

# Competitive Enzyme Linked Immuno-Sorbent Assay (c-ELISA) Based Sero-Prevalence of Bluetongue Virus (BTV) on Small Ruminants in Selected Areas of Wolyita, Southern Ethiopia

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## Abstract

The objective of the present study is to assess the sero-prevalence and associated risk factors for small ruminants' bluetongue infection in selected agro-ecology of wolyita zone, southern Ethiopia. Serum samples were collected randomly from the accessed small ruminates and screened for detection of BTV-specific immunoglobulin G (IgG) antibodies using a competitive enzyme-linked immunosorbent assay (c-ELISA). A total of 476 serum samples were tested and 196 (41.17%) were positive for bluetongue virus antibodies. A prevalence rate ranging from 26.53% for the midland altitude to 73.47% for the lowland was recorded. To disease associated risk factor age, and location was recorded using multivariate analysis of logistic regression model. Within species goat 114 (58.2%) and sheep 82 (41.8%) sero positive reactors was recorded. This is the first report indicating the presence of bluetongue virus infection in the area.

## Introduction

Bluetongue is an infectious arthropod-borne viral disease primarily of domestic and wild ruminants. The geographic restriction is in part related to the climatic and environmental conditions necessary to support the *Culicoides* vectors. Most infections with Bluetongue Virus in wild ruminants and cattle are subclinical. Bluetongue is usually considered to be a disease of improved breeds of sheep, particularly the fine-wool and mutton breeds [1].

Bluetongue virus is the type-species of the genus *Orbivirus* in the family *Reoviridae* [2]. It has a 10-segment double-stranded RNA (dsRNA) genome [2,3]. There is at about 26 serotypes worldwide reported, although not all serotypes exist in any one geographic area; for example, 13 serotypes (1, 2, 3, 5, 6, 10, 11, 13, 14, 17, 19, 22, and 24) have been reported in the USA; 8 serotypes (1, 2, 4, 6, 8, 9, 11, and 16) in Europe [1]; and ten Bluetongue virus serotypes (1, 2, 3, 7, 9, 15, 16, 20, 21, and 23) have been detected in Australia through national surveillance programs [3]. Distribution of Bluetongue Virus throughout the world parallels the spatial and temporal distribution of vector species of *Culicoides* biting midges, which are the only significant natural transmitters of the virus, as well as the temperatures at which Bluetongue Virus will replicate in and be transmitted by these vectors [1].

The course of the disease in small ruminates can vary from peracute to chronic, with a mortality rate of 2%–90%. Clinical signs in young lambs are more apparent, and the mortality rate can be high up to 30% [1]. The major production losses include deaths, unthriftiness during prolonged convalescence, wool breaks, and reproductive losses. It causes vascular endothelial damage, resulting in changes to capillary permeability and subsequent intravascular coagulation. This results in edema, congestion, hemorrhage, inflammation, and necrosis. Some affected sheep have severe swelling of the tongue, which may become cyanotic ("blue tongue") and even protrude from the mouth [4].

In many areas of the world, Bluetongue Virus infection in sheep, and especially in other ruminants, is subclinical. Laboratory confirmation is based on virus isolation in embryonated chicken eggs or mammalian and insect cell cultures or on identification of viral RNA by PCR. The identity of isolates may be confirmed by the group-specific antigen-capture ELISA, group-specific PCR, immunofluorescence, immunoperoxidase, and serotype-specific virus neutralization tests, serotype-specific PCR, or hybridization with complementary gene sequences of group- or serotype-specific genes [5].

There is no specific treatment for animals with bluetongue apart from rest, provision of soft food, and good husbandry. Complicating and secondary infections should be treated appropriately during the recovery period. Prophylactic immunization of sheep remains the most effective and practical control measure against bluetongue in endemic regions. Attenuated and inactivated vaccines against Bluetongue Virus are commercially available in some countries. Three polyvalent vaccines, each comprising five different Bluetongue Virus serotypes attenuated by serial passage in embryonated hens' eggs followed by growth and plaque selection in cell culture, are widely used in southern Africa and elsewhere, should epizootics of bluetongue occur. A monovalent (BTV type 10) modified-live virus vaccine propagated in cell culture is available for use in sheep in the USA. Control of vectors by using insecticides or protection from vectors may lower the number of *Culicoides* bites and subsequently the risk of exposure to Bluetongue Virus infection. However, these measures alone are unlikely to effectively halt a bluetongue epidemic and should be regarded as mitigation measures to be used alongside a comprehensive and vigorous vaccination program. Therefore, the objective of the present study are to assess the sero-prevalence and associated risk factors for small ruminants' bluetongue infection in selected agro-ecology of wolyita zone, southern Ethiopia.

## Methodology

### Study area

Study was conducted in wolaita zone two districts namely Humbo ( at Abala Gaffata & Abala Maraka) representing lowland altitude of **GPS N06° 35' 12.1" and E0370 50' 05.0"** Altitude of **1269 mas** and Damota sore ( at Duge Anchucho & Gununo) representing midland altitude of **GPS N06° 57' 43.4" and E037° 40' 53.0"** Altitude of **1995 mas**. Wolaita Zone have a total of 4471.3km<sup>2</sup> land size, of which 51.7% cultivated land, 6.4% cultivable land, 11.9% grazing land and 30% others [6]. The average crude population density is 425 Person/km<sup>2</sup> [7]. Wolaita is located between **N6.40-7.20 and E37.40-38.20**, about 200 K.m.s from the regional town Hawassa and 390 K.m.s south of the capital Addis Ababa on the way to Arba Minch. The area is characterized by bimodal rainfall with highest from July to September and the rest from March to May. The temperature of the area is 20-35oc.

Using Thrusfield, (1995), formula the sample size was calculated on the basis of 50% prevalence with the expected precision of 5%; at 95% confidence interval to increase our precision a total of 476 serum sample was collected from the accessed small ruminates [8]. All the sera were transported on ice and submitted to the Sebeta National Animal Health Diagnosis and Investigation Center (SNAHDIC) where stored at -20°C until tested.

The Bluetongue Competitive ELISA Kit: (BDSL; Biological Diagnostic Supplies Ltd., Surrey, UK) were used. The test is based on the detection of antibodies specific to the highly conserved segment 7 (VP7) of Bluetongue Virus. It is therefore designed to detect infection by any type of BTV and/or vaccination by any vaccine presenting theVP7 antigen. The test was carried out as described in the protocol supplied by the manufacturers, and the percentage inhibition (PI) values were calculated as described by [9]. Samples with PIs equal to or greater than 50% were considered to be positive, and those with PIs of less than 50% were taken as negative.

The data were entered in computer using statistical package for social studies (SPSS) software package for Windows (version 20.0). The data collected were entered into a computer on a Microsoft Excel spread sheet. Associations between the outcome variable (status of Bluetongue Virus infection in small ruminates) and its potential risk factors were first screened in a univariable analysis using Chi-square test. The significant results of the univariable analysis were re-entered in the final model using multivariable analysis. A multivariable model for the outcome variable was constructed using manual stepwise forward logistic regression analysis. BTV infection was considered as the dependent variable and the risk factors as independent variables. Finally, odd ratios and 95% confidence interval (CI) were calculated, and risk factors with a p-value<0.05 were taken as significant association to Bluetongue Virus infection.

### Result

A total of 476 serum samples were collected from Wolyita zone two districts. The serum samples were screened for detection of BTV-specific immunoglobulin G (IgG) antibodies using a competitive enzyme-linked immunosorbent assay (c-ELISA). Serological evidence of BTV infection was observed in 196 out of 476 animals about 41.17% prevalence rate was recorded. For age group 124 (26.1%) pre weaning age of <3 month and 352 (73.9%) weaning age of >3 month serum sample was investigated, and statistical significant test result was

recorded among the age group. Weaned age of >3 month were 1.8 times more likely to be infected with BTV (OR=1.764, CI=[1.091255-2.851325], p-value=0.021). Regarding animal source (location) as a risk factor, it was shown that small ruminates managed under lowland representing agro- ecology (Humbo) 0.27 times at higher risk of being infected with BTV (OR=0.27, CI =[0.1454435 - 0.501151], p value = 0.000).

Risk factor	Number of animals tested	ELISA positive	χ <sup>2</sup>
Sex			
Male	116	48	0.003NS
Female	360	148	
Age			
Pre weaning age of < 3 month	124	38	7.7678**
Weaned age of > 3 month BCS	352	158	
BCS			
Emaciated	87	44	19.893***
Medium	298	100	
Good	91	52	
Location			
Humbo	235	144	59.001***
D/sore	241	52	
Species			
Goat	211	114	23.974***
Sheep	265	82	
***P, 0.001; **P, 0.01; NS-P >0.05			

**Table.1.** Univariate analysis for the association between potential risk factors and BTV infection among small ruminates using Chi-square test.

Risk factor	OR	95.0% CI	P-value
Sex	1.490185	0.9155448-2.425496	0.193
Age	1.763951	1.091255-2.851325	0.021
BCS	1.238733	0.8977044-1.709315	0.193
Location	0.2699799	0.1454435-0.501151	0.000
Species	1.169823	0.6801587-2.012011	0.571

**Table.2.** Multivariate analysis, using logistic regression model, for significant association of risk factors and BTV infection among small ruminates in the areas.

## Discussion

In recent years, the global distribution and nature of BTV infection has changed significantly. Climate change has been implicated as a potential cause of this dramatic event observed globally [10,11,12]. Bluetongue infection constitutes one of the major unresolved veterinary problems in certain breeds of sheep and in North American white-tailed deer [13]. A lot of research efforts have been made to facilitate rapid molecular detection and differentiation of Bluetongue Virus and BTV-related viruses in susceptible ruminants [14]. But there is very little information is available about the epidemiology of Bluetongue Virus in the East Africa including Ethiopia.

The results presented here record the first confirmation of BTV antibody in small ruminates from Wolyita, area in Ethiopia. Occurrence of precipitating antibodies to bluetongue virus in Ethiopia first reported by using c-ELISA in sheep sera collected from different agro-climatic areas was about 46.67% prevalence rate [15]. This is almost similar to the present study. Also a study in West Azarbaijan in Iran 35.9% of samples was sero-positive [16]. A similar situation has been reported in India, where the highest number of BT cases occurred in districts lying in close proximity to BTV affected areas of neighboring states [17]. With prevalence levels of between 57.6% Aradaib and Shringi, (2005) and 9.3% Arun et al., (2014) among sheep flock and other domestic ruminates [18].

The presence of BTV infection in Wolyita and the risks these infected small ruminates pose for native sheep, necessitates the importance of improved surveillance system for this viral pathogen in southern and other part of Ethiopia. The BTV infection rates increased with the increasing of age in the studied flock. When assessing age as a risk factor, there was a significant association between the BTV infection rate and the age of the animal. It was shown that the lambs and kids started to get infected with BTV after the age of above 3 month /weaned age group. At this age, the animals are usually released into the pasture for grazing, where they are likely to be exposed to infected vectors and subsequent BTV infection. We believe that the association of BTV infection and age is probably attributed to frequent exposure of older animals to infected *Culicoides* vectors. Young age groups are usually kept indoors and are well taken care of by the owners from contracting infectious diseases, particularly the insect and tick-borne infections [19]. Our result is in agreement with previous epidemiological surveys, which reported higher risks of older animals for BTV infections [20,21,16]

Environmental changes can influence the incidence, distribution and evolution of infectious diseases, particularly those transmitted by arthropod vectors [22,23]. In our investigation we have recored that agro-ecology is one of the predisposing factor for sero-positivity for small ruminates BTV infection and our result agree with Gaire et al., (2014) report for eco zone.

## Conclusion and Recommendation

The present study indicated that age and the intensity of the insect vector (location) are influential risk factors for Bluetongue Virus infection in small ruminates in the area. Surveillance for Bluetongue Virus infection should be extended to include other susceptible ruminants and to study the distribution of the insect vectors to better predict and respond to a possible Bluetongue Virus outbreak in the region. A major limitation of the current study was that we did not determine circulating serotype(s) of the BTV. A high priority for future studies on BT in the area will be to evaluate the circulating serotype(s)

as well as temporal variation of sero-prevalence by longitudinal studies. So we recommend strengthening of the surveillance system for BT within Ethiopia and to educate farmers about the management and control of this disease.

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