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Review Article

**A REVIEW ON SERO-EPIDEMIOLOGICAL INVESTIGATIONS  
OF BLUETONGUE VIRUS IN SMALL RUMINANTS WITH  
EMPHASES ON QUETTA BALOCHISTAN.**Najeeb Ullah<sup>1</sup>, Zafar Ahmad<sup>1</sup>, Muhammad Shafee<sup>1</sup>, Muhammad Rizwan<sup>1</sup>, Muhammad Naem<sup>1</sup>, Hafiz Muhammad Arif<sup>1</sup>, Shafiq Ahmad<sup>2</sup><sup>1</sup> Center for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta, Pakistan.<sup>2</sup> Balochistan Agricultural Research and Development Center (BARDC), Quetta., Pakistan**Abstract:**

*Bluetongue is a disease of almost all animals particularly of domesticated and small ruminants such as goats and sheep. BT is caused by a virus known as Bluetongue virus which is a dsRNA virus and belongs to a genus Orbivirus and to a family Reoviridae, as it is transmitted by biting midges (Culicoides) therefore it is insect borne disease. Bluetongue is disease of socioeconomic importance, as it has a great impact on trade at international level of livestock and its yields. In the previous era Bluetongue was only limited to the endemic areas between latitudes 40°N and 35°S; however, this traditional limit has been crossed now and bluetongue has extended to vast areas. Spread of the bluetongue disease is mainly due to the presence of specific vector in the area such as C. imicola, a significant vector of the virus in the "Old World". Bluetongue in South Asia countries such as Iran, India, China, Afghanistan and Pakistan, is endemic and has higher prevalence rates which are recently reported. This is a review which demonstrates selective information on this harmful disease including its diagnosis by different techniques, such as c-ELISA and Real-time PCR based genotyping. Its causative agent, history, spread, routes of transmission and range of target host, pathogenesis and diagnosis of the disease.*

**Key words:** *Bluetongue, Quetta, c-ELISA, Orbivirus, Pathogenesis***Corresponding Author:****Muhammad Rizwan,**Center for Advanced Studies in Vaccinology and Biotechnology,  
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## 1. INTRODUCTION:

Bluetongue is an infection with no contagious type transmission history and it affects large number of animals especially small ruminants like Sheep and goats, though cattle and goats are vulnerable, the infection is commonly subclinical or inapparent in these species. Cattle serve as the most crucial reservoir host of BTV[1]. Bluetongue is caused by a virus known as bluetongue virus (BTV). BTV virus belongs to a genus Orbivirus and to a family Reoviridae [2, 3] Bluetongue virus has been accommodated in the Office International des Epizooties. Because of its importance it is globally known virus. Bluetongue virus is widely spread as it is present almost all over the world and circulates in around 26 serotypes known until today [4,5]. The demonstrations of bluetongue range from an unapparent to a fatal outcome depending on the serotype and strain of the virus and the species, breed and age of the infected animal; older animals are generally more prone to the infection [6]. The losses in economy due to the Bluetongue disease cannot be denoted by certain digits but it has been estimated that this disease contributes in economic loss of almost three(03) billion US\$ per annum globally [7]. Direct losses include (fatality, abortions, loss in animal weight or decreased milk production and meat quality) and, the more important thing is that indirect losses are of concern as animals exportation becomes more restricted, puts worse impacts on the other vital products of animals like their semen and foetal bovine serum. The cost consumptions in terms of prevention and control strategies must also be considered.

## 2. Historic and distributive background:

BTV was primarily identified at end of 18<sup>th</sup> century in South Africa when a well known finewool sheep were imported from Europe [8]. This disease was initially named with different names such as epizootic malignant catarrhal fever, malarial catarrhal fever of sheep [9]. Bluetongue was for the first time identified in cattle in 1933 [10] this disease (BT) was called as pseudofoot-and-mouth disease because the clinical signs and symptoms were similar to seer beck or sore-mouth, foot and mouth disease, [11]. The current name "Bluetongue", is derived from the African "bloutong", which was used by South-African grangers who observed cyanosis on tongue in severely infected animals [12]. Cases of

Bluetongue occur mostly in Asia, Africa and Europe because of the existing of Culicoide imicola vector. Existence of BTV, its target host and Presence of bluetongue virus, its susceptible host and adequate vector at the same time allow the spread and transmission of Bluetongue virus. In the past history, the virus was present in a geographic band between the latitudes 40°N and 35°S where its vectors, certain species of biting midges, were living [13]. The BTV spread even further in North America and china, up to 50°N [14]. The presence of bluetongue disease was just restricted to South Africa before 1940s.

## 3. Sero-Epidemiology of Bluetongue in Pakistan and neighboring countries:

### 3.1 Pakistan:

#### 3.1.1 Sero-Epidemiology of Bluetongue in NWFP (KPK)

Among the animal diseases in Pakistan, bluetongue has important contribution in badly affecting livestock sector and it's a main hindrance in the development of livestock sector. Live stock sector has contributed 55.4% in 2012-2013 in the agriculture sector as compared to the 55.3% in year 2011-2012. (Economic Survey of Pakistan, 2013). Bluetongue was for the first time investigated in Pakistan in 1995. Study was conducted to know the prevalence of serum antibodies against bluetongue virus in 38 sheep flocks in the northern areas of Pakistan previously known as North West Frontier Province of Pakistan and to identify demographic and productivity variables that are linked with BTV seropositivity. Akhter et al (1997) collected blood sample from ewes adopting random sampling strategy in April 1995. Samples of blood were analyzed by using (c-ELISA) for BTV specific immunoglobulins. Bluetongue seropositivity rates of 184 (48.4%) out of 380 tested sera, and in 89.5% (34/38) of the flocks were obtained. Seropositivity ranged from 12.5 to 100% in the 34 BTV positive flocks (median = 47), [16].

#### 3.1.2 Sero-Epidemiology of Bluetongue in Quetta, Balochistan.

The largest city and provincial city of Balochistan is Quetta. According to the 2017 census Quetta has a population of 1,001,205 heads. It is Situated at an average elevation of 1,680 meters (5,510 ft) above sea level, its geographical coordinates are 30° 12' 0" North, 67° 0' 0" East; the city is a major stronghold along the western frontier of the country (figure 1).



**Fig.1: Location of Quetta and its surrounding areas.**

There is very little data available for the investigation of the BT disease in Pakistan especially Balochistan. Therefore a study was conducted to know the disease burden and circulating serotypes in the area. A study was carried out by (Shabbir *et al.*, 2018), to investigate seroconversion and prevalence of serotypes in different districts of Balochistan. A total of 876 samples were subjected to the different techniques like (c-ELISA) and real-time polymerase chain reaction (RT-PCR). Blood samples collected from different districts included (Quetta = 300), (Mastung = 201), (Killa Saifullah = 75) and (Kech = 300) from combined Goats and sheep. Almost all herds under study were found to be BTV seropositive (herds = 97), overall Seroprevalence at the individual level was 47.26% ( $n = 414/876$ , 95% CI = 43.92%–50.63%). Goats were comparatively highly seropositive for anti-VP7 antibodies (50.87%, 95% CI = 45.99%–55.73%) than sheep (44.21%, 95% CI = 39.81%–48.70%). In the study Serotype 8 was the most prevalent (26.82%, 95% CI = 14.75%–43.21%) followed by an equal prevalence of serotypes 2 and 9 (7.31%, 95% CI = 1.91%–21.01%). This study was the first ever study conducted in the province Balochistan and results warn that further investigations need be done and strategies need to be made to control BT from being spread more. Seroprevalence of 49.60% with serotypes 8,9 has been identified in Quetta which clearly suggests that this disease is prevalent in the area which is alarming to the farmers [17]. As 70% of the people in Quetta, Balochistan depend on the livestock regarding source of income so, it is needed to pay full attention towards this alarming disease and further studies need to be conducted. Bluetongue is found to be a

transboundary disease so a programmed surveillance system needs to be developed.

### 3.2 Iran:

A study was conducted to define the distribution and seroprevalence of bluetongue virus (BTV) disease in sheep in Kohgiluyeh and Boyer-Ahmad province of Iran, and to investigate associated risk factors with the exposure of these sheep to BTV infection. A total of 262 blood samples were collected from apparently healthy sheep during the year 2011. The samples were analyzed for BTV specific antibodies with competitive enzyme like immunosorbent assay (c-ELISA). Out of 262 samples 203 (77.48%) sera were found positive for BTV specific antibodies, there were observations like important variations found in the seroprevalence of BT, between sex and age of sheep ( $p < 0.001$ ). No statistically significant differences were noted in BTV seroprevalence among different seasons, nor among recently aborted and normally delivered [40].

In another study in Iran conducted, between 2011 and 2012, sheep and goats screened for BTV specific antibodies. Samples were subjected to competitive Enzyme Linked Immunosorbent Assay. BTV prevalence was about 67.7% in goats and it was also concluded that animals seropositivity ranged from 33.3% to 100% [18].

### 3.3 India:

A research study was carried out to know the status of Bluetongue virus in Jharkhand an eastern state of India. Goats, sheep and cattle were subjected for the screening of BTV specific antibodies. 480 blood samples were tested and results revealed that out of

480 sera samples, 83(43.68%) of sheep, 91(43.33%) of goats and 46(57.50%) of cattle sera were declared as positive for anti-BT antibodies. The percentage of Bluetongue seropositivity ranged between 41% and 51% in various agro-climatic zones [19]. During a research study of bluetongue disease in Tripura state of India, it was concluded that BTV was present there with high Seroprevalence rate. Healthy goats and cattle were selected for sampling in Tripura state and 195 (136 goats, 59 cattle) blood samples were collected. Blood was subjected to ELISA for BTV antibodies detection and it was observed that 59 goats (43.88%) and 25 cattle (42.37%) were having anti-BT antibodies in their sera. Consequently Bluetongue infection was present in the Tripura state of India [20].

### 3.4 China:

Prevalence of bluetongue was investigated in western China, in Tibetan sheep and yaks. 3771 blood samples were collected from the targeted animals. Samples were analyzed for BTV seropositivity by using ELISA kit. Their results revealed a Seroprevalence of 17.34%, 654 samples were positive for BTV out of 3771. BTV specific antibodies were detected in (443/2187) 20.3% of Tibetan sheep where as in yaks BTV positivity was about (211/1584) 13.3%. They also reported seasonal factors which contributed in the disease transmission like 16.5% to 23.4% in sheep. In the yak group, BTV Seroprevalence was 12.6%, 15.5%, and 11% in Tianzu, Maqu, and Luqu respectively [21].

### 4. Etiology:

BTV belong to a genus; Orbivirus in the family Reoviridae. Morphology of Bluetongue virus is same like that of certain Orbiviruses, such as equine encephalosis virus, epizootic hemorrhagic disease virus or African horse sickness virus. BTV has no envelop around its capsid and it's of 90 nm diameter size, capsid has three layered icosahedral protein structure [22]. The genome comprises of (10) double-stranded (ds) RNA segments which code for (07) structural proteins "VP1–VP7" and (04) non-structural proteins (NS1–NS3 and NS3A). External coating of BTV is consisting of (2) main proteins, (VP2 and VP5) [ 22]. VP2 protein identifies the serotype, and is responsible for receptor bonding, haemagglutination and provoking (host-specific immunity).

### 5. Transmission:

#### 5.1. Biting of Culicoides midges

Biting midges are the main source of transmitting bluetongue virus and transmission of bluetongue mainly depends on the presence of competent vector in the area and also vulnerable hosts. Culicoides genus comprises of (1300 – 1400) species currently [14], merely 30 of the species of culicoides are BTV

vectors that spread the virus [25]. Midges are often there in temperate, moist, and muddy places and also where organic matter is plentiful, presence of compatible host also matters in the prevalence of culicoides species. Midges are mainly energetic from about one hour before sundown until one hour after sunup [14]. life cycle of midges takes almost (02-06) weeks to complete, life cycle of a midge comprises of four stages which includes an egg, 04 larval instars, a pupa and an adult [14]. Whole life span of adult midges is only ten to twenty days, but they can live up to ninety (90) days when climate is colder than usual [14, 26].

### 6. Host Range:

Susceptibility of to infection with BTV ranges to almost all ruminants, but in sheep the clinical symptoms appears more often seen [27]. Cattle are important as they play a major role in the BTV epidemiology because they show an extended viraemia, in the past BTV disease has been seen to have subclinical course [28]. During the outbreak in the Central Europe and Western Europe [29] there were clinical symptoms even easily observed in the cattles [30]. Though bluetongue disease is mainly observed in small ruminants and camelids, under some conditions it can also spread and infect carnivores. The use of contaminated vaccines with BTV has been reported to infect dogs [31]. Lynxes kept in Belgian zoo, Eurasia have observed to carry the Bluetongue; where it's also observed in the cats that were fed on the aborted or stillborn foetuses of ruminants raised on neighboring farms [32]. Immunoglobulins against BT virus have been seen to be present in African animals like cheetahs, lions, jackals, hyenas, wild dogs and large-spotted genets [33].

### 7. Pathogenesis

As soon as the virus is taken up by the host through the bite of a midge which carry BTV, the dendritic cells transport the virus from the site of infection such as skin to the nearby lymph nodes [34], which is the first site where virus replicates [15]. Later, it enters into the blood circulatory system causing a primary viraemia which later pips secondary organs, i.e., lymph nodes, spleen and lungs [35]. The virus proliferates in macrophages, vascular endothelial cells, and lymphocytes [15, 35]. During the initial stage virus is associated with the blood elements, where in the later stages it's completely associates with red blood cells [36, 15]. The virus molecules seem to be confiscated in infoldings of membrane of the RBCs [15], permitting extended viraemic conditions in the company of the neutralizing antibodies. Necrosis and apoptosis generally results when host cells are infected with BTV [38] and, by the activation of the p38MAP kinase, the virus

enhances its vascular permeability [39]. Furthermore, it elicits the production of cyclooxygenase-2, IL-1, TNF $\alpha$ , IL-8, IFN-I, and IL-6; where it increases the plasma concentration for the thromboxane and prostacyclin, therefore it results in the increase in the amount of inflammatory response. During this disease the blood vessel are being injured of the infected tissues and as a result of the injury it ends up in tissue infarction and vascular occlusion.

### 8. Clinical signs:

Bluetongue in sheep causes acute chronic and subclinical conditions; where wool breeds are much more sensitive to the infection caused by the bluetongue virus. The symptoms appear after the four to eight days of infection [28] which gradually results in the apathy, hyperthermia, tachypnea, hyperaemia of the lips, nostrils with rise salivation and serous nasal discharge. The nasal way is clear in the beginning but later it becomes mucopurulent and it forms crust around the nostril once it get dries. Oedema of the lips, tongue, sub mandibulum and sometimes ears appears petechiae develop on the conjunctiva and ulcers on the oral mucosa. Cyanotic tongues are found in rare cases. In certain conditions profuse haemorrhagic diarrhea, dyspnoe, or vomiting which results in the aspiration pneumonia. At the end of the pyrexia stage the infected sheep may have laminitis, coronitis, and necrosis of striated muscles; and as a result, stand with an arched back and are reluctant to move. Dermatitis, torticollis out breaks can happen in the wool [28,30,6].

### 9. Diagnosis:

The initial diagnosis is very important and it can be done using laboratory examination that includes results of post-mortem, epidemiological assessment and clinical signs. The sample that can be selected for investigation comprise of blood serum, tissue samples of spleen, lungs, bone marrow, blood serum, liver, , lymph nodes, non-coagulated blood (EDTA or heparin); Sometimes if asked so fetus skeletal and heart muscles along with brain tissue can be taken as well[28]. When collecting the blood serum so it should be freeze down to -20°C before its being transported, where rest of the samples should be placed in the ice [28]. Complete blood can be placed at -4 °C for longer time period but once the blood cells are being isolated so then it's kept in 10% dimethyl sulphoxide at -70 °C.

### 9.1 Antigen identification:

There are many molecular tools that can be used to identify the BTV but one of the most reliable and direct technique is the reverse transcription-polymerase chain reaction (RT-PCR). Through this

technique you can quantitatively and efficiently detect RNA from the blood or tissues samples even after 6 month of the infection [37]. There are some other techniques as immunofluorescence tests, antigen-capture ELISA and immunospot that are rarely used.

### 9.2 Antibody identification:

The best known method to detect the BTV is the ELISA test in which we use the Serogroup-specific antibodies against the BTV by detecting the VP7 protein. This rapid test allows you to determine the BTV even after six days of infection [47]. In the market some other kits also available in which they have the bulk milk samples that can detect the antibodies against it [48]. In addition to the previous technique, agar gel immunodiffusion test is also available but through this method you can produce cross reaction with the other orbiviruses , where a complement-fixation test can be used or haemagglutination-inhibition test . The uppermost sensitive test which is very specific to it is the serum neutralization but the drawback of it is that it consumes time and expensive.

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