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Phylogenetic Analysis of Viral Protein 2 of Bluetongue Virus

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Abstract

Bluetongue is a highly infectious vector born viral disease, and it is a disease of wild and domestic animals (ruminants). Bluetongue is a non-contagious disease of animals and spread by the biting midges (Sperlova. A. and Zendulkova. D. 2011). The name Bluetongue is given by Spreull in 1905 (Spreull,1905). Bluetongue disease is mild in goats and severe in sheep as sheep is the primary host of bluetongue virus. Cattle act as the reservoir of bluetongue virus (Browne, 1971). The Bluetongue virus is first reported by hutcheon in 1881 During the introduction of European sheep breeds in Southern Africa (Hutcheon,1902). Later in 1948 it was reported in North America as a sore muzzle disease (Hardy and Price, 1952).). Spare in 1964 reported outbreak of bluetongue disease in India (Spare, 1964). There are several clinical symptoms of Bluetongue disease have been found in ruminants like fever, viraemia, sore muzzle, facial oedema, hyperaemia and congestion, erosion of mucous membrane, haemorrhages, vascular permeability (OIE, 2014). Symptoms are more severe and easily detectable in sheep and these signs are high fever upto 5-7 days, loss of wool, depression and haemorrhages in the coronary band, difficulty in standing and lameness because of painful hoof, excessive salivation, swollen tongue, swelling in nasal and buccal mucosa, pneumonia and death (Tabachnick et.al., 2009). The Severity of clinical signs of bluetongue disease in sheep influenced by the type and strain of infecting virus (Verwoerd & Erasmus, 2004; OIE, 2014). The bluetongue virus is hypervariable in nature therefore, there are 24 serotypes of bluetongue virus are well recognised with two newly proposed serotypes BTV 25 from Switzerland and BTV 26 from Kuwait. In India 22 serotypes have been reported of Bluetongue virus (Prasad *et al.*, 2009; Kumar, 2009). Bluetongue virus belongs to family Reoviridae and genus Orbivirus (Tabachnick *et al.*, 2009). Blue-tongue has a serious economic impact on dairy and wool industry mainly due to high morbidity, mortality and mandatory trade barrier on the movement of BT infected livestock and germ-plasm. BT is evolving into newer challenges and poses ever increasing the threat to associated environment. An Unnatural host like canines have in the past contracted BT infections. Many species of Culicoides have been reported to spread infections. Recently BT has been categorized as multispecies disease by OIE (2014).

Keywords: Bluetongue, mucosa, pneumonia, sore muzzle disease, sheep breeds, virus, reoviridae, genus orbivirus.

Bluetongue is a highly infectious vector born viral disease, and it is a disease of wild and domestic animals (ruminants). Bluetongue is a non-contagious disease of animals and spread by the biting midges (Sperlova.A. and Zendulkova. D. 2011). The name Bluetongue is given by Spreull in 1905 (Spreull, 1905). Bluetongue disease is mild in goats and severe in sheep as sheep is the primary host of bluetongue virus. Cattle act as the reservoir of bluetongue virus (Browne, 1971). The Bluetongue virus is first reported by hutcheon in 1881 During the introduction of European sheep breeds in Southern Africa (Hutcheon, 1902). Later in 1948 it was reported in North America as a sore muzzle disease (Hardy and Price, 1952).). Spare in 1964 reported outbreak of bluetongue disease in India (Spare, 1964). There are several clinical symptoms of Bluetongue disease have been found in ruminants like fever, viraemia, sore muzzle, facial oedema, hyperaemia and congestion, erosion of mucous membrane, haemorrhages,

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Transmission

BTV is vector born disease, transmitted by biting *Cullicoide* midges (CFSPH & IICAB 2015). *Cullicoides* are mostly energetic one hour before sunset and one hour after sunrise, and these are present in muddy, humid and warm area (Mellor *et al.,* 2000; Chand *et.al.* 2015). Climate change is responsible for transmission of BTV for longer periods, and Global warming has increased the activity of *Cullicoides* (Tweedle and Mellor, 2002; Chand *et al.,* 2015). BTV has been isolated from *Culicoides* vector in India (Jain et al., 1988; Dadawala *et al.,* 2012; Chand *et al.,* 2015). The virus is conventionally believed to be transmitted only through the bite of infected vector and not by contact or through infected products (Mellor *et al.,* 1984; Chand *et al.* 2015). Some serotypes of BTV have been reported to be transmitted via oral and by the vertical route (Darpel *et al.,* 2009; Chand *et al.,* 2015). Isolation of BTV from aborted fetus of dogs has also been reported (Dubovi *et.al.,* 2013; Chand *et.al.,* 2015).

Life cycle of BTV

There is two outer capsid protein VP2and VP5. VP2 is the outermost protein and responsible for cell entry into the vertebrate host cell. VP2 is also responsible for haemagglutination (lysis of RBCs) (Bhattacharya *et al*., 2007; Forzan *et al*., 2007). By a clathrin-dependent endocytosis pathway VP2 is internalised in endosomes after the interaction of BTV with target cell surface (Forzan et al., 2007; Pandrangi .A . 2012). After getting entry into the cell VP2 of BTV gets degraded in acidic medium of endosome exposing VP5 (Forzan *et al*., 2004, 2007).

Transcription takes place in the core of Bluetongue virus (Boyee et al., 2004). The Core of the virus consists of three minor (VP1, VP4 and VP6) and two major (VP3 and VP7) structural proteins (Verwoerd *et al*., 1970, 1972). From each of the ten BTV genome segments, the VP1 transcribe positive sense ssRNA copies (Boyee et al., 2004; Pandrangi .A . 2012). After synthesis of negative strand RNA by VP1 of Bluetongue virus dsRNA is produced by the negative strand RNA (Boyee et al., 2004). All dsRNA segments gets autonomously linked with different transcription complex (VP1, VP4 and VP6) situated inside VP3 along with a fivefold alignment (Nason et al., 2004; Pandrangi.A. 2012). Transportation of mRNA and metabolites induced by channels of VP7 of BTV during the core transcription (Roy, 2005). Fragile VP3 subcores provide scaffold for the addition of VP7 trimers and increases the rigidity and stability of cores. VP2 and VP5 of BTV added to the progeny core particle; mature progeny virus particle are transported on microtubules involving VP2/vimentin interactions within the cytoplasm (Han and Harty, 2004; Pandrangi A. 2012). VP5 is the second most variable protein of BT virus which is indirectly involved in the induction of immune response. It enhances the protective neutralizing

activity of VP2 protein and induces higher serotype specific antibody titer than the VP2 alone (Huismans *et al*., 1983; Roy *et al*., 1990). The activity of NS3 for cell membrane destabilisation is responsible for release of virions from the infected cell resulting in cell breakdown or cell death (Han and Harty, 2004; Pandrangi .A . 2012).

Structure of BTV

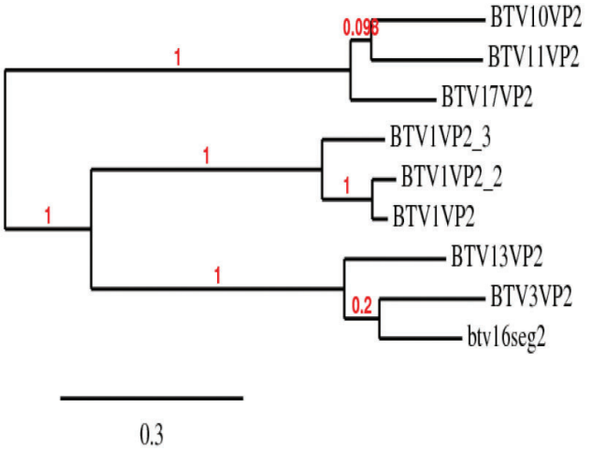
Bluetongue virus is a non-enveloped virus. The density of Bluetongue Virus is 1.337g/cm^3. BTV is composed of 10 dsRNA. The core of Bluetongue virus is surrounded by two outer capsid protein VP2 and VP5, outermost protein out of these two is VP2 (Bhattacharya *et al*., 2007; Forzan *et al*., 2007). The Inner capsid of the virus consist of 3 minor (VP1, VP4 and VP6) and two major (VP3 and VP7) proteins (Verwoerd *et al*., 1970, 1972). X-ray Crystallography and Cryo electron microscopic study of Bluetongue virus revealed that it is composed of two major structural protein on outer shell 60 trimers or 180 copies of VP2 (111 kDa) and 120 trimers or 360 copies of VP5 (59 kDa) (Cornil *et al*., 2008). The inner capsid of virus consist of major immunodominant structural protein VP7 organised in 260 trimers forming a T=13 icoshederal lattice covering the subcore (Cornil *etal*., 2008; Nason eta.,l 2004). Second major core protein VP3 encoded by segment 3 (vp3 gene), exists as dimers and forms the scaffold for the deposition of VP7 protein. VP3 protein also interacts with VP1 and VP4 proteins which are encoded by L1 and M4 genomic segments respectively. VP1 is the largest protein of 150 kDa having RNA dependant RNA polymerase activity (Roy *et al*., 1988; Roy, 2008). The capping and methyl transferase activities of VP4 proteins enable it to cap the nascent mRNA as it emerges out of the polymerase (Roy, 2005; Sutton *et al*., 2007). The dsRNA segment S9 encodes the ssRNA and dsRNA binding VP6 protein, which performs the helicase and NTPase activities of the virus.

Materials and Methodology

The 24 protein sequences of nucleocapsid of Bluetongue virus were retrieved from the biological database- National Centre for Biotechnology Information (NCBI) cited at http://www.ncbi.nlm.nih.gov and the UniProt KB Database in ExPASy Proteomics Server available at http://www.uniprot.org/. Multiple sequence alignments of the given NP sequences were performed by using the Clustal W Program with default parameters in MEGA 4.0.2 version. The phylogenetic tree was built by Maximum Parsimony method in MEGA 4.0.2 version.

Result and Discussion

Phylogenetic tree of VP2



Phylogenetic analysis of VP2

VP2 protein of BTV16 resembled with that of VP2 of BTV13. The VP2 sequence of BTV3 seems to have evolved later from VP2 of BTV16. Interestingly BTV1 has got 3 of genes. These genes are similar to VP2 of BTV13, BTV3 and BTV16 group and have diverged farther back in time from BTV10, BTV11 and BTV17. One more explanation of this seemingly distant relationship could be due to segmented genome and cross infection by more than one type of viron particle. Such an event could lead to drift of a later evolved gene to a different genomic background carrying viron. It would ultimately lead to formation of newer virus types. The considerable genetic diversity as seen in the Bluetongue Virus family is an indication of similar phenomenon.

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