JIMMA UNIVERSITY
COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE
SCHOOL OF VETERINARY MEDICINE

SEROPREVALENCE OF BLUETONGUE IN SMALL RUMINANTS IN SELECTED DISTRICTS OF SOUTH WESTERN ETHIOPIA

M.Sc. THESIS

BY

TEMESGEN ABERA

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Submitted to Jimma University College of Agriculture and Veterinary Medicine, School of Graduate Studies, in Partial Fulfillment of Masters of Science (M.Sc.) in Veterinary Epidemiology

Major advisor: Dr. Molalegne Bitew (PhD, Associate Professor)
Co-advisor: Dr. Yosef Deneke (PhD, Associate Professor)

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APPROVAL SHEET

JIMMA UNIVERSITY

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SCHOOL OF GRADUATE STUDY

As Thesis Research advisors, we here by certify that we have read and evaluated this thesis prepared, under our guidance, by Temesgen Abera entitled “seroprevalence of bluetongue in small ruminants in selected districts of south western Ethiopia”. We recommend that it be submitted as fulfilling the thesis requirement.

Major Advisor: Dr. Molalegne Bitew Signature _____ Date _____

Co-Advisor: Dr. Yosef Deneke Signature _____ Date _____

As member of the Board of Examiners of the M.Sc. Thesis Open Defense Examination, we certify that we have read and evaluated the Thesis prepared by Temesgen Abera Jelebo and examined the candidate. We recommended that the Thesis be accepted as fulfilling the Thesis requirement for the Degree of Master of Science in Veterinary Medicine (Vet. Epidemiology).

_____	_____	_____
Chairperson	Signature	Date
_____	_____	_____
Internal Examiner	Signature	Date
_____	_____	_____
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STATEMENTS OF THE AUTHOR

I, hereby declare that this thesis is my original work and that all source of materials used for this thesis have been well acknowledged. This thesis has been submitted in partial fulfillment of the requirements for master of degree in Veterinary epidemiology from Jimma University College of Agriculture and Veterinary Medicine and is deposited at the university library to be made available to borrower under the rules of the library. I kindly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

Name: Temesgen Abera

Signature: ----------

Place: Jimma University College of Agriculture and Veterinary Medicine

Date of submission: September, 2016

BIOGRAPHY

Dr. Temesgen Abera was born in Hadiya zone South Nation Nationalities People Regional State of Ethiopia on 28 June, 1987 GC. He started his primary and secondary school at Morsito elementary and secondary school from 1994-2003, Misha district. He attended his preparatory education from 2004 to 2005 at Wachamo preparatory school. He joined Haramaya University, College of Agriculture and Veterinary Medicine, School of Veterinary Medicine in 2006 and he was awarded DVM degree in 2010. From 2010 to 2013 he served in South Nation Nationalities People Regional State in Hadiya zone Shashogo district agriculture office animal health and quarantine main work process. He attended his M.Sc. in Veterinary Epidemiology at Jimma University College of Agriculture and Veterinary Medicine, School of Veterinary Medicine, from 2014 to 2016.

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LIST OF ABBRIVIATIONS

ABADU	Arthropod-borne animal diseases research unit
AGID	Agar gel immunodiffusion
ARS	Agricultural research service's
BCS	Body condition scores
BHK-21	Baby Hamster Kidney fibroblasts
BTV	Bluetongue virus
c-ELISA	Competitive enzyme-linked immunosorbent assay
CFT	Complement fixation test
CLPs	Coat like proteins
CSA	Central statistical agency
DISC	Disabled infectious single cycle
DNA	Deoxyribonucleic acid
ds-RNA	Double stranded RNA
EHDV	Epizootic hemorrhagic disease virus
ESGPIP	Ethiopia Sheep and Goat Productivity Improvement Program
NS	Non-structural protein
NVI	National veterinary institute
PAs	Peasant associations
PCR	Polymerase chain reaction
RT-PCR	Reverse transcription polymerase chain reaction
SNNPR	Southern nation nationalities and people's region
ss-RNA	Single stranded RNA

ABSTRACT

Bluetongue (BT) is an infectious, non- contagious disease of ruminants transmitted by Culicoides biting midges and the causative agent is a member of the genus Orbivirus in the family Reoviridae. A cross sectional study was conducted to determine the seroprevalence and associated risk factors of bluetongue infection in small ruminants in the selected districts of South Western Ethiopia: between December 2015 to August 2016. A simple random sampling technique was used to select sheep and goats for serum sample collection. A total of 422 small ruminants serum samples were examined using competitive enzyme-linked immunosorbent assay (c-ELISA) test, 129 (30.6%) at 95% CI (26.2 - 35) of them were found to be seropositive to bluetongue virus. Multivariate analysis indicated that statistically significant in goats (AOR = 2.4, 95% CI (1.538 – 3.91), P = 0.001), adult (AOR= 3.1, 95% CI (1.909 – 5.148), P = 0.001), lowland (AOR = 3.1, 95% CI (1.525 – 6.476), P =0.002) and midland (AOR =2.4, 95% CI (1.320 – 4.319), P = 0.004). Also statistically significant in Bedele (AOR = 2.3, 95% CI (1.213 – 4.340), P = 0.011) were significantly influences the occurrence of bluetongue virus infection in present study districts. Sex and body condition have no statistically significant difference with the seroprevalence of bluetongue virus (P > 0.05). From this study it is concluded that the bluetongue antibodies virus presence in the sheep and goats in all examined districts which indicate serological evidence of exposure to infection was widely distributed all over the study area. These results clearly show the occurrence of the BTV that emphasize the necessity to a well-defined control strategy for preventing and controlling in South Western Ethiopia.

Keywords: Bluetongue, c-ELISA, Risk factors, South West Ethiopia, Seroprevalence, Small ruminants

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1. INTRODUCTION

Ethiopia is believed to have the largest livestock population in Africa with estimated to be 183.04 million of which 56.71 million cattle, 29.33 million sheep, 29.11 million goats, 2.03 million horses, 0.4 million mules, 7.43 million donkeys, 1.16 million camels and 56.87 million chicken. This livestock sector has been contributing considerable portion to the economy of the country, and still promising to rally round the economic development of the country. It is eminent that livestock products and by-products in the form of meat, milk, honey, eggs, cheese, and butter supply etc. provide the needed animal protein that contributes to the improvement of the nutritional status of the people (CSA, 2014/15).

Livestock also plays an important role in providing export commodities, such as live animals, hides, and skins to earn foreign exchanges to the country (CSA, 2014/15). However, the economic benefit derived from the livestock sector in Ethiopia is not commensurate with the potential and the sub-sector remained untapped due to number of constraints, such as climate change, inadequate nutrition, infectious diseases, lack of support services such as extension services, insufficient data with which to plan improved services and inadequate information on how to improve animal breeding, marketing and processing (UN, 2002; Asfaw, 2003).

In addition, shortage of capital, poor infrastructure, lack of enabling policy and legislation, poor animal handling and inadequate facilities at the export level decrease the economic benefit of the country (Alemu and Markel, 2008). The occurrence of infectious and economically important animal diseases in Ethiopia excludes the country from profitable international markets, thereby greatly reducing the country foreign exchange earnings (Livestock Marketing Authority, 2005).

Among the different challenges of livestock, an infectious disease was one of the major. Bluetongue (BT) is an infectious, non- contagious disease of ruminants transmitted by *Culicoides* biting midges and the causative agent is a member of the genus *Orbivirus* in the family *Reoviridae.* It is caused by the bluetongue virus (BTV) and it is classified as a reportable disease by the World Organization for Animal Health (OIE). Clinical disease is often observed in sheep, occasionally in goats, and rarely in cattle (Maclachlan, 2010). Bluetongue virus genome consists of ten double-stranded (ds-RNA) segments coding for seven structural proteins (VP1–

VP7) and four non-structural proteins (NS1–NS3 or NS3A, and NS4). Currently 26 serotypes have been reported throughout the world (Maan *et al.,* 2007; Bhanuprakash *et al.,* 2009) with recent additions of the 25th serotype ("Toggenburg orbivirus") from Switzerland in goat and 26th serotype from Kuwait in sheep and goat (Hofmann *et al*., 2008; Maan *et al*., 2011; Maan *et al*., 2012). There is only low level of cross-protection among the BT virus serotypes and making vaccination strategies and control programmes a daunting task (Hofmann *et al.,* 2008; Eschbaumer *et al.,* 2009; Bitew *et al*., 2013). Bluetongue is multiple vector species disease to the World Organization for Animal Health (Eschbaumer *et al.,* 2009).

Among 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 (Merck, 2014); 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 (David *et al*., 2012). 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 (Merck, 2014). Due to the large number of circulating BTV serotypes in the Mediterranean Basin, it is generally very difficult to predict the serotype for a specific region (Saegerman *et al*., 2008).

The course of the BT disease in small ruminants can vary from per-acute to chronic, with mortality rate of 2% – 90%. Clinical signs in young lambs are more apparent, and the mortality rate can be high up to 30%. 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 (Merck, 2014).

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 (OIE, 2014).

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.

There are insufficient numbers of seroprevalence reports of bluetongue virus in Ethiopia. Even though, these reports from Ethiopia were very few and only focus on sheep except in Wolyita Southern Ethiopia report which included goats and covers small agro-ecological area vis-à-vis huge livestock potential and large landmass of the country.

Bluetongue (BT) disease has socioeconomic importance for community, national and global perspective. Even though, the disease can cause serious costs on sheep, goats and cattle production in different countries for instance in one study in the United States an estimated $125 million annually, manages to survive the winter by reproducing in the insect that transmits it (Merck, 2010). There are no vaccination strategies at hand for the country (Yilma and Mekonnen, 2015).

The significance of the current study is determining occurrence of bluetongue virus infection because seroprevalence of bluetongue was insufficient in Ethiopia and absolutely absent in the current study area. Present study helps to create awareness for occurrence of bluetongue in study area and country in generally. Also helps to differentiates disease from other synonyms diseases.

These all the above underlined facts are the rationale to initiate this research work. Therefore, the present study was undertaken with the following objectives:

Objectives

- To determine the seroprevalence of bluetongue virus in sheep and goats in selected districts, South Western Ethiopia
- To identify the potential risk factors in relation to bluetongue virus infection.

2. LITERATURE REVIEW

2.1. History and Background

Bluetongue virus can be found worldwide within tropical and subtropical climates from approximately latitudes 35° S to 40° N where its vectors, certain species of biting midges, were living (Wilson and Mellor, 2009). However, in North America and China the virus spread even further up to 50^{o}N which is revealed in figure 1 below (Mellor *et al.*, 2008, Bitew *et al*., 2013). The northward extension may be attributable to the effects of global climate change (Purse *et al.*, 2005) and involvement of Palaearctic vectors. But BTV is not found in continent of Antarctica. Endemic areas exist in Africa, Europe, the Middle East, North and South America and Asia, as well as on numerous islands (e.g., Australia, the South Pacific, and the Caribbean). Multiple serotypes can be found in many regions. Outbreaks can occur outside endemic areas, but in most cases, the virus does not persist once cold weather kills the *Culicoides* vectors. Unusually, a serotype 8 virus overwintered for multiple years in central and northern Europe.

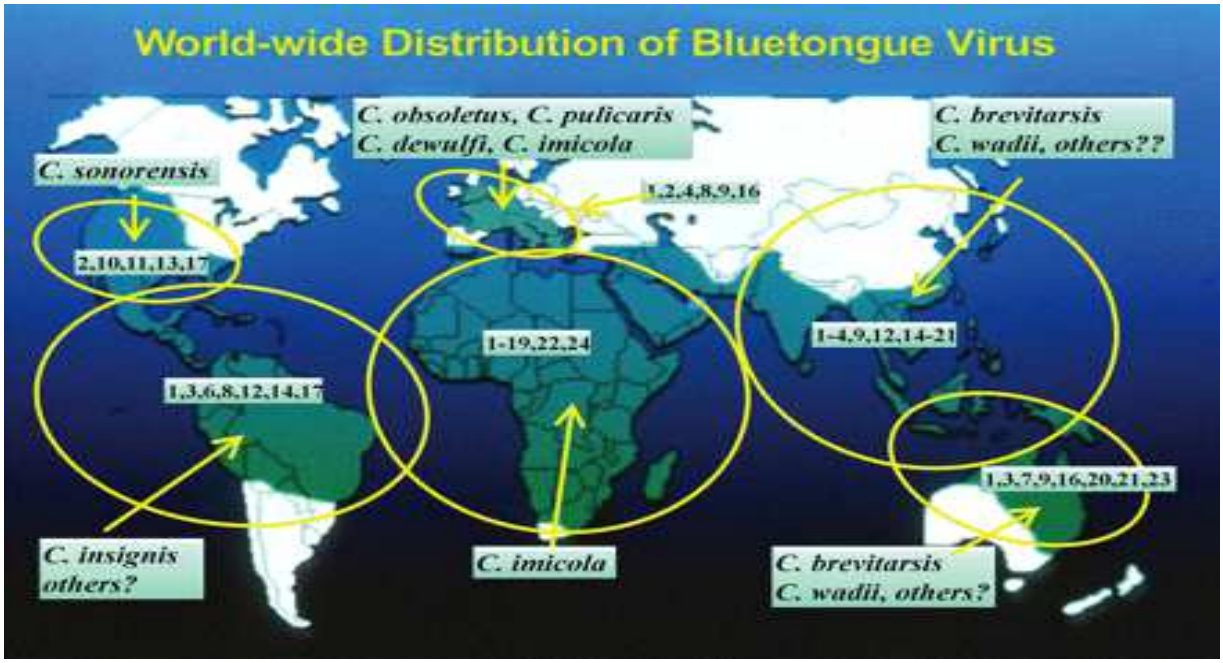

Figure 1: The worldwide distribution of bluetongue virus (BTV) serotypes and the primary *Culicoides* vectors in different geographical regions denoting six predominant BTV episystems (Tabachnick, 2004).

Bluetongue (BT) is a non-contagious, insect-transmitted disease of ruminants, particularly sheep and certain species of non-African wild ruminants (Verwoerd *et al*., 2004, MacLachlan *et al*., 2009). The disease was first described in the Cape Colony of southern 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, the Americas, and Asia. Twenty six (26) serotypes of BTV are recognized globally (Hofmann *et al*., 2008), and the virus has now been isolated on all continents except Antarctica. The traditional global range of BTV has been considered to reside between latitudes of approximately 40–50 N and 35 S; thus the northern limits previously were considered to be southern British Columbia in North America and Kazakhstan and Mongolia in Asia, and the southern limits in Africa, Australia and South America were, respectively, southern South Africa, northern New South Wales, and northern Argentina. The species of Culicoides insects that serve as the principal vectors of the virus differ between regions, as do the constellations of BTV serotypes and genetic strains (topotypes) that occur within each (Tabachnick, 2004; MacLachlan *et al*., 2006).

Bluetongue is characterized by BTV-induced vascular injury that results in haemorrhage and ulceration of the mucous membranes in the upper portion of the gastrointestinal tract; coronitis and laminitis; facial and intermuscular oedema; pleural and pericardial effusion; pulmonary oedema and necrosis of skeletal and cardiac muscle (Verwoerd *et al*., 2004, Schwartz-Cornil *et al*., 2008, MacLachlan *et al*., 2009). BT was infrequently described in cattle prior to emergence of BTV serotype 8 in Europe, however sheep, cattle, goats, camelids (llamas, alpacas), bison, yaks, deer and even zoo carnivores such as Eurasian lynx have all been affected during this ongoing pandemic (Elbers *et al*., 2006, Darpel *et al*., 2007, Jauniaux *et al*., 2008, Mauroy *et al*., 2008, Schwartz-Cornil *et al*., 2008). In addition to causing clinical disease in domestic and wild ungulates, BTV serotype 8 infections has caused reproductive failure amongst pregnant cattle and sheep and a high rate of vertical virus transmission with teratogenic defects in con- genitally infected calves (Wouda *et al*., 2008, Wouda *et al*., 2009, De Clercq *et al*., 2009). BTV infection of ruminants results in prolonged, but not persistent cell associated viraemia (MacLachlan *et al*., 2009).

Highly sensitive and specific serological (c- ELISA) and virus detection (quantitative PCR) assays are now widely available to confirm the presence or absence of BTV infection in animals (Hoffmann *et al*., 2009). Furthermore, infection with one BTV serotype confers long-term (likely life-long) immunity to the homologous serotype. Thus, livestock can safely be moved into BTV-free regions regardless of the status of the exporting region, but only with stringent adherence to appropriate testing and quarantine practices as prescribed by the world organization for animal Health (OIE) (Anonymous, 2009).

2.2. Evolution

There is extensive genetic heterogeneity of field strains of BTV, and this genetic variation is likely responsible for differences in the virulence and other biological properties of individual field strains of the virus. This diversity arises as a consequence of genetic shift and genetic drift, the latter as a result of reassortment of viral genes during mixed infections of either the vertebrate or invertebrate hosts following infection with more than one virus sero- type or strain (Bonneau *et al*., 2004). Variation in the nucleotide sequence of each of the 10 dsRNA segments of the BTV genome occurs as a result of the complex process of quasi species evolution and founder effect during alternating passage of BTV in its ruminant and insect hosts (Bonneau, 2001). Furthermore, negative (purifying) selection of individual BTV genes occurs over time following the incursion of novel viruses into new regions, creating genetically distinct region-specific clusters (topotypes) of certain virus genes (Balasuriya *et al*., 2008). Genetic differences are responsible for clearly documented differences in phenotypic properties (such as virulence) of BTV strains, even of the same serotype, and can complicate the design and use of nucleic acid based virus detection methods such as reverse transcriptase polymerase chain reaction (RT-PCR) assays. The genetic determinants of BTV virulence remain uncharacterized, however, as do those that presumably are responsible for, or associated with, emergence of the virus in new regions and in novel vector species (Balasuriya *et al*., 2008).

2.3. Global emergence

The global distribution and nature of BTV infection has changed significantly in recent years, and climate change has been implicated as a potential cause of this dramatic event, especially in Europe (Purse *et al*., 2005, Purse *et al*., 2008, Gale *et al*., 2009).

2.3.1. Europe

According to MacLachlan and Guthrie (2010), until recently, only transient incursions of single serotypes of BTV had occurred in Europe but, since 1998, at least 8 different serotypes of BTV have emerged and persisted in Europe causing substantial economic losses through mortality and reduced productivity of affected animals, the requirement for mass vaccination of susceptible livestock, and restrictions on the movement and trade of animals from affected regions (Wilson *et al*., 2009). A current distribution map of restriction zones for individual BTV serotypes within Europe is available at http://ec.europa.eu/food/animal/diseases/control measures/ bt restricted zones map.jpg. Five BTV serotypes (1, 2, 4, 9 and 16) initially invaded the Mediterranean basin by extension from adjacent regions of either North Africa or the Middle East. Although these 5 serotypes have persisted in extensive portions of the Mediterranean basin, only BTV serotype 1 has since spread to northern Europe. Subsequent to the spread of BTV serotypes 1, 2, 4, 9 and 16 within the Mediterranean basin, BTV serotypes 6, 8 and 11 all appeared in northern Europe (Benelux) after 2006; whereas the strains of BTV serotypes 6 and 11 are closely related to South African live attenuated vaccine viruses (De Clercq *et al*., 2009), the origin of the highly pathogenic strain of BTV serotype 8 that has now spread throughout much of Europe remains unknown. Since its appearance in northern Europe, this virus has now spread to the Mediterranean basin, Scandinavia, and the Middle East. This strain of BTV serotype 8 exhibits several distinctive features that are unusual amongst other field strains of BTV, notably its ability to cross the ruminant placenta with high frequency (Wouda *et al*., 2009).Vertical transmission has previously been considered to be largely or exclusively a property of BTV strains modified by growth in embryonated eggs or cell culture (MacLachlan *et al*., 2009).

An additional remarkable feature of the BT pandemic in Europe is the recognition of several apparently new vector species, including Palaearctic species such as *C. chiopterus*, *C. dewulffi*,

C. obsoletus, C. scotius and *C. pulicaris* (Wilson *et al*., 2009). These insects were all resident in Europe long before the emergence of multiple serotypes of BTV, suggesting that environmental changes may have been responsible for their recent ability to serve as efficient vectors of the virus (Purse *et al*., 2005, Purse *et al*., 2008). While it is proposed that recent environmental changes have facilitated expansion of the range of known vectors such as *Culicoides imicola*, the traditional Asian/African vector, this opinion is strongly disputed by others. Importantly, several of the Palaearctic *Culicoides* species that are now efficiently transmitting BTV in the absence of known vectors such as *C. imicola*, also occur throughout the Holarctic region that includes North America (Borkent *et al*., 2009). This raises the unnerving possibility that the viruses that have recently spread throughout extensive portions of Europe, especially the unusual and highly virulent strain of BTV serotype 8, might even further expand their global range in the future.

2.3.2. North America, Australia, Asia and the Middle East

Sharma *et al*. (2016) estimated the seroprevalence of antibodies to bluetongue virus among domestic ruminants of Grenada. Sera samples collected from 133 cattle, 314 goat and 481 sheep were tested using competitive Enzyme linked immunosorbent assay (c-ELISA). Of the total of 928 samples tested, the overall BTV seroprevalence was 78.4% (95% confidence interval). The seropositivity of ovine, caprine and bovine was found to be 71.7%, 80.2% and 98.5%, respectively. Based on this study it is evident that bluetongue virus is endemic in Grenada.

Bluetongue virus is the prototype of the genus *Orbivirus,* family *Reoviridae* (Oryan *et al*. 2014). Bluetongue (BT) occurs throughout the temperate and tropical regions of the world, in an area that parallels the distribution of the competent vector, *Culicoides* spp. There is considerable genetic variability within the serogroup of BTV so that 26 serotypes have been recognized worldwide. A total of 1,010 blood samples from 820 sheep and 190 goats, with history of abortion and mucosal diseases, from 25 Counties of Fars Province, southern Iran, using competitive enzyme-linked immunosorbent assay (c- ELISA), for anti-bluetongue virus antibodies. A total of 772/ 1,010 (76.4 %) samples, 162/190 (85.3 %) goats and 610/820 (74.4%) sheep, were found seropositive for BTV. The enzootic nature of BTV in southern areas of Iran is supported by climatic factors that favour the maintenance and recirculation of the virus in its

vertebrate and non- vertebrate hosts. This investigation evaluates the present status of BT in southern Iran.

Bitew *et al*. (2013) determined seroprevalence on the basis of antibodies to VP7 protein of bluetongue virus by competitive enzyme linked immunosorbent assay. Ninety one sera sample were obtained from sheep and goats. The animals were observed for clinical signs of BTV infection and serum samples were obtained from all animals for c- ELISA. Further, blood samples were collected from the c-ELISA positive animals. Out of 91 animals tested, 26 (28.6%) were found to be seropositive by c-ELISA. Goats showed more seropositivity to bluetongue as compared to sheep. It was worth enough to conclude that higher seroprevalence among goats indicated that goats would be the most important animals in the epidemiology of BTV with less clinical manifestation due to development of acquired immunity as the result of continuous exposure.

Yapici *et al*. (2014) determined antibodies against Bluetongue Virus (BTV), Border Disease Virus (BDV) and Peste des Petits Ruminants virus (PPRV), for the first time in Kyrgyzstan. Six hundred fifty-five sera samples were collected from healthy sheep (Jaydara breed) from different regions of Kyrgyzstan (144 from Issik Gol region, 208 from Narin region, 189 from Talas region and 114 from Çuy region). Commercially available competitive ELISA kits were used to detect antibodies from samples. Seroprevalence was found to be 36.94%, 7.32% and 35.11% against BTV, BDV and PPRV infections, respectively. This is the first report on seroprevalence of viral infections for BTV, BDV and PPRV in the Jaydara breed of sheep within Kyrgyzstan in central Asia.

According to Khezri & Azimi (2013), epidemiology of Bluetongue virus infection was referred as poorly defined in many parts of the world, including a wide range of Asia and the Middle East. These authors reported the results of a Bluetongue serological survey in sheep from some provinces of Iran during 2007-2008. Nine hundred ninety-six sheep sera were collected from eight provinces in Iran and tested for Bluetongue virus specific antibodies using c-ELISA. The results showed that the Bluetongue virus seroprevalence of sheep over the entire study areas was 34.93%, with the highest and lowest prevalence seen 64.86% in West-Azerbaijan and 12.1% in Qom, respectively. The results demonstrated a high prevalence of Bluetongue antibodies in

Iranian sheep, giving serological evidence of extensive exposure to Bluetongue virus infection in some provinces of the country.

Khezri *et al*. (2016) determined seroprevalence of bluetongue virus (BTV) in sheep in West and Northwest provinces of Iran. Due to its economic impact, bluetongue (BT) is an Office of International des Epizooties (OIE)-listed disease. Seven hundred fifty six sera samples collected during 2007-2008, were available. Sera were tested with competitive enzyme-linked immunosorbent assay (C-ELISA). The seroprevalence in sheep was 40.87%. The rate of positivity in sheep in West and Northwest was 46.10% and 33.75%, respectively. The highest prevalence of antibodies in serum was in West Azerbaijan (64.86%), and lowest was in Ardabil (23.77%).

Sabaghan *et al*. (2014) bluetongue (BT) is a viral disease of ruminants transmitted by Culicoides biting midges and has the ability to spread rapidly over large distances. Among the numerous diseases of ruminants, BT has gained considerable importance in recent years as one of the best examples of the effects of climate change on disease spread. Sheep are major livestock species in Iran, but studies of BT have not gained the priority compared to other diseases. Two hundred sixty two apparently healthy sheep sera were collected during the year 2011. The collected sera of the animals were screened with competitive enzyme like immunosorbent assay (c- ELISA). Reported from these authors 203 (77.48%) out of 262 sera tested were seropositive to BTV antibodies.

Mozaffari *et al*. (2014a) reported 84.62% an overall seroprevalence of bluetongue antibodies in small ruminants in Southeast Iran. From 852 sera from sheep and goats within five districts in Southeast Iran was tested using competitive enzyme linked immunoassay (c-ELISA). When computed with in species seroprevalence of 48.7% and 92.7% in sheep and goats respectively. Results show that exposure to infection is widely distributed in southeast Iran.

Reported by Lundervold *et al*. (2004) from Kazakhstan from 958 sera collected from livestock animals (cattle, sheep and goats) with focused on seroprevalence for BT virus was 23%, and seropositive animals were widespread suggesting endemicity, despite the disease not having being previously reported.

According to Hasanpour *et al*. (2014), from this study was conducted on 198 sheep blood samples from 20 sheep flocks in Tekab city in the West Azarbaijan province in the West North of Iran with the objective was to determined seroprevalence and distribution of serum antibodies to Bluetongue virus (BTV) in a sample. Competitive ELISA was applied to detect antibodies and an overall seroprevalence were reported that 35.9%. From this study it is concluded that the bluetongue antibodies presence in the sheep sera from Tekab area and can to create a disease.

The report revealed by Mozaffari *et al*. (2014b) to describe the seroprevalence rate of bluetongue virus in goat flocks in southeast of Iran. The blood samples were collected randomly from herds of southeast of Iran. A total of 93 sera samples were collected between 2011 and 2012. Antibodies to BTV in sera were detected by using a commercial competitive ELISA 3 according to manufacturer's instructions. The seroprevalence rates were 67.7% for goats. Within a herd, prevalence of BTV seropositive animals ranged from 33.3% to 100.0%. All goat flocks were positive to BTV antibodies. This study describes a high seroprevalence rate of bluetongue virus in goat flocks in southeast of Iran for the first time.

Five BT virus serotypes have long been recognized as enzootic in North America, specifically serotypes 10, 11, 13 and 17 occur throughout much of the continent coincident with the distribution of C. sonorensis, whereas serotype 2 is restricted to the south-eastern USA apparently because of its reliance on C. insignis for transmission (MacLachlan *et al*., 2006, Tabachnick *et al*., 2004). Since 1998 ten additional previously exotic serotypes (1, 3, 5, 6, 9, 12, 14, 19, 22 and 24) have also been isolated in the south-eastern USA, but without any obvious associated disease outbreak. The majority (but not all) of these virus serotypes were previously identified in the Caribbean/Central America ecosystem, as determined by either virus isolation or serological studies on ruminants in the region (Tabachnick *et al*., 2004). Thus, the BTV serotypes that recently have encroached into the south-eastern USA have likely originated in the adjacent tropical ecosystem to the immediate south, where C. insignis and possibly other species such as *C. pusillus, C. furens, C. filarifer and C. trilineatus* are the vectors of numerous BTV serotypes (MacLachlan *et al*., 2010). The importance of vector species other than C. sonorensis is largely undetermined in North America, and there has been little or no comprehensive, prospective surveillance of the viruses and vectors present in the USA and Caribbean basin/Central America for several decades.

Additional novel serotypes of BTV have also recently invaded Israel and Australia, countries that previously had stable annual cycles of BTV infection (virus serotypes and distribution), as shown by ongoing surveillance over many years. Specifically, BTV serotypes 2, 4, 6, 10, 16 previously were recognized in Israel, but serotypes 5, 8, 15 and 24 have all recently emerged in the country and the occurrence of serotype 24 in particular was associated with very high mortality in infected sheep. Interestingly, BTV serotype 24 also recently appeared in the south- eastern USA without any associated disease out- break, further confirming that serotype alone does not determine the virulence of individual field strains of BTV (MacLachlan *et al*., 2009). Similarly, 8 serotypes (1, 3, 9, 15, 16, 20, 21, 23) of BTV previously have been identified in Australia, of which only two (serotypes 1 and 21) annually spread into eastern Australia as far south as northern New South Wales; however, BTV serotypes 2 and 7 were recently identified for the first time in the Northern Territory of Australia.

Finally, BTV infection is enzootic in the Indian subcontinent with the presence of at least 21 serotypes of the virus; however there are multiple recent, largely unexplained, reports of the emergence of virulent BT disease amongst indigenous breeds of sheep.

Table 1: Summary review of seroprevalence of BT virus in abroad or out of Africa continent.

Authors and year	Study animals	Total sample	Test	Seroprevalence (%)	Country
Bitew *et al*. (2013)	Small ruminants	91	c-ELISA	28.6	Uttar Pradesh, India
Hasanpour *et al*. (2014)	Sheep	198	c-ELISA	35.9	Tekab area in Iran
Mozaffari *et al*.(2012a)	goat		c-ELISA	67.7	Southeast Iran
	sheep		c-ELISA	6.57	
Mozaffari *et al.* (2014a)	overall	852	c-ELISA	84.62	Iran
	Sheep			48.7	
	Goats			92.7	
Mozaffari & khalili (2012)	Small ruminant		c-ELISA	48.72	Iran
Moneer (2013)	Overall		c-ELISA	16.5	

	sheep			23.5	
	goats			10.9	
Sabaghan *et al.* (2014)	sheep	262	c-ELISA	77.48	Iran
Khezri & Azimi, (2012a)	sheep		c-ELISA	51.85	Kurdistan province of Iran
Khezri & Azimi, (2013)	sheep	996	c-ELISA	34.93	Iran
Khezri & Azimi, (2016)	sheep	756	c-ELISA	40.87	Iran
Lundervold *et al*. (2004)	Rum.LSK	958	c-ELISA	23.0	Kazakhstan, central Asia
Yapici *et al*. (2014)	Sheep	655	c-ELISA	36.94	Kazakhstan, central Asia
Sharma *et al*. (2016)	Overall	928	c-ELISA	78.4	
	Ovine	481		75.73	Grenada
	Caprine	314		84.61	
	Bovine	133		98.5	
Shoorijeh *et al*. (2010)	Sheep		c-ELISA	37.7	west Azerbaijan, Iran
Sadri (2012)	Small ruminant	981	c-ELISA	55.9	Azerbaijan
Sreenivasual (2004)	Small ruminant			45.7	India
Gur (2008)	Small ruminant			29.5	Turkey
Yousef *et al*.(2012)	Sheep	962		54.1	Saudi Arabi
	goat	783		53.3	
	Cattle	321		44.8	
	Camel	228		25.7	
	Overall	4845		47.3	
Garcia *et al*. (2009)	Small		c-ELISA	41.0	Spain

	ruminant				
Oryan *et al*. (2014)	Small ruminant	1010	c-ELISA	76.4	Fars provinces, Iran

Source: Own study 2016

2.3.3. Africa

Bluetongue was first reported at the end of the 18th century in South Africa after an import of fine-wool sheep from Europe. It was first referred to as fever, malarial catarrhal fever of sheep or epizootic malignant catarrhal fever of sheep. In 1933 it was first diagnosed in cattle and, because its clinical signs were similar to those of foot and mouth disease (FMD), it was called pseudo-foot-and-mouth disease, seerbeck or sore-mouth (Vellema, 2008). “Bluetongue”, the name used today, has been derived from the African “bloutong“, which was coined by South-African farmers who noticed tongue cyanosis in seriously diseased animas (MacLachlan *et al*., 2009). It was the first to report that the infecting agent was a filterable virus (Mehlhorn, 2008; Vellema, 2008).

Bluetongue viruses from the Western lineages circulate in Africa, the Caribbean and the Americas, whereas those from eastern lineages are endemic in Asia, Indonesia and Australia. In Africa, Bluetongue has been known in South Africa for over a hundred years and endemic in wild ruminants since antiquity (Maclachlan, 2011). To date 21 of the 26 known BTV serotypes have been isolated from sheep in South Africa. In Africa, *C. imicola* is the reported vector of serotypes 1-15, 18, 19, 22, 24 and 25.

The invasion or attack of BTV serotype 2 into North Africa (Algeria, Libya, Morocco and Tunisia) has been confirmed. The way that BTV entered and spread within the North African region remains unclear (Maclachlan, 2011).

In Eastern Africa the information is still unclear. In one study by Toye *et al.* (2013) in western Kenya reported the presence of bluetongue virus (BTV) and Epizootic Haemorrhagic Disease virus (EHDV) in calves. Serum was analyzed for BTV and EHDV antibodies. The population seroprevalence for BTV and EHDV for calves at 51 weeks of age were estimated to be 94.2% and 63.7%, respectively, indicating high levels of circulating BTV and EHDV in this study area.

When 99 calves were tested for BTV and EHDV RNA by real-time RT-PCR, 88.9% and 63.6% were positive, respectively. Eight samples positive for BTV RNA were serotyped using 24 serotype-specific real-time RT-PCR assays. Nine BTV serotypes were detected, indicating that the cattle were infected with a heterogeneous population of BT virus.

According to Moustafa *et al*. (2016), from a total of 2871 blood samples were collected (450 cattle, 1932 sheep and 489 goats) from 225 randomly sampled herds. The results show an overall herd level seroprevalence of 16.44%, recorded 21.3% for cattle and 13.33% for small ruminants. At the individual level; our results revealed an overall seroprevalence of 6.96% (200/2871); 13.7% (62/450) for cattle and 5.70% (138/2421) for small ruminants by c-ELISA was used to detect antibodies from sera as an indicator of exposure to bluetongue virus (BTV).with aim to indicated that transhumance, mixed herds, presence of wetlands nearby the herds and lack of Culicoides controls strategies were the major risk factors for bluetongue seropositivity in Algerian ruminant herds.

According to Adam *et al*. (2014), bluetongue virus (BTV) causes febrile disease in sheep and a fatal hemorrhagic infection in North American white tailed deer. However, in cattle the disease is typically asymptomatic and no clinical overt disease is associated with bluetongue infection. Bluetongue virus activity has been detected in Khartoum, Sennar and South Darfur states of the Sudan. Currently, no information is available in regard to previous exposure of livestock to Bluetongue virus in North Kordufan State, the largest livestock producing region in the country. The present study was conducted to determine the prevalence of bluetongue antibodies and to identify the potential risk factors associated with the presence of bluetongue antibodies among cattle in North Kordufan State, Sudan. A total of 299 bovine blood samples were collected randomly from six localities in North Kordufan State and were tested by enzyme linked immunosorbent assay (ELISA) for detection of BTV specific immunoglobulin G (IgG) antibodies.

The serological evidence of Bluetongue virus infection was observed in 58 out of 299 cows, accounting for a 19.4% prevalence rate among cattle in North Kordufan State. Older cattle (> 2 years of age) had four times the odds to be infected with BTV compared to young cattle. Application of preventive measures, such as spraying or dipping with insecticide protects cattle

against Bluetongue infection. Application of vector control measures decreased the odds for bluetongue seropositivity by seven times. The results of this study indicated that age and application of routine insecticides are influential risk factors for seroprevalence of Bluetongue in cattle. Surveillance of Bluetongue virus should be extended to include other susceptible animals and to study the distribution of the insect vectors in the region to better predict and respond to BTV outbreak in the State of North Kordufan, Sudan (Adam *et al*., 2014).

Table 2: Summary review of seroprevalence of BT virus in Africa

Authors and year	Study animals	Total sample	Test	Seropreval ence (%)	Country
Toye *et al*. (2013)	Calves	99	real-time RT-PCR	94.2	East Africa, Western Kenya
Moustafa *et al*. (2016)	Small ruminants	2421	c-ELISA	5.70	Algeria
Steyn *et al*. (2015)	cattle	1260	c-ELISA	95.7	Mnisi, South Africa
Adam *et al*. (2014)	cattle	299	c-ELISA	19.4	North Kordutan state, Sudan

Source: Own study 2016

2.3.4. Ethiopia

In Ethiopia the status of bluetongue virus and culicoides vectors involvement and transmission has not been well explored. This could be due to the bluetongue disease is misdiagnosed with other highly prevalent diseases like Orf (contagious pustular dermatitis), Foot and mouth disease, Acute haemonchosis, Facial eczema, *Oestrus ovis* infestation, Pneumonia, plant poisoning, Salmonellosis, Sheep pox and Peste des Petits Ruminants (PPR).

According to Woldemeskel *et al*. (2000), 90 sera samples collected from indigenous sheep were tested in which 42 (46.67%) were positive for bluetongue virus antibodies. This was first reported seroprevalence of bluetongue virus infection in Ethiopia by using competitive enzyme-linked immunosorbent assay (c-ELISA). A prevalence ranging from 9.67% for sheep sampled in the highland to 92.85% for sheep sampled in the lowland was recorded. The prevalence correlated with the probable distribution of the *Culicoides* vector.

The report revealed by Gulima (2009) who conducted seroepidemiological survey of bluetongue virus in indigenous Ethiopian sheep in northwestern Ethiopia using competitive enzyme-linked immunosorbent assay was used to detect anti-bluetongue virus antibodies. From 860 sera samples were collected from indigenous sheep and three agro-ecological zones including lowland, mid-altitude and highland. An overall seroprevalence of bluetongue virus antibodies in the indigenous sheep in the study areas at individual animal and flock levels were 34.1% and 65.4%, respectively. Individual animal level bluetongue virus antibody seroprevalence was highest in lowland 53.4%, followed by mid-altitude 37.2%, and highland 15.5%. Regarding to age specific seroprevalence lower in yearlings less than 2 years with 24.2% to higher in old sheep greater than 5 years with 48.0%

According to Yilma and Mekonnen (2015), a total of 476 serum samples tested that were collected from small ruminants in selected areas of Wolyita, Southern Ethiopia in which an overall 196 (41.17%) seropositive for bluetongue virus antibodies were reported. Serum 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. When compared with agro-ecology, 26.53% for the midland altitude to 73.47% for the lowland was recorded.

Table 3: Summary review of seroprevalence of BT virus in Ethiopia

Authors & year	Study animals	Total sample	Test	Seroprevalence (%)	Region
Gulima (2009)	Sheep	860	c-ELISA	34.1	Amhara region, Ethiopia
Woldemeskel *et al*. (2000)	Sheep	90	c-ELISA	46.67	Central Ethiopia
Yilma & Mekonnen (2015)	Sheep & goats	476	c-ELISA	41.17	Wolyita zone, Southern Ethiopia.

Source: Own study 2016

2.4. Environmental Persistence

Bluetongue virus cannot persist in the environment, as it is unable to persist outside of the vector species or host species. Researchers at the Agricultural Research Service's (ARS) Arthropod-Borne Animal Diseases Research Unit (ABADRU) have found that the biting midges are either unable to survive or are dormant during the cold winter months. Table 4 presents other environmental persistence characteristics of BTV (OIE, 2009).

Table 4: Resistance of bluetongue virus to physical and chemical action

Action	Resistance
Temperature	Inactivated by 50°C/3 hours; 60°C/15 minutes
PH	Sensitive to pH < 6.0 and > 8.0
Chemicals/Disinfectants	Inactivated by β-propiolactone, iodophores and phenolic compounds
Survival	Very stable in the presence of protein (e.g., has survived for years in blood stored at 20°C)

Source: Technical Disease Card for Bluetongue (OIE, 2009)

2.5. Overwintering

The survival of virus from one "vector season" to the next is called "overwintering", but the mechanism involved is still poorly understood. However, BTV can survive in the absence of adult vectors for 9 to 12 months of cold weather in an infected host with no detectable viraemia, disease or sero-conversion (Osmani *et al*., 2006; Wilson *et al*., 2007). One way in which overwintering may be achieved is by the infection of adult vectors (Wilson *et al*., 2008). Although the average life span of these is usually 10 to 20 days, they can occasionally live for up to 3 months (Lysyk and Danyk, 2007). This suggests that under favorable conditions some biting midges can live long enough to survive the period between two vector seasons (Wilson *et al*., 2008). Possibilities for BTV to survive at different stages of the *Culicoides* life cycle have also been investigated. In some instances, the virus could overwinter in cattle owing to prolonged BTV viraemia, which can occasionally last up to 100 days, or due to latent BTV infection. Another mechanism suggested for BTV overwintering is transplacental infection (De Clercq *et al*., 2008; Menzies *et al*., 2008; Backx *et al*., 2009; Darpel *et al*., 2009; Lewerin *et al*., 2010; Santman-Berends *et al*., 2010). Pregnancy in cattle is long enough for BTV to survive during a period free of competent insect vectors (Wilson *et al*., 2008). Mechanical vectors may also be involved in virus overwintering; BTV has been isolated from the sheep ked and some tick

species (Bouwknegt *et al*., 2010), which are arthropod species living much longer than *Culicoides* midges. In addition, the trans-stadial passage found in hard ticks and transovarial passage in soft ticks suggests their role in virus transmission (Bouwknegt *et al*., 2010). Mechanical vectors should therefore be regarded as potential reservoirs for BTV (Wilson *et al*., 2008; Bouwknegt *et al*., 2010).

2.6. Transmission

Bluetongue virus is transmitted by biting midges of the *culicoides* genus (*Diptera: Ceratopogonidae*). Among 1,400 species of midges only 20 *culicoid* species are known to be involved in transmission of bluetongue disease (Figure 3).

Figure 2: Blood feeding *culicoides* Midges (Wilson *et al*., 2008).

In addition to biting midges, BTV has been isolated from some arthropods, for example, sheep ked (*Melophagus ovinus*) or some species of ticks (Bouwknegt *et al*., 2010) and mosquitoes. However, these are mechanical vectors with only a negligible role in disease epidemiology. It can be directly transmitted from one animal to another through semen and transplacentally. Bluetongue can also be spread by live attenuated vaccines against BTV, or even by vaccines against other antigens contaminated with BTV (Evermann, 2008). To date 26 distinct internationally recognized serotypes (based on the lack of cross neutralization) of the virus have

been identified. Cattle and goats are major hosts of the virus, but in these species infection is usually asymptomatic despite high virus levels, allowing the disease to circulate in the absence of any symptoms. Sheep and deer are usually the only species to exhibit symptoms of infection. The manifestations of bluetongue range from an unapparent to a fatal outcome depending on the serotype and strain of the virus, the species, breed and age of the infected animal; older animals are generally more susceptible (Elbers *et al*., 2008). Bluetongue typically occurs when susceptible animal species are introduced into areas with circulating virulent BTV strains, or when virulent BTV strains extend their range to previously unexposed populations of ruminants (Zientara *et al*., 2010).

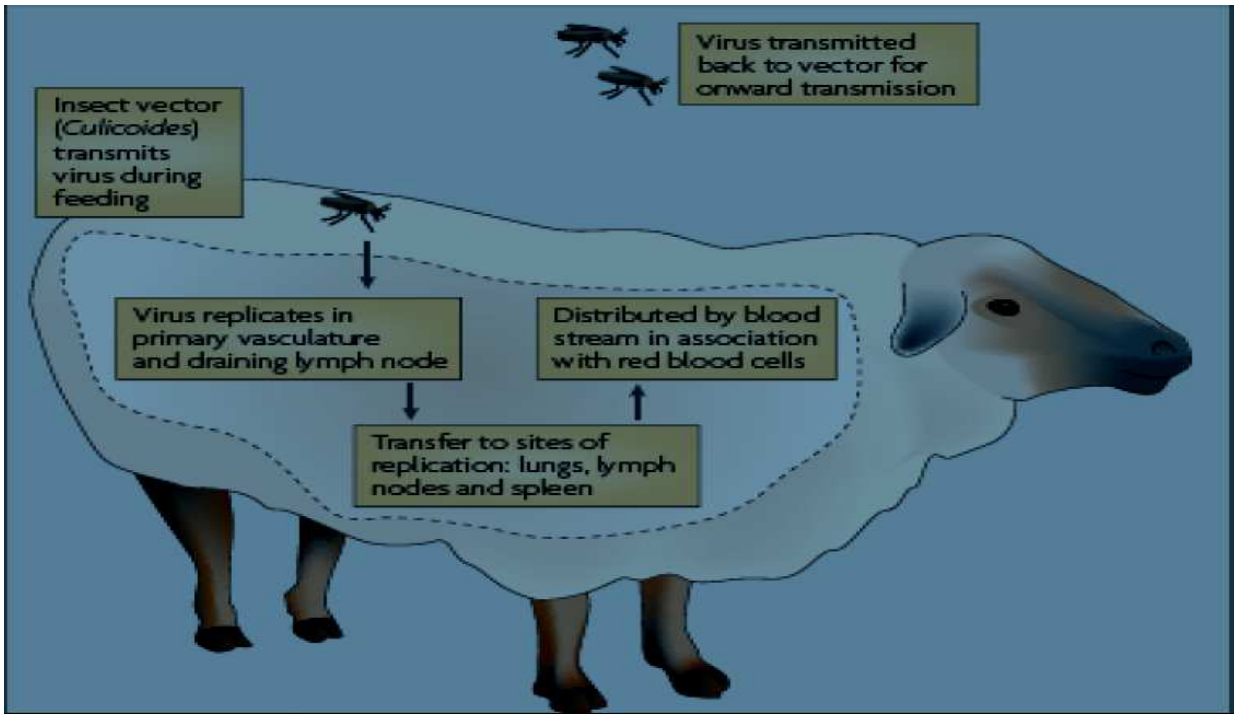

Figure 3: Transmission and replication of bluetongue virus (Roy *et al*., 2009).

2.7. Viraemia and Immune Response

Viraemia in infected animals has a prolonged course, but is not persistent. Its duration depends on the longevity of erythrocytes to which virus is bound, in contrast to the other blood cells, even at the late stage of infection. It is also related to the species and breed of the infected animal. Viraemia lasts 14 to 54 days in sheep and 19 to 54 days in goats. In cattle, viraemia may last as

long as 60 or, even 100 days, which makes this animal an important host, from the epidemiological point of view (MacLachlan *et al*., 2009).

The infected animals react to BTV with interferon production and humoral and cell-mediated immune responses. Serotype-specific neutralizing antibodies against the VP2 protein confer protection against homologous strain reinfection (Schwartz-Cornil *et al*., 2008). Neutralizing antibodies are also induced, to a lesser degree, by the VP5 protein. The sera of infected ruminants also contain serogroup specific antibodies induced by the VP7 protein, as well as antibodies against other structural and non-structural proteins. The cell-mediated immune response to BTV can probably reduce the spread of virus in the organism early after infection, but cannot eliminate the virus completely. By producing a cytotoxic effect in infected cells, CD8+ T-lymphocytes play most an important role (Schwartz-Cornil *et al*., 2008).

2.8. Diagnosis

Various techniques have been used to detect antibodies against BTV. Only Agar Gel Immunodiffusion (AGID) and c-ELISA are recommended as prescribed tests for international trade in the OIE Manual of Standards for Diagnostic Tests and Vaccines (OIE, 2013). A preliminary diagnosis based on clinical signs, post-mortem findings and epidemiological assessment should be confirmed by laboratory examination. Samples to be examined in the laboratory should include non-coagulated blood (use of ethylene diaminetetraacetic acid or heparin is preferred), blood serum, post-mortem tissue samples of spleen, lymph nodes, lungs, liver, bone marrow and, when indicated, heart and skeletal muscles; in addition, brain tissue is collected in foetuses. For transport, blood serum samples should be frozen at –20 °C and the other samples should be kept on ice (Tweedle and Mellor, 2002). Full blood samples can be stored at +4 °C for a long time; isolated blood cells in 10% dimethyl sulphoxide require storage at a temperature of –70 °C.

2.8.1. Virus isolation

Bluetongue virus can be propagated in embryonated chicken eggs, cell cultures or in sheep. Nine to 12 days old Embryonated eggs, are used for BTV isolation (Anonymous, 2004). This method is 100- to 1000-fold more sensitive than yolk sac inoculation, but is demanding in terms of

technical skills and experience. The material obtained from embryonated eggs can either be further propagated in cell culture or directly examined using molecular methods (PCR or in situ hybridisation) (Clavijo *et al*., 2000).

Bluetongue virus can also be isolated in cell lines of insect origin, such as the KC line derived from *Culicoides* sonorensis cells or the C6/36 line from Aedes albopictus (AA) cells; the mammalian BHK-21, Vero cell lines can also be used. The cytopathic effect produced by BTV is observed only on cell lines of mammalian origin at three to five days after inoculation and appears as foci of rounded and refractile cells. The isolation of virus in cell culture is usually preceded by its passage in embryonated chicken eggs which are more susceptible to BTV than cell lines (Mecham, 2006).

Sheep can provide a sensitive and reliable system for BTV isolation; however, today they are used only occasionally, e.g., in cases when a sample contains a very low virus titre (Anonymous, 2004).

2.8.2. Antigen identification

A direct identification of BTV in blood or tissue samples is possible with use of the reverse transcription polymerase chain reaction (RT-PCR) method that allows for serotyping and can detect BTV RNA in samples as late as six months after infection. A quantitative assessment of RNA in an examined sample is possible by real time-RT-PCR (Shaw *et al*., 2007; Toussaint *et al*., 2007). The identification of a BTV serotype is carried out in the virus neutralization test. Other available diagnostic methods include antigen-capture ELISA, immunospot and immunofluorescence tests, but they are rarely used.

2.8.3. Antibody identification

Serogroup specific antibodies against BTV can be detected by a competitive ELISA test targeted to the VP7 protein. This is a rapid method permitting determination of serum or plasma antibody as early as the 6 th post-infection day. There are other commercial ELISA kits developed recently by which early antibodies or antibodies against BTV in individual or bulk milk samples can be detected (Mars *et al*., 2010). In addition, serogroup-specific antibodies can be identified by an

agar-gel immunodiffusion test (AGID), which, however, may produce cross-reactions with other orbiviruses, a complement fixation test (CFT) and a haemagglutination-inhibition test. The serum neutralisation test has the highest specificity and sensitivity of all the tests, but is also most expensive and time-consuming (Hamblin, 2004).

2.9. Prevention and control

There is no specific treatment available, other than supportive care. Symptomatic therapy includes gentle handling of affected animals, their stabling and, if indicated, administration of non-steroidal drugs. An immediate ban on animal import from countries with bluetongue is the priority measure, followed by the monitoring of farms raising domestic ruminants which include clinical examination and serological and virological testing, and a monitoring of insect vectors. Prophylactic immunization and the removal of vectors or prevention of vector attacks can also be used (Tweedle and Mellor, 2002).

2.9.1. Prophylactic immunization

Vaccination can prevent clinical bluetongue or at least make less severe its course by interrupting the BTV cycle in the environment; it thus reduces the economic losses due to animal infection and makes transfer and trading of animals from BTV enzootic regions possible (Savini *et al*., 2008; Bhanuprakash et al., 2009). Bluetongue vaccines are serotype-specific and therefore, before use in a given area, the serotypes present in the environment should be taken into account. Two types of vaccines, inactivated and live attenuated, are currently available (Bhanuprakash *et al*., 2009).

2.9.2. Vector Control

Understandably, it is impossible to completely eliminate *Culicoides* midges in the natural environment. It is possible, however, to reduce midge populations to ineffective levels, or to prevent vector attacks by stabling susceptible animals overnight since midges have nocturnal feeding habits. In addition, the protection of animals in stables can be improved by door and window screens made of a fine mesh or a coarse fabric impregnated with insecticide (Calvete *et al*., 2010). Alternative approaches involve moving the animas from insect resting and breeding

sites or complete elimination of such sites. The species *C. imicola*, *C. obsoletus* and *C. pulicaris* breed in wet or not yet having dried soils rich in organic matter and such grounds should be drained and dried (Calvete *et al*., 2010).

The control of adult midges can be carried out by use of approved insecticides (Schmahl *et al*., 2009a, b) sprayed outside or inside (in areas with *C. dewulfi* occurrence) the stable or directly to the susceptible animals. The latter approach is allowed only with agents of low toxicity to mammals such as synthetic pyrethroids (deltamethrin, cyfluthrin, permethrin and fenvalerate); these agents provide protection for 3 to 5 weeks and can be used in the form of insecticide-impregnated ear tags. Animals can also be protected by systemic invermectins administered intradermally or subcutaneously. The larvicide abate (5% temephos granulated with gypsum) can be applied to midge breeding grounds. Insect repellents can also be used for direct protection of animals; diethyl toluamide (DET), for instance, is effective for up to four hours (Schmahl *et al*., 2009a,b) and can be used in the form of insecticide-impregnated ear tags.

3. MATERIALS AND METHODS

3.1.Description of Study Area

Study was conducted in selected districts of South western Ethiopia namely Jimma town, Bonga town and Bedele woreda. From each districts the following peasant associations were selected: Shobe, Sidisa, Qare and Yabela from Bedele, Ifa Bula, Bore and Qofe from Jimma and Bonga zuria 01 and 03 from Bonga were selected.

Jimma town has 17 kebeles and located in Jimma zone, Oromia region at 352 km West direction of Addis Ababa, Ethiopia. Mixed crop-livestock production system is the main form of agriculture in the area. Although the area is mainly known for its coffee production, crop and livestock production are also important agricultural activities as well. The zonal elevation is 1744 meters above sea level (m.a.s.l.) which is categorized as mid-altitude and found at 7º 41'0.012" N latitude and 36 º 50'59.880" E longitudes (Figure 5). The mean annual minimum and maximum temperature are 6ºC and 31ºC respectively, with an overall average of 18.7ºC. The mean annual rainfall is about 1530 millimeters which comes from the long and short rainy seasons (Central statistics agency Jimma branch office, 2015).

Bedele district has 40 peasant associations and located at Illubabor zone, Oromia region at about 145 km Jimma town to Northwest and 480 km from Addis Ababa in Southwest direction, Ethiopia**.** The estimate elevation is 1497-1795 m.a.s which is categorized as lowland and mid-altitude and found at 8° 14' 44" to 8° 38' 4.90" North latitude and 36^{o}19' 55" to 36^{o}25' 31.90" East longitude (Figure 5). The mean annual rainfall is about 950 millimeters which comes from the long and short rainy seasons (Central statistics agency Bedele branch office, 2015).

Bonga town in order to facilitate the socio- economic development of the town, it is structured in to three kebeles. It is administrative center of the Kaffa zone, SNNPR, Ethiopia and situated at a distance of 449 Km south west of Addis Ababa. Topographically it lies at an altitude of 1650-1840 m.a.s which is categorized as midland and highland altitudes. Its average annual rainfall is 1750 it has been categorized as Woyena dega type of climate. It is one of the 22 towns identified

as autonomous provisional city in the region. It has latitude and longitude of 7°16′N 36°14′E coordinates. It is surrounded by Ginbo woreda and administrative center of the Kaffa Zone (Figure 5) (Central statistics agency Bonga branch office, 2015).

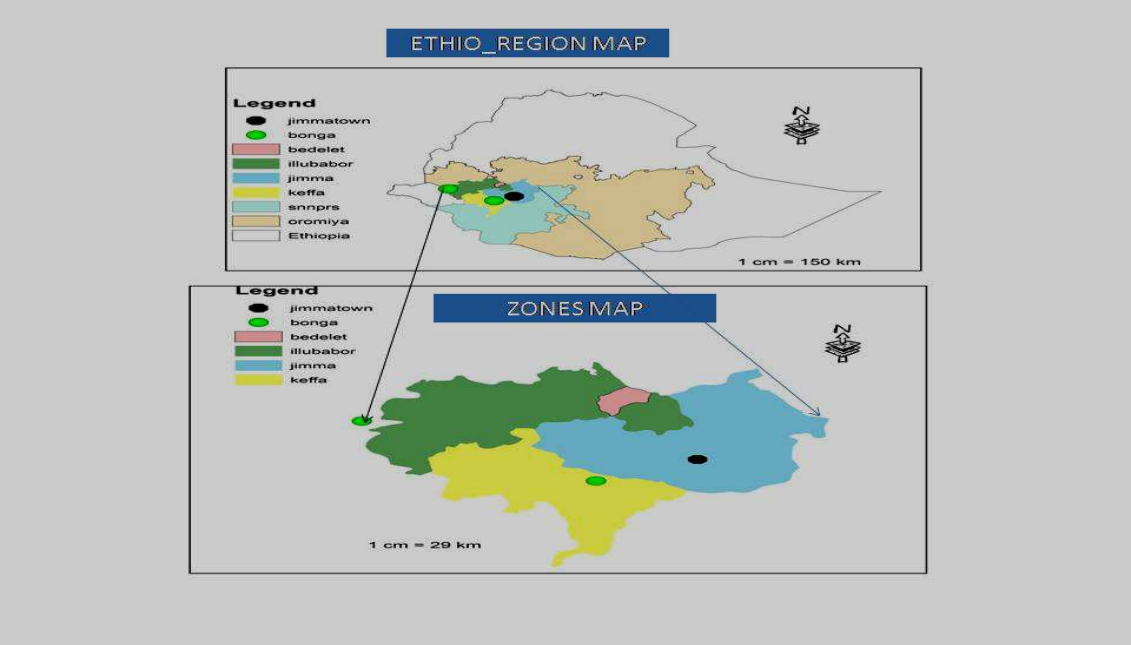

Figure 4: Map of study area

3.2.Study Population

The study population was small ruminants at different agro-ecological zones found in different Peasant Associations (PAs) of selected districts'. Total livestock population in Jimma town is 28650 cattle, 13390 sheep, 5765 goats, 2143 horses, 152 mules, 285 donkey and 70000 poultry (Jimma town agricultural office, 2014). Total livestock population in Bedele district is 37870 cattle, 26450 sheep, 11450 goats, 11290 horses, 489 mules, 2045 donkeys, 1170634 poultry, and 598361 beehives (Bedele woreda agricultural office, 2015). Total livestock population in Bonga town is 17467 cattle, 11245 sheep, 10254 goats, 4020 horses, 150 mules, 1450 donkeys, 45237 poultry and 45378 beehives (Bonga town agricultural office, 2014).

3.3.Study Design and Period

A cross-sectional study was conducted to estimate the seroprevalence of bluetongue virus (BTV) from December, 2015 to August, 2016 in small ruminants at South Western Ethiopia.

3.4.Sample Size Determination

Simple random sampling technique was used to select the study animals in the study area. The sample size was determined according to Thrusfield (2005) with 95% confidence interval and 5% absolute precision, since there was no previous research work done in the study area, the expected prevalence rate was taken as 50%. Therefore, 384 small ruminants were obtained for the study using formula. But to increase precision and to get more accurate, the sample size was taken as 422 of which 248 sheep and 174 goats due to proportionality of study population in the study districts were included. So, from each district samples were taken proportional to total study population in the study districts of which 141 from Jimma district, 101 from Bonga district and 180 from Bedele district.

$$n = 1.96^2 P\exp(1 - P\exp) \Big/ d^2$$

Where,

n = sample size,

P_{exp} = expected prevalence, and

d = desired absolute precision.

A total of 422 small ruminants were selected from the target population, and were sampled (50% prevalence and d = 0.05% was considered).

3.5.Sampling Technique

Convenience sampling was used for selection of study districts from different agro-ecological zones. Simple random sampling was used in selection of sampling animals and peasant associations (PAs). Body condition scores was done as described as annex 2 (Technical bulletin no.8) and age determination described as annex 3 (Vatta *et al*., 2006).

3.6.Sample Collection

About 5 ml of blood was collected aseptically from the jugular vein of each sheep and goats using plain vacutainer tubes and needles. The blood was allowed to clot for 1-2 h at room temperature, stored horizontally overnight at 4°C within refrigerator and then the serum was separated. All the sera were stored at -20°C until tested and transported on ice and submitted to the national veterinary institute (NVI) to test by c-ELISA.

3.7.Laboratory Analysis

The competitive ELISA was carried out in this study because it is able to discriminate BTV from another closely associated Orbivirus such as Epizootic Hemorrhagic Disease virus (EHDV). Competitive ELISA referred to highly sensitive (100%) and specific (99%) serological and now widely available to confirm the presence or absence of BTV infection in animals (Maclachlan and Guthrie, 2010). Test detailed procedure of c-ELISA for BTV antibody detection is described in annex 1.

The detection of BTV specific antibodies in serum was carried out using competitive ELISA assays (IDvet, 310, rue Louis Pasteur- Grabels- FRANCE) according to the manufacturer's instructions. Results were reported as positive or negative and percent competition based on optical density readings at greater than or equal to 40% are considered negative and less than 40% are considered positive. The microplate was read spectrophotometrically at 450nm.

3.8.Data Management and Analysis

The data was collected and entered in computer using Microsoft Excel spread-sheet and analyzed using statistical package for social science (SPSS) software package for Windows (version 20.0). Associations between the outcome variable (status of Bluetongue Virus infection in small ruminants) and its potential risk factors are first screened in a univariable logistic regression analysis. The p-value less than 0.2 results of the univariable analysis are re-entered in the final model using multivariable analysis. A multivariable analysis for the outcome variable is constructed using logistic regression model. Finally, odds ratios and its 95% confidence interval (CI) are calculated and risk factors with a P-value < 0.05 are taken as statistically significant.

4. RESULTS

A total of 422 sera samples were collected from sheep and goats and examined and out of which 129 (30.6%) (at 95% CI = 26.2 - 35) seroprevalence was recorded on the basis of antibodies to VP7 protein of bluetongue virus by competitive enzyme linked immunosorbent assay (c-ELISA).

Among selected PAs Shobe from Bedele district was the highest seroprevalence resulting with 53.3% and lowest prevalence was in Bonga zuria 03 with 7.8% as described in table 5.

Table 5: Seroprevalence of bluetongue virus antibodies in small ruminants from the selected PAs in study area.

Peasant association	**No. tested**	**No. positive (Prevalence %)**
Bonga zuria 01	50	10 (20.0)
Bonga zuria 03	51	4 (7.8)
Bore	47	5 (10.6)
Ifa bula	47	10 (21.3)
Qare	45	14 (31.1)
Qofe	47	20 (42.6)
Shobe	45	24 (53.3)
Sidisa	47	24 (51.1)
Yabela	43	18 (41.9)
Total	**422**	**129 (30.6)**

From this present study, highest seroprevalence of bluetongue virus was recorded in Bedele (44.4%) and lowest from Bonga district (13.9%) as described in table 6.

Table 6: Both univariate and multivariate logistic regression analysis result of bluetongue virus in small ruminants in selected districts of Southwest Ethiopia.

Districts	No. tested	No. positive (%)	P-value	OR	95 % CI	P-value	AOR	95% CI
Jimma *	141	35 (24.8)	-	-	-	-	-	-
Bonga	101	14 (13.9)	0.039	0.487	0.247-0.963	0.151	0.591	0.288-1.212
Bedele	180	80 (44.4)	0.001	2.423	1.496-3.924	0.011	2.295	1.213-4.340
Total	**422**	**129 (30.6)**						

OR = Normal odds ratio, AOR= Adjusted odds ratio *= reference category

Table 7: Univariate logistic regression analysis of bluetongue virus and risk factors in small ruminants in selected districts of Southwest Ethiopia.

Variables	Total	Prevalence (%)	P-value	OR	95% CI
Species					
Sheep*	246	57 (23.2)	-	-	-
Goat	176	72 (40.9)	0.001	2.296	1.505 – 3.500
Total	**422**	**129 (30.6)**			
Sex					
Female*	236	74 (31.4)	-	-	-
Male	186	55 (29.6)	0.785	0.919	0.605 - 1.396
Total	**422**	**129 (30.6)**			
Age					
Young*	181	33 (18.3)	-	-	-
Adult	241	96 (39.8)	0.001	2.969	1.880 - 4.690
Total	**422**	**129 (30.6)**			
Body condition					
Emaciated	89	22 (24.7)	0.175	0.673	0.380 - 1.193
Medium	150	47 (31.3)	0.777	0.935	0.589 - 1.486
Good*	183	60 (32.8)	-	-	-
Total	**422**	**129 (30.6)**			

Location					
lowland	92	48 (52.2)	0.001	5.692	3.107-10.426
Midland	187	58 (26.8)	0.002	2.346	1.363- 4.038
Highland*	143	23 (17.7)	-	-	-
Total	**422**	**129 (30.6)**			

OR = Normal odds ratio, *= reference category

For species as risk factor, it was shown that goats were 2.4 times more likely to be infected with BT virus infection than sheep. In case of age, adult animals were 3 times more likely to be infected with BT virus than young animals. Regarding agro-ecology, small ruminants from lowland altitudes were 3 times more likely exposed for BT virus infection than highland altitudes and midland altitudes were 2.2 times more likely exposed with BT virus infection than highland altitudes. When compared districts, small ruminants in Bedele district are 2.3 times more likely exposed to BT virus infection than Jimma district.

Table 8: Results of multivariable logistic regression analysis of bluetongue virus infection status and risk factors in small ruminants in selected districts of Southwest Ethiopia.

Variables	**Total**	**Prevalence (%)**	**P-value**	**AOR**	**95% CI**
Species					
Sheep*	246	57 (23.2)	-	-	-
Goat	176	72 (40.9)	0.001	2.455	1.538 – 3.917
Total	**422**	**129 (30.6)**			
Age					
Young*	181	33 (18.3)	-	-	-
Adult	241	96 (39.8)	0.001	3.135	1.909 – 5.148
Total	**422**	**129 (30.6)**			
Location					
lowland	92	48 (52.2)	0.002	3.143	1.525-6.476
Midland	187	58 (26.8)	0.004	2.387	1.320- 4.319
Highland*	143	23 (17.7)	-	-	-
Total	**422**	**129 (30.6)**			

AOR=Adjusted odds ratio, statistically significant association ($P < 0.05$), no significant association ($P > 0.05$).

Concluding after multivariable logistic regression analysis of potential risk factors revealed that risk factors like between species, age, location and districts statistically significant ($P < 0.05$) difference was observed in the seroprevalence of BT virus infection. Sex and body condition no statistically significant difference ($P>0.05$) at 95% confidence level.

5. DISCUSSIONS

The results presented here record the first confirmation of BTV antibody in sheep and goats from South west Ethiopia. However, few bluetongue (BT) seroprevalence reports in small ruminants in Ethiopia in the past (Woldemeskel *et al*. 2000, Gulima *et al*. 2009, Yilma and Mekonnen, 2015). Overall seroprevalence finding from this current study was that more than one quarter 129 (30.6%) of small ruminants tested was positive for antibodies to BT virus infection.

These findings were in agreement with the results of Gulima (2009) who reported 34.1% seroprevalence of bluetongue virus from indigenous sheep in northwestern Ethiopia. Other reports from outside from Ethiopia such as Bitew *et al*. (2013) who reported 28.6% from small ruminants in Uttar Pradesh, India; Lundervold *et al*. (2004) who reported 23% from domestic livestock in Kazakhstan. Hasanpour *et al*. (2014) who reported 35.9% from sheep in Iran; Khezri & Azimi (2013) who reported 34.93% from sheep in Iran; Khalid *et al*. (2012) who reported 39.47% in goats in Iran; Gur (2008) reported 29.5% in small ruminants in Turkey. Shoorijeh *et al*. (2010) who reported 37.7% in sheep in Iran; Khezri & Azimi (2016) who reported 33.75 % in sheep in Iran. The test was conducted using c-ELISA.

The currently reported result was found to be lower than the previously reported seroprevalence of bluetongue virus by different authors in different countries such as Yilma & Mekonnen (2015) who reported 41.17% in small ruminants in Southern Ethiopia; Woldemeskel *et al*. (2000) 46.67% in sheep in central Ethiopia. Sharma *et al*. (2016) who reported 78.4% in small ruminants in Grenada; Oryan *et al*. (2014) who reported 76.4% from small ruminants in Iran; Sreenivasulu *et al*. (2004) 45.7% in small ruminant in India; Khalid *et al*. (2012) who reported 43.82% in small ruminant in Iraq. Khezri *et al*. (2016) who reported 40.87% from sheep in Iran; Mozaffari *et al*. (2014) 84.62% from sheep and goats in Southeast Iran, Yousef *et al*. (2012) who reported 47.3% from sheep in Saudi Arabia. Garcia *et al*. (2009) who reported 41.0% from goats in Spain; Mozaffari *et al*. (2012a) who reported 67.7% from small ruminants in Southeast Iran. Sabaghan *et al*. (2014) who reported 77.48% from sheep in Iran; Khezri and Azimi (2016) who reported 46.10% in sheep in West Iran. Khezri and Azimi (2012a) who reported 51.85% in sheep in Iran and Sadri (2012) 55.9% from sheep in Azerbaijan they were reported the seroprevalence

of BTV antibodies. All they were conducted c-ELISA test and kind of sample were serum from blood.

On the other hand, currently reported seroprevalence was higher than the reports of some scholars; Mozaffari and Khalili (2012) who reported 6.57% in sheep in Southeast Iran. Moustafa *et al*. (2016) who reported 6.96% from small ruminants in which between species 13.7% in goats and 5.70% in sheep reported in Algeria. Ravishankar *et al.* (2005) reported 2.63% and Doddamani & HariBabu (2006) reported 5.3% in small ruminants in Kerala and Karnataka, respectively. Tests were conducted by c-ELISA to detect antibodies from sera as an indicator of exposure to bluetongue virus.

The current study result difference might be due to difference in animal species, age of sampled animals, immune status of sampled animals, agro ecology, location and occurrence of *culicoides* vectors.

Seropositivity to BT virus infection reported in current study in goats was higher than sheep. Higher seroprevalence among goats than sheep suggest that goats would be the most important animals in the epidemiology of BTV being asymptomatic due to development of acquired immunity as a result of continuous exposure in agreement with Bitew *et al*. (2013), Yilma and Mekonnen (2015). Sheep which are highly susceptible animals to BT show clinical signs and die due to the infection. However, in the goats clinical manifestation is less and they can be tested positive.

BT virus infection increased with the increasing of age of animals recorded in current report in agreement with Yilma and Mekonnen (2015). When assessing age as a risk factor, there was a statistically significant association between the BTV infection and the age of the animal. It was shown that the younger animals started to get infected with BTV after the age of included at category of adult level. 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. Current study finding 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 (Aradaib *et al.*, 2011). Our result is in agreement with previous epidemiological surveys, which reported higher risks of older animals for BTV infections (Gaire *et al.*, 2014). The increase of the age of animals was associated with seropositive results, probably a reflection of increased duration of exposure (Radostits *et al.*, 2007). Higher seroprevalence of adult animals were likely due to acquired immunity gained over multiple years of exposure to BTV. This figure shows clearly that with increasing age of small ruminants, the risk of seropositivity has increased.

Present result in agreement to Woldemeskel *et al*. (2000) and Yilma and Mekonnen (2015) who reported higher seroprevalence in small ruminants living in lowland altitude than highland altitudes. The prevalence correlated with the probable distribution of the *Culicoides* vector. In addition environmental changes can influence the incidence, distribution and evolution of infectious diseases, particularly those transmitted by arthropod vectors (Jimenez-Clavero, 2012). The current study recorded that lowland agro-ecology is one of the predisposing factors for sero-positivity for small ruminants for BTV infection. This difference of seroprevalence between agro-ecology could be due to the difference in geographic nature and climate condition. The distribution and intensity of infection in regions of the continents is determined by the climate, geography and altitude, since these factors affect the occurrence and activity of the *Culicoides* vectors (Jimenez-Clavero, 2012).

There is very little information available about the seroprevalence of bluetongue virus infection in the East Africa in generally, in Ethiopia specifically and completely in South Western Ethiopia. There is no vaccination program for BT in Ethiopia. The presented results here show that the first confirmation of presence BT virus antibody in sheep and goats from south west Ethiopia.

6. CONCLUSION AND RECOMMENDATIONS

Bluetongue (BT) is a reportable disease of considerable socioeconomic concern and of major importance for the international trade of animals and animal products. It has been reported in few regional states of Ethiopia including this present study area.

The obtained results showed that the occurrence of BT antibodies has been detected among sheep and goats in South West of Ethiopia. This indicates that serological evidence of exposure to infection was distributed all the study area.

It affects a considerable number of small ruminants within Ethiopia and the seroprevalence distribution reported in few regions of the country. Age, species, and location of animals were found to be risk factors of bluetongue virus infection.

This study reveals high seroprevalence of BT in goats than sheep, in adult small ruminants than young, and in lowland than highland. Therefore, based on the above concluding remarks the following recommendations are forwarded:

- ☞ We don't know exactly what the *culicoides* species transmitted bluetongue virus in Ethiopia. Similarly kind of serotypes are not known. Studies should be undertaken to differentiate the culicoides species responsible for the transmission of the disease and serotype identification.
- ☞ No reports indicate for which season of *culicoides* vectors are maximal active to transmit bluetongue virus in Ethiopia. The season identification study should be conducted.
- ☞ Educate farmers about BT management system such as insect control includes, use of insecticides, or moving animals into barns and kept animals indoors and control of this disease.
- ☞ Identification of serotype of BTV and vaccination of susceptible species animals followed by preparation or importation of appropriate vaccine from commercial available source.

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8. ANNEXES

Annex 1: Testing Procedure

Testing Competitive ELISA was applied to detect antibodies against bluetongue virus in the National Veterinary institute (NVI), Ethiopia. For this study (IDvet, 310, rue Louis Pasteur-Grabels- FRANCE) kit was applied. This diagnostic kit is designed to detect antibodies secreted against the bluetongue virus vp7 protein. The samples to be tested and the controls are added to the microwells. The anti-vp7 antibodies, if present, from an antibody-antigen complex which makes the vp7 epitopes. An anti-vp7 peroxidase or horseradish peroxidase (HRP) conjugate is added to the microwells. It fixes to the remaining free vp7 epitopes, forming an antigen-conjugate peroxidase complex. After washing in order to eliminate the excess conjugate, the substrate solution Tetramethylbenzidin (TMB) is added. The resulting coloration depends on the quality of specific antibodies present in the sample to be tested: In the absence of antibodies, a blue solution appears which becomes yellow after addition of stop solution. In the presence of antibodies, no coloration appears. The micro plate is read spectrophotometrically at 450nm. The kit components: micro plate coated with vp7 (8strips of 12 microwells), anti-vp7-conjugate (10x), positive control, negative control, dilution buffer 2, wash concentrate (20x), substrate solution, stop solution (0.5 M).

Allow all the reagents to come to room temperature (21 ± 5 ºc) before use. Homogenize all reagents by inversion or vortex.

1. Add:

- 50 µl of dilution buffer 2 to each well.
- 50 µl of the positive control to wells A1 and B1.
- 50 µl of the negative control to wells C1 and D1.
- 50 µl of each sample to be tested to the remaining wells.

2. Incubate 45± 4 minutes at 21 ± 5 ºc.

3. Prepare anti-vp7 conjugate 1x by diluting the anti-vp7-po conjugate (10x) to 1/10 in dilution buffer 2.

4. Add 100 µl of the anti-vp7-po conjugate to each well.

5. Incubate 30±3 minutes at 21±5 °c

6. Wash each well 3 times approximately 300 µl of the wash solution. Avoid drying of the wells between washing.

7. Add 100 µl of the substrate solution to each well.

8. Incubate 15±2 minutes at 21±5 °c

9. Add 100 µl of the stop solution to each well in order to stop reaction

10. Read and record to O.D. at 450 nm.

The test is validated if:

- The mean value of the negative control O.D. (ODnc) is greater than 70% (0.7) (ODnc>0.7)
- The mean value of the positive control O.D. (ODpc) is less than 30% (0.3) of the ODnc (ODpc/ODnc<0.3)

For each sample, calculate the competition percentage :

Competition % = ODsample/ODnc×100

Samples presenting a competition percentage (PP):

- ☞ Greater than or equal 40% are considered negative.
- ☞ Less than 40% are considered positive.

Annex 2: Sample collection sheet

- ☞ Date …….....month.....year.....
- ☞ Owner name……..Case number.....
- ☞ Sex…….Age.....species…….…body condition score (BCS)…….…...location ….……or others……
- ☞ Name of districts…….peasant association……
- ☞ Serum..... Serology test result.....
- ☞ Others…….……..……..

Annex 3: Body condition scores of small ruminants.

One of the most important management skills of livestock producers is the ability to recognize when their animals are too skinny or too fat or just right in their fat reserves. The body condition or flesh on an animal affects its ability to resist infection and parasites, its ability to grow and produce milk, and its likelihood of breeding.

Condition	**Score**	**Lumbar region**	**Rib cage**	**Sternum**
Emaciated	1	The spinous processes feel prominent but smooth, and individual processes can be felt only as fine corrugations. The transverse processes are smooth and rounded, and it is possible to pass the fingers under the ends with a little pressure. The eye muscle areas are of moderate depth, but have little fat cover.	Some ribs can be seen. There is a small amount of fat cover. Ribs are still felt.	Sternal fat is wider and thicker but can still be grasped and moved slightly from side to side.
Moderate	2	The spinous processes are detected only as small elevations; they are smooth and rounded and individual bones can be felt	Ribs are barely seen; an even layer of fat covers them. Spaces	Sternal fat is wide and thick. It can still be grasped

		only with pressure. The transverse processes are smooth and well covered, and firm pressure is required to feel over the ends. The eye muscle areas are full, and have a moderate degree of fat cover.	between ribs are felt using pressure.	but has very little movement.
Good	3	The spinous processes can just be detected with pressure as a hard line between the fat covered eye muscle areas. The ends of the transverse processes cannot be felt. The eye muscle areas are full, and have a thick covering of fat	Ribs are not seen	Sternal fat is difficult to grasp and cannot be moved from side to side.

Annex 4. Estimated age for sheep and goats with different numbers of erupted permanent incisors

No. of permanent incisors	Estimated age range	
	Sheep	Goat
≤ two pairs	Young	Young
≥ two pairs	Adult	Adult

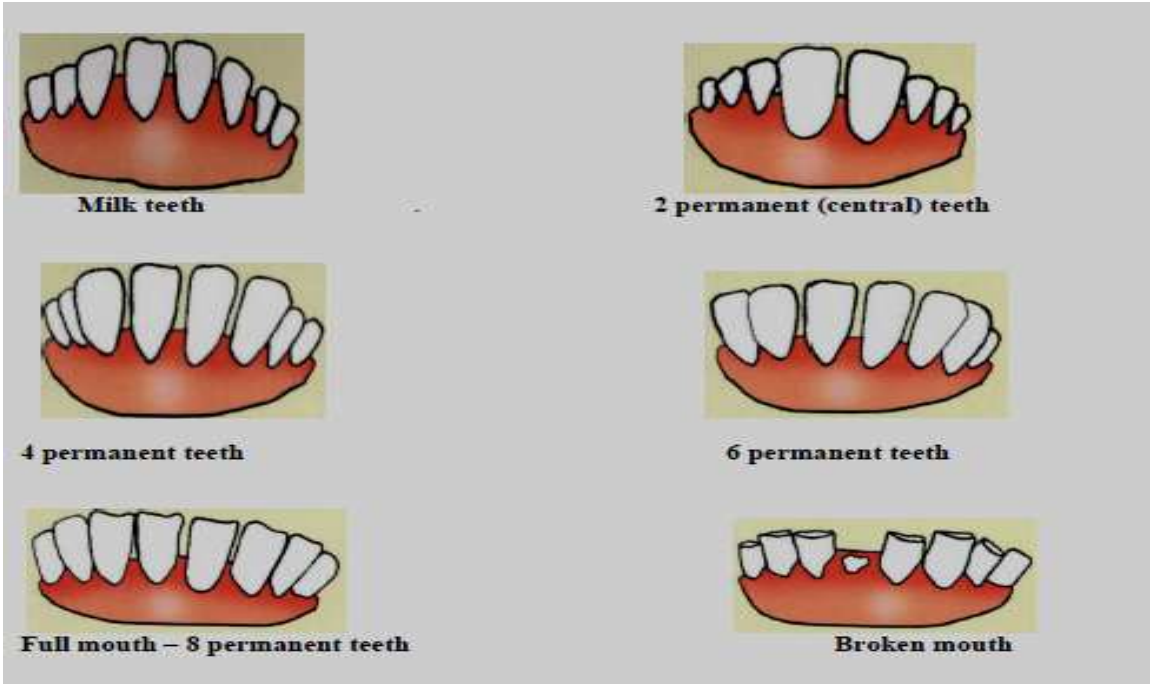

Figure 5. Dentition showing the estimated ages of sheep and goats (Adopted from Vatta *et al*., 2006)