CHAPTER - 1

Introduction
INTRODUCTION

India earns Rs.8500 crores annually through the production of meat, wool and skin from 485 million livestock, of which Tamil Nadu accounts for 24 million livestock (Seventeenth Livestock Census, 2003). Small and medium farmers in Tamil Nadu rear sheep and goats for meat and skin, which fetch Rs.200 for an individual animal, and the current total number of sheep and goats for meat and skin records 5.2 and 6.4 million respectively. The outbreak of the bluetongue disease in Tamil Nadu and its occurrence in many parts of India over the last few decades have affected millions of sheep, goats and other livestocks (Ilango, 2006). Sustained growth of the sheep and goat industry depends on eradication of such emerging disease.

India has significant population of domestic and wild ruminants which are known to be susceptible to bluetongue virus infection. Several exotic breeds of sheep were introduced into the country between 1960 and 1970 for the genetic improvements of national flock by cross breeding with native breeds. In India, the total sheep population is 51 million, accounting to 5 percent of world’s sheep population and 123 million goats accounting for 20 per cent of the total global livestock. (FAO, 2003). The increase in the national susceptible population, along with favourable climatic conditions, appears to have the establishment of bluetongue in the country. Hence bluetongue has become one of the important sheep diseases of the Indian subcontinent. The disease was first reported in India in 1964 (Sapre, 1964). Bluetongue in India is endemic in Tamil Nadu, Andhra Pradesh, Karnataka, Maharashtra, Gujarat, Rajasthan, Haryana, Himachal Pradesh, Jammu and Kashmir. However the recent epidemics in Tamil Nadu were devastating (Wilson et al., 1997).
In Tamil Nadu, 22 out of 24 districts were reported to be affected by the bluetongue virus. The reported case of bluetongue virus among sheep and goats occurs presumable in an epidemic form during the Southwest monsoon season which favours vector population of bluetongue virus (Ilango, 2006). Although the history of reporting was not continuous, the number of outbreaks, attacks and deaths among ruminants reported is of great concern that needs immediate attention for the protection of livestock and economic growth (Wilson et al., 1997). Outbreak of bluetongue in sheep and goats swept in an epizootic form during 1997-98 from 12 districts in Southern Tamil Nadu leaving alarmingly 5.23 lakhs infected and 2.98 lakhs dead (Anonymous, 1998).

The clinical form of the disease is usually observed among sheep. Infection among cattle and goats are generally unnoticeable, although clinical bluetongue has been reported. The clinical signs of the disease range from a mild febrile illness to extensive erosions of the oral mucosa. Bluetongue virus infection causes inflammation, swelling, and haemorrhage of the mucous membranes of the mouth, nose, and tongue. Inflammation and soreness of the feet are also associated with bluetongue during the terminal stages. In sheep, the tongue and mucous membranes of the mouth become swollen, hemorrhagic, and may look red or dirty blue in color, thus giving the disease its name – ‘Bluetongue’ (APHIS, 2003).

1.1 BLUETONGUE DISEASE

Bluetongue (BT) is an infectious, non contagious arthropod-borne disease of ruminants caused by Bluetongue virus (BTV), prototype species of the genus Orbivirus, within the family Reoviridae. Twenty four serotypes of bluetongue virus have been identified till date (Davies et al., 1992).
Bluetongue virus is a small (80nm in diameter) icosahedral virus with a ten segmented, double stranded RNA genome. Each of the ten segments codes for at least one of ten distinct viral proteins, wherein seven are structural components of the virus particle, and the rest three are non structural. Out of the 10 viral proteins, VP₂ is serotype specific and VP₇ is serogroup specific (Barber et al., 1975).

The development of laboratory based systems for the investigation of animal disease such as bluetongue is crucial to understand the infectious agent and the disease process globally. As a result, of adaptation of bluetongue virus to grow in laboratory system, such as cell culture and embryonated chicken eggs it has been possible to develop a range of diagnostic tests and vaccines (Bowne et al., 1970). It is essential that diagnostic laboratories use the most appropriate test methods available to achieve the desired result and therefore they must have a clear understanding of the test uses and limitations. Testing sera for serotype presence of bluetongue virus antibody may be required for the following purposes:

1. to facilitate safe international trade in live animals, animal products and germplasm
2. for serological surveillance
3. for monitoring vaccination campaigns and
4. for serotype identification of field strains.

Identification of BTV antibody is an essential part of the laboratory confirmation of bluetongue virus infections. This may be achieved in three different ways, namely a) identification of antibody by serological assay, b) identification of the virus antigen by virological assay, c) identification of the specificity of nucleic acids by reverse
transcriptase polymerase chain reaction (RT—PCR) and d) sequence analysis (JOE, 2000).

Due to the complexity of the serotypes of bluetongue virus, current procedures for monitoring the prevalence of bluetongue infection are generally based on the determination of the serotype specific antibodies in animal serum samples. These procedures are cumbersome for highly serotype specific BT virus and time-consuming. Therefore, it is imperative to use simplified tests for the purpose of seromonitoring of bluetongue virus in a particular animal population in order to demonstrate that the population has been exposed to bluetongue virus infection. Until recently, tests such as agar gel immunodiffusion have been in use. However, apart from being less sensitive, these tests have the major drawback of being unable to consistently distinguish between antibodies against bluetongue virus and the closely related epizootic haemorrhagic disease virus. Competitive ELISA (cELISA) has been used as highly specific and sensitive test for detection of bluetongue virus group blue tongue immuno diffusion test (BTID) specific antibodies. cELISA is now recommended as an official test by Office International Disease Epizootics (OIE) for serological monitoring of bluetongue virus antibodies in small ruminants like sheep and goats.

Against the above backdrop, in this current investigation focus has been given to seasonal influence on the BTV outbreak, isolation, characterization and adaptation of BTV, cultivation of different native isolates in different routes, titration, identification of BTV by different techniques and their comparison of sensitivity and enumeration of restriction sites.
1.2 OBJECTIVES OF THE PRESENT STUDY

The specific objectives of the study include the following:

- Influence of rainfall and wind current on the BTV outbreak.

- Isolation and adaptation of BTV isolates to embryonated chicken egg in different routes as yolk sac and intravenous.

- Cultivation of BTV in established cell lines like BHK-21 and Vero to assess the cytopathic effect, titration, plaque morphology and serum neutralization test.

- Studying the physico-chemical characteristics of the BT viral isolates.

- Identification of bluetongue virus antigen and antibody using Agar gel precipitation test (AGPT), and Immunofluorescent antibody test (IFAT).

- Identification of BTV using RT-PCR and Nested PCR analysis and enumeration of restriction sites of BTV isolates.

- Comparison of the sensitivity of AGPT, IFAT, RT-PCR and Nested PCR.