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SEROLOGICAL SURVEY ON BLUETONGUE VIRUS INFECTION IN CAMELS AT TWO CAMEL-REARING REGIONS OF SOMALILAND

(With One Table and One Figure)

By

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مسح سيرولوجى لعدوى فيروس اللسان الأزرق في مقاطعتان لتربية الابل في شمال الصومال

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لتحديد وجود ومدى انتشار ألعدوي بفيروس اللسان الازرق فى مناطق مختلفة من ارض الصومال اجرى اختبار الاليزا للكشف عن الاجسام المناعية فى سيرم الابل فى مقاطعتان هما توغادير واكوياجالبيد وأجريت هذه الدراسة في القترة من يوليو 2008 إلى أبريل 2009 في شمال الصومال. كانت الجمال تحت الدراسة جميعها سليمة ظاهريا وبدون تاريخ مرضى فى الفترات السابقة ولم يظهر عليها أي اعراض مرضية. تم تسجيل نتيجة العدوى بالفيروس وحجم القطعان وموقعها ومدى اختلاطها بحيوانات اخرى وطرق التربية. تم اختبار عدد 30 قطيع فى كلا المقاطعتان وكانت 7 قطعان سالبة لاختبار الاليزا بنسبة 23و33% و 23 قطيع موجبا لاختبار الاليزا بنسبة 76و67% وبنسبة اصابة كلية و 96و13% (عدد 93 حيوان) وكانت عدد الابل المحتبرة 537. بالنسبة للجنس كانت الذكور المختبرة وعددها الكلى 286 كانت 9 قطعان بنسبة 30% سالبة لاختبار الاليزا وعدد 21 قطيع موجبة بنسبة 70% وبمعدل انتشار للعدوى بنسبة 64و13% (عدد 39 حيوان موجب) .بالنسبة للاناث المختبرة وعددها الكلى 326 كان معدل انتشار للعدوى بنسبة 21و14% (عدد 54 حيوان موجب) كانت نتائج الاختبار إيجابية في نسبة 76.7% من قطعان الجمال المفحوصة (23 قطيع). كانت نسبة الإصابة بين الذكور 70% بينما كانت73.3% في الإناث على مستوى القطعان تحت الفحص. بالنسبة لمدى الانتشار بالنسبة للمناطق تبين انتشار المرض في منطقة توغادير كان عدد القطعان المصابة من اصل 16 قطيع تحت الفحص فكانت 4 قطعان سالبة (25%) وعدد 12 قطيع موجبة بنسبة (بنسبة 75%) وبنسبة اصابة كلية 32و14% (عدد 54 حيوان موجب) من اصل 377 جمل تحت الاختبار بينما كانت فى واجويا جالبيد كانت

عدد القطعان المفحوصة 14 قطيع وكانت 3 قطعان منهم سالبة بنسبة 43و21% و11 قطيع موجبة بنسبة 57و78% لاختبار الاليزا. من هذه الدراسة تبين تواجد وانتشارالعدوى بفيروس مرض اللسان الأزرق في الجمال الصومالية. الجمال المصابة قد لا تظهر أعراضا إكلينيكية. تعتبر هذه هي الدراسة الأولى التى تؤكد تواجد أجسام مناعية لمرض اللسان الأزرق في الجمال في أرض الصومال بشمال الصومال.

SUMMARY

To determine the presence and prevalence of bluetongue virus infection in camels at different geographical regions of Somaliland, a competitive enzyme-linked immune-sorbent assay (cELISA) for the detection of serum antibody against BTV in clinically healthy camels has been carried out at northern Somalia in two main districts of camel-rearing regions namely, Togdheer, and Waqoyi Galbed in the period between July 2008 to April 2009. Results for bluetongue infection, herd size, and herd location, mixing with other animal species with various other associations were detected among demographic, husbandry and disease variables. All camels tested were apparently normal without showing clinical signs and without history of any specific clinical signs for BTV infection. Out of 30 camel/herds investigated, 7 (23.33%) herds were serologically-negative and 23 (76.67%) were serologically-positive by cELISA to BT virus infection with an overall prevalence of 13.96% (n=93). According to sex, for 286 males tested, 9 (30.0%) herds were found serologically-negative and 21 (70.0%) with an overall total prevalence of 13.64% (n=39). For 380 she camels tested, 54 (14.21%) camels were serologically-positive and 326 (85.79%) camels were serologically-negative. According to districts investigated, for Togdheer district, out of 16 camel/herds investigated, 4 (25.0%) herds were serologically-negative and 12 (75.0%) were found to be serologically-positive by cELISA with overall prevalence of 14.32% (n=54) out of 377 tested camels. For Waqoyi Galbed district, Out of 14 camel/herds investigated, 3 (21.43%) herds were serologically-negative and 11 (78.57%) were serologically-positive by cELISA to BTV infection with a total prevalence of 12.33% (n=39) out of 289 camels tested. The results of the present investigation indicate that the bluetongue virus exists within the camel herds. The findings suggest that the disease is widely distributed in most investigated parts of the Somaliland where possible insect vectors may prevail and may suggest disease endimicity which is probably subclinical or in-apparent in camels of the Somaliland. The results presented here may consider the first confirmation of bluetongue virus antibody in camels in Somaliland.

Key words: *Virology, blue tongue, camel, serology.*

INTRODUCTION

Bluetongue virus infects all species of domestic and wild ruminants (MacLachlan, 2004; and Stallnecht and Howerth, 2004) with similar pathogenesis and clinical signs which obviously lead to poor welfare in the animal population (Barret-Bayes and Maclachlan, 1995; MacLachlan, 2004; Verwoerd and Erasmus, 2004). However, Wernery and Kaaden (2002) encountered bluetongue virus infection in Camelids as a non pathogenic virus. BTV is the prototype member of the genus *Orbivirus*, family *Reoviridae* (Mertens *et al.*, 2004). There are to date, 24 distinct serotypes of BTV have been described that all share common group antigens but distinguished on the basis of serotype-specific virus neutralization assays (Bonneau *et al.*, 1999; Pritchard *et al.*, 2004; Bréard *et al.*, 2007b). BTV infection of ruminants occurs throughout much of the temperate and tropical regions of the world, coincident with the distribution of specific species of *Culicoides* biting midges that are biological vectors of the virus (Gibbs and Greiner 1994; Tabachnick 2004). The global distribution of BTV has historically been between latitudes of approximately 40 – 50 °N and 35 °S but the virus recently (Since 1999) has spread northward in parts of the Mediterranean Basin and in North-Western Europe in 2006 (Hawkes 1995). BT occurs as a clinical disease of small ruminants in many countries of Africa, the Middle East, the Indian subcontinent, China, the United States, Mexico. BTV is also present in Southeast Asia, northern Australia, Papua New Guinea and northern South America, normally without associated clinical disease. Between 1998 and 2003, an unprecedented BT epidemic has occurred (Baylis 2002), affecting many countries in both the east and west Mediterranean areas.

Thirty-two species of *Culicoides* are considered to be involved in the transmission of BTV (Meiswinkel *et al.*, 2004a). In Africa at least 10 species of *Culicoides* breed exclusively in the dung of indigenous herbivores (Meiswinkel *et al.*, 2004b). *Culicoides imicola* appears to be the major vector of BTV in the Old World**.** The persistence between years should only be possible where adult *Culicoides* vectors are present year-round and are of sufficient abundance and competence to permit continual host-midge cycling of BTV over winter where overwintering of BTV was possible. (Mellor and Boorman 1995). However, no clinical signs or pathological lesions in camels caused by bluetongue virus have ever been reported, although some serologically-positive camels have been reported from a number of countries (Wernery and Kaaden 2002). Ruminants naturally infected with one serotype of BTV have a solid,

lifelong immunity to the homologous serotype but only partial or no protection against other (heterologous) serotypes (Verwoerd and Erasmus 2004), thus recovered animals pose no threat for transmission of infection if they are confirmed to be virus-free prior to their movement. However, BTV infection of ruminants is prolonged but not persistent (MacLachlan 2004; Melville *et al.*, 2004; White and Mecham 2004; Lunt *et al.*, 2006). A true persistent BTV carrier state occurred in some animals (Barratt-Boyes and MacLachlan 1995; MacLachlan 2004; Lunt *et al.*, 2006). The serotypes are differentiated by serum neutralisation tests, but there are cross-reactions between some serotypes. All BTV's share group antigens, which can be demonstrated by agar gel diffusion tests, fluorescent antibody tests and the group reactive ELISA. Prevalence of camel bluetongue in Somaliland has not been discussed. Therefore, the aim of the present study was to explore the serologic-prevalence and potential risk factors of camel BTV in the Somaliland using a commercially available competitive enzyme-linked immune-sorbent assay (C-ELISA).

MATERIALS and METHODS

1- Study Area

The present study was conducted during the period from July 2008 to April 2009 in the northern part of Somalia. Two main districts were covered in this study namely, Waqoyi Galbed, and Togdheer, which constitute (Figure1).

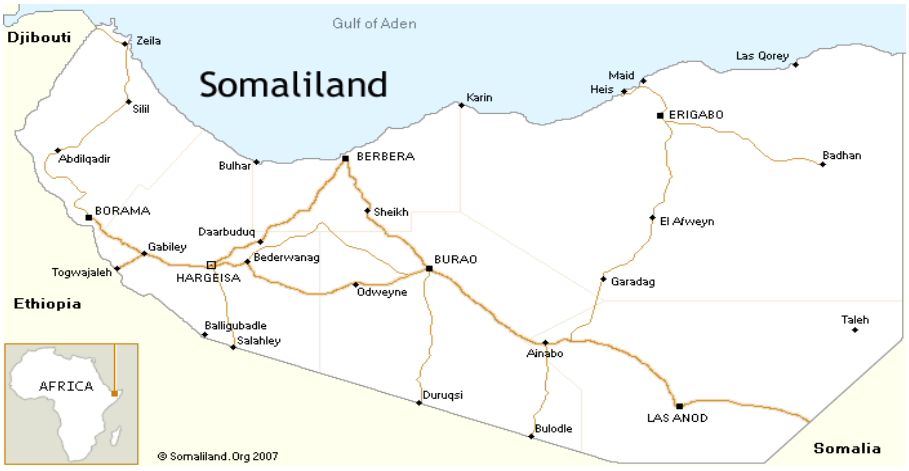

Fig. 1: A map of Somaliland showing locations of sample collection from camels for serosurvey of BTV

2- Study Animals

A total of 666 one humped unvaccinated camels blood sera were examined in this study and involved 30 herd/flocks (16, and 14 herds from Togdheer and Waqoyi Galbed respectively. The number of camel heads per herd/flock ranged from 14-31. Information of each camel sampled were obtained including, location, herd size, sex, age, health status, history of disease, whether reared individually, with other species or in a camel herd. Camel's sera were collected as 377 samples from Togdheer (162 males and 215 females) and 289 samples from Waqoyi Galbed (124 females and 165 females).

3- Sample collecting

A 10 ml blood samples were collected and transferred to the laboratory of the Gulf International Veterinary Quarantine in Berbera city of Somaliland. The sera were separated and stored at -20°C until testing

4- Serological test

Bluetongue virus antibody kit, cELISA Assay. Catalog No. 287-5, 5. Product Code 5010.20- VMRD, Inc. P.O.Box 502 Pullman, WA, 99163; USDA Veterinary License No. 332, Serial No. P071008-004. solid plates 460. VMRD's competitive enzyme-linked immune-sorbent assay (cELISA) detects antibody to bluetongue virus in ruminant sera. It has been demonstrated to detect all 24 known serotypes of Bluetongue Virus (BTV). The serologic procedure of cELISA was carried-out according to the instruction of the enclosed pamphlet and the final plates was read on a plate reader (MR-96A Micro-plate Reader produced by Shenzhen mindary bio-medical electronics CO., LTD. Mindray Building, Keji 12th Road South, High-Tech Industrial Park, Nanshan, ShenZhen518057, and P.R. China). Set the optical density (O.D.) reading wavelength to 620, 630 or 650 nm. Test Validation: The mean of the Negative Controls must produce an optical density greater than 0.300 and less than 2.000. The mean of the Positive Controls must produce an optical density less than or equal to 50% (one-half) of the mean of the Negative control. 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

Overall Serologic prevalence of the two districts (Table 1)

Out of investigated camel herds (n=30), 23 (76.7%) were found serologiclly positive to BTV infection and the remained herds (7, 23.3%) were negative. For 286 males included in the 30 herds, 9 (30%) herds were found negative and 21 (70%) were positive with a total district serologic prevalence of 13.64% (n=39). For 382 females included in the 30 herds, 8 (26.7%) herds were serologic negative and 22 (73.3%) herds were found positive with a total district serologic prevalence of 14.21 % (n=54) (Table 1)

For Togdheer (Table 1)

Out of 16 camel herds investigated, 4 (25.0%) herds were serologic-negative and 12 (75.0%) were serologically positive by cELISA to Bluetongue virus infection. For 162 males included in the 16 herds, 5 (31.25%) herds were found negative and 11 (68.75%) were positive with a total prevalence of 13.58% (n=22). For 215 females included in the 16 herds, 4 (25.0%) herds were serologic-negative and 12 (75.0%) herds were found positive with a total prevalence of 14.88% (n=32). All camels were apparently normal without showing clinical signs and without history of a specific clinical signs for BT vial infection.

For Waqoyi Galbed (Table 1)

Out of 14 camel herds investigated, 3 (21.43%) herds were serologic-negative and 11 (78.57%) were serologically positive by cELISA to Bluetongue virus infection with a total prevalence of 12.33% (n=39). For 124 males included in the 14 herds, 4 (28.57%) herds were negative and 10 (71.4%) were positive with a total prevalence of 13.71% (n=17). For 165 females included in the 14 herds, 4 (28.6%) herds were negative and 10 (71.4%) herds were positive with with a total prevalence of 13.33% (n=22). All camels were apparently normal without showing clinical signs and without history of any specific clinical signs for BT viral infection.

Table 1: Serological survey results on district and herd levels at Somaliland by cELISA.

District	Herd No.	Herd size	Male Camels			Female Camels			Total			Contact with other species
			cELISA			cELISA						
Togdheer			Total Males	+ve	% -ve %	Total Females	+ve	% -ve %	+ve	% -ve %		
	1	22	10	1	10.0 9 90.0	12	1	8.33 11 91.67	2	9.09 20 90.91		Cattle
	2	26	10	0	0.0 10 100.0	16	0	0.0 16 100.0	0	0.0 26 100.0		Sheep, goats
	3	25	11	4	36.36 7 63.64	14	3	1.43 11 78.57	7	28.0 18 72.0		Cattle, sheep, goats
	4	30	16	1	6.25 15 93.75	14	2	14.29 12 85.71	3	10.0 27 90.0		None
	5	22	9	3	33.33 6 66.67	13	4	30.77 9 69.23	7	31.82 15 68.18		Cattle
	6	17	8	0	0.0 8 100.0	9	1	11.11 8 88.89	1	5.88 16 94.12		None
	7	25	12	2	16.67 10 83.33	13	4	30.77 9 69.23	6	24.0 19 76.0		Cattle
	8	28	12	0	0.0 12 100.0	16	0	0.0 16 100.0	0	0.0 28 100.0		None
	9	21	9	2	22.22 7 77.78	12	3	25.0 9 75.0	5	23.81 16 76.19		Sheep, goats
	10	20	7	3	42.86 4 57.14	13	4	30.77 9 69.23	7	35.0 13 65.0		Cattle, sheep, goats
	11	25	10	1	10.0 9 90.0	15	3	20.0 12 80.0	4	16.0 21 84.0		None
	12	31	13	0	0.0 13 100.0	18	0	0.0 18 100.0	0	0.0 31 100.0		None
	13	26	12	1	8.33 11 91.67	14	2	14.29 12 85.71	3	11.54 23 88.46		None
	14	18	8	2	25.0 6 75.0	10	3	30.0 7 70.0	5	27.78 13 72.22		Cattle
	15	21	8	0	0.0 8 100.0	13	0	0.0 13 100.0	0	0.0 21 100.0		None
	16	20	7	2	28.57 5 71.43	13	2	15.38 11 84.62	4	20.0 16 80.0		Cattle
Total		377	162	22	13.58 140 86.42	215	32	14.88 183 85.12	54	14.32 323 85.68		
Waqoyi Galbed	1	23	10	1	10.0 9 90.0	13	2	15.38 11 84.62	3	13.04 20 86.96		sheep, goats
	2	21	9	2	22.22 7 77.87	12	3	25.0 9 75.0	5	23.81 16 76.19		cattle, sheep, goats
	3	23	9	0	0.0 9 100.0	14	0	0.0 14 100.0	0	0.0 23 100.0		None
	4	20	8	1	12.50 7 87.50	12	2	16.67 10 83.33	3	15.0 17 85.0		sheep, goats
	5	24	9	2	22.22 9 77.87	15	3	20.0 12 80.0	5	19.23 21 80.77		cattle, sheep, goats
	6	18	8	0	0.0 8 100.0	10	0	0.0 10 100.0	0	0.0 18 100.0		None
	7	17	7	2	28.57 5 71.43	10	2	20.0 8 80.0	4	23.53 13 76.47		sheep, goats
	8	14	6	1	16.67 5 83.33	8	1	12.50 7 87.50	2	14.29 12 85.71		None
	9	23	11	2	18.18 9 81.82	12	3	25.0 9 75.0	5	21.74 18 78.26		sheep, goats
	10	22	9	0	0.0 9 100.0	13	2	15.38 11 84.62	2	9.09 20 90.91		None
	11	23	11	0	0.0 11 100.0	12	0	0.0 12 100.0	0	0.0 23 100.0		None
	12	19	8	1	12.50 7 87.50	11	1	9.09 10 90.91	2	10.53 17 89.47		None
	13	17	8	2	25.0 6 75.0	9	0	0.0 9 100.0	2	28.57 15 71.43		None
	14	25	11	3	27.27 8 72.73	14	3	21.43 11 87.57	6	24.0 19 76.0		cattle, sheep, goats
Total		289	124	17	13.71 107 86.29	165	22	13.33 143 86.67	39	12.33 250 87.67		

DISCUSSION

Somaliland had never previously recorded the presence of BTV in camels and the present study revealed an overall serologic-prevalence of 13.96%. The differences observed between geographic areas are not significant as in Togdheer it was 14.32% while in Waqoyi Galbed was 12.33% (Table 1). The presence of BTV antibodies in these sera had previously been demonstrated by the c-ELISA. In endemic areas, serologic-prevalence's of 46–52% in sheep, 44% in goats and 33–95% in cattle have been reported (Formenty *et al.*, 1994; Thevasagayam *et al.*, 1996). Our findings of 12.33%-14.32% serologic-prevalence are relatively low, however, they suggest that BTV is widespread and endemic in the country**.** This result may the first confirmation of BTV antibody in camels from Somaliland. Our interviews with farmers and officials highlighted the fact that vaccination against BTV diseases as a major economic and public health importance is not performed in Somaliland. Epidemic disease may constitute a serious problem for Somaliland's rural economy in future, and the situation is likely to worsen in the next few years. The overall prevalence of the BTV antibodies in camels in the two districts was 12.33% - 14.32%, and serologic-positive animals were detected in both districts sampled. Failure to detect serologic-reactors in these serologic-negative herds may due to the relatively low number of animals screened. In addition, mild disease may go unnoticed and/or unreported. This may be attributed to the nearness of these districts to the states of Awdal, where BTV is considered endemic in Ethiopia as a border country, and to the unrestricted movement of relatively large numbers of camels, cattle, sheep and goats from these 'endemic' regions into the Somaliland districts. One of the factors that might contribute to these higher levels in tested herd camels. The cELISA described here detected positive BTV antibody in sera from camels in the two studied districts comprising 30 herds. This assay has proved reliable for the detection of BTV antibody at the farm level and could be used with confidence. Smriti and Shringi (2005), found that cELISA being highly sensitive and specific followed by CCIE and AGID test. BT, it is essential to develop methods for accurate prediction of BT risk in space and time. Persistence of BTV within a particular geographical area does not mean "static". Once a vertebrate host is infected with BTV it either dies or mounts an enduring antibody response and so becomes resistant to further infection. This means that within any small geographical area (a farm or village) most

or all of the initially susceptible hosts are likely to be infected and thus become "unavailable" to the virus within a fairly short space of time. BTV can only survive under such constraints by continually moving to new locations occupied by naïve vertebrate hosts. These movements are via the agency of viraemic hosts or infected vectors. BTV is therefore a peripatetic virus and even within its enzootic zones its activity may be envisaged as a pattern of endlessly shifting viral "hot spots". Where annual bouts of BT occur, they may represent new introductions (from adjacent infected areas) or may be the visible evidence of low-level persistence from year to year. However, neither clinical signs nor gross pathological alterations caused by Bluetongue virus in all species of camelids (Wernery and Kaaden, 2002) with exception in only one llama was found with signs with respiratory manifestation in association with abortion (Fowlar, 1998). Based on our results a further studies on prevalence of BTV should carried-out to avoid the epidemic for this disease in Somaliland. Serotyping of the existence and persistence of BTV in Somaliland's camels is extremely urgent . Moreover, epidemiological studies need be done to explore the current status of the disease in other ruminants and other animals to enable the public veterinary authorities to construct concrete program for prevention of the disease within animal herds in Somaliland or transmission of the disease via animal trading to the other countries.

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