INTRODUCTION
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The poultry industry is one of the fastest growing sectors in India with a phenomenal rate of 12 to 15 per cent every year. The poultry sector in India has undergone a paradigm shift in structure and operation during the past 25 years transforming what was largely a backyard venture before the 1960s into a vibrant agribusiness contributing about $230 million to the GNP of India as per the information released by the Ministry of Food Processing, Government of India. With an annual production of 53,000 million eggs and 3.2 million tonnes of poultry meat, India is now among the biggest egg and broiler producers in the world (World poultry, 2009). To sustain the current growth rate of poultry industry in India, it is imperative to protect the poultry from contagious, non contagious, endemic, emerging and reemerging diseases. Of these, the Marek’s disease (MD) is considered as a major reemerging viral disease affecting intensive poultry rearing areas of India with a greater impact in Maharashtra (Yeshwant, 2006) and Haryana (Kamaldeep et al., 2007).

Marek’s Disease is a T-cell lymphoma of chickens caused by a herpesvirus known as Marek’s Disease Virus (MDV) (Osterrieder et al., 2006). It is a significant concern in commercial poultry production due to its highly contagious nature and prevalence in the field. The pathogenesis and economic significance of MD has been well established and is universally accepted. The disease is characterized by T cell lymphomas of visceral organs, unilateral or bilateral enlargement of sciatic and brachial nerves due to infiltration of lymphoblasts, graying of iris due to lymphoblastoid cell infiltration and
cutaneous form characterized by nodular lesions at the base of feather follicles (Witter and Schat, 2008).

The disease is named after Professor Jozsef Marek, who described it a century ago (Marek, 1907) as a polyneuritis. The causative agent of MD, a cell associated herpesvirus, was discovered and isolated in cell culture by Churchill and Biggs (1967).

Marek’s disease virus strains that induce disease in chicken are classified as serotype-1 under alpha herpesviruses. The genome of MDV has been shown to be a linear double-stranded DNA with a molecular weight of $110 \times 10^6$ and a buoyant density of $1.705 \text{ g/cm}^3$ in neutral CsCl$_2$. Serotype-2 MDV and serotype 3 (Turkey Herpes Virus, HVT) are naturally occurring, infectious viruses but are non oncogenic to chicken.

Serotype-1 strains are further divided into pathotypes, which are often referred to as mild (m) MDV, virulent (v) MDV, very virulent (vv) MDV and very virulent plus (vv+) MDV (Witter, 1998a).

Soon after the isolation of MDV serotype-1 (MDV-1), the first vaccine became available based on either highly passaged attenuated MDV-1 (Churchill et al., 1969) or the serologically related herpesvirus of turkey (HVT) serotype-3 of MDV like viruses (Okazaki et al., 1970). The introduction of vaccination in the 1970s was a major milestone in the history of vaccinology as it was the first demonstration of an effective and widespread use of vaccination to prevent a virus induced neoplasm in any species.

The major potential disadvantage inherent in MD vaccines is their inability to prevent the infection and shedding of the virus, although vaccines can effectively reduce
tumor formation by the virus in MDV infected birds. Due to the inability of vaccines to confer sterile immunity upon the chicken host, MDV has increased its virulence against the selection pressure imposed by vaccination (Schat and Baranowski, 2007) and that has been evidenced by the emergence of virulent strains in vaccinated flocks. The reappearance of MD, in this manner, highlights the need for the potential vaccine that induces better immunity against both MDV infection and clinical Marek's disease.

Several different types of MD vaccines are in common use, both individually and in various combinations. The most widely used vaccines are attenuated serotype-1 MDV and naturally avirulent HVT or Serotype-2 viruses. The serotype-2 virus was able to protect against pathogenic strains of MDV 1 on its own, but when administered with HVT it had synergistic activity, providing improved protection (Witter et al., 1984). Vaccine failures can be due to challenge from highly virulent MDV strains, especially in areas of chicken density (Bublot and Sharma, 2004). The use of multivalent vaccines can compensate for the lower protection induced by HVT and the delayed protection induced by CVI988, a vaccine strain of MDV-1 (Gerlings et al., 1999).

Though the morbidity and mortality due to MD receded but often vaccine breaks are reported and increased virulence of challenge viruses recorded within ten years from the advent of vaccine. The subsequent introduction of more aggressive vaccines and vaccine regimens has driven MDV to evolve to even greater virulence over the last 30 years (Pastoret, 2004).

During the second decade after the introduction of MD vaccines, an increasing number of vaccine breaks were reported possibly due to the adaptation of the field virus to selective pressures due to widespread and intensive vaccination. However in USA, this
emerging disease was controlled by bivalent vaccine comprising of Serotype-2 (SB-1 strain) and Serotype-3 (HVT) (Morrow and Fehler, 2004).

The current level of controlling MD using HVT monovalent vaccine seems to be fragile, because, significant disease outbreaks have been noted in HVT vaccinated flocks and the occurrence of these outbreaks are unpredictable (Yashwant, 2006). As the CVI988 vaccine has not been licensed in India for commercial production and the superiority of HVT+SB-1 bivalent vaccine over monovalent HVT vaccine in our system need to be established.

In order to maintain the potency of the vaccine, it is crucial that the right procedures are followed while thawing and dilution of the cell associated MD vaccine. MD cell associated vaccines are most delicate vaccines in veterinary use since they need to be maintained at -196°C during transport and until used. Thawing the ampoules at temperature of more than 28°C, or prolonged incubation can cause destruction of the vaccine virus. Similarly there is a necessity to determine the optimal temperature for thawing of HVT+SB-1 live vaccine as the conditions recommended by manufacturers differ greatly.

Antiviral immunity against herpes viruses is principally mediated by CD8 αβ cytotoxic T lymphocytes (CTL) and CD4 helper cells that secrete cytokines. Depletion of CD8 cells leads to higher MDV titres in CD4 cells, the target cells for MDV, suggesting that CD8 cells exert an important antiviral effect that influences the course of the disease (Davison and Kaiser, 2004).
The Real Time PCR was used to confirm the success of vaccination and to derive how the vaccine load correlates with protection against challenge (Baigent et al., 2006). PCR based assay correlated significantly with subsequent development of disease and was a better predictor than plaque assay for the likelihood of development of pathological disease in birds studied (Bumstead et al., 1997). Virus load of CVI988 in feather tips is predictive of virus load of lymphoid tissues, where immune response will occur (Baigent et al., 2005b). Load of challenge MDV but not that of HVT DNA in blood and buffy coat are indicative of protection and can be used as a criterion for early diagnosis of MD (Gimeno et. al., 2008).

MD vaccines which protect against the disease do not prevent the super infection with pathogenic MDV (Zelnik, 2004). Histopathology is required to establish the cause of the tumour than the mere virological examination.

Keeping in view of the above background, the present study was taken up to evaluate bivalent HVT+SB-1 vaccine in comparison to HVT vaccine alone against Marek’s disease by considering the following objectives.

1. Estimation of titre of Marek’s disease vaccine virus by in vitro plaque assay, to determine optimum thawing conditions of bivalent vaccine.
2. Evaluation of the safety and potency of bivalent HVT+SB-1 and monovalent HVT Marek’s disease vaccines.
3. Evaluation of the immune response against bivalent HVT+SB-1 and monovalent HVT Marek’s disease vaccines.