Competitive ELISA test for detection of bluetongue virus antibodies in cattle, sheep and goats

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Summary
Competitive enzyme linked immunosorbent assay kit was used to detect bluetongue virus group specific antibodies in 179 serum samples collected from apparently healthy adult native breeds of sheep, goats and cattle of both sexes from Aswan, Menia, Alexandria and Matrouh Governorates. Out of 179 tested animals 80 (50.3%) were positive for bluetongue virus group specific antibodies where 68 bovine serum samples were positive with 51.1%, 16 Ovine serum samples were positive with 51.6% and 6 caprine serum samples were positive with 40%. As no vaccination program is adopted in Egypt and C-ELISA is a prescribed test for international trade due to its sensitivity and accuracy, seropositive samples reflect subclinical infection of the tested animals. In conclusion C-ELISA provides rapid, simple and accurate method for screening of group-specific antibodies against bluetongue virus infection among cattle, sheep and goats for routine monitoring to know the status of bluetongue infection in Egypt. Also C-ELISA is recommended for examination of imported ruminants to Egypt.

Keywords: Bluetongue, C-ELISA, Antibodies.

Introduction
Bluetongue (BT) is an arthropod-transmitted virus disease of domestic and wild ruminant species caused by bluetongue virus (BTV) (Mc Vey et al., 2013). It is listed under category "A" of OIE and consequently restrictions depend on movement of ruminants from BT endemic regions to BT free zones. So it is of major importance to international trade (Alexander et al., 1996).

It related to a member of the Orbivirus genus, one of nine genera classified in the family Reoviridae (OIE, 2004). Medges of the genus culicoides act as biological vectors of BTV (Mellor et al., 2000). BT is endemic throughout much of the world (Gibbs and Greinder, 1994).

Definitive diagnosis of BTV infection relies on laboratory techniques for isolation and Identification of BTV antigens, viral nucleic acid and antibodies. Since BT infection is often subclinical in domestic and wild ruminant (Afshar, 1994), 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 indicated the presence of viral infection (Jain et al., 1992).

BTV has been detected serologically in regions where culicoides vectors are present (OIE, 2002). Serological tests used to detect BTV antibodies are group specific as agar gel precipitation test (AGPT) (Jochim and Chow, 1969), complement fixation test (CFT), indirect enzyme linked immunosorbent assay (IELISA) (Afshar et al., 1987 and Afshar, 1994) and serotype specific as haemagglutination inhibition (HI) test and serum neutralization test (SNT) (Appleton and Letchworth, 1983).

AGPT was the standard method for 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 (C-ELISA) has solved this problem (OIE, 2004). C-ELISA is highly specific and sensitive in detecting BT group specific antibodies and should be routinely used for detection of antibodies against BTV in ruminants (Smirti and Shringl, 2005).

ELISA may be an alternative for large scale sero-diagnosis of BTV infection (Charan and Gautam, 1984) than AGPT which lacks sensitivity and specificity due to cross reacting antibodies to other Orbivirus specially EHD which may cause false positive reaction (Della-Porta et al., 1985).

Current procedures to determine the serotype of antibodies in sera are cumbersome because they require determination of the capacity of tested sera and infectivity of panels of known virus serotypes (OIE, 2004).

In Egypt, the disease is generally mild in indigenous sheep because the classical symptoms are not seen (Ayoub and Singh, 1970). except for abortion syndrome (Iman Bastawecy 1990), 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 may indicates the the presence of viral infection (Jain, et al., 1992).

The aim of the present study is the detection of BTV antibodies in sera of apparently healthy sheep, goats and cattle by using competitive ELISA commercial kit.

Materials and Methods

Samples:
179 Blood samples were collected from February to May, 2016 from apparently healthy animals. They belong to Aswan, Menia, Alexandria and Matrouh Governorates. (31 sheep, 15 goats and 133 cattle ) of both sexes of adult native breeds (Table 1).

Table 1. Serum samples collected from different Governorates.

<table>
<thead>
<tr>
<th>Gov.</th>
<th>Species</th>
<th>Cattle</th>
<th>Sheep</th>
<th>Goats</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aswan</td>
<td></td>
<td>25</td>
<td>5</td>
<td>5</td>
<td>35</td>
</tr>
<tr>
<td>Menia</td>
<td></td>
<td>55</td>
<td>6</td>
<td>3</td>
<td>64</td>
</tr>
<tr>
<td>Alexandria</td>
<td></td>
<td>31</td>
<td>9</td>
<td>-</td>
<td>40</td>
</tr>
<tr>
<td>Matrouh</td>
<td></td>
<td>22</td>
<td>11</td>
<td>7</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>133</td>
<td>31</td>
<td>15</td>
<td>179</td>
</tr>
</tbody>
</table>

ELISA for detection of BT.
The ELISA kit was used for detection of group specific BTV antibodies in serum samples, commercial Kit (Ingezim, Spain).
The kit has been designed to detect antibodies specific for BTV in sheep, goats and cattle being able to detect a very low titers of antibodies in sera, and plasma samples of infected animals. The commercial Ingezim BTV kit is based on the blocking immunoenzymatic assay where the solid phase is plates coated with monoclonal VP7 protein of BTV

Test procedure:
All reagents must be allowed to come to room temperature before use.

Addition of samples:
Add 50µl of diluent to each well. Add 50µl of each serum sample to be assayed.
For controls add 50µl diluents and Ve+ serum on We recommend to assay the samples by duplicate
Seal the plate and incubate over night (18-24 hours) at room temperature or alternatively 2.5 hours at 37ºC.
Wash 6 times following the described procedure.
Add 100µl of conjugate ready to use to each well. Seal the plate and incubate for 30 min at 37ºC.
Wash 6 times following the described proce-
Add 100μl of substrate to each well. Keep the plate for 10 min at room temperature. Add 100 μl of stop solution to each well. Read the OD of each well at 450nm just after 5 min after the addiction of stop solution.

**Reading and result interpretation**

In case that the samples had been run in duplicate, it has to be considered the mean of both OD values. In the same way, the mean of the values obtained in the two well of positive and the two well of negative control has to be made.

**Blocking % calculation**

\[
\text{Blocking % of sample} = \frac{\text{Sample OD} \times 100}{\text{Negative control OD}}
\]

**Results interpretation**

Samples will be considered Positive (there are antibodies specific for BTV), when the OD at 450 nm was equal or lower than the positive cut off (60% of negative control). Samples will be considered Negative (there are no antibodies specific for BTV in the sample) when the OD value at 450 nm was equal or higher than the negative cut off (65% of negative control). Samples with percentages of binding between both cut off have to be considered Doubtful. In these cases a new samples of the animal is recommended to be analyzed in two weeks.

**Results**

Result of detection of BTV by C-ELISA the results obtained with the BTV antibody detection by the C-ELISA (Table 2) as follows:

- Out of 133 bovine sera, 68 (51.1%) were positive for BTV-antibodies.
- Out of 31 ovine sera, 16 (51.6%) were positive for BTV-antibodies.
- Out of 15 caprine sera, 6 (40%) were positive for BTV-antibodies.

**Table (2). Results of C-ELISA for detection of BTV antibodies in sera of the tested cattle, sheep and goats.**

<table>
<thead>
<tr>
<th>Species of tested animals</th>
<th>No. of serum samples</th>
<th>Positive</th>
<th>% of positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>133</td>
<td>68</td>
<td>51.1</td>
</tr>
<tr>
<td>Sheep</td>
<td>31</td>
<td>16</td>
<td>51.6</td>
</tr>
<tr>
<td>Goats</td>
<td>15</td>
<td>6</td>
<td>40</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>179</strong></td>
<td><strong>90</strong></td>
<td><strong>50.3</strong></td>
</tr>
</tbody>
</table>

The result obtained with PTV antibody in different governorates were out of 35 sera sample 12 (34%) were positive in Aswan. out of 64 sera sample 22 (48.4%) in Menia. out of 40 sera sample 22 (55%) in Alexanderia. Out of 40 sera sample 25 (62.5%) in Matrouh.

**Table (3). Results of C-ELISA for detection of BTV antibodies in different Governorates.**

<table>
<thead>
<tr>
<th>Gov.</th>
<th>Total Exam.</th>
<th>Total Pos.</th>
<th>% of positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aswan</td>
<td>35</td>
<td>12</td>
<td>34%</td>
</tr>
<tr>
<td>Menia</td>
<td>64</td>
<td>31</td>
<td>48.4%</td>
</tr>
<tr>
<td>Alexanderia</td>
<td>40</td>
<td>22</td>
<td>55</td>
</tr>
<tr>
<td>Matrouh</td>
<td>40</td>
<td>25</td>
<td>62.5%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>179</strong></td>
<td><strong>90</strong></td>
<td><strong>50.3%</strong></td>
</tr>
</tbody>
</table>

Table (4) figured that in Aswan gov. out of 25 Bovine Sera sample 10 (40%) were positive, out of 5 Ovine Sera sample 1 (20%) were positive, out of 5 Caprine Sera sample 1 (20%) were positive.
sample 17 (54%) were positive, out of 9 Ovine Sera sample 5 (55%) were positive, Caprine Sera sample was negative.

in Matrouh gov. out of 22 Bovine Sera sample 13 (59%) were positive, out of 11 Ovine Sera sample 8 (72%) were positive, out of 15 Caprine Sera sample 6 (57%) were positive.

From the above mentioned that, out of 133 Bovine Sera sample were examined for BTV antibodies 68 (51%) were positive. out of 31 Ovine Sera sample 16 (32%) were positive. out of 15 Caprine Sera sample 6 (40%) were positive.

Table (4). Results of C-ELISA for detection of BTV antibodies in different species.

<table>
<thead>
<tr>
<th>Gov.</th>
<th>Sp.</th>
<th>Cattle</th>
<th>%</th>
<th>Sheep</th>
<th>%</th>
<th>Goats</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aswan</td>
<td></td>
<td>10/25</td>
<td>40</td>
<td>1/5</td>
<td>20</td>
<td>1/5</td>
<td>20</td>
</tr>
<tr>
<td>Menia</td>
<td></td>
<td>28/55</td>
<td>50.9</td>
<td>2/6</td>
<td>33</td>
<td>1/3</td>
<td>33</td>
</tr>
<tr>
<td>Alexandria</td>
<td></td>
<td>17/31</td>
<td>54</td>
<td>5/9</td>
<td>55</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>matrouh</td>
<td></td>
<td>13/22</td>
<td>59</td>
<td>8/11</td>
<td>72</td>
<td>4/7</td>
<td>57</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>68/133</td>
<td>51</td>
<td>10/31</td>
<td>32</td>
<td>6/15</td>
<td>40</td>
</tr>
</tbody>
</table>

Discussion
BT is related to the live stock industry where it has direct and indirect effects on economic losses mainly attributed to high morbidity, mortality, abortions, fetal deaths and fleece losses.

Bluetongue affects both domestic and wild ruminants, and its origin is probably African ruminants. It was first identified in South African Merino sheep in the late 18th century (Gerdes, 2004). 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 (World Organization for Animal Health 2010).

The present study included bovine sera where cattle are the most important in the epidemiology of BT than sheep and goats because they are considered to be attractive to the vector, rarely exhibit clinical signs for the disease and they have viraemia of longer duration than sheep and goats which is up to 100 days (MacLachlan et al., 1991) enabling the virus to "bridge" vector free periods (Koumbati et al., 1999).

Sheep is the most susceptible of the domestic ruminants to BTV, it serve as an indicator host for the virus (Anthony and Werner, 1992). The current study used C-ELISA which is 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).

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 (Iman, and EL-Fayoumy, 2006). Sheep is the most susceptible of the domestic ruminants to BTV and serve as an indicator host for the virus (MacLachlan, et al., 2009).

This study estimates the prevalence and distribution of antibodies to BTV in different domesticated animals in 4 Governorates of Egypt. Our results revealed (50.3%) of BTV infection in all species as shown in (Table 2) which was comparable to that has been described amongst ruminants in regions of Saudi Arabia (47.3%) (Yousef, et al., 2012), Turkey (29.5%) (Gür 2008), India (up to 45.7%) (Sreenivasulu et al., 2004) and Pakistan (48.8%) (Akhtar et al., 1997).
In our study BTV antibodies were detected in sera of cattle (68 of 133 by 51.1%), sheep (16 of 31 by 51.3%) and goats (6 of 15 by 40%) in all Governorates (Table 2).

The highest proportion of seropositive was in Matrouh, Alexandria Menia then Aswan (Table 3), this 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 Governorates. 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 (Dungu et al., 2004) and also there were possibility of windborne carriage of infected Culicoides from distant endemic areas (Gibbs, et al., 1988).

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 (Verwoerd and Erasmus 2004).

In conclusion C-ELISA provides rapid, simple and accurate method for screening of group-specific antibodies against BTV infection among cattle, sheep and goats for routine monitoring to know the status of BT infection in Egypt. Also C-ELISA is recommended for examination of imported ruminants to Egypt specially those imported from Europe.

References


