Sero-prevalence study of bluetongue infection in sheep and goats in selected areas of Ethiopia

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Abstract

Bluetongue is an infectious, a non-contagious, arthropod borne viral disease of ruminants and has been reported from most of the tropical and subtropical regions of the world. Seroprevalence study was carried from July, 2013 to January, 2015 to understand bluetongue virus infection in selected areas of sheep and goats found in and around small ruminant breed improvement center. A total of 1420 sera samples from sheep and goats were collected and screened for the presence of group specific bluetongue virus antibody using competitive Enzyme-Linked Immuno-Sorbent Assay(c-ELISA) with sensitivity of 100% and specificity of 99%. The overall seroprevalence of bluetongue virus antibody was 69.01% and 60.53% in sheep and goats, respectively. Seroprevalence of bluetongue ranges from 14.5% (Adami Tulu Research Center) to 91.43 % (Benestemay). Antibody to bluetongue virus was detected from both sheep and goats in all study areas. Result of this study showed that small ruminant dwelling in and around the small ruminant breed improvement centers are exposed to bluetongue virus. In the present study areas there were no observation of clinical cases in any species of animals. This indicates that local breed of animals are resistant to clinical disease of bluetongue infection and or there may be circulation of mild virus strain in the population and so further studies are required to determine the bluetongue serotypes that are circulating in sheep and goats.

Keywords: Bluetongue Virus, ELISA, Seroprevalence, Sheep and Goats, Ethiopia

Introduction

Bluetongue was first described in the Cape Colony of South Africa after merino sheep were introduced into the region in the late 18th century and was subsequently recognized in other parts of Africa, Europe, the Middle East and Indian subcontinent, Americas, Asia, Australia and several islands in the tropics and subtropics (Howell, 1979). Twenty four (likely 25) serotypes of bluetongue virus (BTV) are recognized globally and the virus has now been isolated on all continents except Antarctica (Hofmann *et al*., 2008). Bluetongue virus (BTV) is an arthropod borne Orbivirus in the family *Reoviridae*. There is considerable genetic variability within the serogroup. This arises either by genetic drift of individual gene segments or by reassortment of gene segments when ruminants or the vectors are infected with more than one strain.

The distribution and intensity of infection is determined by the climate, geography and altitude, as these affect the occurrence and activity of the *Culicoides* vectors and the presence of susceptible mammalian hosts. It usually occurred where sheep industries have been established by the introduction of European fine wool and mutton breeds (Davies and Walker, 1974; Howell, 1979; Maclachlan *et al*., 2009). Native sheep have a high level of innate resistance rendering most infections totally benign. Cattle and goats are involved in maintaining the virus wherever it occurs but disease is seldom seen in any breed. Although bluetongue does not appear to be a major economic constraint to African livestock breeders and it adversely affect the success of livestock improvement projects (Haresnape *et al*., 1988).

In endemic areas the infection is always present but clinical disease of the indigenous species is unusual. It can occur with new BTV strains and when non indigenous susceptible species are introduced to the area. Infection occurs in a number of animals but significant disease occurs only in sheep. Infection of bluetongue is also seen in cattle but also recorded in elk, white-tailed deer, pronghorn antelope, camels and other wild ruminants. The disease is not contagious and is transmitted biologically by certain species of *culicoides* (Du Toit, 1944). Bluetongue infection is seasonal because *Culicoides* life depends on the climate change. *Culicoides* breed in damp, wet areas including streams, irrigation channels, muddy areas and fecal runoff areas around farms and habitats for them exist on the majority of farm environments. Cattle are the reservoir

and amplifying host and have a high titer viremia (Sperlova and Zendulkova, 2011).

Cattle appear to be much more attractive to *Culicoides spp*. and this may enhance the importance of cattle as carriers. Seroprevalence increases with age and probably a reflection of increased duration of exposure. All breeds of sheep are susceptible but to varying degrees. Merinos and British breeds are more susceptible than native African sheep (Davies and Walker, 1974; Howell, 1979; Maclachlan *et al*., 2009). The objective of the serosurveillance was to determine the seroprevalence of bluetongue virus in goats and sheep in and around selected sheep and goat breed improvement centers in Ethiopia.

Materials and Methods

Study areas

Melka Warer Agricultural Research Center in Afar Regional State keeps local and exotic goat breeds. Adami Tulu Agricultural Research Center of Oromiya Regional State keeps local and exotic goat breeds, Areka and Jinka Agricultural Research Centers in Southern Nations Nationalities and Peoples Regional State (SNNPRS) which keep local and exotic sheep and goat breeds, respectively, Fafan Agricultural Research Center in Somali Regional State also keeps sheep and goats. These sheep and goats are intended for breed improvement of the local sheep and goats, respectively. Farmers owning sheep and goat in three to four kebeles adjacent to the breeding centers were also included in the survey.

Study population

The populations of interest were those animals which were kept by breed improvement centers for small ruminant in different parts of the country. The breed improvement center kept both local and cross breed sheep (*Ovis iris)* and goats (*Capra hircus*). Local and cross breeds of sheep and goats that were kept tin surrounding kebels were also included.

Study design

This study was carried out from January, 2013 to January, 2015. The animals were sampled using systematic random sampling technique whereas the first

animals selected randomly from the flock and then n^{th} number of animals was sampled until the samples for that flock was reached. Age was determined by observation of the erupted permanent incisors (De launta and Habel, 1986). Animals' less than one year were considered as young while those greater than or equal to one years were considered as adult.

Sampling procedure

About 5 to 7 ml of blood were collected from jugular vein in plain vacationer tube from each animal and put in slightly inclined position in order to separate serum from blood. Then sera were extracted and labeled and stored immediately at 4°C until they arrived at the laboratory. Sera were stored at minus 20°C until testing. Epidemiological data related to clinical syndrome of bluetongue were also collected using questionnaire format prepared for this purpose.

Laboratory test

Sera samples were examined for the presence of BTV group specific antibodies using a competitive enzyme-linked immunosorbent assay (c-ELISA) which was manufactured by (VMRD INC, P.O. box 502, Pullman WA 99163 USA). It has been demonstrated to detect all 24 known serotypes of bluetongue virus (BTV) and not to detect antibody to serotypes 1 or 2 of epizootic hemorrhagic disease virus (EHDV). It has sensitivity and specificity of 100% and 99%, respectively. The test protocol briefly described as 25µl of control and test sera were dispensed according to the test plate lay out and incubate at 21-25°C at room temperature for 15 minutes. After 15 minute25µl peroxidase conjugate was added and incubate for additional 15 minute at 21-25°C at room temperature. The plate was washed three times and then50µl substrate solution was dispensed to each well. The plate was incubated at 21-25°C at room temperature for 10 minute. The reaction was stopped by adding 50µl of stop solution and the plate was read at 650nm optical density. Test sera that were turned optical density greater or equal to 50% mean of negative controls were considered negative while those less than 50% were considered positive.

Data management and analysis

Data collected were entered in to Microsoft Excel spreadsheet and descriptive statistic was done using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Chi-square test and Fisher's exact tests were applied to analyze the association between seroprevalence status among age, sex, breed and specie values less than or equal to 0.05 were considered to be significant.

Results

A total of 1420 goats and sheep sera samples were tested from different part of the country. The overall seroprevalence was 60.53 and 69.1% in caprine and ovine, respectively. Seroprevalence in the centers and surrounding kebeles ranges from 23.65% in Adami Tulu to 91.43% in Benetsemay (Table 1). Seroprevalence of the bluetongue in sheep and goats in the centers was 64.69% (621/960) while in the surrounding kebeles it was 66.3% (305/460). There is no significant variation in seroprevalence of the bluetongue between centers and surrounding kebeles (p=0.6).

Table 1: Seroprevalence of bluetongue in selected areas of Ethiopia

Centers or site	No. of tested	No. positive	Percentage Positive	95 % confidence interval	
Adami tulu	241	57	23.65	15.09	32.21
Ambara	354	266	75.14	70.40	79.88
Areka	144	70	48.61	42.68	54.54
Arsi Negelle	50	34	68.00	63.02	72.98
Bene tsemay	70	64	91.43	87.13	95.73
Doyo Gena	76	52	68.42	63.44	73.40
G/Mekeda	30	24	80.00	75.41	84.59
Fafan	280	205	73.21	68.40	78.02
Jinka	175	154	88.00	83.62	92.38
Total	1420	926	65.21	60.05	70.35

There were significant variation in seroprevalence of bluetongue among age, sex and species but there were no variation in seroprevalence of bluetongue in local and cross breed (p<0.05) (Table 2).

Table 2: Seroprevalence of the bluetongue in different age, sex and breed groups

Category	No of sample tested	No of sample positive	Percentage Positivity	95 % confidence interval		Pearson Chi-Square
Age						
Adult (≥1year)		955	643	67.33	66.23	P-value 0.018
Young(<1year)		465	283	60.86	59.70	
Sex						
Female	Female	1151	784	68.11	65.54	P-value 0.000
Male	Male	269	142	52.79	49.85	
Breed						
Cross	767	455	59.32	57.01	61.63	P-value 0.406
Local	653	471	72.13	70.04	74.22	
Species						
Caprine	636	385	60.53	59.01	62.05	P- value 0.001
Ovine	784	541	69.01	67.60	70.42	
Total	1420	926	65.21	60.05	70.35	

Seroprevalence in kebeles or sites ranges from 14.5% in Adami Tulu Agricultural Research Center to 91.43% at Oleka Kibra in Benetsemay wereda (Table 3).

Table 3: Seroprevalence of bluetongue in selected kebele and farms in agricultural research centers

Farm /Kebele	No. of tested	No. of positive	Percentage positivity	95 % confidence interval	
Rafu Aregesa	50	34	68.0	62.8	73.2
Areka Agricultural Research Center	144	70	48.6	42.4	54.8
Adami Tulu Agricultural Research Center	145	21	14.5	3.0	26.0
Awra Ara	76	52	68.4	63.2	73.6
Fafan Agricultural Research Center	142	110	77.5	72.6	82.4
Golatobi	43	18	41.9	35.2	48.6
Halla Hago	43	25	58.1	52.4	63.8
Jinka Agricultural Research Center	175	154	88.0	83.4	92.6
Kubajane	53	42	79.2	74.4	84.0
Kudhac	42	28	66.7	61.4	72.0
Marta	30	24	80.0	75.2	84.8
Oleka Kibra	70	64	91.4	86.9	95.9
Melka Warer Agricultural Research Center	354	266	75.1	70.1	80.1
Weyso konchoso	53	18	34.0	26.6	41.4
Total	1420	926	65.2	60.05	70.35

Discussion

The detection of antibodies against bluetongue virus in different part of the country in our studies indicates that bluetongue virus infection is endemic among small ruminants. Serological tests indicate that bluetongue virus is circulating in all studied areas. Relatively higher seroprevalence of bluetongue was reported from different areas of the world. The seroprevalnce of 60.53 and 69.1% in caprine and ovine, respectively in our study was lower than that study of Mulabbi *et al*. (2013) reported 90% seroprevalence in goats in Uganda. There were similar studies and findings by Yousef *et al*. (2012) who reported seroprevalence of 54.1% and 53.3% in sheep and goats, respectively, in Saudi Arabia. Different studies show that bluetongue virus infection widely distributed and reported from many countries in the world including Turkey 29.5% by

Gur (2008), India 45.7% by Sreenivasulu *et al*. (2004), Pakistan 48.8% Akhtar *et al*. (1997) and in Iran 89.2% by Vahid and Mahin (2013).

There was higher seroprevalence of bluetongue in ovine (69.1%) than in caprine (60.53%) in our studies. Similarly higher seroprevalence of bluetongue in sheep (17.5%) than goat (14.7%) was reported by Mahmoud and Khafagi (2014) in Egypt. In contrast to our findings higher seroprevalence in goat 67.7 % was reported in Iran Ali *et al*. (2014). This may be due to difference in reaction of ovine and caprine to vector of bluetongue virus. In this study seroprevalence of 72.13% in local breed and 59.32% in cross breed was reported however, there was no statistical variation in seroprevalence of bluetongue in local and cross breed ($p<0.05$). This may be due to equal exposure of both cross and local breed to bluetongue vectors since they managed similarly.

There was significant variation of seroprevalence among age, sex and species ($p < 0.05$). Evidence of rising antibody seroprevalence with age suggests that there were a series of years in which a widespread of infection with bluetongue was occurred Hawel, 1079 and Mohammadi *et al*. (2012). However, this evidence could result either from the continuous presence of the virus or annual re-infections of an external source.

The seroprevalence of bluetongue has been detected among sheep and goats in different areas of Ethiopia indicating serological evidence of exposure to infection to be widely distributed all over the country. Antibody to bluetongue virus was detected in low land (Benestemay) to high land (Doyo Gena). The result of competitive ELISA indicates that bluetongue is prevalent in all studied areas. A clinical case of bluetongue was not observed among animals sampled during clinical examination and so far no cases have been reported in Ethiopia. The absence of clinical disease suggests that indigenous breed of sheep and goats have a high degree of innate immunity Haresnape *et al*. (1988). This phenomenon could be changed if more virulent strains are able to gain entry or if host resistance is to be lowered by cross-breeding. As blood level of the exotic breed increased either through breed improvement or introduction of susceptible population bluetongue could be assumed to be one of the threats to livestock development.

Conclusion

This study indicates that local breed of animals are resistant to clinical disease of bluetongue infection and or there may be circulation of mild virus strain in the population and so further studies are required to determine the bluetongue serotypes that are circulating in sheep and goats in Ethiopia in order to devices control strategy.

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