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A cross sectional study to determine the seroprevalence of bluetongue virus antibodies in sheep, goats, cattle and camel in the eastern region of Kingdom Suadi Arabia

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The aim of the present work was planned to determine the sero-prevalence and to analyze the risk factors associated with BTV infection in the eastern region in the Kingdom of Saudi Arabia in a cross sectional study. A total of 1476 serum samples were collected equally from cattle, sheep, goats and camel and were examined for the presence of specific antibodies against BTV by competitive ELISA (c ELISA). The association between seropositivity and categorical predictor variables; sex, age, breed for each animal species was identified using a univariate logistic regression analysis. The overall BTV sero-prevalence among sheep, goat, cattle and camel in the eastern province of KSA were 48%, 50%, 8.2% and 0.8%. Animal species showed highly significant effect on the prevalence of antibodies to bluetongue virus ($p<0.0001$). Goat and sheep are highly susceptible to the infection with bluetongue virus followed by cattle and camel. Breed had higher odd's ratio for BTV in sheep, goat, cattle and camel (OR>1) but without significance except for Swakni sheep and Swmali camels which were considered at higher potential risk for BTV. Sex was associated with a non-significant seroprevalence of BTV in sheep, goat and camels. Age had a non-significant with BTV in goat while a significant association (OR>1) was found in case of sheep and camel population however, the association was found non-significant ($p<0.05$) based on chi-square. Cattle of > 3 years old were associated with significant (OR>1; $p<0.05$) higher seroprevalence other than young cows. The obtained results showed that BTV antibodies has been detected among sheep, goat cattle, and camels in the eastern region of Kingdom of Saudi Arabia. The effect of breed, sex and age as risk factors associated with BTV differed from one Spp. to another.

Keywords: Bluetongue virus; risk factors; c-ELISA; Seroprevalence

INTRODUCTION

Bluetongue (BT) is a disease transmitted by insect to domestic and wild ruminants that is characterized by elevation of body temperature, respiratory distress, edema of the face, lameness, and oral ulceration and hemorrhage (MacLachlan, 2004; Verwoerd and Erasmus, 2004). The severity of the disease in sheep is highly variable, ranging from subclinical to severe depending on virus strain and host susceptibility (Alexander et

al., 1996). The severity of the clinical disease varies among hosts as the disease is much severe in sheep and some species of deer (Elbers et al., 2008). Blue tongue disease is a notifiable disease causing important losses such as deaths, drop in milk production, infertility and abortion, beside the indirect losses as restrictions of international trade, surveillance costs and mass vaccination, vector control and treatment (Bluetongue, 2014; Coetzee et al., 2012; Wilson & Mellor, 2009). Bluetongue virus (BTV), the causative agent of BT, has the potential for serious and rapid spread, irrespective of national borders, and is of major importance to the international trade of livestock and livestock products (Alexander et al., 1996). Trade restrictions can have a devastating impact in areas where BTV is endemic, regardless of the incidence of clinical disease (MacLachlan, 2004).

There is widespread of bluetongue antibodies in the sera of ruminants in Saudi Arabia. During the 1980s and before, activity against BT virus serotypes 6, 14, 17, 18 and 19 was detected (Hafez & Taylor, 1985); while during the 1990s these serotypes were no longer present. Thereafter, mono-specific antibodies for BT virus serotypes 10, 12, and 15 were detected for the first time in sentinel and other ruminants in Saudi Arabia (Abu Elzein et al., 1998). A varying prevalence of BTV has already been reported in neighboring countries like Pakistan in sheep, goat and cattle population originating from Orissa, India a seroprevalence of 26.66%, 31.25% and 52.27% has been recorded (Joardar et al., 2014). The prevalence of antibodies in sheep (20.3%) and yaks (13.3%) has been reported from Tibetan Plateau ,China (Ma et al., 2017).

Blue tongue virus infection causing a wave of abortions, stillbirths and deformities in sheep at Al-Ahsa in the eastern region of Saudi Arabia in the second half of 1999 (Housawi et al., 2004). BTV has been reported in several Middle Eastern countries (Egypt, Jordan, Syria, Turkey, Cyprus, Iraq, Iran, Oman, Qatar, Yemen and Saudi Arabia) since 1951 (Abu Elzein et al., 1998; Al-Busaidy & Mellor, 1991; OIE, 2009). Also, a novel BTV 44.8% and 25.7% in sheep, goat, cattle and camel serotype has been identified in Kuwait in 2010 (Maan et al., 2011). Yousef et al. (2012) estimated the prevalence and distribution of serum antibodies to BTV in different domesticated animals in different localities of Saudi Arabia. They found that the overall BTV antibody prevalence was 54.1%, 53.3%, 44.8% and 25.7% in sheep, goat, cattle and camel respectively (at 95% confidence level). They concluded that the Jizan and Eastern Province districts were the regions with the highest prevalence.

The transmission of BTV between countries may be through active or passive flight of carrier vector (Culicoides spp.) (Saegerman et al., 2008) and the trade of animals and animal's products (Raziq et al., 2010; Zahur et al., 2011).

The aim of the present work was planned to determine the sero-prevalence and to analyze the risk factors associated with BTV antibodies in the eastern province in a cross sectional study.

MATERIALS AND METHODS

Study area:

The study area is the Eastern Region of the Kingdom of Saudi Arabia located between longitudes 44.81, 55.69 East and latitudes 17.10 and 29.10 North, occupying an area of 672,522 km2. The Eastern Province is boarded by Iraq and Kuwait in the north; Arabian Gulf, Qatar, and the United Arab Emirates in the east; Riyadh and Najrān Provinces in the west and Oman in the south. The largely uninhabited Rub' al Khali (Empty Quarter) desert occupies more than half of the province. The total human population in Eastern Province is approximately 5.1 million. The temperature ranges from 10-20 in winter and from 30-45 in summer. The relative humidity percentage ranges from 30 -60 %.

Sampling

Sera samples were collected from cattle, sheep, goats, and camels during the period from January-December 2011. The number of samples were calculated according to (Thrusfield, 2005).

The sample size were calculated after the formula:

$$n = \frac{t^2 \times p(1-p)}{m^2}$$

n = required sample size

t = confidence level at 95% (standard value of 1.96)

p = estimated prevalence of Bluetongue disease.

m = margin of error at 5% (standard value of 0.05)

In summary each species in the Eastern province were considered a separate population that means we have 4 species (cattle, sheep, goats and camel). Totally, there were four population from which each sample size was 369 that means totally 1476 serum samples.

Collection of blood and Separation of Serum

Blood was collected from the jugular vein in glass tubes and left for 1 hour at room temperature to allow them to clot. After that, samples were left at 4°C overnight to allow the clot to contract. The tubes were centrifuged at 4000 rpm for 20 minutes at 4°C. After that, the serum was removed from the clot by gently pipetting off into a clean tube using a glass Pasteur pipette. Finally, the sera were stored at20°C with complete data label till use.

Detection of BTV antibodies by cEISA:

The IDEXX Bluetongue Competition Ab Test (Scorpius 60 Building F Hoofddorp, 2132 L The Netherland) was used for detection of antibodies specific to the bluetongue virus (BTV) in individual sheep, goat and cattle sera. It is based on competition between the serum to be tested and a monoclonal antibody, which is coupled to the peroxidase and directed to the N-terminal part of the VP7 protein, a major core protein of the BTV (specific for the BT serogroup). Sample sera was added and incubated with antigen coated ELISA plate. After washing and with the addition of conjugate, conjugated antibodies showed color in the absence of anti-VP7 antibodies in test sera when incubated with respective substrate. The optical density (OD) values were measured at 450nm by iMark™ Microplate Absorbance Reader.

Calculation of the sample to negative percentage (S/N%) for each sample was followed according to the following equations:

Calculation of negative control mean absorbance (NC×)	Calculation of S\N percentage for each sample
$NC\times = \frac{NC1\ A450 + NC2\ A450}{2}$	$SN\ \% = 100 \times \frac{sample\ A450}{NC\times}$

Interpretation of results are based on, samples with S/N percentage greater than or equal to 80% are considered negative, samples with S/N percentage greater than 70% and less than 80% are considered doubtful and must be retested and samples with S/N percentage less

than or equal to 70% are considered bluetongue virus antibody positive.

Prevalence and Relative risk estimation

Prevalence of BT among different species was estimated by dividing the number of positive animals by the number of tested animals. The 95% confidence interval (CI) for the prevalence was estimated using the Wald method (Vollset, 1993) as follows:

$$CI = P \pm Z^* \sqrt{\frac{P*(1-P}{N}}$$

Where P is the seroprevalence, Z = 1.96 and N is the number of samples.

The relative risk (RR) of BT infection among different species of examined animals was estimated using the prevalence of infection among cattle as the baseline groups as follows (Hegazy et al., 2009) :

(D1/N1)*(N2/D2)

Where D1 is the number positive animals in a particular animal species and N1 is the total number tested animals of this animal species. D2 is the number of positive cattle and N2 is the total number tested cattle.

Risk factors identification

Data on putative risk factors were obtained at the time of blood sampling. The association between seropositivity and categorical predictor variables; sex, age, breed for each animal species was identified using a univariate logistic regression analysis that was carried out in IBM SPSS statistics for Windows version 21.0. (IBM SPSS Inc, Armonk, NY).

RESULTS

Sero-prevalence of BTV

The overall BTV sero-prevalence among sheep, goat, cattle and camel in the eastern province of KSA are shown in table 1. The study found 8.2% (30/367, CI=5.7 – 11.6) BTV positive cases in cattle. The prevalence of BT was the highest in small ruminants; 50% (CI=45–55) and 46% (CI=41-51) among goats and sheep, respectively. The relative risk of infection among these small ruminants was 6.1 and 5.7 times the risk among cattle. On the other hand, camels showed the lowest prevalence (0.8%, CI=0.2–2.5), the risk of infection among them was 0.1 the risk among cattle.

Risk factors associated with BTV

The univariate logistic regression declared that breed had higher odd's ratio for BTV in sheep, goat and cattle (OR>1). Swakni breed only of sheep was associated with a higher significant seroprevalence of BTV in sheep population ($p<0.05$). Among camels, Swmali breed was considered at higher potential risk factor for BTV other than Baladi breed ($p<0.05$).

Table 1: Prevalence of BTV among sheep, goat, cattle and camel

Species	Number tested	Number positive	Prevalence %	95% CI*	RR** (95% CI) (P-value<)
Sheep	366	169	46	41 - 51	5.7 (3.9 – 8.1) (0.001)
Goat	360	179	50	45 -55	6.1 (4.3 – 8.1) (0.001)
Cattle	367	30	8.2	5.7 – 11.6	1
Camel	370	3	0.8	0.2 – 2.5	0.1 (0.03 – 0.3) (0.001)

* Confidence interval ** Relative risk

Table 2: Univariate logistic regression of BTV among sheep, goat, cattle and camel

Species	variable		Number tested	Number positive	P Value	OR	95% CI
sheep	Breed	Namai	241	102	-	-	-
		Nagdi	22	9	0.90	0.94	0.39 - 2.29
		Swakni	41	26	0.01	2.36	1.19 - 4.69
		Barbari	25	13	0.36	1.50	0.65 - 3.37
		Hari	21	10	0.64	1.24	0.51 to 3.03
		Austurali	16	9	0.28	1.75	0.63 - 4.86
	Sex	Male	308	142	-	-	-
		Female	58	28	0.76	1.1	0.62 - 1.91
	Age	< 1 year	177	78	-	-	-
		> 1 year	189	92	0.88	1.2	0.80 - 1.82
Goats	Breed	Ardia	282	137			
		Shami	42	25	0.19	1.56	0.81 - 3.01
		Gabily	34	17	0.88	1.06	0.52 -2.16
	Sex	Male	300	153	-	-	-
		Female	60	26	0.27	0.74	0.42- 1.28
	Age	< 1 year	236	118	-	-	-
		> 1 year	124	61	0.88	0.97	0.62 - 1.50
Cattle	Breed	Baladi	39	2	-	-	-
		Friesian	328	28	0.47	1.70	0.40 - 7.54
	Sex	Male	290	17	-	-	-
		Female	77	13	0.003	3.26	1.51 - 7.05
	Age	< 1 year	193	13	-	-	-
		1- 3year	112	6	0.63	0.78	0.29 - 2.12
		> 3 years	62	11	0.01	3.0	1.26 - 7.06
Camels	Breed	Baladi	342	0	-	-	-
		Swmali	28	3	0.003	94.01	4.72 - 1870.44
	Sex	Male	251	3	-	-	-
		Female	119	0	0.42	0.30	0.02 - 5.80
	Age	< 1 year	127	0	-	-	-
		1- 3year	175	2	0.40	3.67	0.17 - 77.20
		> 3 years	68	1	0.29	5.67	0.23 - 141.01

Sex was associated with a non-significant seroprevalence of BTV in sheep, goat and camels. Sex was found a significant factor in case of cattle as female cattle was associated with significant (OR>1; p<0.05) higher seroprevalence other than males.

Age had a non-significant with BTV in goat while a significant association (OR>1) was found in case of sheep and camel population however, the association was found non-significant (p<0.05) based on chi-square (Table 2). Cattle of > 3 years old were associated with significant (OR>1; p<0.05) higher seroprevalence other than young cows.

DISCUSSION

This study is considered the 1st cross-sectional large-scale study on the prevalence status of BTV antibodies with assessments of risk factors in the eastern province of Kingdom Saudi Arabia. The reported prevalence rate of bluetongue virus in different species in this study was ٪46 50%, 8.2% and 0.8% in sheep, goat, cattle and camel respectively. Statistical analysis showed highly significant effect of different species on the prevalence of antibodies to bluetongue virus (p<0.0001). It is clear that sheep and goat are the highly susceptible followed by cattle where camel is the least susceptible species to the infection. These results are supported by (Chandel et al.,

2004) who demonstrated that high susceptibility to bluetongue virus infection was reported in sheep and goat while camels are less susceptible. Meanwhile, our results differs completely from that reported by (Yousef et al., 2012) who recorded bluetongue seroprevalence of 65.3% in sheep, 62.5% in goat, 53.4% in cattle and 28.5% in camel in the same study area using the same diagnostic technique. The difference between the two studies can be attributed to the sample size that considered one of the main limitations of this study. The total prevalence of bluetongue virus antibodies in sheep ()46(%in this study ranged from (26.6 – 65.8%) was in contact with (Sreenivasulu et al., 2004) in India (45.7%), (Akhtar et al., 1997) in Pakistan (48.8%), (Yavari et al., 2018) in Iran (46%), (Khezri and Azimi, 2012) (40.87%) in Iran and (Halder et al., 2016), in Nepal (25%). Moreover, 73%, 73.12% and 69.01% were reported by (Housawi et al., 2004) in Saudi Arabia and (Çelik and Şahin, 2019) in Turkey as well as (Gizaw et al., 2016) in Ethiopia respectively. However , a relative lower prevalence rates 34%, 29.59%, 28% and 21.4% were listed in studies of (Shoorijeh et al., 2010) in Iran, (Gür et al., 20008), in Turkey, (Eisa et al., 1979) in Sudan, and (Lundervold et al., 2003) in Kazakhstan respectively.

In this study the apparent prevalence rate in goat was 50% ranged from (24.2 – 68.2%). Similar findings were obtained in other studies of (Gaire et al., 2014; Halder et al., 2016 ; Armin Elbers et al., 2008; and Gizaw et al., 2016) who recorded a prevalence of 31.3% in Nepal, 30.24% in South Bengal, 49.9% in Netherlands, 47% and 60.53% in Ethiopia respectively. On the other hand lower seroprevalence of 5.3% in Sudan, 5.61% and 1.12% in India were estimated by (Eisa et al., 1979; Ravishankar et al., 2005; Shringi, 2005) respectively. The higher prevalence of BTV antibodies in goat than sheep in our study was in line with studies of Mulabbi et al. (2013) 90% seroprevalence in goats in Uganda and (Vahid and Mahin, 2013 and Mozaffari et al. (2014) reported 89.2% and 67.7% positive seroprevalence of goat to BTV in Iran and Afghanistan respectively. In contrast, these findings were different from results of (Gizaw et al., 2016 and Mahmoud and Khafagi, 2014) as they recorded higher seroprevalence of ovine BTV antibodies than in caprine in Nepal and Egypt respectively. This difference in susceptibility to BTV between sheep and goat may be attributed to variation in response to vector of BTV.

Concerning to the lower seroprevalence of bluetongue virus antibodies in cattle in this study (8.2%), it is in contact with (Eisa et al., 1979) 8 % in Sudan. On the opposite side a relatively high prevalence of 67% in Sudan (Hadia, 2014;) and 51.1% & 44.8% in Saudi Arabia were recorded by (Abu Elzein et al., 1998; Yousef et al., 2012) respectively.

Regarding to the seroprevalence of bluetongue virus antibodies in camel was 0.8% in this study. Nearly higher results (1.5%) recorded by (Al-Afaleq et al., 2006) in Saudi Arabia. On the opposite side a relatively higher prevalence rates in camel of 38.34% and 25.7% were recorded in India, 9.33%, in Sudan, 25.7%, in Saudi Arabia, 27.97 % and in Egypt (Chandel et al., 2004; Chauhan et al., 2004; Eisa et al., 1979; Yousef et al., 2012; AbouElnag, 2012) respectively. The lower prevalence of BTV in camels in this study may be explained the lower susceptibility of camel to bluetongue than other species. The variation of the prevalence of antibodies to bluetongue virus between animal species may be attributed to genetic factors and the ecology including the geographical distribution, insect vectors, beside the sensitivity and specificity of the test used in different studies, moreover cannot be ignore, sample size and others factors.

The prevalence and distribution pattern of BTV infection are influenced by several risk factors such as the climate condition, geographical locations and activity of insect vectors. In the present study, the risk factor of breed sex and age were investigated and statistically analyzed in association with BTV. The results revealed non-significant association of different breeds of sheep, goat and cattle and the prevalence of antibodies to bluetongue virus. However, Swakni and Swmali breeds of sheep and camel respectively have positive significant correlation with seroprevelance of BT infection with ($p<0.05$). These findings were in agreement with (Gizaw et al., 2016) who recorded BTV seroprevalence of 72.13% and 59.32% in local and cross breed of sheep respectively, with no significant variation ($p<0.05$). Moreover, Gaire et al., (2014) suggested that foreign breeds of sheep appeared to be more susceptible to BTV compared to indigenous breed although non-significant variation was recorded in breed susceptibility with [OR = 9.04 (95% CI: 3.08- 24.46)]. The discrepancy in seroprevalence of BT infection in correlation with breeds between studies may be attributed to that most of the investigated breeds of sheep and goat in our study are local indigenous breeds which are seem

to be equal in susceptibility to bluetongue virus infection except for swakni sheep which was associated with a higher significant seroprevalence of BTV in sheep population ($p<0.05$).

Considering the effect of the sex on the seroprevalence of BT antibodies, the current study reported that non-significant effect in sheep, goat and camel, where, a significant effect was observed only in cattle. On the other hand, previous reports described that animal sex has a significant influence on the seroprevalence of bluetongue among examined sheep and goats (Sodhi et al., 1981; Formenty et al., 1994; Lundervold et al., 2004; Miller et al., 2010; Gizaw et al., 2016)as they concluded that females had higher seroprevalence of bluetongue virus antibodies. Furthermore, (Gaire et al., 2014) considered that sex is a significant risk factor associated with BT in female sheep and goats.

The univariate logistic regression statistical analysis revealed non-significant effect of the age on the prevalence of antibodies to bluetongue virus in sheep, goat and camel. Similar findings was obtained in sheep by (Shringi, 2005) who concluded that the prevalence of BT antibodies increase with the advanced age of sheep. Additionally, Yavari et al., (2018) found a negative correlation between advances in age and the seroprevalence of BTV antibodies. This is properly due to the more frequent exposure of the animals to bluetongue infection.

CONCLUSION

The current results in cattle concerned a significant effect on seroprevalence of BT in old cattle. Several surveillance were in line with our results as Mohammadi et al., (2012) described that a significant variation in cattle over 3 years old.and Elhassan et al., (2014) as they recorded highly risk correlation of seroprevalence of antibodies to bluetongue virus with advance of cattle age. On the other hand Taylor and Mellor (1994) in Turkey mentioned that the prevalence of BTV infection was less common in sheep up to 2 years and attributed this to the endemic status of the disease in this area. In a different comparative study Mozaffari et al. (2012) in Iran, the rate of seropositive animals decreases in old age animals. The Australian serotypes of the BTV infect sheep higher than 3 years old (Radostitis et al., 2007). There is a positive relation between age and BTV serotype and thus can be explain that the endemic status of the disease and the high maternal immunity level provided to the newborn lambs. The non-significant effect of age reported in between studies may be attributed to the difference of age in the groups is not quite large to demonstrate a significant effect of the age.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

All listed authors have made substantial contributions to the research design, the acquisition, analysis, or interpretation of data; and to drafting the manuscript or revising it critically; and that all authors have approved the submitted version

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