DISCUSSION
V. DISCUSSION

Marek’s Disease is still considered as one of the most economically devastating poultry diseases worldwide. Billions of dollars are lost in clinical cases as well as in the form of condemnation of carcasses in the slaughter plants. The discovery and eventual application of current vaccines for MD have greatly reduced the occurrence of the “typical” MD in the field. Of late, the situation with MD, however, is changing in India and there have been vaccination failures leading to outbreaks. There are concerns that the MD virus continues to change to a possibly more virulent status, against which the current cell free HVT vaccines poorly protect against vvMDV (Witter, 1998b). These concerns warrant the introduction of more potent vaccines to counteract very virulent MD viruses.

In the United States of America, HVT+SB-1, bivalent vaccine was used to control the MD vaccine breaks arising from HVT vaccination. It is mainly due to the adoption of the field virus to the selective pressure caused by widespread and intensive vaccination during the second decade after the introduction of MD vaccines (Morrow and Fehler, 2004). One of the barriers to limit the potential of single point mutations leading to virulent pathotypes of MDV-1 is by using polyvalent vaccines used at sufficient dose to provide robust immunity (Witter, 1998b). In India, there is a need to replace the current monovalent HVT vaccine with HVT+SB-1 vaccine to prevent the losses due to MD as there were sporadic reports of vaccine failures. Before introduction of any such bivalent vaccine, there is a need to evaluate and validate the bivalent HVT+SB-1 vaccine before releasing for field application, in terms of virus titer, safety and potency as per the OIE standards. The immune competence of a host can be evaluated by several parameters,
including subpopulation of circulating T lymphocytes. The ability of vaccine to mount robust immune response, particularly the CMI response consisting of CD4 and CD8 cells and virus efficacy in reducing the load of challenge virulent virus in a bird is of substantial index to prove and compare the efficacy of vaccines.

Thawing of MD cell associated vaccine is a critical step in the handling of the vaccine and thawing at inappropriate temperature would make the vaccine ineffective on an account of loss of virus titer. Cell-associated MD vaccines have to be handled carefully to make sure that the intended live vaccine dose gets delivered into the chicks. Landman and Verschuren (2003) attributed the MD outbreaks in The Netherlands to loss of titers in MD vaccine and they showed the usefulness to assess the PFU per chicken dose of reconstituted MD vaccine and vaccine ampoules to unravel true vaccine failures, which could result in disease outbreaks in the field. Upon review of the literature to date, regarding the thawing temperature advocated by researchers and vaccine manufacturers, which is listed in the chapter 2.5, it was observed that there was a difference of opinion on the optimum thawing temperature used to thaw cell associated MD vaccine. It is worthwhile to note that most of the work on this issue has been done in temperate countries where the average ambient temperature is less than that of the Indian subcontinent which can also influence the virus titre of diluted vaccine. There is a need to check and ascertain the optimum thawing temperature of the cell associated MD vaccine before release to the field use for the benefit of poultry farmers. Histopathology is of immense help in diagnosing MD and the ability of bivalent HVT+SB-1 vaccine in reducing lymphoproliferative lesions in visceral organs, in comparison to monovalent vaccines (Pruthi et al., 1989).
By considering these factors the current work was undertaken to evaluate a bivalent MD vaccine containing MDV-3+MDV-2 (HVT+SB-1) against monovalent vaccine having MDV-3 (HVT) alone, \textit{w.r.t.} its safety, potency, T cell count, virulent virus load in feather tip pulp and lymphoproliferative lesions in visceral organs and nerves by histopathology.

\subsection*{5.1 Determination of optimum thawing temperature of the cell associated bivalent vaccine}

MD vaccines are amongst the most delicate vaccines in veterinary use and this is especially the case with the widely used cell-associated MD vaccines. The main reason for the occurrence of increased mortality is the improper handling of the cell associated vaccines currently in use (Morrow and Fehler, 2004). Halvorson and Mitchell (1979) reported that even a subtle deviation from the correct handling of MD vaccine can result in 14 to 97 per cent loss of vaccine titer. Thawing temperature and holding period of the vaccine after the dilution of vaccine constitute an important component of handling of MD cell associated vaccine.

In the present study, the highest titer for HVT+SB-1 vaccine was obtained at a thawing temperature of 35°C for 45 sec held on ice is in accordance with the findings of Geerligs and Hoogendam (2007) who investigated the effect of the thawing procedure on live virus titer of the CVI988 vaccine and reported that the highest titers were found with diluent at a temperature of 30°C to 37°C. The reconstituted vaccine after thawing at 35°C for 45 sec when held at RT, there was a drastic loss of virus titre at 120 min of holding period, which is in accordance with the observation of Gerlings and Hoogendam (2007)
who also reported that the diluted vaccine should be stored at cold conditions to prevent the loss of titer. It was found that when the vaccine was kept on ice after thawing at 35°C for 45 sec there was no significant loss of titer up to two hour holding period, which is in agreement with the opinion of Morrow and Fehler (2004) who recommended that MD vaccine should be used within two hours after reconstitution. M/s Venkateshwara Hatcheries Pvt. Ltd. which produces HVT+SB-1 vaccine in India recommends that reconstituted vaccine should be stored on ice and to be used completely within one hour.

On thawing at 26°C for 45 sec, there was a significant reduction in virus titer, compared to 35°C; however the vaccine titre was more than the minimum PFU required per bird and still can be used for vaccination. Morrow and Fehler (2004) opined that thawing the ampoules at temperatures of more than 28°C, or prolonged incubation before dilution, can cause damage to MD vaccine virus. HVT+SB-1 bivalent vaccine produced by Bioimmune Company, Kansas USA, recommends thawing temperature of 26.5°C and usage of the diluted vaccine at least within one hr to avoid loss of titer. In our studies we have found that, contrary to the opinion of Morrow and Fehler (2004), thawing at more than 28°C but within the 35°C does not have any deleterious effect on virus activity. At 20°C thawing for 60 sec, the virus titre was significantly reduced compared to 35°C and 26°C thawing but still the titer was above the standard required titre per bird recommended by the OIE. It was found that 45 sec duration of thawing at 20°C was not sufficient to completely dissolve the vaccine and minimum of 60 sec duration was required for complete thawing at 20°C. In North America, MD vaccine manufacturing companies prescribe a wide range of thawing temperatures ranging from 15°C to 30°C and it looks the vaccine manufacturers are averse to increasing the thawing temperature.
more than 30°C fearing damage to virus and loss of virus titre. Our results showed that 40°C and 45°C thawing for 45 sec is undesirable as it lead to loss of titer, especially at 45°C where there was no viral plaques at all, an indication of loss of viral infectivity which is in agreement with the claim of M/s Venkateshwara hatcheries Pvt. Ltd. India, that thawing more than 37°C would cause damage to the virus.

Fifteenth Western Meeting of Poultry Clinicians and Pathologists held at USA in 2004 has suggested thawing of MD vaccine at around 27°C for 90 sec. However in the present study, thawing at 26°C for 45 sec yielded lesser PFU than thawing at 35°C for 45 sec. The optimum thawing temperature of 35°C for 45 sec found in the present study is in accordance with the recommended thawing temperature of M/s Venkateshwara hatcheries Pvt. Ltd. in India. In many parts of India, during summer season, the temperature exceeds 40°C and this necessitates proper handling of the MD cell associated vaccines.

5.2 Evaluation of CD4 and CD8 cell responses in vaccinated and control birds

The life cycle of oncogenic MDV can be divided into four phases (Calnek, 2001). Early productive replication occurs between 3 and 7 dpi, followed by the onset of latency starting at 6 to 8 dpi. The third and fourth phases are secondary productive replication and transformation, respectively, in susceptible chickens infected with virulent MDV. In chickens infected with avirulent MDV or HVT, only productive replication and latency occur. During the productive infection, the vaccine viruses productively replicate as non-enveloped virions and antigens are synthesized, but during latency, MDV stays nonproductive and no viral or tumor-associated antigens are produced (Sugaya, 1990).
In general, cellular immune response plays a crucial role in protection from herpesvirus infections (Rickinson et al., 1997). Morimurara et al. (1998) suggested that CD8 T cell responses induced by the MD vaccine are essential for anti-virus but not anti-tumor effects. This type of response is important in providing protection from infection with MDV especially due to the strict cell-associated nature of MDV infection. Further, in MDV infected chickens, CD8 cytotoxic T lymphocytes are expanding when the virus enters latency and then significantly decrease to a level that of in uninfected chickens (Schat and Markowski-Grimsrud, 2001).

In the present investigation it was observed that CD8 lymphocytes were significantly increased in HVT+SB-1 and HVT vaccinated birds on 7th and 14th dpc compared to unvaccinated birds. Though there is a production of CD8 T cells in response to MDV-1 challenge in unvaccinated birds, these cells are of little use due to down regulation of MHC molecules on account of lytic infection (Schat and Markowski-Grimsrud, 2001 and Osterrieder, 2006). There was a significant difference in CD8 counts between HVT+ SB-1 and HVT vaccinated birds on 23rd dpv indicating a better CMI response of bivalent vaccine. These observations are also in agreement with that of Kano et al. (2009) who suggested that, CD8α^high TCR1^+ cells might be primed by the vaccination. Further, these cells are specifically induced by the challenge with virulent strain of MDV during the latent phase of MD infection, thus making CD8α^high TCR1^+ cell population one of the key factors involved in the protective mechanism induced by a vaccine strain, CVI988. The increase in CD8 population in HVT+SB-1 vaccinated and challenged birds on subsequent to 21st dpc is also in agreement with the findings of Kano et al. (2009) who reported a significant increase in CD8 cells in spleen particularly
in vaccinated-challenged chickens at 21 dpi compared to unvaccinated-challenged chickens. They opined that this increase in CD8 counts was due to vaccination.

The study is also in accordance with the observation of Changxin et al. (2009) who reported that, birds which received single MD vaccine showed an increase in CD8 and CD4 cell counts by seventh day and reached a peak by 14 dpv, and thereafter declined to a level that of in unimmunized chickens. The down regulation of CD8 cells in unvaccinated challenged birds compared to vaccinated birds was in accordance with the observation of Morimura et al. (1995), though not significant after 21st dpv. The reduction of CD8 cells in unvaccinated controls on 16th dpv with MDV-1 did not find its agreement with Li et al. (2011) who reported such a reduction of CD8 T cells on 21 and 28 dpi with GX101, a virulent MDV-1 cloned in bacterial artificial chromosome that the percentage of CD8 T cells was drastically reduced in MDV virulent GX101, cloned in bacterial artificial chromosome (bac-GX0101).

In the current study it was observed that there was a significant increase of CD4 cells on 16th dpv in unvaccinated control birds in comparison to HVT+SB-1 and HVT vaccinated birds indicating lympho-proliferation in unvaccinated birds. These results were in agreement with the findings of Parvizi et al. (2009) who studied the T cell population in spleen at 4, 10 and 21 dpi and reported that increased number of CD4 T cells, as well as a significant increase in the expression of viral Meq gene in CD4 T cells, which coincided with the presence of tumors in various organs of infected bird. The result of increased CD4 T cells in the control unvaccinated group is in accordance with the works of Li et al. (2011) who reported increased CD4 cells on days 14, 21 and 28 dpi in bac-GX0101-infected chickens.
5.3 Safety Test

World Organization for Animal Health (OIE), the regulatory body of the world animal health in its *Terrestrial Manual* 2010 has specified the safety requirement of MD vaccines. OIE *Terrestrial Manual* 2010 and Code of Federal Regulations (2007) prescribe 25 numbers of one day old birds for the safety test for MD vaccine whereas, European Pharmacopoeia (2005) recommends not fewer than 10 birds and the test will be invalid when more than twenty per cent birds show abnormal clinical signs or die from causes not attributable to the vaccine.

Both the HVT and HVT+SB-1 vaccine complied the safety test requirements as per OIE norms as none of the bird either in HVT or HVT+SB-1 vaccinated group showed any adverse reactions during a 21 day observation period. The results were on the expected lines since HVT and SB-1 viruses are non pathogenic to chicken. However, Friedman et al. (1992) reported that HVT+SB-1 bivalent vaccine caused temporary B-lymphocyte dysfunction and reduced resistance to infection in chicks against *E.coli*, during the 25 day observation period. The vaccines in the current study also passed the safety test conditions mentioned in 9CFR113.330 Code of Federal Regulations (2007) of USA since there were no deaths or lesions attributable to the vaccine and all the birds survived the 21 days observation period.

5.4 Potency test

In the present study of potency test, the control group had acute MD with enlargement of visceral organs in MD affected control birds. HVT+SB-1 bivalent vaccine and HVT vaccine gave protective index of 96 and 92.6 per cent respectively, passing the
potency test as per OIE norms (OIE, 2010) which say that protective indices should be more than 80 per cent.

The Protective index of HVT+SB-1 vaccine was 3.6 per cent more than HVT vaccine and both the vaccines showed the PI of higher than 80. There are many reports suggesting HVT+SB-1 vaccine gave better protection against MD than HVT alone. Witter (1982) showed that a polyvalent vaccine containing HVT+SB-1 virus protected chicken better against a battery of five highly virulent MD challenge virus than the monovalent HVT vaccine. Further, Calnek et al. (1983) reported that HVT+SB-1 bivalent vaccine was superior to vaccine containing HVT alone and they found a mortality range of 0.39-1.26 per cent in HVT+SB-1 vaccinated group against 1.92-7.44 per cent in birds that received HVT alone. Further, MD losses were reduced approximately by 3-13 folds in groups vaccinated with HVT + SB-1. Similarly, Witter et al. (1984) reported that HVT needed to be supplemented with small amounts of SB-1 to obtain the benefits of protective synergism. Pruthi et al. (1987) compared the efficacy of the bivalent vaccine with HVT and inactivated MDV separately and observed the bivalent vaccine appreciably delayed mortality resulting from MD and elicited the highest protective efficacy as judged on the basis of MD specific mortality and percentage of occurrence of lesions. They concluded that the protective efficacy of bivalent vaccine was higher than either HVT or inactivated MD vaccine. Houclot et al. (1993) found that the mortality of laying hens vaccinated with bivalent vaccine (SB-1 + HVT) was 9.8 per cent in contrast to 15.2 per cent in hens receiving HVT alone. Young (1993) observed that no protective benefit could be demonstrated for the Type 2 vaccine used alone.
Buscaglia et al. (2004) reported that HVT didn't protect satisfactorily against any of the virulent strains characterized as very virulent (vv) and very virulent plus (vv+) in Argentina but found that Serotype-2 plus Serotype-3 provided a significant protection when challenged with the virulent strains.

There are also reports of genetic variation among the birds susceptible to MD. It is very well documented that the occurrence of MD varies with the genetic makeup of chicken strain used to evaluate the vaccinal efficacies. Cho (1977) reported that differences in viremia titres in two strains of birds despite the same dose of MD vaccine due to their genetic differences in MD susceptibility. Chang et al. (2010) reported that protective indices against MD varied greatly among the chicken lines with a range of zero to 84 per cent. They also reported that non-MHC host genetic variation significantly affects MD vaccine efficacy in chicken. However, in the present experiment, birds with similar genetic makeup were used to test the potency of the vaccine, thus nullifying any effect of genetic difference in MD susceptibility.

In the present study protective index of HVT+SB-1, bivalent vaccine was 3.6 per cent more than HVT vaccine. Our findings were in accordance with the results of the previous works mentioned above though the difference in the protective index between HVT+ SB-1 and HVT is marginal.

5.5 Quantification of Marek’s Disease Virus by real time PCR

This study was designed to quantify relative load of MDV in feather pulp using Real time PCR. Monitoring of MD protection in the field is extremely difficult because MDV is ubiquitous and mere infection does not mean disease. Moreover, despite a strong
neutralizing antibody response after MD vaccination, these antibodies do not protect against development of tumors. Several attempts have been made to develop methods to monitor the efficacy of MD vaccines in the field. Baigent et al. (2006 and 2007) reported that virus load in feather tips was predictive of virus load in lymphoid tissues where immune responses would occur. They proposed to measure MDV DNA load in feather pulp to evaluate vaccine administration and monitor the protection obtained thereof. Gimeno et al. (2008) showed that measurement of challenge MDV DNA but not HVT DNA in blood is an adequate criterion for an early diagnosis of MD under experimental conditions.

In the present study to quantify relative amount of MDV-1 in HVT+SB-1 and HVT group in comparison to unvaccinated control birds, Meq gene was employed in real-time PCR. Meq gene is unique and present only in MDV-1 and absent in MDV-2 and HVT. Studies on deletion mutants suggest that Meq is likely to be the principal oncogene for MDV associated with MD tumour formation, with other MDV genes serving auxiliary function. Jones et al. (1992) mapped the Meq gene to the long repeats of MDV genome and reported that it was highly expressed in MDV-induced T-cell tumors. Lupiani (2004) showed that the rMd5DeltaMeq virus was fully attenuated in chickens because none of the infected chickens developed MD associated lymphomas, suggesting that Meq is involved in lymphocyte transformation. Li et al. (2011) suggested that the Meq gene played an important role not only in tumor formation but also in inducing immunosuppressive effects in MDV-infected chickens.
Feathers are living tissues that can be collected easily from live birds with minimal damage and bleeding. Necropsy can be avoided and repeated feather sampling can be done from the same bird. Baigent et al. (2005a and 2005b) quantified MDV genome in feather tips by measuring the Meq gene through real time PCR and they opined that MDV-1 quantification using feather tips could significantly further our understanding of vaccinal control of MD. Baigent et al. (2005b) further opined that feathers are the ideal tissues for PCR confirmation of successful vaccination in commercial chicken. Davidson and Borenshtein (2002) found that PCR for MDV using DNA from feather tips was more effective for the diagnosis of naturally infected commercial chicken than using the liver and spleen. Handberg et al. (2001) reported that detection of MDV in feather tips by PCR appeared to be as sensitive as co-cultivation of buffy coat cells in primary CEF.

In the present study, it was found that the relative feather pulp MDV-1 load in HVT+SB-1 vaccinated group was lower than the unvaccinated control group at all intervals of study i.e., 7th, 14th, 28th, 43rd and 61st days pc. But the difference of MDV-1 load between these two groups was non significant on 7th dpc, while the MDV-1 load was significantly decreased from 28th dpc onwards (P<0.001) in HVT+SB-1 vaccinated group. This observation is in accordance with the studies of Islam et al. (2006) who found a significant reduction in MDV load in PBLs of vaccinated chickens at 28 and 35dpc.

In the present study we found that on 7th dpc, the MDV-1 load in feather tips of HVT+SB-1 vaccinated birds, was declined by 1.71 folds and the decrease was steady up
to 28\textsuperscript{th} dpc reaching to 142.85 folds. After 28\textsuperscript{th} dpc, there was a drastic reduction in MDV-1 load in feather tip with 1000 fold the folds decrease on both 43\textsuperscript{rd} and 63\textsuperscript{rd} dpc. HVT vaccinated birds also showed a significant reduction in MDV-1 load compared to controls on 14\textsuperscript{th}, 28\textsuperscript{th}, 43\textsuperscript{rd} and 63\textsuperscript{rd} dpc. However HVT+SB-1 vaccinated birds had less quantity of MDV-1 load in feather pulp than HVT vaccinated birds indicating probable synergistic activity of SB-1.

The high relative MDV-1 load in control birds correlated with severe MD histopathology lesions specific to MD, whereas low MDV-1 load in HVT+SB-1 and HVT vaccinated birds showed very mild microscopic lesions on histopathology. This is in accordance with the studies of Gimeno \textit{et al.} (2008) who found that chickens that develop tumors have higher load of challenge MDV-1 DNA in both whole blood and buffy coat samples, and the differences can be detected as early as three wpc. In the present study the significant difference in MDV-1 load was noted on 7\textsuperscript{th} dpc itself in feather tips which is in accordance with the observation of Abdul-Careem \textit{et al.} (2006) and Baigent \textit{et al.} (2007) who reported that MDV DNA was detected in the feather tips of MDV infected birds commencing on day seven pi. The superiority of HVT+SB-1 vaccine over HVT vaccine in terms of reduction in MDV-1 load in feather tip was demonstrated in the present study.

Difference of HVT virus load in HVT+SB-1 vaccinated birds and HVT vaccinated birds was non significant (P>0.05) which do not correlate with the presence of microscopic lesions in birds. This result is in agreement with the findings of Gimeno \textit{et al.} (2008) who reported that HVT virus load in blood or buffy coat was not related to
development of tumors. Nevertheless the presence of HVT DNA in feather tips indicates successful vaccination.

5.6 Histopathology studies

Vaccination against MD does not preclude infection in MD and mere virological diagnosis is not sufficient to rule out disease. Virus isolation does not provide much information because of the ubiquitous nature of the virus. Isolated virus need to be characterized and further investigation requires experimental challenge studies in chickens, which are difficult to carry out. On the other hand, histopathology plays a vital role in confirmatory diagnosis of MD. In the present investigation a comparative study has been done to determine the efficacy of HVT+SB-1 bivalent vaccine based on microscopic lesions on challenge with virulent MDV-1.

Pruthi et al. (1989) studied the pathology of MD in chickens vaccinated separately with three different vaccines, viz., HVT, inactivated MDV and a bivalent vaccine and observed that the MD-specific mortality following challenge was significantly lower (2.0 %) in bivalent vaccinated birds than those vaccinated with either HVT (5.9 %) or inactivated MDV (13.7 %) alone. They also showed that the occurrence, extent and severity of gross lymphomas and lymphoproliferative lesions in visceral organs were lower in the bivalent vaccinated birds than in other groups. It is also noted that atrophy and degenerative changes were seen in unvaccinated and challenged birds but not in challenged and bivalent vaccinated birds. In the present investigation also a comparative study was carried out to determine the efficacy of HVT+SB-1 bivalent
vaccine and HVT vaccine, based on microscopic lesions on challenge with virulent MD virus.

On 7th day pc, all the samples from unvaccinated birds showed multifocal infiltration of lymphoid cells in liver and spleen but the same was not noticed in HVT+SB-1 vaccinated birds. The HVT vaccinated birds showed very mild infiltration of lymphoid cells in liver and spleen. The microscopic lesions indicated that the bivalent vaccine was effective in preventing early infiltration of lymphocytes into visceral organs than HVT vaccine alone owing to synergistic activity of SB-1.

The vaccine viruses prevent the replication of MDV-1 in early cytolytic phase irrespective of vaccination status. Proventriculus and kidney were affected with lymphoid cell infiltration but the numbers of affected birds were less than vaccinated birds. The bursa of unvaccinated birds was very much affected showing follicular degeneration with depletion of cells indicating widespread cytolytic infection caused by virulent MDV-1. These changes were in agreement with the observation of Jakowski et al. (1970) who reported the early necrosis and loss of architecture in the bursa of Fabricius in MDV infected birds. Grzegoraz et al. (2010) found highest dynamics of MDV replication in the lymphoid organs such as bursa of Fabricius, indicating stringent association of MDV-1 with lymphoid cells and tissues.

On the 14th day pc, the infiltration of lymphoid cells was seen in the liver and spleen of both vaccinated and unvaccinated group but Bursa was very much affected in unvaccinated group indicating severe B cell cytolytic infection which indicates late cytolytic infection (Calneck, 1986) arising out of the secondary wave of
immunosuppression. The infiltration was mild in the vaccinated group compared to unvaccinated group. Mild infiltration of lymphocytes into sciatic nerves was noticed in unvaccinated birds but not in vaccinated birds indicating both the vaccines protected the nerves from lymphoid cell infiltration.

On 28th day pc, there was an extensive infiltration of lymphoid cells in liver, spleen, kidney and proventriculus in unvaccinated birds compared to HVT and SB-1 vaccinated birds which showed very mild infiltration. Infiltration of lymphoid cells in liver was similar to unvaccinated birds but only three out of six birds showed the lesions. Thus the bivalent vaccine performed comparatively better than HVT vaccine in preventing lympho-proliferation.

On 43rd day pc, unvaccinated control showed extensive infiltration of lymphoid cells in liver and spleen whereas bivalent vaccinated birds showed minute aggregation of lymphocytes in liver and spleen. The HVT vaccinated birds showed mild aggregates of pleomorphic lymphocytes in liver whereas; spleen had extensive infiltration of lymphocytes. The microscopic lesions were mild in bivalent vaccinated group compared to HVT vaccinated birds and unvaccinated controls.

On 61st day pc, severe lympho-proliferation was seen in visceral organs compared to vaccinated groups. All the birds in the vaccinated group showed mild lymphoid cell infiltration. Nerve infiltration was seen only in control birds.

The study revealed that HVT+SB-1 vaccinated birds had less lymphocytic infiltration compared to HVT vaccinated group and invariably the control unvaccinated
group. The lesions in the Bursa of Fabricius was also noted by Rana et al. (1984) who observed that microscopic changes in HVT vaccinated and challenged birds characterized by mild paucity of lymphocytes and mild to moderate degree of retrogressive changes in cortex and medulla that were confined to Bursa of Fabricius with a mild degenerative changes in nerves of a few cases. Contrary to the observations of Rana et al. (1984), in the present study, neither HVT vaccinated birds nor HVT+SB-1 vaccinated birds showed any lymphoproliferative or degenerative changes though a few unvaccinated birds showed a mild lympho-proliferation in nerves. The severity of lymphoproliferative lesions in HVT and HVT+SB-1 vaccinated birds was in accordance with the findings of Pruthi et al. (1989) who found that the severity, intensity and frequency of gross and histological lesions in bivalent-vaccinated birds following challenge was less compared to HVT vaccinated and unvaccinated control birds.