

## Observations on the Epidemiology of Bluetongue Disease

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**Abstract:** Bluetongue disease (BT) is an arthropod transmitted disease that affects, mainly ruminants causing great global economic losses. The disease is caused by a double stranded RNA virus member of the genus Orbivirus; family Reoviridae. Bluetongue virus (BTV) evolves through a process of quasispecies evolution that is driven by genetic drift and shift as well as intragenic recombination. Quasispecies evolution has over time led to the establishment of 26 genetically distinct strains of the virus in different epidemiological systems throughout the world. The intrinsic molecular determinants that influence the phenotype of BTV have not clearly been characterized. It is currently unclear what contribution each of the viral genome segments have in determining the phenotypic properties of the virus and it is also unknown how genetic variability in the individual viral genes and their functional domains relate to differences in phenotype. Moreover, BTV field strains may differ substantially from each other with regards to their phenotypic properties (i.e. virulence and/or transmission potential). There are various techniques which are involved in BTV diagnosis; however, it is of prime importance to use recent techniques for detection of BTV in infected animals due to the expected high sensitivity, accuracy and specificity. Molecular characterization of BTV is currently based on the detection of virus molecule. Sequencing of BTV has lead scientists to know more accurate information about the origin and serotypes of the virus and to plane for a proper vaccine production strategy. In conclusion, it is of prime importance to recommend starting a proper vaccine strategy against BTV in Egypt science all vaccine protocol has ceased due to the high risk of incoming out breaks which may result in great.

**Key words:** Bluetongue disease • Bluetongue disease virus • BT • BTV

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### INTRODUCTION

Bluetongue (BT) is an infectious viral disease of domestic and wild ruminants. It is listed as notifiable disease by OIE 2015 (<http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2015/>) to monitor the disease and provides early warning of possible outbreaks [1].

Bluetongue virus (BTV) is transmitted by adult female hematophagous midges, belonging to species of the genus *Culicoides*. In cattle and goats, bluetongue virus (BTV) causes very mild, self-limiting infections with only minor clinical consequences. On the other hand, in sheep the clinical symptoms are sever represented by depression, fever, excessive salivation, nasal discharge, facial oedema, hyperaemia, ulceration of the oral mucosa, coronitis and torticollis. Oedema of the head and neck in sheep restricts blood flow to the tongue, leading to a

cyanotic 'blue' appearance and hence the name given to the disease [2].

**Virus Evolution, Serotypes and Outbreaks:** It is believed that BTV evolves through genetic drift/shift as well as intragenic recombination [3]. These evolutionary processes result in the generation of quasispecies populations in both host and vector cells, from which variants with optimal fitness may be selected under different environmental conditions [4].

There are 26 BTV serotypes currently recorded and the 25<sup>th</sup> serotype is proposed to be a Toggenburg virus [5]. The disease was firstly described in Africa in the early 19<sup>th</sup> century and from there BT was transmitted to Europe, the most serious incursion caused by the strain of BTV serotype 8 (BTV-8) [6]. Due to consequence of climate change, the global distribution of individual BTV serotypes is not uniform (Fig.1) [7].

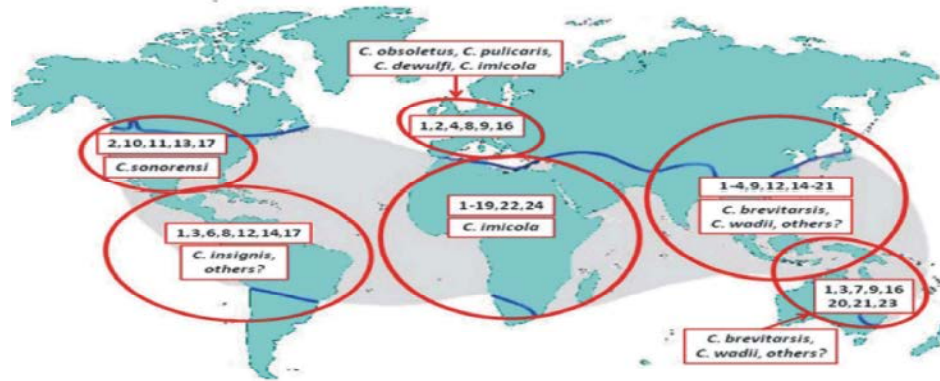


Fig. 1: Global distribution of serotypes of BTV and *Culicoides* spp. [11].

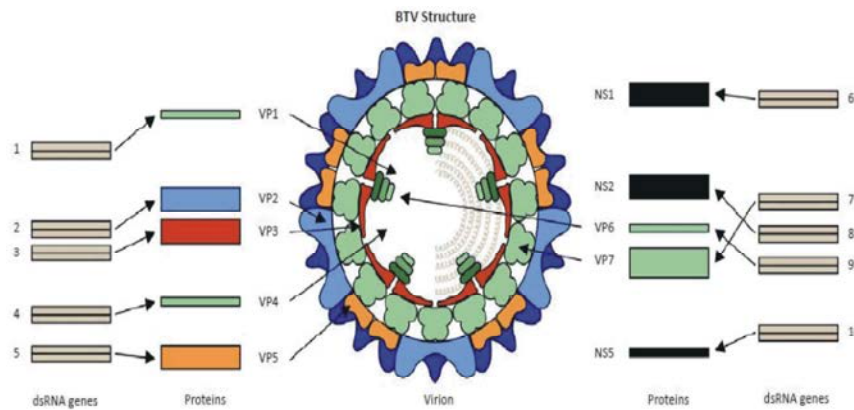


Fig. 2: Coding the viral proteins VP1-VP7 and non-structural NS2- NS3 by the 10ds-RNA genes of Bluetongue virus [26].

Several BTV serotypes (serotypes 1, 2, 4, 6, 8, 9, 11 and 16) have invaded Europe since 1998 [8]. BTV serotypes that invaded the Mediterranean basin after 1998 have all originated in adjacent regions of either Africa or Asia [9]. Similarly, BTV-10, 11, 13 and 17 have long occurred throughout extensive portions of North America and serotype 2 was first documented in Florida nearly 30 years ago, some 10 additional BTV serotypes (BTV- 1, 3, 5, 6, 9, 12, 14, 19, 22 and 24) have been identified in the southeastern United States since 1998. Eight serotypes (1, 3, 9, 15, 16, 20, 21 and 23) of BTV have been identified in Australia [7].

Interpretation of the BTV status of other regions of the world is complicated by the lack of adequate surveillance in many areas, particularly throughout much of Asia and the Middle East e.g. Egypt. BT killed more than 1.5 million native and genetically highly susceptible European sheep [10].

**Bluetongue Disease in Egypt:** The disease was firstly reported in Egypt as early as 1974 by Ismail [12] and Mohamed [13] whereas the virus was isolated on Vero cells and embryonated chicken eggs (ECE). The virus was

titrated and found in erythrocyte sediment. The identified serotype was BTV-16 [12-13]. In 1980 BT clinical signs appeared to be more aggressive. The virus was identified by neutralization, complement fixation, agar gel precipitation and passive haemagglutination tests [14]. In 1991, the virus was isolated from animal slaughter houses and propagated in BHK-21 cells and ECE and identified by complement fixation test, fluorescent antibody technique and passive haemagglutination test [15] then BTV was found circulating in blood of all ruminants [16]. The disease remains circulating until it was isolated again and identified according to Habashi [17]. Moreover, Habashi *et al.* [18], isolated BTV and applied vaccinal trial from isolated serotypes. In 2012 and 2013, the disease was isolated from different governorates where it caused focal outbreaks [19, 20].

**Structural and Molecular Biology of Bluetongue Virus:** BTV is a double stranded RNA virus and a member of genus Orbivirus, family Reoviridae [21]. Genome of BTV is composed of double-stranded RNA, divided into 10 linear segments (Fig. 2). The RNA codes for 5 non-structure proteins (NS1, NS2, NS3, NS3A and NS4) and

Table 1: Recent structural analyses of the major protein components of BTV

Genome segment (size: bp)	ORFs bp	Protein nomenclature	Location	Number of Amino acids Da	Properties and Functions
1(3954)	12-3917	VP1 (Pol)	within the sub-core at the 5 fold axis	1302	RNA dependent RNA polymerase
2(2926)	20-2887	VP2	outer capsid	956	Trimmer, controls virus serotype, serotype specific antigen cell attachment protein, outer layer of the outer capsid, contains neutralizing epitopes, most variable protein, involved in determination of virulence cleaved by proteases.
3(2770)	18-2720	VP3 (T2)	sub-core capsid layer	901	Innermost protein capsid shell, sub-core capsid layer, self assembles, retains icosahedral symmetry by itself, controls size and organization of capsid structure, RNA binding, interacts with internal minor proteins. Highly conserved protein. Physical organization of genome.
4(1981)	8-1940	VP4 (Cap)	within the sub-core at the 5 fold axis	644	Dimers, capping enzyme, guanylyltransferase, transmethylase 1, transmethylase 2.
5(1769)	35-1690	NS1 (TuP)	Cytoplasm forms tubules	552	Forms tubules of unknown function in the cell cytoplasm. These tubules are a characteristic of orbivirus replication.
6(1638)	30-1607	VP5	outer capsid	526	Inner layer of the outer capsid, glycosylated, helps control virus serotype, variable protein, trimmer, causes membrane fusion indicating a role in membrane penetration during initiation of infection.
7(1156)	18-1064	VP7 (T13)	Outer core	349	Trimmer, forms outer core surface, in some species it can form flat hexagonal crystals made up of layers of hexameric rings of trimmers, involved in cell entry, involved in high core infectivity in vector insect and cells, reacts with "core neutralizing" antibodies, Immuno dominant major serogroup specific antigen.
8(1124)	20-1090	NS2 (ViP)	Cytoplasm, viral inclusion bodies	357	Important viral inclusion body matrix protein, ssRNA binding phosphorylated by cellular kinase. RNA binding may be affected by phosphorylation state. May be associated with outer capsid Nucleotidyl phosphatase activity.
9(1046)	16-1002	VP6 (Hel)	Within the sub-core at the 5 fold axis	329	ssRNA and ds RNA binding, helicase, NTPase. Two forms of VP6 can be generated by initiation from two in frame initiation codons
10(822)	20-706 59-706	NS3 NS3a	Cell Membranes	229 216	Glycoproteins, membrane proteins, involved in cell exit in some genera these are variable proteins and may be involved in determination of virulence. Cytotoxic, can disrupt cell membranes

[ Mertens *et al.*,: [http://www.reoviridae.org/dsrna\\_virus\\_proteins/BTV.htm](http://www.reoviridae.org/dsrna_virus_proteins/BTV.htm)]

7 structure proteins (VP1–VP7). It has actually 26 serotypes which show no cross protectivity [22, 23]. The mature BTV virion is non-enveloped, spherical in appearance and can be divided into an outer diffuse protein layer and an icosahedral sub-core. The sub-core is composed of 60 dimers of VP3 (coded by segment 3). The sub-core is covered by 260 trimmers of VP7 (coded by segment 7) [24]. Epitopes of VP7 are exposed through the outer protein layer of the BTV. The outer diffuse protein

layer of the mature virion is composed of 60 VP2 trimmers (coded by segment 2) that are interspersed with 120-VP5 trimmers (coded by segment 6) [25]. The function of each protein is summarized in Table 1.

### Virus Diagnosis

**Virus Isolation and Propagation:** Samples are from different sources from blood of sheep and cattle and some internal organs from dead animals as spleen.

The following samples are to be taken at the peak of fever or at postmortem for virus isolation if bluetongue is suspected: 15–20 ml of heparinized blood and spleen, liver, kidney, lung and heart tissues. Isolation of the virus can be performed via inoculation of blood samples in ECE (embryonated chicken egg) and/or tissue culture BHK-21 (Baby hamster kidney cells) [27].

**Virus Identification:** A major problem in the diagnosis of BTV infection by immunological methods is the cross-reactivity with proteins from other orbiviruses, although this may be circumvented by the use of competitive ELISA (c-ELISA) [28]. Commercial c-ELISA depends on the presence of antibodies targeting VP7 in serum of infected animals [19]. To avoid these problems, PCR-based assays were developed and evaluated for the detection of BTV serotypes based on nucleotide sequences of different genome segments [29]. Thomas *et al.* [30] have discovered non-structural protein 1 (NS1) tubules, viral inclusion bodies (VIBs) and virus particles using Electron microscope. Cells infected with BTV were prepared for immunocytochemistry by freeze substitution technique and incubated with specific monoclonal antibodies and colloidal gold probes to detect virus antigens of varying copy number; these BTV proteins were structural proteins VP2 and VP7 and the non-structural protein NS2. These proteins were pre-fixed with minimum concentrations of glutaraldehyde to inactivate the virus. Results showed that cryo-sections yielded the best signal-to-noise ratio for all proteins examined and were therefore the most sensitive method for the detection of low copy number proteins [31]. This method was modified later by Yin *et al.* [32] on VP7 using gold nanoparticles (GNPs) coated with polyclonal antibodies and used in antigen captured ELISA as a sensitive method for detection of very low amounts of VP7 and other viral proteins. Sequencing of RNA coding genes of viral proteins of VP2, 5 and other non structural proteins is a very accurate method for virus serotyping. Phylogenetic analysis of each serotype was estimated and relations between BTV serotypes were made clear.

**Virus Vaccine:** A suitable live attenuated vaccine is used for vaccination against BT in Europe against BTV-1, 2, 4 and 8. Some European countries (Germany, Belgium, Switzerland, Spain and Italy) have used vaccination strategy against BT since 2008. It is worth mentioning that BT emergence vaccination is a must during outbreaks because it is not contagious disease [33].

Egypt has ceased vaccination program against BTV since 2008. BTV has been diagnosed lately in the last few years; also the disease has been reported in many adjacent countries so the chance that BTV will form a disastrous outbreak in Egypt is most likely. Therefore, it is recommended to produce a vaccine from the local serotypes isolated from Egypt.

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