

# Overview of Bluetongue disease in small ruminants: A review

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## ABSTRACT

Bluetongue is a severe viral disease of domestic and wild animals, distributed worldwide, highly harmful in sheep and is caused by the infectious virus of the family reoviridae, genus of *Orbivirus*. The fever, depression, serous to mucopurulent nasal discharge, hyperaemia of the muzzle, oral and nasal mucous membranes, conjunctive and coronary band of the hooves are among the clinical signs of the disease and it is transmitted by an intermediate host, commonly biting *culicoides*. The diagnosis of bluetongue disease, more importantly, depends on the clinical signs, and advanced laboratory techniques such as serological, viral isolation and Molecular detection. The Bluetongue disease is becoming a major concern of small ruminant production and productivity worldwide at the present day. To solve these constrict, many researchers conducted viral detection and isolation by using advanced techniques including molecular characterization. But, more scarcity of study was in the eastern part of Africa, in Ethiopia. In general, the disease status is still unclear and there is limited evidence of it in many parts of the country's agroecological areas, especially in sheep and goats. Thus, an extended study should be conducted in different geographical areas of Ethiopia, to combat the problem of disease evidence, to enhance small ruminant production, success of control, and prevention.

**Keywords:** Bluetongue disease, Bluetongue virus, diagnosis, epidemiology, small ruminants.

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## INTRODUCTION

Bluetongue is a severe viral disease of domestic and wild animals, in different regions worldwide. Common hosts are; shoats, cattle, dromedaries, deer, buffalos, llamas and antelopes. The disease is highly harmful in the ovine (Jilo et al., 2016) and is caused by the infectious virus of the family reoviridae, genus of *Orbivirus* (Abera et al., 2018). Bluetongue virus possesses more serotypes, up to 35 are already investigated. Out of these serotypes, eleven are typical and the remaining 24 species are considered classical. The virus is also known for its ten segments of double-stranded RNA and can have a large degree of mutation in infected animals (Ries et al., 2022).

The Bluetongue disease was included in the world's notifiable diseases list by the world organization for animal health (OIE), because of its causing harmful effects economically and more being threat to animal

health (OIE, 2010). In the late eighteenth century, the bluetongue was declared in Cape Town of South Africa and the disease was ultimately distributed to other African nations, Europe, Asia and America (Hofman et al., 2008). Recently, this virus was being identified all over the world rather than the bare land in Antarctica (Eticha and Mekonnen, 2019). In Ethiopia, there is a large number of goats and sheep population. In detail, the country has been populated with a 24.06 and 53.99 population of goats and sheep, respectively (CSA, 2013). However, there is an absence of common status of Bluetongue virus (BTV) evidence, due to a lack of diagnosed indications in the country (Abera et al., 2018).

The virus has dissimilar clinical signs based on its strain, breeds of hosts and also husbandry factors (Verwoerd and Erasmus, 2004). Accordingly, hyperaemia

and congestion, which resulted to face oedema, eyelids and ears, and haemorrhages and happening erosion in mucus membrane are observed during acute cases in sheep. In chronic cases, clinical signs of muscle degeneration, unable to move and disruptions in wool can cause severity in relation to infection through sheep hair follicles. The fatality behavior of the disease may occur as the formation of froth in bronchial trees, internal alveolar hyperaemia in the lungs, and different amounts of fluid in pericardial and thoracic cavity commonly occur. For instance, the unvaccinated sheep mortality rate rises to 70% when the infection is influenced by virulent species of BTV (MacLachlan and Mayo, 2013). The transmission of BTV is by the means of blood-feeding mosquito species. More specifically, the matured female of culicoides species known as haematophagous midges is a solely important vector to transmit the disease from infected to healthy animals (Meiswinkel et al., 2008). Out of a huge number of species of culicoid, only thirty are identified so far in the transmission of bluetongue (Coetzee et al., 2012).

Isolation of the virus, serological techniques; such as competition Elisa, AGID and Molecular detection by PCR and sequencing techniques are very important in diagnosing BTV. The better and more specific diagnostic methods are molecular detection and characterization of the virus from the appropriate samples of infected animals (Mertens et al., 2007). The sophisticated control and prevention of the disease can be performed in a very important manner by an efficient vaccine. Although, the restriction of animal movement and vector controls are somewhat useful in different countries (MacLachlan and Mayo, 2013). Bluetongue is becoming a major concern in small ruminants' production and productivity worldwide. To solve this constricts, many researchers conducted viral detection and isolation by using advanced techniques including molecular characterization (Merck, 2014). But more scarcity of study was in the eastern part of Africa, as Ethiopia is a member with less bluetongue disease status unclear (Aradaib et al., 2005). Recently, Eticha and Mekonnen. (2019), identified that there is limited evidence of this disease in many parts of the country's agroecological areas, especially in sheep and goats. In addition, there recommended further epidemiological studies to know the status of diseases throughout the country. Thus, the general objective of this review is to assess the Epidemiological status, diagnosis methods and control measures of the Bluetongue disease for the success of the control and prevention plan.

## LITERATURE REVIEW

### Background of the disease

Bluetongue has been not known well from the beginning.

It was commonly considered as "epizootic catarrh" first, 'malignant malarial catarrhal fever' later in sheep in and identified in South Africa in the late eighteenth century after the entry of new sheep with good wool from Europe (MacLachlan et al., 2009). The word "Bloutong" was an Anglicized form of the Africans which was coined by Boer farmers to define the characteristic cyanotic tongue of highly diseased sheep and served as the origin of Bluetongue (Jilo et al., 2016). Since 1905, the disease was recorded as "Bluetongue" in relation to its effect of causing cyanotic tongues of sheep to occur irregularly (Coetzee et al., 2012).

The disease was later renamed "bluetongue" (1905), with reference to the characteristic cyanotic tongues that were occasionally observed in infected sheep (Meiswinkel et al., 2008).

United States (US), South America, Israel, and Australia became endemic to the virus and identified the new BTV virus through extended studies. In addition, they indicated that the dispersion of the virus in the inter-continent is fast rising (Mayo et al., 2020).

### Epidemiology

The disease can occur in all ruminants, while sheep are highly susceptible to it; also, it is severe for white-tailed deer (*Odocoileus virginianus*) (Johnson et al., 2006). Recently, a study identified that there is evidence of transmission to Carnivores and the infection is also described in Canines (Evermann, 2008).

The distribution of BTV is global, through climates of tropical and subtropical regions from nearly 35° S to 40° N and in some areas outside of this region (in parts of California). It is endemic in Africa, Europe, the Middle East, North and South America and Asia. In addition, the disease is commonly occurring in Australia, South Pacific and the Caribbean islands. In many cases, BTV can occur at extra of its endemic places. However, it cannot survive in cold regions as the virus is vulnerable to this type of weather. Although, the strain of BTV, serotype-8 is uncommonly stagnated in northern and central parts of Europe (Howerth et al., 2001).

The vector species *Culicoides imicola*, which is active at the environmental temperature of 13°C to 35°C is highly distributed throughout the world and played a great role in the transmission of the disease in the "Old World". Furthermore, Africa, south of Asia, Portugal, Spain, Greece, Cyprus, Corsica, Italy, Israel, Turkey, Yemen, Oman and Jizan, and Najran, districts to the horn of Africa such as Ethiopia, Eretria, Djibouti, Somalia and Sudan where the enzootic nature of BTV is larger in the region of Africa, five serotypes namely serotypes 1, 2, 4, 5 and 16) were identified in Sudan due to the huge presence of this vector species (Aradaib et al., 2005).

## Etiology

The bluetongue disease is an infection caused by double-stranded RNA Bluetongue virus, which is a family of Reoviridae, genus of *Orbivirus*. This RNA of BTV encodes to 7 structure proteins (VP1–VP7) and also 3 non-structure proteins. The virus consists of 26 infectious strains with higher genetically change as a result of genetic drift of individual gene segments as well as by re-assortment of gene segments during infection of vectors and hosts, especially ruminants by more serotypes (Eticha and Mekonnin, 2019).

## Clinical signs

The fever, depression, serous to mucopurulent nasal discharge, hyperaemia of the muzzle, oral and nasal mucous membranes, conjunctive and coronary band of the hooves is obvious clinical signs of the Bluetongue disease caused by BTV. Other clinical signs of the disease are muzzle, periocular region and edematous on the faces of infected animals. In severely diseased animals, sudden cyanotic of the tongue, swelling and can protrusion out of the mouth. Stillborn, abortion and lesions of the central nervous system, retinal lesions and skeletal malformations commonly occur in pregnant ewes. Pulmonary edema or secondary complication can lead to life-threatening, and lead to death as a result of the prolonged disease. The viral serotypes and species hosts can make a direct influence on the mortality rate (Maclachlan et al., 2009).

## Transmission

Bluetongue is transmitted by an intermediate host, commonly biting culicoides. In addition, the disease can be transmitted through direct vertical, oral, and venereal transmission and indirectly by reused sharp materials such as needles. Although, there is uncertain epidemiological significance in this transmission way (Darpel et al., 2016; Belbis et al., 2017). On the other hand, this type of transmission is exclusively important to produce persistent infection in caprine, when it was BTV-25, BTV-26 and BTV-27 serotypes (Maclachlan et al., 2015).

## Diagnostic methods

The diagnosis of bluetongue disease, more importantly, depends on the clinical signs, and advanced laboratory techniques such as serological, viral isolation and molecular detection (Radostits et al., 2006; Eticha and Mekonnin, 2019).

## Clinical diagnosis

During diagnosis, hyperaemia of lips, pyrexia, bloody nasal fluids and congested conjunctiva in the eyes were observed. In addition, swelling and mule oedema mare appeared on infected ruminants. Another clinical diagnosis of BTD is based on the presence of swelling and oedema on the face, tongue and ears. Also, oral erosions and ulcers, cyanosis of the tongue, extreme salivation (hyperptyalism), coronitis, lameness, abortion, stillbirth, and congenital deformities (cerebral malformations) are considered the main clinical signs of the disease (Saminathan et al., 2020).

## Laboratory diagnosis

The present laboratory diagnosis methods of bluetongue disease are serological tests, viral isolation and molecular “polymerase chain reaction” (Radostits et al., 2006).

## Serological tests

BTV can be detected after seven to fourteen days of infection. The common serological diagnosis methods are; competitive ELISA(c-ELISA), Indirect ELISA (i-Elisa), Complement fixation test, and antibody neutralizing tests (Munmun et al., 2022).

**Competitive ELISA:** This method was developed to detect unique antibodies to BTV from the beginning, as explained so far by Lunt et al. (1988). This technique is very useful in identifying the organism at the beginning (6<sup>th</sup>) days of infection rapidly (Mars et al., 2010). It can detect specific antibodies by using serogroup known as Mabs which are specific to BTV. Accordingly, the serum antibody is compete with the Mabs to make binding with Antigen (Eticha and Mekonnin, 2019).

**Indirect ELISA (i-ELISA):** This method is very important to detect bluetongue virus from the sample of whole milk by using VP7 protein as substrate and IgG anti-ruminant as conjugate. It is highly specific up to 96% and its sensitivity is reached 98%. I-ELISA is advanced in identifying whether the infection is due to the vaccination (DIVA) system or not dependent on vaccination, in the detection of Bluetongue virus NS3 –antibodies (Barros et al., 2009) and also, better than c-Elisa by its ability to detect the virus in the sample of animals that have been taken deactivated vaccine (Rojas et al., 2019).

**Complement fixation test:** This test was applied for the first time in the identification of the Bluetongue virus from isolates in Australia, historically (George et al., 1978).

However, it is not more used in the detection of BTV antibodies from the sample of diseased animals. In 1975, the modified and direct method of CFT was introduced by Carrier and Boulanger and this technique is important to identify the organism anti-body in the infected sample of ovine and bovine. For instance, modified indirect CFT has limited Sero-typing of bluetongue virus isolates (Saminathan et al., 2020).

**Agar gel immuno-diffusion (AGID):** This method is principally characterized by the precipitation of soluble antigens after the addition of an antibody that is specific in a sterile medium. As a principle, three test serums are put in separate wells with three positive sera and the central well is completed by unpolished preparation of BTV-infected BHK or Vero cells. Following this, the immune complex will be formed as a result of passive diffusion of antigen (soluble) and antibody in the gel, on the way to each of them. The importance of this test is its simplicity to give the needed result and ease to use. On the other hand, the lower sensitivity and specificity are among the problem of this test which made it be not used for the aim of intercontinental trade (OIE, 2018).

**Serum neutralization test (SNT):** This diagnostic test is among serological methods, used in the identification of Bluetongue disease virus antibodies which are designed for dissimilar strains. In this method, the reaction of the serum used in the test is take place differently in a permanent manner for each strain. Then, the inoculations of mammalian culture are applied to measure the quantity of neutralization of the virus not treated with the serum, in relation to that of the treated one. As a result of its advanced specificity and sensitivity, SNT is an important method to avoid cross-reaction with another species of *Orbivirus*. Although the disadvantage is its expensiveness, the challenge of results clarification in the immersion of greater than one strain as the sheep can develop more heterotypic-serotype antibodies for many strains of BTV during infection by additional strains needs reference serotype and the habit of time taking. Hence, this test is little application across globally (OIE, 2018).

### Viral isolation

The Bluetongue virus can be isolated from the appropriate samples of diseased animals. Accordingly, the blood, tissues and semen samples are used for virus isolation. Also, the virus can be found in a mixture of the infected organs of animals (Rojas et al., 2019).

The inoculation of chicken embryonated egg and that of Kc cell lines (a cell line derived from *C. sonorensis* midges) is usually used for the isolation of BTV. The most widely important one is the KC cell line, because of its high sensitivity (McHolland and Mecham, 2003).

The animal cell culture is believed to be better and more suitable. Although, the degree of realization is smaller than that of both embryonated chicken egg and KC cell schemes. Following the first inoculation in ECE, The preceding step is the passage of passage in the *Aedes albopictus* (AA) clone C6/36 insect cell culture or primary isolation in cells resulting from *Culicoides sonorensis* (free of BT viruses) and which was labeled as KC (CuVa cells). After this, the hamster kidney (BHK 21) or African green monkey kidney (Vero cell) is applied as the next passage. This passage is very important in developing CPE and indicating replication of the BTV. The presence of CPE is after five to seven days of inoculation and monolayers of the cell are stored at 37°C in 5% CO<sub>2</sub> through moisture. In the end, the absence or appearance of cytopathic effect will confirm either polymerase chain reaction (PCR) or antigen detection ELISA diagnostic methods (Munmun et al., 2022).

### Molecular diagnostic tests

The reverse polymerase chain reaction (RT-PCR) with great sensitivity and specificity has been known by detecting the genome of BTV by using different types of specimens and is better a method when compared with serum neutralization test (SNT), and Viral inhibition (VI) (Saminathan et al., 2018).

De Leeuw et al. (2015) underlined another importance of RT-PCR is the ability to identify viral RNA, even after six months of infection. The specific primers (Seg-2/ VP2 centered) were designed to identify the specific strain of the bluetongue virus. Accordingly, RT-PCR can detect the virus through amplification of 101bp (base pair) nucleotide sequence of BTV genome Seg-6/ VP5 (Bandyopadhyay et al. (1998) and by amplifying 274 bp of Seg-5/NS1 gene for identification of BTV-RNA in cell cultures with sensitivity boundary of ten infectious particles of bluetongue virus as standardized by Prasad et al. (1999).

### Conventional RT-PCR

By differentiating the genome of unlike strains of BTV, Conventional RT-PCR is considered a more important method than other types of serological tests (Saminathan et al. 2018).

### Nested PCR

It is a very important method by having greater sensitivity, about a hundred times (100\*) than small PCR in the detection of the very little genome (0.1 femtograms; 5 particles) of BTV and by its advanced role of

Epidemiological investigation. By using VP7 and NS1-based primers, this nested PCR can easily identify the BTV from the tissue specimen and culture of cell (Ayanur et al., 2016).

### **Duplex PCR**

This is the advanced technique produced to decrease the untrue results by using the host  $\beta$ -actin RNAs. It is highly useful in detecting organisms by having more specificity and sensitivity than every polymerase chain reaction technique, while the presence of complicated infection was produced by Billinis et al. (2001) for the first time.

### **Reverse transcriptase-polymerase chain reaction (RT-PCR)**

Maan et al. (2012) established a trustworthy and rapid method with high sensitivity. This technique is used typing assay for each BTV serotype, through amplifying outer-capsid protein VP2 next to nucleotide sequencing and phylogenetic analyses of 26 BTV serotypes and 26 BTV strains are detected by these highly specific primers. And also, there is no cross-reaction observed during amplification with outstanding twenty-five strains. But, the importance of this RT-PCR was limited in detecting the virus in raw specimens (Å et al., 2020).

### **Quantitative real time - Polymerase chain reaction (qRT-PCR)**

This is the type of molecular technique in identifying the BTV based on Seg-1/VP1, Seg-5/NS1, Seg-2/VP2, and Seg-10/NS3 primers. Although, Seg-5 is preferred in the detection of all BTV as a result of being conserved. It is highly advanced in detecting the virus in all samples including culture, tissues and semen of infected animals by having great sensitivity than the conventional method and uses Taq-Man fluorescence-probe which is also very sensitive (Umeshappa et al., 2011; Saminathan et al., 2020).

### **Differential diagnosis**

Bluetongue disease is complicated clinically, with the infections such as; acute photosensitization, acute haemonchosis, Oestrus ovis infestation, pneumonia, plant poisoning, salmonellosis, sheep pox and Peste des Petits Ruminants (Wilson and Mellor, 2009). On the other hand, BTV is known for its similarity with pododermatitis and epizootic hemorrhagic disease of deer by clinical

signs (Eticha and Mekonnen, 2019).

### **Control and prevention**

In controlling BTD, vaccination is playing a great role, both in live and attenuated form. It is highly effective to decrease the loss of sheep and cattle, eradicating and permitting the better movement of livestock globally. Conversely, the recombinant BTV, which is dependent on different tactics still failed to be included in use, because of the absence of a licensed organization (Van Rijn, 2019).

The live-attenuated vaccine is confirmed for the prevention of Bluetongue disease and is effective in the production of immunity in vaccinated animals. In addition, this vaccine is cheap and very protective after solitary inoculation. For instance, live attenuated vaccine is common and served for more than fifty years, encouraging real and lifelong immunity. In contrast, it has been known by causing side effects such as; low milk production, abortion/embryonic death, teratogenesis and congenital defects (Ranjin et al., 2019).

Other methods are used to prevent the virus. These are the proper management of diseased animals, using non-steroidal anti-inflammatory drugs, controlling transmitting vectors, and also movement restriction of animals are important (Eticha and Mekonnen, 2019).

### **CONCLUSION**

Bluetongue is a disease that can occur in all ruminants, while the sheep are highly susceptible to it and the distribution of BTV is global. It is a disease caused by double-stranded RNA Bluetongue virus, which is a family of Reoviridae, genus of *Orbivirus*. The fever, depression, serous to mucopurulent nasal discharge, hyperaemia of the muzzle, oral and nasal mucous membranes, conjunctive and coronary band of the hooves are obvious clinical signs of the Bluetongue disease caused stillborn, abortion and lesion of the central nervous system, retinal lesions and skeletal malformations in pregnant ewes. It is transmitted by biting *culicoides* and through direct vertical, oral, and venereal transmission and indirectly by reused sharp material such as needles. The diagnosis of bluetongue disease, more importantly, depends on the clinical signs, and advanced laboratory techniques such as serological, viral isolation and molecular detection. The disease is becoming a major concern of small ruminant production and productivity worldwide. To solve these constrict, many researchers conducted a study on viral detection and isolation by using advanced techniques including molecular characterization. But, still, there is more scarcity of study evidence and disease status is unclear in the eastern part of Africa, in Ethiopia. Hence, an extended study is desired.

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