Introduction
I. INTRODUCTION

The White revolution in India during the past three decades has revived the Indian rural economy by giving its populace the much needed sustainable monetary security and a source of livelihood. As per existing agrarian circumstances in India, even if crops let down the farmer, milk has never failed him. Today, India is the largest milk producer in the world with an annual production of 121.8 million tons in 2011, while the world milk production stood at 700 million tons. Approximately 25% of the Indian agricultural gross domestic produce is contributed by the livestock sector and this tremendous growth in dairy sector is primarily attributed to the extensive cross breeding programme undertaken during this period. Though increased milk production is a boon from cross breeding programme, a curse accompanying it was, an increased susceptibility of thus produced crossbred cattle and emergence of new diseases, purely because cross breeding was made possible by introducing high yielding new animals or their germplasms from foreign countries which paved the way for entry of several unheard, new diseases to India. Bovine Herpes Virus -1 (BoHV-1), is an example for such an emerged pathogen. The first report of BoHV-1 in India was by Mehrotra and co workers in 1976 and as the milk production multiplied so was the virus, which has taken mammoth strides to spread to 31% of Indian cattle in 1996 to 42% in 2010 with an overall prevalence of 36% on cumulative study (PD_ADMAS Annual Report, 2010-2011).

Bovine Herpes Virus -1 is one of the very few pathogens which has been recorded in almost every country in the world, demonstrating its widespread nature owing to its unique survival strategies in the host. Worldwide, it is responsible for
significant economic losses in the form of decreased milk production, decreased conception rate, infertility, still births, abortions. More importantly, presence of BoHV-1 has become major trade barrier under new international trade regulations causing significant economic losses at the international market (Gibbs and Rweyemamu, 1977; Kilari et al., 2000 and Dhand et al., 2002).

Bovine herpes virus -1 is an enveloped, double stranded DNA virus having an icosahedral nucleocapsid consisting of 162 capsomeres. Bovine herpes virus -1 is a member of the genus Varicellovirus, subfamily Alphaherpesvirinae, Family Herpesviridae under the order Herpesvirales (ICTV, 2012). The virus infects the respiratory and genital tracts of cattle causing diverse ailments viz., infectious bovine rhinotrachitis (IBR), infectious pustular vulvovaginitis (IPV), infectious balanoposthitis (Gibbs and Rweyemamu, 1977), keratoconjunctivitis (Mehrotra, 1977) and neurological disorders (Caron et al., 2002). All BoHV-1 strains belong to one single viral species and are classified in three subtypes BoHV-1.1, BoHV-1.2a and BoHV-1.2b. Subtypes 1.1 and 1.2a have been associated with severe diseases including infection of the fetus and abortion. The subtype 1.2b was not associated with abortion. However, there is no molecular basis supporting the tropism of BoHV-1 for genital or respiratory epithelial cells (OIE, 2010).

The severity of the disease caused by BoHV-1 is influenced by several factors such as the virulence of the BoHV-1 strain (Kaashoek et al., 1996), resistance factors of the host, especially the age, and potential concurrent bacterial infection. Subclinical BoHV-1 infections are common. Clinically the virus produces high fever, red appearance
of nasal mucosa, serous to mucopurulent nasal discharge and in severe cases, heavy breathing at inspiration. Ocular signs such as conjunctivitis and mucopurulent ocular shedding are common. Naturally occurring BoHV-1 abortion are usually observed at four to eight months of gestation but can also cause embryonic deaths. As stated above, only BoHV-1.1 and BoHV-1.2a strains have been so far associated with abortigenic potentials. The BoHV-1 genital form is usually transmitted at mating. The names given to the diseases affecting the cow (infectious pustular vulvovaginitis, IPV) and the bull (infectious pustular balanoposthitis, IPB) describe clearly the clinical pictures observed following the primary infection.

Cattle are able to set up an effective immune response following the primary infection with BoHV-1 allowing in most cases recovery from the disease and the arrest of virus excretion. Cattle once infected by BoHV-1 are never able to eliminate the infection. Primary BoHV-1 infections always lead to a life-long latent infection, the latency - reactivation cycle is responsible for the maintenance of BoHV-1 in a cattle population and deep epidemiological impact (Gabev et al., 2009). After primary infection, breeding bulls may shed BoHV-1 in their semen (under stress conditions) and must be regarded as lifelong potential shedders. Transmission of BoHV-1 is also possible by artificial insemination; wherein semen of a single infected ejaculate may be inseminated to thousands of females leading to clinical consequences of the disease. To prevent this, bovine semen should mandatorily be screened for BoHV-1 by a sensitive test before it is used for artificial insemination purposes (Smits et al., 2000).
Bovine herpes virus-1 is able to infect, multiply, spread and sustain very long in the infected animals because of its unique immune evasion strategies. Diagnosis of BoHV-1 infection has always been a challenge considering its subclinical nature, immune evasion strategies and most importantly the latency it establishes in the host. World Organization for Animal Health (OIE, 2010), prescribes Indirect ELISA, virus isolation and real time PCR as approved tests for diagnosis BoHV-1. Serum neutralization test and different versions of Indirect ELISA have been employed for detection of antibodies against BoHV-1. In India, extensive studies have been done on seroprevalence of BoHV-1, with recording higher seroprevalences of BoHV-1 (Suresh et al., 1999). Since the virus remains in latent form with several immune evasion mechanisms, the relevance of serology in detecting an active infection has never been worked out.

Though, virus isolation is a gold standard test for the diagnosis of BoHV -1, it has got several drawbacks. Poor sensitivity and requirement of skilled personnel with modern cell culture infrastructure limits its application in developing countries. Further, virus isolation from semen samples is extremely difficult because of the natural cytotoxicity of semen and its overshadowing effect on the viral cytopathic effect (Smits et al., 2000). With the advent of modern technologies, viral nucleic acid detection by PCR represents an excellent diagnostic tool for the rapid and sensitive detection of BoHV-1 in biological and clinical specimens. Various PCR assays for detection of BoHV-1 have been described using gB, gC and gD specific primers (Wiedmann et al., 1993; Kibenge et al., 1994; Vilcek et al., 1994 and Ranganatha, 2011). Since there is a high risk of cross contamination while handling post amplified PCR products, conventional PCR, has not been recommended as an approved test for BoHV-1 diagnosis.
However, OIE (2010) recommends Real time PCR in which the amplified PCR products are detected directly during the amplification cycle, with better sensitivity and specificity, as an approved test for BoHV-1 detection in semen and other clinical samples. Inspite of extensive seroprevalence of BoHV-1, no systematic study has been made on the molecular epidemiology of the virus in India. Further, in India no diagnostic test has been concretely prescribed for diagnosis of BoHV-1. Comparison and evaluation of available OIE approved tests and of their true clinical relevance in Indian context where there is no vaccination but record high seropositivity is the need of the hour.

Keeping in view of the unique pathogenesis of BoHV-1 and its impact on international trade and absence of a much needed antigen detection tool for BoHV-1 detection, the present study was undertaken with the following objectives;

1. Isolation of Bovine herpes virus -1 (BoHV-1) from semen and clinically suspected samples in cell culture system and screening of serum samples by Indirect ELISA.

2. Molecular characterisation and phylogenetic analysis by cloning and sequencing of partial glycoprotein B gene of BoHV-1 isolate/s.

3. Standardisation of Real time PCR targeting glycoprotein B gene of BoHV-1.

4. Comparative evaluation of Real time PCR with Virus isolation and Indirect ELISA.

5. Development and validation of Real Time PCR based antigen detection kit for diagnosis of BoHV-1.