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Molecular Characterization of Bluetongue Disease

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Abstract: Bluetongue disease (BT) is a viral disease affect ruminant. Bluetongue disease virus (BTV) non- is an enveloped, spherical double-stranded RNA virus, belongs to genus Orbiviruss and family Reoviridae. The RNA codes for 5 non-structure proteins (NS1, NS2, NS3, NS3A and NS4) and 7 structure proteins (VP1–VP7). VP7 reacts with core neutralizing antibodies, Immuno dominant major serogroup specific antigen. While the outer diffuse protein layer of BTV is composed of VP2 which controls virus serotype and considered as serotype specific antigen cell attachment protein. NS3/NS3a is responsible for non-lytic release of BTV from insect cells. On the other hand, it is believed that BT virus from mammalian cells is through lytic mechanism. BTV can be isolated from blood, semen or tissue sample as liver, brain, spleen, lymph node & mucosal epithelium. The virus can be propagated on specific pathogen free- Embryonated chicken egg (SPF-ECE) via yolk sac or intravenous rout. BT virus may also propagate through cerebral inoculation of suckling mouse. The virus can then be adapted to mammalian cell culture (as BHK-21 or Vero cells) or insect cell culture (as KC cells). The virus can be diagnosed by E, Electron Microscopy, X- ray crystallography, dot immunobinding assay (DIA), immunoelectron microscopy, different types of ELISA and different types of RT-PCR specially Real Time PCR. Sequencing of genes encoding variable protein and constructing phylogenetic trees reveal relation between different BTV serotypes.

Key words: Bluetongue disease · Bluetongue disease virus · BT · BTV · Phylogenetic Analysis · ELISA

INTRODUCTION

Bluetongue (BT) is a viral disease with great economic importance for ruminants specially sheep and goat causing high morbidity and mortality. Bluetongue disease virus (BTDV) is non- contagious as it is transmitted by cullcoides. Bluetongue virus (BTV) belongs to genus Orbiviruss and family Reoviridae [1]. The disease is manifested by sever clinical signs such as lameness, fever, swelling and cyanosis of lips and tongue. The disease possess high tendency to spread among large and small ruminants and therefore it is listed as a multiple species disease by Office International des Epizooties [2]. There are twenty six serotypes of BTV [3]. Most recently, a novel serotype was detected, BTV-n (boldface), during sequencing the gene encoding VP2 in goats, Corsica, France, 2014 [4].

The disease was firstly described in Africa in the early 19th century. There have been multiple incursions of BTV into Europe from Africa, the most serious incursion caused by the strain of BTV serotype 8 (BTV-8). The outbreak spread across Europe between 2006 and 2009 and caused clinical signs in cattle, goats and sheep. BTV serotypes tend to re-emerge in France and other parts of Europe after a period of disappearance [5]. As limited sequence information is available for BTV strains circulating across Africa, it was not possible to conclude with certainty the origin of this virus. However, full genome sequence analysis indicated that it may have originated from sub-Saharan Africa [6].

Virus Molecular Structure: BTV is a double stranded RNA divided into 10 linear segments. The RNA codes for 5 non-structure proteins (NS1, NS2, NS3, NS3A and NS4) and 7 structure proteins (VP1–VP7). BTV 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) and it controls size and organization of capsid structure, RNA binding, interacts with internal

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minor proteins. The sub-core is covered by 260 trimmers of VP7 (coded by segment 7) and it reacts with core neutralizing antibodies, Immuno dominant major serogroup specific antigen. The outer diffuse protein layer of the mature virion is composed of 60 VP2 trimmers (coded by segment 2) and it 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 also it is interspersed with 120-VP5 trimmers (coded by segment 6). VP5 causes membrane fusion indicating a role in membrane penetration during initiation of infection [1].

BTV is released from insect cell with non-lytic release not only by budding, acquiring a temporary envelope, but also via disruption of the cell membrane, whereas cell lysis is the prominent release mechanism in mammalian cells. NS3/NS3a comprises a long N-terminal domain, two transmembrane domains with a short extracellular domain in between and a shorter C-terminal cytoplasmic domain. Recent researches confirmed the interaction between the p11 cellular calpactin complex subunit and recruitment of the ESCRT-I TsgI protein that facilitate virus release as non-lytic mechanism [7].

Virus Isolation and Propagation: BTV can be isolated from blood, semen or tissue sample as liver, brain, spleen, lymph node & mucosal epithelium. The virus can be propagated on specific pathogen free- Embryonated chicken egg (SPF-ECE) via yolk sac or intravenous rout. BTV may also propagate through cerebral inoculation of suckling mouse. The virus can then be adapted to mammalian cell culture (as BHK-21 or Vero cells) or insect cell culture (as KC cells) [8].

Group-Specific Antigen Detection Assays: Several specific virus-induced structures have been discovered, including non-structural protein 1 (NS1) tubules, viral inclusion bodies (VIBs) and virus particles [9]. These structures have subsequently been identified in every BTV-infected mammalian and insect cell type that has been studied, including primary mammalian cells such as monocytes and lymphocytes (undergoing blastogenesis) [10]. X-ray crystallography is another technique that is revealing detailed information about the three-dimensional structures of BTV proteins. The structure of each protein is very specific e.g. BTV encodes a single capping protein, VP4, which catalyzes all reactions required to generate cap1 structures on nascent viral transcripts. Further, structural analysis by X-ray crystallography

Fig. 1: The Crystal Structure of Bluetongue Virus VP7 [13].

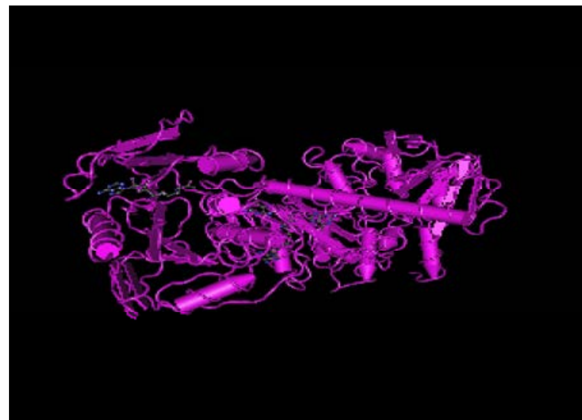

Fig. 2: The Structure of Bluetongue Virus VP4 [14].

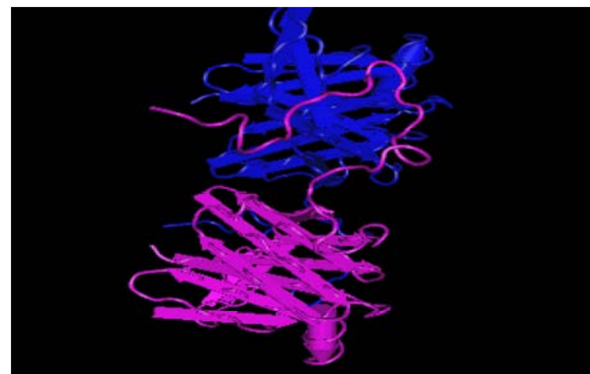

Fig. 3: Crystal Structure of the RNA Binding Domain of BTV Non-structural Protein 2(NS2) [15].

indicated each catalytic reaction is arranged as a discrete domain [11]. Mertens *et al*., [12] has determined the atomic structure of the BTV core using X-ray crystallography, demonstrating how the major core proteins are assembled and interact. Fig. 1-4 show X-ray crystallography of different BTV proteins.

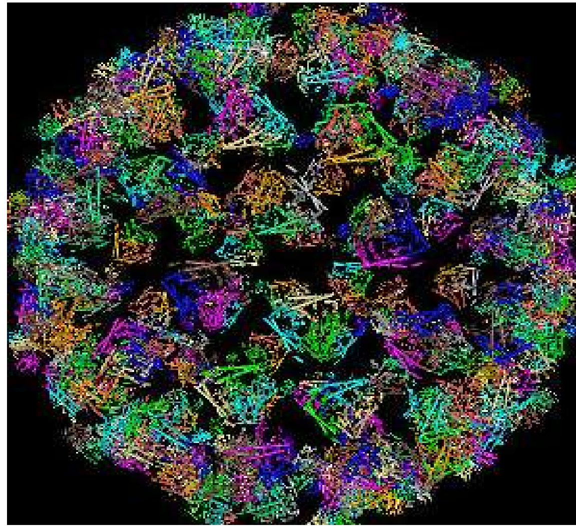

Fig. 4: Bluetongue Virus Structure Reveals a Sialic Acid Binding DOM Amphipathic Helices and a Central Coiled-Coil in Proteins VP5 and VP2 [16].

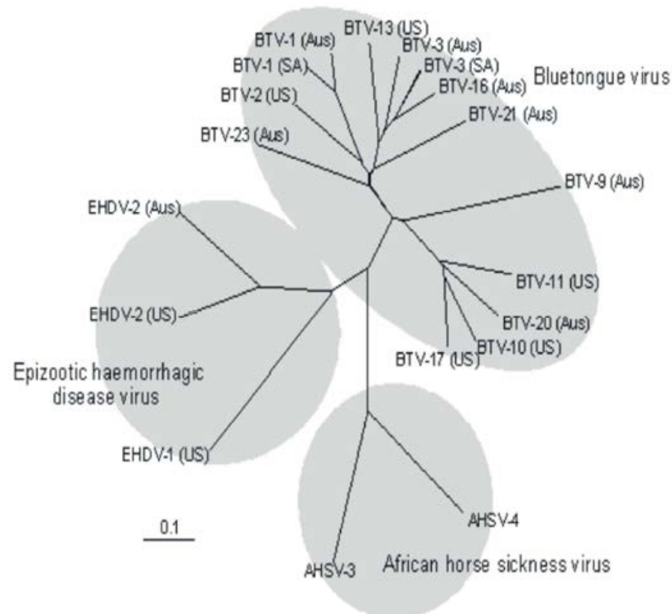

Fig. 5: Phylogenetic tree for the orbiviruses constructed using partial VP2 sequences (amino acids 303-464). VP2 is the larger outer capsid protein and major neutralisation antigen. Amino acid sequences derived from the nucleotide sequence of genome segment 2 were aligned by using the CLUSTALW 1.60 program The tree was prepared using Clustal X. [29]

Group-Specific Antibody Detection Assays: Many types of ELISA showed high sensitivity as well such as ELISA [17], dot immunobinding assay (DIA) [18] and immunoelectron microscopy [19]. The use of antigen capture ELISA for the detection of BTV in the blood of infected ruminants has either been unsuccessful [20], has detected antigen only in animals with high viremia [21], or was not consistent enough to allow for the reliable diagnosis of BTV [17]. A major problem in the diagnosis of BTV infection by immunological methods is also the cross-reactivity with proteins from other orbiviruses [22], although this may be circumvented by the use of c-ELISA [23]. 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.

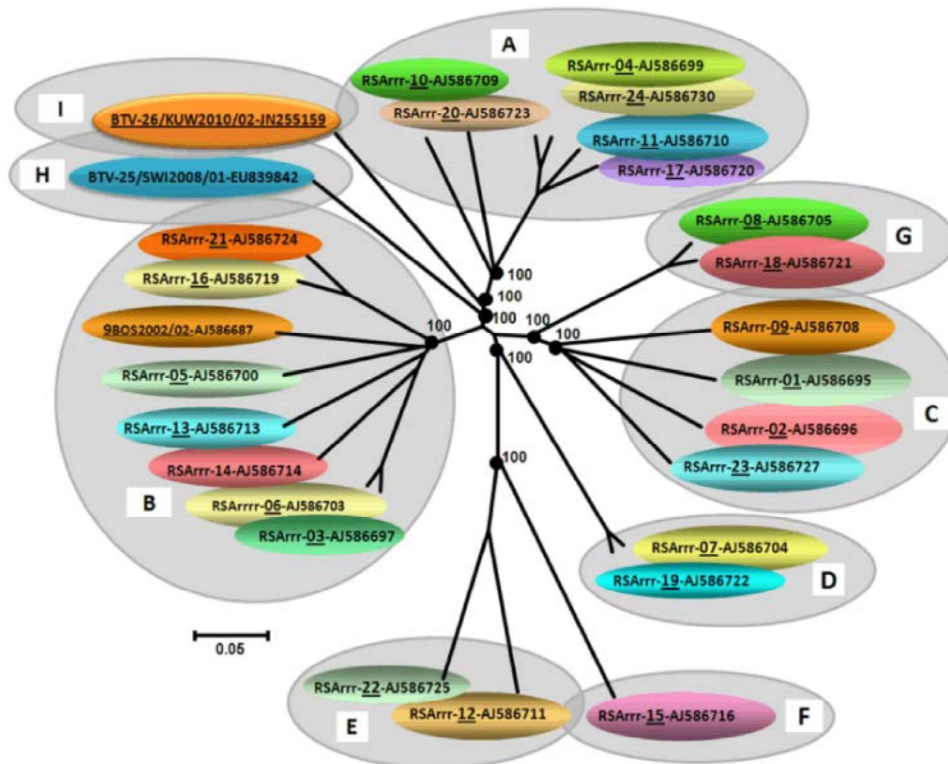

Fig. 6: Neighbour-joining tree showing relationships between Seg-6 from KUW2010/02 with the twenty five reference strains of different BTV serotypes.The eight evolutionary branching points are indicated by black dots on the tree (along with their bootstrap values), dividing the sequences into nine 'Seg-6 nucleotypes' designated 'A-I'. In previous studies, eight Seg-6 nucleotypes were identified. Members of the same nucleotype show >76% nt identity in Seg-6, while members of different nucleotypes show <76% nt identity. However the analyses of BTV-26 (KUW2010/02) described here indicate that it forms a new 9th Seg-6 nucleotype (I), as it shows a maximum of 73.0%/79.3% nt/aa identity with previously existing BTV serotypes. Seg-6 accession numbers used for comparative analyses: AJ586695 - AJ586699, AJ586700, AJ586703 - AJ586711, AJ586713, AJ586714, AJ586716, AJ586719, AJ586720 - AJ586725, AJ586727, AJ586730, EU839842. doi:10.1371/journal.pone.0026147.g002 [30].

Genotyping and Fingerprinting Assays: Molecular techniques that depend on genes encoding BTV proteins such as RT-PCR, Real time PCR and sequencing are used for detection and serotyping of BTV. There are varieties of PCR used for detection of BTV-RNA e.g. Duplex- RT-PCR [24]**,** Multiplex RT-PCR [25], Nested PCR [26] and Real Time-PCR [27].

RT-PCR directed to amplify certain sequence of a gene encoding conservative protein such as VP7 identify the virus ad BTV while sequencing genes encoding variable protein (VP5 and VP2) reveals the different serotypes of the virus. Phylogenetic analysis of reveals the relationship between the different BTV serotypes [28]. Fig 5, 6 show different phylogenetic tree of BTV and the relation between different serotypes.

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