Chapter - 1

INTRODUCTION

Bluetongue is an International office of Epizooties multiple species disease (OIE, 2000) and described as an economically devastating affliction of sheep. The vast economic losses of BTV infection is due to high morbidity, mortality, abortions, stillbirths, loss of fleece, reduced meat production in affected animals and the restrictions placed on trading of animals and animal products from the endemic areas. Small ruminants like sheep and goats contribute significantly to the economy of developing country like India. The sheep are closely identified with poor people in pastoral system ranging from arid and semi-arid tropics to complex crop-livestock system in humid tropics. These animals are bestowed with sound innate capacity to thrive under extreme conditions with better economic returns than larger ruminants. Small ruminants are integral part of the poor section of Indian farming community. India has a sizeable population of approximately 58 millions (FAO statistics, 2001) of sheep contributing to 1.12% of GDP to national economy. In view of its importance, state and central governments initiated variety of sheep improvement programmes. However, the programmes/production is hampered by a number of infectious diseases. Among them, Bluetongue (BT) is one of the most important viral diseases of sheep from the aspect of animal health, economic losses and germplasm.

Bluetongue is a non-contagious, insect-transmitted viral disease of domestic and some species of wild ruminants. Bluetongue occurs principally in sheep and in some species of wild ruminants and infection in cattle, goat and most
wild ruminant species is typically asymptomatic or sub clinical (Verwoerd and Erasmus, 2004). In sheep the characteristic symptoms of the disease have any combination of fever, anorexia and malaise, respiratory distress, excessive salivation, serous to bloody nasal and ocular discharge, oral lesions and ulcers, lameness and/or stiff gait, hyperemia and haemorrhage of coronary bands, oedema of head and neck, reproductive disorders leading to abortion or congenital abnormalities, swollen and cyanotic tongue that gives the disease its name is uncommon (MacLachlan, 1994). Mortality rates vary from 0% in mild outbreaks to 30% or even higher in outbreaks caused by virulent strains of the causative agent in highly susceptible breeds of sheep. Most animals that succumb to acute BT die within 14 days of infection.

The disease was first depicted in late 18\textsuperscript{th} century in the African continent (Hutcheon, 1902 and Spreull, 1902 and 1905). For many decades the disease was bound to African continent. However, the virus crossed its native boundaries and expanded to many tropical, sub-tropical and temperate regions of the world. In India, the disease was first reported in 1963 (Sapre, 1964). Initially the disease was observed in exotic breeds of sheep and later it appeared in native sheep. During last decade the disease outbreaks have become a regular feature every year in Indian native sheep especially in the southern parts of India. A network project was initiated in 2001 to isolate and characterize the circulating viruses with long term objective of development of suitable vaccine for BT control in India.

There are 24 confirmed serotypes of BTV, and two more have been recently proposed (Hofmann \textit{et al.}, 2008, Maan \textit{et al.}, 2009 and 2011). Out of these 24 confirmed serotypes recognized worldwide, a total of 24 serotypes were
reported to existing in India (Jain et al. 1992, Bommineni et al., 2008 and Susmitha et al., 2010) either by demonstration of serotype specific antibodies or virus isolation. The demonstration of antibodies against a serotype is of limited value due to cross reactivity between serotypes observed. Hence demonstration of virus by molecular methods like RT-PCR is the method of choice for confirmation of different serotypes. The BTV genome is composed of 10 double stranded RNA segments, which encode 7 structural proteins (VP1-VP7) and four non-structural proteins (NS1, NS2, NS3 & NS3A). The most divergent of the 10 segments are L2 and M5, which encode VP2 and VP5 proteins respectively. Although segment S7 is more conserved than L2 and M5 segments, it was still the third most variable of the BTV genome segments compared by Maan et al., 2008. Of the 10 genomic dsRNA segments of BTV the S7 is true representative of the BTV genome as it encodes the group specific antigen VP7 which is the major core protein of BTV (Huismans and Erasmus, 1981). Sequence analysis of S7 gene will be useful in understanding the relationship of BTV with other Orbiviruses. The smallest of the BTV non-structural genes is S10, encoding NS3 protein, represents the fourth most variable of the BTV genes/proteins. (Maan et al., 2008).

Earlier methods for detection of BTV include isolation of the virus from blood samples, chicken embryo inoculation, adaptation to cell culture and subsequent confirmation by neutralization tests, which are time consuming. Capture-ELISA has also been employed routinely to detect BTV antigen in blood and tissues of infected animals. The demonstration of antibodies against a serotype is of limited value due to cross reactivity between serotypes observed.
Hence demonstration of virus by molecular methods like PCR is the method of choice for confirmation of virus/specific serotype.

As the NS3 gene of BTV possesses the highly conserved regions among all the existing serotypes it can be used to detect the virus in field samples. Hence, the present study aimed to detect the virus in field samples from Andhra Pradesh and to characterize those isolates based on S7 (VP7) and S10 (NS3) genes and to develop molecular diagnostics with the following objectives:

- Standardization RT – PCR for the detection of bluetongue virus in field samples from Andhra Pradesh
- Propagation and Purification of BTV-2/Tirupati isolate and other field isolates in BHK 21 cell line
- Optimization of RT-PCR for the full length amplification of S7 (VP7) and S10 (NS3) genes
- Cloning and Sequencing of VP7 and NS3 genes of BTV isolates
- Phylogenetic Characterization of bluetongue virus isolate(s) based on S7 and S10 genes