A survey of Bluetongue Virus Antibodies and Associate Risk Factors among Camels in Khartoum State, Sudan

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ABSTRACT

The current study was serological survey it was conducted in Khartoum State, Sudan to determine the prevalence of BTV antibodies and to identify the potential risk factors associated with the disease among camels. To achieve that total of 184 blood samples were collected randomly from six localities in the Khartoum, State, the serum samples screened for the presence of BTV specific immunoglobulin (IgG) antibodies using a competitive enzyme linked-immunosorbent assay (cELISA) and all camels included in this study were subjected to a questionnaire to determine the potential risk factors associate with the disease. The result of test showed the serological evidence in 123 camels out of total 184 camels tested, with overall prevalence (66.8%). It was found the source of animals and present of other animals in herd of camels is important risk factor associated with the disease, the prevalence of infection is higher in camels population mixed with other animals (68.6%) than camels population rearing alone (57.1%). also the prevalence of infections is higher in camels purchased from market (73.6%) than camels raised in farm (66.7%), when analysis this risk factors showed the significant association between the source of animals and BTV infection (p-value = 0.040) and significant association to present of animals and BTV infection (p-value = 0.01). Finally the present study confirmed that BTV does exist in camels with the high prevalence in Khartoum state, to diminish that we should improve the control measured to infection, and provide the more study about BTV in camels and identify the potential risk factors associated with the disease and role of camels to the spread of infection.

Keywords: Blue Tongue, Risk factors, Camel.

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INTRODUCTION

The one humped camels (Camelus dromedarius) have been served the needs of people for thousands of years and have provided them with food, fiber and fuel. In many parts of world they have also served as beast of burden. They secured trade and communication throughout wide arid and semi-arid expanse to Bedouin of the Arabian Peninsula and North and East Africa, the dromedary was, and still is in some parts, vital for survival in a most in hospitable environment (Wernery and Kaaden, 2002). According to the United Nation Food and Agriculture Organization (FAO), the total world camel’s population is approximately 23 million animals more than 98% of which are found in the developing countries. Sudan is the...
second most densely populated country in the world by camels falling in Somalia (http://faostat.fao.org).

In Sudan, the current dromedary population is estimated to be around 4.6 million concentrated mainly in the Eastern and Western regions of the country (Kordofan, Darfur, and Butana). Dromedaries in Sudan are distributed in the arid and semi-arid parts of the country which lie North of latitude where the annual rainfall is less than 350 mm. (Sudan ministry of animal Resources & Fisheries, 2010). Despite the general reputation for hardiness and resilience, camels are however vulnerable to many disease. Viruses such as African horse sickness, rinderpest, foot and mouth disease, rift valley fever, bovine viral diarrhea and bluetongue virus have been isolated from camelids, some of them have caused mild infections. Little is known about the unusual orbiviruses in camelids, which have been identified through serological investigation. (Wernery, Kaaden, 2002). Bluetongue (BT) is an infectious, non-contagious arthropod-born disease. It infects ruminants and camels caused by Bluetongue virus and transmitted between vertebrate hosts via the bites of certain species of the biting midges of the genus Culicoides (Mellor, 1990). Bluetongue virus (BTV) is a double stranded (ds) RNA orbivirus of the family Reoviridae (Fenner et al., 1974). There are 26 distinct serotypes including serotypes 25 and 26 which were identified recently (Maan et al., 2011). In Sudan, Bluetongue disease was first reported in 1953, when samples from the Blue Nile province were confirmed by the veterinary Reasearch Laboratory at Ounderstepoorts, South Africa, to contain bluetongue virus (Anon, 1953). Culicoides imicolatus was reported to be the principal insect vector for transmission of the virus (Mohammed and Mellor, 1990 and Tabacknick, 2004). At least, five BTV serotypes designated as serotype 1, 2, 4, 5 and 16 are enzootic in different states of the Sudan. These serotypes were recovered from sentinel calf herds at the Khartoum University from, Shambat, Nyala South Darfur state, as well as from Um benin, Sennar State, Sudan (Mohammed and Taylor et al., 1987 and Aradaib et al., 2005). Bluetongue virus may cause a severe hemorrhagic disease in certain breeds of sheep and often fetal hemorrhagic infection in North American white tailed deer, other sheep breeds, such as Sudanese ecotypes of sheep, may develop only febrile disease while cattle and camels mostly develop subclinical infections (Hoff and Trainer, 1974). The indirect losses associated with decreased body weight and condition, drop in milk production, and poor subsequent reproductive performance were thought to have a greater economic effect than occasional overt disease (Abuelzein, 1986, Mohammed and Taylor, 1987 and Gorman, 1992). Currently little is known about the prevalence of BTV infection in camels in the Sudan, and no information is available in regard to potential risk factors associated with the infection. Previous prevalence of BTV sero-positive dromedaries identified by the presence of BTV specific antibodies using serological tests. This prevalence confirmed that camels were infected by BTV and suggested that camels may play an important role in the epidemiology of the disease (Abuelzein, 1985).

A serological survey of the one-humped camel in the Sudan to BTV antibodies in Sudanese dromedaries use done by using agar immunodiffusion test, total of 442 camels serum samples were collected.
from the various camel breeding region of Sudan. The prevalence of BTV group-specific antibody varied from 0 to 40.2% of the samples from the various localities, with overall prevalence of 16.6%. The highest prevalence rate were recorded in Western and Central Sudan, where BT is endemic, a relatively low incidence was recorded for samples from the Eastern region. (Abu Elzein, 1985)

MATERIAL AND METHODS

Study area: The study was conducted in six localities in Khartoum state, which is located in North Eastern of the centre of Sudan, the state is located between latitude 15, 16˚N and 32, 34˚East, the state cover 22.142KM, the climate is semi-desert dry and hot in summer (Maximum temperature is 47.1°C, and Minimum temperature 22.7°C) the range of rain fall is 150mm per year.

Study design: A cross sectional study was conducted to estimate the prevalence of BTV in the one humped camels (Camelus dromedaries) and to determine associated risk factors of disease (Martin et al., 1987)

Sampling Methods: The sampling method was a probability sampling methods using multistage random sampling methods. Six localities was selected randomly from seven localities of the state. Thirty camels were selected from each locality by simple random sampling to choose animals from each herd.

\[
\text{Prevalence Rate} = \frac{\text{No of camels with BTV} \times 100}{\text{Total No of camels tested at particular time}}
\]

Figure 1: Location of study area in Khartoum state, Sudan

Sample size determination

The sample size was calculated by the formula:

\[
n = \frac{(1.96)^2 \times P \times Q}{L^2}
\]
n = sample size, \( P = \) expected prevalence, \( Q = (1 - P) \).

From the previous study carried out in Sudan the seroprevalence of BTV in camels was (16.6%) 

The sample size was equal:

\[
\frac{(1.96^2) \times 0.166 \times (1 - 0.166)}{0.0025} = 188 \text{ samples}
\]

**Questionnaire surveys:** All animals included in this study were subjected to a questionnaire, which was filled out by the animal owners and or managers of farms. The questionnaire included individual risk factor attributes including age (younger animals less than 3 years, older animals 3 years and above), sex (male, female), breed (indigenous, cross), body condition (thin, emaciated, fat), and management risk factor attributes including herd size (small, medium, large) grazing system (nomadic, semi-nomadic, stationary), insect vector (presence or absence), the source of each animal in the herd (raised on farm, purchased from other farms or purchased from local market).

**Data analysis:** The data collected were entered into a computer on a Microsoft Excel spread sheet. Statistical analysis was performed using ‘Statistical Package for Social science’ (SPSS), version 16 (for windows). Associations between the outcome variable (status of BTV infection in camels), and its potential risk factors were first screened in a univariable analysis using Chi-square test. Potential risk factors with P-value less than 0.35 (two tailed, \( \alpha = 0.35 \)) were initially considered significant in \( \chi^2 \) test. The significant result of the univariable analysis, a multivariable model for the outcome variable was constructed using manual step wise forward logistic regression analysis. BTV infection was considered as the dependent variable and the risk factors as independent variable.

Finally, add ratios and 95% confidence interval (CI) were calculated and risk factors with a \( P < 0.05 \) were considered as significant association to BTV infection.

**Blood sample collection:** A total of 184 sera of camels were collected randomly from six localities (ShargElNile, Bahri, Um Durman, Um badah, Karary, Jabal Aulya), in Khartoum state, Sudan. Between November 2014 to March 2015. Blood samples were collected from jugular vein in sterile test tubes (Vaccutainers). the tube were labeled with animal number, date, sex, and then transferred immediately to the laboratory diagnostic laboratory in department of medicine and therapeutic, faculty of veterinary medicine, university of Khartoum, the blood were centrifuged at 3000rpm for 5minutes to separate serum. Sera were stored at -20 until used.

**Competitive Enzyme-Linked ImmunoSorbent Assay (cELISA):** A Competitive enzyme linked immunosorbent assay (cELISA) was performed using a commercially available BTV antibody cELISA kit (Veterinary Medical Research and Development, USDA product code 5010,20Pullman WA,USA) .the sera was screened for BTV specific IgG antibodies basically as described by the
manufactures specification. cELISA was performed in 96well antigen coated micro plate unless stated otherwise ,25or50microliters(µl) test volumes were used in the cELISA assay the incubation was performed for 15 min at room temperature (23±2°C) the plate was washed three times with provided washing buffer briefly aliquots of 25µl test sera as well as positive and negative controls sera was transferred undiluted to the BTV antigen coated plates ,using multi-channel pipette after incubation the plate was washed and 25µl of Ab peroxidase conjugated was added each well the plate was then incubated at15 min at room temperature the plate will then washed and 50µl the substrate was added to each well ,the reaction was stopped using 50µl of the stopping solution, the results were read either visually or by using ELISA reader set at 450nm .Presumptive diagnosis were made when the tested samples produced an optical density <50% of the mean of the negative controls the test samples were considered negative if the optical density >_50%of the mean of the negative controls.

**Statistical analysis:** All data were coded in master sheets, checked, entered and analyzed by using Statistical Package for Social Science (SPSS) version 16.The level of statistical significance was set at 5% (p< 0.05).

**RESULTS**

A serological survey in camels was conducted in Khartoum state, Sudan to screened the BTV-specific igG antibodies by using a cELISA detected 123 (66.8%) from total 184 camels serologically positives to BTV infection in the study. The highest prevalence rate recorded in Jabal Aulya locality (81.8%), while the Sharg Elnile recorded the lowest rate of infection (57.1%). Using univariate analysis chi-square test was conducted for the association between the potential risk factors and BTV infection . In Table 1 showed that independent variables including sex, animals source, presence of other animals, farm yard were initially significant (P-value less than 0.25) to BTV infection .The significant results of the univariate analysis were re-entered into final multivariate models using logistic regression analysis and take the independent variables statistically significant if the p-value less than 0.05.The results of the final models multivariate analysis in Table 2 showed only two independent variables statistically significant risk factors to BTV infection included (animals source and present of other animal), and they was no significant association between rate of infection among camels and potential risk factors included (localities, sex, breed, grazing system, farm yard, herd size, vector control, age, insect control and body condition).In contrast, when potential risk factors were measured against BTV infection rate, mixed rearing of camels with other ruminant were higher infected with BTV to compare with camels rearing alone, and camels purchased from market were higher infected than camels raised on farm.
### Table 1: Univariate analysis for the association between potential risk factors and BTV infection among camels in Khartoum state, Sudan using Chi-square test.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Animals tested</th>
<th>Animals affected (%)</th>
<th>Df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ShargElNile</td>
<td>28</td>
<td>16 (57.1%)</td>
<td>5</td>
<td>0.356</td>
</tr>
<tr>
<td>Bahrain</td>
<td>32</td>
<td>20 (62.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Om Durman</td>
<td>29</td>
<td>19 (65.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Um badah</td>
<td>28</td>
<td>17 (60.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karari</td>
<td>34</td>
<td>24 (70.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JabalAulya</td>
<td>33</td>
<td>27 (81.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td>1</td>
<td>0.596</td>
</tr>
<tr>
<td>Old</td>
<td>243</td>
<td>97 (67.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>41</td>
<td>26 (63.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>1</td>
<td>0.110</td>
</tr>
<tr>
<td>Male</td>
<td>40</td>
<td>23 (57.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>144</td>
<td>100 (69.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breed</td>
<td></td>
<td></td>
<td>2</td>
<td>0.440</td>
</tr>
<tr>
<td>Bushari</td>
<td>71</td>
<td>45 (63.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anafi</td>
<td>19</td>
<td>15 (78.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western</td>
<td>94</td>
<td>63 (67.05%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body condition</td>
<td></td>
<td></td>
<td>2</td>
<td>0.847</td>
</tr>
<tr>
<td>Fat</td>
<td>99</td>
<td>68 (68.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thin</td>
<td>82</td>
<td>26 (4.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emaciate</td>
<td>53</td>
<td>53 (66.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of animals</td>
<td></td>
<td></td>
<td>2</td>
<td>0.213</td>
</tr>
<tr>
<td>Raised on farm</td>
<td>72</td>
<td>53 (73.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purchase from other farm</td>
<td>23</td>
<td>16 (69.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purchase from local market</td>
<td>89</td>
<td>54 (60.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grazing system</td>
<td></td>
<td></td>
<td>1</td>
<td>0.632</td>
</tr>
<tr>
<td>Stationary</td>
<td>122</td>
<td>83 (68.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nomadic</td>
<td>62</td>
<td>40 (64.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmyard</td>
<td></td>
<td></td>
<td>1</td>
<td>0.245</td>
</tr>
<tr>
<td>Indoor</td>
<td>65</td>
<td>47 (72.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outdoor</td>
<td>119</td>
<td>76 (63.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present of other animals</td>
<td></td>
<td></td>
<td>1</td>
<td>0.236</td>
</tr>
<tr>
<td>No</td>
<td>28</td>
<td>16 (57.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>156</td>
<td>107 (68.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herd size</td>
<td></td>
<td></td>
<td>2</td>
<td>0.266</td>
</tr>
<tr>
<td>Small</td>
<td>11</td>
<td>9 (81.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>22</td>
<td>12 (54.45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>151</td>
<td>102 (67.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vector control</td>
<td></td>
<td></td>
<td>1</td>
<td>0.565</td>
</tr>
<tr>
<td>No</td>
<td>152</td>
<td>103 (67.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>32</td>
<td>20 (62.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insect control</td>
<td></td>
<td></td>
<td>1</td>
<td>0.565</td>
</tr>
<tr>
<td>No</td>
<td>152</td>
<td>103 (67.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>32</td>
<td>20 (62.5%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In Table (2): the final models of BTV infection indicated that only two independent risk factors were statistically significant to the BTV infection in camels by using logistic regression analysis of risk factors, that risk factors including (Animals source, present of other animals). There was no
significant association between rate of infection among camels and potential risk factors including animal sex, locality, breed, grazing systems, farmyard, herd size, vector control, and insect control. In contrast, when potential risk factor were measured against BTV infection rate ,mixed rearing of camels with other ruminant were higher infected with BTV (C I= 0.43-0.771 ,P-value = 0.016). Camels purchased from market were higher risk factors to BTV infection (CI = 0.223-1.071, P-value = 0.040).

Table 2: Multivariate analysis using logistic regression model, for significant association (P < 0.05) of risk factors and BTV infection among camels in Khartoum state, Sudan

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Animal affected (%)</th>
<th>OR</th>
<th>95.0% C.I for OR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal source</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Raised on farm</td>
<td>53(73.6%)</td>
<td>0.424</td>
<td>0.223-1.071</td>
<td>0.040</td>
</tr>
<tr>
<td>- purchased from other farm</td>
<td>16(69.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- purchased from market</td>
<td>54(60.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present of other animals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Yes</td>
<td>107(68.6%)</td>
<td>0.176</td>
<td>0.43-0.771</td>
<td>0.016</td>
</tr>
<tr>
<td>- No</td>
<td>16(57.1%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

BTV has become of great veterinary concern to diary producers, wild life managers and veterinary diagnosticians because of the frequent occurrence of outbreak among domestic and wild ruminants in geographical regions previously known to be BTV free. (Lander voldet al 2003., Purse et al 2005 Saegerman et al.,2008). In Sudan BTV is endemic (Eisa et al 1979) and one of major disease in ruminants specially in goats while cattle and camels develop subclinical infections of BTV. Very little information is known about BTV in camels and what the role of camels to epidemiology of the disease. To advance beyond the current knowledge of the epidemiology of this disease we conducted this study to determine the prevalence of BTV in camels and potential risk factors associated with the disease in Khartoum state; one of areas where BTV is endemic in Sudan (AbuElzein, 1983). Many seropositive BTV in dromedaries have been reported from many countries. In Sudan, where BTV is endemic, it had been found that, 4.9% and 16.6% of dromedaries were BTV positive by Eisa et al., (1980), and AbuElzien (1985) respectively. In Botswana NF (1979) found prevalence of 81% of the dromedaries population showing antibodies to the BTV .In Iran Mozaffari et al. (2013) found 67.8% respectively positive to BTV .In the present study the prevalence of BTV group specific antibodies in camels in Khartoum state was 66.8% is markedly higher than previous study in Sudan, in contrast with the result of Abu Elzien (1985) and Eisa (1979) ,the reason for the great difference in the prevalence rate may be attributed to climatic change in environment that lead to increase of vector midges to transmission of disease . In contrast with study of AbuElzien (1985) he was conducted his study in Sudan in many regions that appears varied of rate 0-40.2% but the overall prevalence 16.6% the highest rate recorded in central Sudan and western regions of the Sudan . Also Abuelzien and Eisa detected the BTV antibodies by Agar Gal
Immunodiffusion Test (AGID), while in present study detected the BTV antibodies by the Competitive Enzyme-Linked Immunosorbent Assay (cELISA). In the present study the prevalence of BTV infection 66.8% is similar to those reported in Iran 67.8% and Saudi Arabia 67%. The results of our study showed that BTV infection rate increased in camels herd rearing with other animals 68.6%, while camels rearing alone show the lowest rate of infection 57.1%, when assessing the present of other animals in herds of camels as a risk factors there was a significant association between the BTV infection rate and present of other animals. That explaining the high prevalence of BTV in other animals include, sheep, goats, cattle, determined by the previous study, Abu Elzien (1985) found the highest rate of infection 93% of cattle, 86% of sheep and 73% of goat exhibit positive titers. Also Khair et al. (2014) recorded 67% of cattle infected by BTV in Darfur. This percentage may explained the susceptibility of other animals to infections more than camels, while camels always develop subclinical infection of the disease, in present study the camels purchased from other markets are more infected than camels raised on farm, that means the trade camels purchased from out of Khartoum may increase the prevalence rate of infection, when assessing the source of camels as risk factor, there was no significance association between BTV infection. The present study the prevalence rate various from locality to other, the highest prevalence rate of infection recorded in Jabal Aulya (81.8%), that attributed to present of other animals in herd of camels, and suitable climatic condition to survival of vector in this locality in contrast with Sharg El Nile and Om Durman locality recorded the lowest rate of infection of BTV in camels may attributed to separation of camels population from other animals and unsuitable climatic condition to present of vector control.

In conclusion Bluetongue is highly endemic in Khartoum state, Sudan, as determined by the presence of BTV specific IgG antibodies in camels serum collected from all locality of the state, and highly prevalence rate recorded was (66.8%) respectively positive. Camels harbor the virus, without showing symptoms, and thus can spread the infection to other ruminant. To diminish the spread of infection we should rearing camel’s separation from other ruminant, and reduce purchased of camels from endemic area to the Khartoum state.

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