and our results agreed with *Mellor (1996)* who mentioned that goats could be infected but with no clinical signs and it disagree with *Spreull (1905)* who stated that goats are resistant to BTV infection under natural condition.

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 result denotes that these lambs were of infected dams. This agreed with results obtained by *Livingston and Hardy (1964)* who found that antibodies passively transferred in the colostrums of BT – immune dams persisted in lamb sera for as long as three months.

In conclusion, the present study revealed that there was exposure to BTV infection among the indigenous breeds of sheep, goats and cattle in Alexandria, Behera and Matrouh governorates during 2004-2005. VMRD, s C-ELISA provides a reliable, rapid, simple and accurate method for detecting

group-specific antibodies against BTV in serum of ruminants and could be used for routine monitoring for the presence of BTV antibodies to keep track on the status of BTV infection among sheep, goats and cattle in Egypt. We should not ignore the possibility of occurrence of some clinical cases as the virus is circulating and detailed investigations have to be done repeatedly if not continuously to know the situation in Egypt and to determine the most prevalent and incriminated virus serotypes.

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Table (1): Results of competitive ELISA test for detection of BTV antibodies in serum samples of sheep, goats and cattle.

Species of tested animals	No. of serum samples	Positive	% of positive	Negative	% of negative
Sheep	145	85	58.6	60	41.4
Goats	21	8	38.1	13	61.9
Cattle	18	7	38.9	11	61.1

Table (2): Seasonal distribution of BTV antibodies among sheep, goats and cattle.

Season	No, of serum samples collected	No. of positive samples	% of positive serum samples
Summer	50	24	48.0
Autumn	60	41	68.3
Winter	30	20	66.7
Spring	34	15	44.1

Table (3): BTV antibody values detected by competitive ELISA in sheep, goats and cattle sera samples.

Species of animals	High		Moderate		Low	
	Number	%	Number	%	Number	%
Sheep	30/145	20.7	48/145	33.1	7/145	7.8
Goats	1/8	12.5	3/8	37.5	4/8	50.0
Cattle	4/7	57.1	3/7	42.9	0/8	0

Table (4): Number and percent of positive serum samples for BTV antibodies in lambs up to three months as well as adult sheep.

Age	Number of serum samples	Positive sera	% of positive	Negative	% of negative
Up to three months lambs	49	31	63.3	18	36.7
Adult sheep	96	54	56.3	42	43.8

Fig. (1): Percent of positive sheep, goats and cattle sera for BTV antibodies detected by C-ELISA.

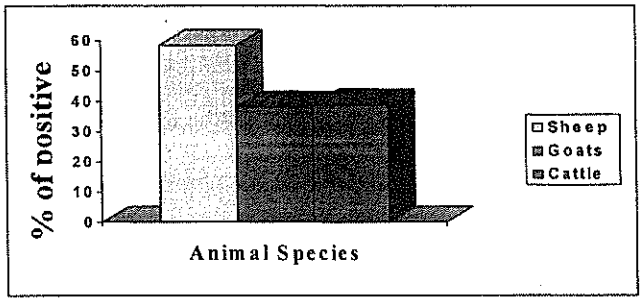

Fig. (2): Seasonality of BTV antibodies detected.

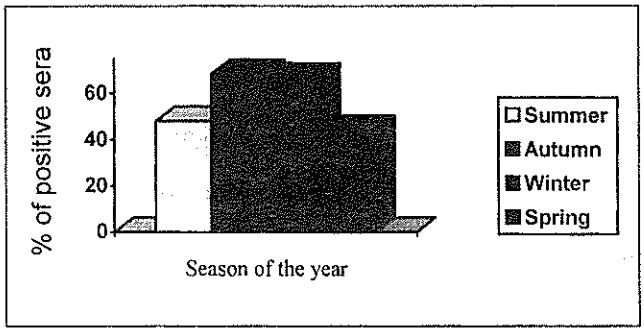

Fig. (3): BTV antibody values in sheep, goats and cattle sera.

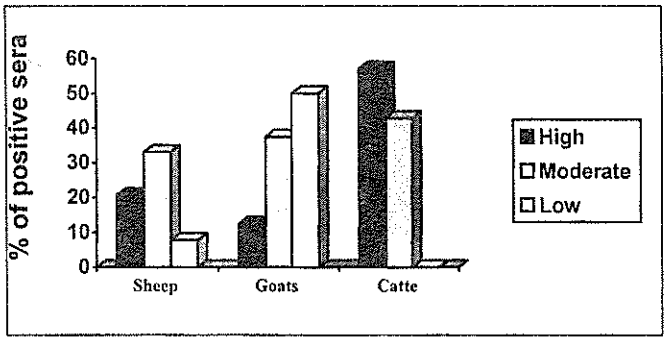

Fig. (4): Percent of positive sera for BTV antibodies in lambs up to three months and adult sheep.