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Review Article

Economically Important Non-oncogenic Immunosuppressive Viral Diseases of Chicken—Current Status

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ABSTRACT

Immunosuppressive viral diseases threaten the poultry industry by causing heavy mortality and economic loss of production, often as a result of the chickens' increased susceptibility to secondary infections and sub-optimal response to vaccinations. This paper aimed to present an up-to-date review of three specific economically important non-oncogenic immunosuppressive viral diseases of chickens, viz. chicken infectious anaemia (CIA), infectious bursal disease (IBD) and hydropericardium syndrome (HPS), with emphasis on their immunosuppressive effects. CIA and IBD causes immunosuppression in chickens and the socio-economic significance of these diseases is considerable worldwide. CIA occurs following transovarian transmission of chicken anaemia virus and has potential for inducing immunosuppression alone or in combination with other infectious agents, and is characterized by generalized lymphoid atrophy, increased mortality and severe anemia. The virus replicates in erythroid and lymphoid progenitor cells, causing inapparent, sub-clinical infections that lead to depletion of these cells with consequent immunosuppressive effects. The IBD virus replicates extensively in IgM^+ cells of the bursa and chickens may die during the acute phase of the disease, although IBD virus-induced mortality is highly variable and depends, among other factors, upon the virulence of the virus strain. The sub-clinical form is more common than clinical IBD because of regular vaccination on breeding farms. Infection at an early age significantly compromises the humoral and local immune responses of chickens because of the direct effect of B cells or their precursors. HPS is a recently emerged immunosuppressive disease of 3–6-weeked broilers, characterized by sudden onset, high mortality, typical hydropericardium and enlarged mottled and friable livers, with intranuclear inclusion bodies in the hepatocytes. The agent, fowl adenovirus-4, causes immunosuppression by damaging lymphoid tissues; the presence of IBD and CIA viruses may predispose for HPS or HPS may predispose for other viral infections. Synergism with CIA or other virus infections or prior immunosuppression is necessary to produce IBH-HPS in chickens and the susceptibility of chickens infected with fowl adenovirus varies throughout the course of CIA infection. The mechanism of immunosuppression has been studied in detail for certain chicken viruses at molecular levels, which will provides new opportunities to control these diseases by vaccination.

Keywords: viruses, CAV, IBDV, FAV, immunosuppression, chicken

Abbreviations: AC-ELISA, antigen-capture enzyme-linked immunosorbent assay; AGPT, agar gel precipitation test; ALV, avian leukosisvirus; AMV, avian myeloblastosis virus; BF, bursa of Fabricius; CAV, chicken anaemia virus; CEF, chicken embryo fibroblast; CEL, chicken embryo liver; CIA, chicken infectious anaemia; CMI, cell-mediated immunity; CTL, cytotoxic T cell; DPI, days post infection; FAV, fowl adenovirus; FPV, fowl poxvirus; HI, haemagglutination inhibition; HPS, hydropericardium syndrome; IBD, infectious bursal disease; IBDV, IBD virus; IBH, inclusion body hepatitis; IFN, interferon; Ig, immunoglobulin; IL, interlukin; ILTV, infectious laryngiotrachitis virus; INIBs, intranuclear inclusion bodies; LD_{50}, dose of virus

that kills 50% of birds on average; MDV, Marek disease virus; NDV, Newcastle disease virus; NK, natural killer; PCV, packed cell volume; qPCR, quantitative PCR; REV, reticuloendotheliosis virus; RT-PCR, reverse transcription-polymerase chain reaction; SPF, specific pathogen-free; $TCID_{50}$, tissue culture infective dose 50%; TGF, transforming growth factor; VNT, virus neutralization test

INTRODUCTION

With the breeding of superior genetic stock of poultry and high production pressure, the birds have become more susceptible to many diseases. As a result, many hitherto unknown and latent form of infections have emerged besides the prevailing diseases. Changes in the host–parasite relationship have resulted in the emergence of variants of existing viruses or the development of new diseases/syndromes, impeding the profitability of the poultry industry, often as a result of the chickens' increased susceptibility to secondary infections and sub-optimal response to vaccinations. Among the various emerging/re-emerging/prevailing diseases, viral diseases in general and immunosuppressive viruses in particular have been incriminated as the aetiological agents for a variety of clinical conditions in poultry. The emergence of such diseases not only threatens the economy of poultry production but also poses a challenge to the scientific community. The mechanism of this immunosuppression has been studied in detail for certain chicken viruses that have both direct and indirect effects on the cells of the immune system (Lutticken, 1997). Recent advances in molecular biology, genomics and immunology are revolutionizing our approach to managing infectious diseases of humans, livestock and poultry. One of the most interesting additions to the accumulated knowledge for research focusing on controlling infectious diseases has been a better understanding of how the host's immune system recognizes danger signals (Babiuk *et al*., 2003). The viruses that cause immunosuppressive effects in chickens are oncogenic viruses such as Marek disease virus (MDV), reticuloendotheliosis virus (REV) and avian leukosis virus (ALV), certain respiratory viruses such as infectious laryngiotrachitis virus (ILTV), Newcastle disease virus (NDV), and reoviruses, besides the infectious bursal disease virus (IBDV), chicken anaemia virus (CAV), and adenoviruses (Van den Berg, 1996; Islam *et al*., 2002), which are of major interest because of the widespread occurrence of the infections in commercial chickens and the severe economic losses they cause.

From the historical occurrences of theses diseases, Ellerman and Bang in Denmark in 1908 demonstrated avian leukosis; the first oncogenic virus could be transmitted from chicken to chicken by tumour tissue-derived cell-free filtrate. Subsequently, Rous demonstrated similar effects with avian sarcoma virus in 1911 (Murphy *et al*., 1999). The outcome of avian reovirus infections in birds ranges from inapparent to fatal and in some cases may be sub-clinical and produce focal necrosis and inflammatory infiltrations in bursae and other lymphoid tissues. Newcastle disease has been one of the most important diseases of poultry worldwide ever since the advent of high-density confinement husbandry systems. It was first observed in Java in 1926 and in the same year it spread to England, where it was first recognized in Newcastle, hence the name. In the same year, a specific viral disease of chickens in the United States, infectious laryngiotrachuitis (ILT), caused by gallid herpesvirus 1, occured among chickens worldwide. Infectious bursal disease (IB), in chickens was reported by Cosgrove in 1962 in an outbreak in Gumboro, Delaware, USA, and subsequent outbreaks were named 'Gumboro disease' after the location of the first recorded

outbreaks (Muller *et al*., 2003). Marek disease was described for the first time by Marek in 1907, but the herpesvirus aetiology was not established until 1967; prior to the introduction of vaccination in 1970, it was the most common lymphoproliferative disease of chickens and occurs worldwide. Much of the impact of this disease in broiler chickens is considered to be due to immunosuppression induced by MDV (Islam *et al*., 2002). CAV disease was first recognized in Japan in 1979; it is now known to occur worldwide and is problem in all countries with poultry industries (Rosenberger and Cloud, 1998). The aetiological agent of infectious canine hepatitis was demonstrated in 1960 by Cabasso's group; most of the adenoviruses produce only sub-clinical infections with occasional respiratory diseases, but aviadenoviruses are associated with a variety of clinical important syndromes (Rosenberger *et al*., 1974). Among these, hydropericardium syndrome (HPS) emerged during the last decade in broiler chicks as an the important disease and was reported from Angara Goth near Karachi, Pakistan in late 1987 (Jaffery, 1988). Virus-induced immunosuppression is a well-known phenomenon attributed to destruction of lymphoid tissues, poor response of immune cells to mitogens, and depletion of these cells with consequent immunosuppressive effects. Lymphopenia is the index of virus-induced cytolysis and its persistence, even after virus elimination, is the hallmark of immunosuppression. For example, IBDV destroys lymphocytes of the bursa and so causes bursal necrosis leading to immunosuppression. IBD constituted a serious problem for the poultry industry, and the recent 're-emergence' of IBDV in the form of antigenic variants and hypervirulent strains has been the cause of significant losses. This is due to evolution of the virus, which has been ascribed to recombination by reassortment method.

Keeping all the existing knowledge in view, in this paper we provide an overview of existing knowledge on the subject to enhance the available information on non-oncogenic immunosuppressive viral diseases that are of high economic importance. Chicken infectious anaemia (CIA), infectious bursal disease (IBD) and the recently emerged HPS in chickens are briefly summarized with major emphasis on their immunosuppressive effects.

CHICKEN INFECTIOUS ANAEMIA

CIA is highly contagious, is primarily a disease of young chickens, and is clinically characterized by severe anaemia, generalized lymphoid atrophy and increased mortality in young chicks, with concomitant severe immunosuppression enhancing susceptibility to other infectious agents and diminished vaccine responses leading to severe economic losses (Hagood *et al*., 2000). The causative agent of the disease is CAV, belonging to the genus *Gyrovirus* of the family *Circovidae* (Pringle, 1999), and is recognized as an important avian pathogen worldwide (Rosenberger and Cloud, 1998; Todd, 2000). CAV has been proven to be a potent immunosuppressive agent for very young unprotected chicks, thereby increasing their susceptibility to secondary infections, e.g. with viral, bacterial and fungal agents, and depressing vaccinal immunity and production performance under field conditions (Van den Berg, 1996; Adair, 2000; Todd, 2004). The emergence of CIA is posing a severe threat to poultry rearing countries and necessitate the determination of the epidemiological status of the disease, with emphasis on CAV research for diagnosis and developing suitable control measures.

The clinical disease is rare today because of the widespread practice of vaccinating breeders, but the sub-clinical form of the disease is ubiquitous (Sommer and Cardona, 2003). CAV causes clinical symptoms in newborn chickens and sub-clinical symptoms in older ones. However, maternal antibodies prevent the clinical signs of disease but do not prevent infection and transmission of the virus or immunosuppression (Sommer and Cardona, 2003). The disease is acute, with the clinical stage developing after a incubation period of 1 to 14 days; inconsistent pathognomonic symptoms include anaemia on the non-feathered areas, weakness, anorexia, ruled feathers and stunted growth, depending on disease severity (Pope, 1991). The mortality of the disease varies usually between 5% and 10% and the morbidity between 20% and 60%, but up to 60% mortality has also been recorded (McNulty *et al*., 1991). Among the clinical signs, severe depletion of cortical thymocytes and erythroblastoid cells in the bone marrow causes immunodeficiency and anaemia (Noteborn and Koch, 1995).

The chicken is the only natural host for the virus, which is ubiquitous not only in commercial domestic fowl but also in SPF stocks (Cardona *et al*., 2000b). Chickens of all ages are susceptible to CAV infection, but after 2 weeks of age susceptibility to clinical disease decreases rapidly due to the development of an effective humoral response (Hu *et al*., l993a). Both sexes are affected; broilers are found to be more susceptible, with chicks at 1 day of age being most susceptible (Rosenberger and Cloud, 1989). Chicks are at increased risk of infection with CAV and the period of susceptibility to disease may be extended by early exposure to other lymphocidal agents such as IBDV, MDV, REV, adenoviruses or certain avian reoviruses and other agents that interfere with development of the immune system (Imai *et al*., 1999). Since it is transmitted through eggs (Yuasa and Yoshida, 1983), eradication of CIA is virtually not possible in the field; elimination from the SPF flock should be given top priority so as to prevent the risk of vaccine contamination by CAV. Vertical as well as horizontal modes of transmission are evolved in the spread of CAV among chickens, resulting in clinical and sub-clinical infections, respectively (Yuasa *et al*., 1983; McNulty, 1991). Production or transfer stress might enhance susceptibility to CAV infection, or persistent CAV infection may be activated because of hormonal changes leading to vertical transmission and subsequent seroconversion near sexual maturity (Cardona *et al*., 2000a,b). CAV can be propagated and assayed in susceptible chickens, in chicken embryos, or in lymphoblastoid cell lines such as MDCC-MSBI (T-cell, MDV transformed), MDCC-JP2 (T cell, MDV transformed), LSCC-1104/X5 B1 (B-cel1, ALV induced), LSCC-HDII (AMV transformed) and MDCC-CUI 47 (Yuasa, 1983; Jeurissen *et al*., 1992; Noteborn *et al*., 1994; Coombes and Crawford, 1998; Calnek *et al*., 2000).

Bursal atrophy is an important risk factor for the development of CIA (Hagood *et al*.,2000). The target cells of CAV have been identified as erythroid and lymphoid progenitor cells, viz. haemocytoblasts and precursor T lymphocytes and also reticular cells (Jeurissen *et al*., 1992; Smyth *et al*., 1993). However, B cells are not susceptible to CAV infection and are not directly affected by the virus (Adair, 2000). CAV replicates in lymphocytes, causing destruction of thymic lymphocytes, and is directly cytotoxic for bone marrow haematopoietic precursors, leading to transient severe anaemia and immunosuppression (Adair, 2000). This depletion of thymocytes is caused by apoptosis, as evidenced from apoptosis-specific laddering patterns in DNA isolated from infected thymocytes and condensed chromatin and apoptotic bodies in the cytoplasm of epithelial cells (Jeurissen *et al*., 1992). The viral

protein VP3 (apoptin) induces apoptosis in specific lymphoid cells, chicken thymocytes and lymphoblastoid cell lines (MSBI) (Jeurissen *et al*., 1992), and this is also an important phenomenon during the pathogenesis of CAV (Noteborn *et al*., 1994; Noteborn and Koch, 1995). The main cytopathogenic effects of CAV infection are the induction of the apoptotic machinery (reviewed recently by Noteborn, 2004) and the appearance of VP3-induced characteristic 'doughnut-like' structures similar to those observed in CAV-infected cells (Noteborn *et al*., 1994). Apoptin acts as a transcriptional regulator of genes influencing the apoptotic process and this is also a possible mechanism (Noteborn *et al*., 1998). Hemorrhages due to primary destruction of thrombocytes causes thrombocytopenia and impaired clotting (Pope, 1991).

Subclinical infections of chickens (3 weeks old and above) can also result in immunosuppression as evidenced by laboratory findings (Adair, 2000). CAV exerts a destructive effect on both primary and secondary lymphoid tissues and especially suppresses the population of both helper ($CD4^+$) and cytotoxic ($CD8^+$) lymphocytes in the thymus (Hu *et al*., 1993a,b; Adair, 2000). Poor antibody response after CAV infection at 1 day of age is a consequence of depressed T-helper responses in the early phase of infection (Otaki *et al*., l988). There is marked damage to haematopoietic and lymphopoietic tissues, viz. stem cells in bone marrow and precursor T-lymphocytes in the thymus. The bursa, spleen and other lymphoid organs are also depleted of lymphoid cells (Smyth *et al*., 1993; Dhama, 2002; Dhama *et al*., 2002). However, McKenna and colleagues (2003), while studying the immunopathological effects of two attenuated CAV isolates, demonstrated that reduced CAV pathogenicity for 1-day-old chicks correlates with reduced depletion of T-cell populations in the thymus and with reduced severity of lesions in the thymus and bone marrow. Intramuscular inoculation of the virus at 1–7 days of age resulted in reduced levels of lymphocyte transformation responses of splenic, thymic and blood lymphocytes to T-cell mitogens and adversely affected lymphokine production (IL-2, TGF, IFN) at 7–21 DPI (Bounous *et al*.,1995; Dhama, 2002). Destruction of erythroid progenitors in bone marrow results in severe anaemia and depletion of granulocytes and thrombocytes. Destruction of precursor T cells results in depletion of mature cytotoxic and T-helper cells, with consequent effects on susceptibility to and enhancement of the pathogenicity of secondary infectious agents and sub-optimal antibody responses (Adair, 2000). Apoptosis appears to be a feature of the lymphocyte depletion in the thymic cortex, which may be mediated by apoptin. There is a decrease in the ratio between the weight of lymphoid organs, viz. thymus, bursa and spleen, and the whole-body weights. T and B cell proliferation activities decreases in immune organs, resulting in a significant decrease in immunoglobulin (IgG, IgM and IgA) levels in all body fluids of CAV-infected chicks and leading to decreased immunoprotective efficacies (Cloud *et al*., 1992a,b; Dhama, 2002). It has also been observed that there is substantial reduction in macrophage functions such as bactericidal activity, phagocytosis, cytokine (IL-1) production and Fc receptor expression (Adair *et al*., 1993). Inhibition of interleukin (IL-1, IL-2, etc.) and IFN production adversely affects molecular immunoregulatory responses in the cytotoxic activities of macrophages, cytotoxic T-lymphocytes natural killer (NK) cells and expression of surface receptors. Studies conducted with co-infection of IBDV and CAV showed that very virulent IBDV infection inhibited production of neutralizing antibody to CAV in chickens at 7 days post infection (DPI) (Imai *et al*., 1999). Although antibody appeared after 14 DPI, CAV was recovered from blood cells at high titres

($10^{2.5}$–$10^{5.5}$ $TCID_{50}$/0.1 ml), from 7 to 28 DPI in IBDV-induced immunosuppressed chickens (Imai *et al*., 1999). This study first examined the persistence of CAV in blood cells and the effective enhancement of primary CAV infection as a result of immunosuppression caused by IBDV infection. Control measures are directed at limiting vertical transmission and preventing co-infection with other lymphocidal agents (Rosenberger and Cloud, 1998). Hematocrit, histopathology, concanavalin A-induced lymphocyte proliferation, intracellular calcium signalling, and lymphocyte subpopulations were analysed over 6-week period in individual chicks inoculated with the CL-1 isolate of CAV (Bounous *et al*., 1995). Lymphoid depletion/atrophy was observed in the thymus and bone marrow at 11 DPI anemia at 14 DPI. The mean lymphocyte proliferation stimulation index (SI) of the inoculated group was significantly lower than that of the control group by 11 DPI, with a reversion at 18 DPI. Percentages of $CD3^+$, $CD4^+$, $CD8^+$ and NK cells decreased significantly at 18 and 25 DPI (Bounous *et al*., 1995).

CAV-infected birds develop a profound immunosuppression in the presence of concurrent infection with other viruses such as MDV (Miles *et al*., l999), IBDV (Imai *et al*., 1999), FAV (IBH/HPS) (Toro *et al*., 2001), reoviruses (McNeilly *et al*., 1995) and NDV (De Boer *et a1*. 1994), leading to synergistic effects of both agents (Pope, 1991). High doses of aflatoxin resulted in elevated mortality and immunosuppression and also possibly vertical transmission of CAV (Von Bulow, 1991). CAV infection causes decreased immune response against several vaccine viruses, viz. NDV, MDV, ILTV and FPV, leading to vaccination failures or aggravation of the residual pathogenicity of attenuated vaccine viruses, and could even lead to the emergence of variant viruses (Cloud *et al*., 1992ab; Dhama *et al*., 2002). As an example of this, birds immunized against coccidiosis in the presence of CAV infection showed a lower level of protection (Ibrahim, 1998). The impairment of specific CTL after natural and experimental infections of chickens with CAV and MDV or REV has also been reported (Markowski-Grimsrud and Schat, 2003). IL-1β and IL-2 mRNA levels were not significantly affected by CAV infection at 7 or 14 DPI. Similar assays for IFN-γ transcripts demonstrated a 10-fold increase in IFN-γ mRNA levels at 7 DPI following infection with REV or REV with CAV, while CAV alone caused a 2-fold to 4-fold increase (Markowski-Grimsrud and Schat, 2003). These results showed a strong link between CAV antibody status, CAV replication, and the ability to generate REV-specific CTL. Because these viruses cause immunosuppression by inapparent, subclinical infections, there is need for assessment of the immune status of chickens. Interference with induction of transcription of chicken IFN-α and IFN-γ was noted after sub-clinical infection with CAV or IBDV (Ragland *et al*., 2002).

Gross lesions in lymphoid tissues and bone marrow are most pronounced at 12–16 DPI. Lesions consist of transient severe bone marrow aplasia and pancytopenia, with reduction in haematocrit values ranging between 6% and 27% (PCV $<$ 25%) due to severe anaemia. Bone marrow characteristically changes from a red to a pale to whitish colour and might have a fatty consistency. The liver becomes pale, discoloured and enlarged. There is intense atrophy of the lymphoid organs at the peak of the anaemic phase, including the thymus, bursa of Fabricius and spleen. Other visceral organs might show areas of congestion and haemorrhages (Dhama *et al*., 2002). Bursal atrophy is generally modest and transitory (Yuasa *et al*., 1979; Goryo *et al*., 1987). Histologically, lesions appear first in bone marrow and thymus at 6 DPI and then in the bursa, spleen and liver (Goryo *et al*., l987; Smyth *et al*.,

1993). The thymus becomes atrophic, with hydropic degeneration of residual cells and occasional necrotic foci. Lesions in the bursa consist of atrophy of the lymphoid follicles with occasional small necrotic foci, infolded epithelium, hydropic epithelial degeneration, and proliferation of reticular cells. In the liver, kidneys, proventriculus, duodenum and caecal tonsils, lymphoid foci are depleted of cells, making them smaller and less dense than those in unaffected birds (Dhama, 2002). Intracytoplasmic botryoid inclusions within macrophages and depletion of T and B cells are common. Inclusions can be considered pathognomonic, but because they are transient and small in size, identification is difficult and they are of limited diagnostic value (Goryo *et al*., 1987; Pope, 1991).

Tentative diagnosis of CAV infection can usually be made on the basis of flock history, clinical signs/presentation and haematological and pathological findings but these are seldom conclusive (Goodwin and Brown, 1992). The major breakthrough reported by Yuasa (1983), indicating CAV-induced cytopathogenicity in MDCC-MSBI, enabled *in vitro* virus isolation, which facilitated virus purification and progress in development of diagnostic assays (McNulty *et al*.,l990; Todd *et al*., 1990). Isolation and identification of the agent (Yuasa *et al*., 1983), detection of viral antigens by electron microscopy, immunoassays such as IPT, FAT (McNeilly *et al*., 1991), PCR, nucleic acid hybridization, RE mapping, and serological assays such as SNT, IIFT, IIPT, and ELISA were employed for confirmatory diagnosis of the disease (Dhama *et al*., 2002). PCR and dot blot hybridization (DBH) tests have been developed for the diagnosis CIA (Todd *et al*., 1991) and a novel circovirus infection of geese (GoCV) (Ball *et al*., 2004). A rapid method in which CAV genomes in infected cells are quantitated by qPCR at 3–4 DPI, without passaging virus in cells has also been reported (Van Santen *et al*., 2004). Typical combinations of clinical signs, haematological changes, gross and histopathological lesions and flock history are suggestive of CIA. Differential diagnosis should be made from Marek disease, IBD, avian erythyroblastosis, osteoporosis, IBH-aplastic anaemia syndrome and intoxications with mycotoxins and sulphonamides (hemorrhagic syndrome), sulphur and trichloroethylene toxicity (Dhama *et al*., 2002).

Good management practices, good hygiene and strict biosecurity practices are of immense value/help in protecting young chicks against early exposure to CAV as well as co-infections with other lymphocidal agents, especially IBDV and MDV following suitable vaccination programmes, so as to limit immunosuppression and reduce economic losses (Engstroms, 1999). Acquired immunity from the use of vaccine efficiently prevents vertical transmission in the flock. Chicken embryo-propagated Cux-1 isolate live vaccine ($10^{4.5}TCID_{50}$/bird), although it gave effective protection, harboured risks of reversion to virulence (Vielitz *et al*., 1987). Cell culture-propagated CAV in adequate titre can also serve as an effective drinking-water vaccine; however, reversion to virulence even after many passages (P170) has also been reported (Von Bulow and Schat, 1997). Vaccination should be performed at about 13–15 weeks of age, 3–4 weeks prior to the onset of lay to avoid the hazard of spreading vaccine virus through eggs (Vielitz *et al*., 1987; McNulty, 1991). Koch and colleagues (1995) developed an effective recombinant subunit vaccine (VP1 and VP2 proteins of CAV) using a baculovirus insect cell culture system to provide an alternative safe and cost-effective vaccine. Yamaguchi and colleagues (2001) recently showed that a single amino acid change at residue 394 of VP1, a major determinant of pathogenicity, can generate a low-pathogenity CAV. These approaches can provide good potential candidates for development of a safe and stable genetically modified, live-attenuated CAV vaccine,

which might help in disease control. However, research investigations should be focused on the comparative pathogenicity of CAV isolates, molecular studies to better understand mechanism of pathogenicity, the immunosuppressive properties, and sub-clinical disease and its interactions with other viruses/pathogens in the field.

INFECTIOUS BURSAL DISEASE

IBD is an important immunosuppressive viral disease of chickens. It has been described throughout the world, and its socio-economic significance is recognized worldwide. When IBD appeared in chickens in 1962, the disease was designated 'Gumboro disease' after the location of the first recorded outbreaks (Muller *et al*., 2003). Although first observed about four decades ago, this disease continues to pose an important threat to the commercial poultry industry. The causative agent of the disease is IBD virus (IBDV), which belongs to genus *Avibirnavirus* (Leong *et al*., 2000) of the family *Birnaviridae* (Dobos *et al*., 1979). It has two molecules of linear dsRNA (segments A and B) enclosed in a non-enveloped icosahedral capsid. There are two serotypes of IBDV, which can be differentiated by VNT (McFerran *et al*., 1980). Serotype 1 contains the strains pathogenic for chickens, whereas serotype 2 strains, mainly isolated from turkeys, cause neither disease nor protection against serotype 1 strains in chickens. Infections caused by IBDV may exacerbate infections with other aetiological agents and reduce the ability of chickens' responses to vaccination. Strain of virus, susceptibility and breed of flock, intercurrent primary and secondary pathogens, and environmental and managemental factors influence the economic impact of IBD. In recent years there has been greater research activity on IBD, leading to significant progress in the understanding of the structure, morphogenesis and molecular biology of IBDV, reflecting the economic importance of the species affected and the recent changes in the antigenic make-up and pathogenicity of IBDV (Saif, 1998).

Various forms of the disease have been described, but typing remains unclear, since antigenic and pathotypic criteria are used indiscriminately, and the true incidence of different types is difficult to determine. Moreover, the infection, when not fatal, leads to a degree of immunosuppression that is often difficult to measure (Van den Berg *et al*., 2000). In the classical form of outbreaks, the mortality rate may range from 1% to 50%. IBDV causes immunosuppression in broilers in addition to mortality; this is denoted by a high prevalence of viral respiratory infections and elevated mortality due to airsacculitis and or colisepticaemia at the 6–8 weeks of age; birds may become refractory to live attenuated vaccine against respiratory diseases (Muller *et al*., 2003). The virus is ubiquitous and, under natural conditions, chickens acquire infection by the oral route and IgM^+ cells serve as targets for the virus (Sharma *et al*., 2000). The major target cells of IBDV serotype 1 strains are lymphoid cells in the bursa of Fabricius. Chickens are highly susceptible to the virus when the bursa reaches maximum development, i.e. between 3 and 6 weeks of age. Infection results in lymphoid depletion and the final destruction of the bursa as the predominant feature of the pathogenesis of IBD. 'Very virulent' (vv) strains of IBDV can cause up to 70% flock mortality in laying pullets (Chettle *et al*., 1989; Van den Berg *et al*., 1991). These strains can cause lesions typical of IBDV and are antigenically similar to the 'classical' strains (Eterradossi *et al*., 1992). Remarkably, vvIBDV can establish infections in the face

of levels of maternally derived antibodies that were previously protective against 'classical' strains. vvIBDV infections have been observed in Africa, Asia, Europe (Nakamura *et al*., 1992; Indervesh *et al*., 2003; Domanska *et al*., 2004; Owoade *et al*., 2004; Tan *et al*., 2004) and, only recently, in South America (Ikuta *et al*., 2001) and Egypt (Hassan, 2004).

IBD of chickens can run an acute lethal course, or death can result from a B cell-dependent immunodeficiency due to destruction of the bursa of Fabricius following infection with IBDV. Depending on the host cell in which the virus replicates, various types of defective particles are formed and various degrees of pathogenicity appear. Pathogenic properties of the virus are also influenced by both genomic segments and cannot be attributed to a single gene (Becht and Muller, 1991; Kong *et al*., 2004b). During the acute phase of the disease, which lasts for about 7–10 days, the bursal follicles are depleted of B cells and the bursa becomes atrophic. Abundant viral antigen can be detected in the bursal follicles and other peripheral lymphoid organs such as the caecal tonsils and spleen. $CD4^+$ and $CD8^+$ T cells accumulate at and near the site of virus replication. The virus-induced bursal T cells are activated, exhibit upregulation of cytokine genes, proliferate in response to *in vitro* stimulation with IBDV and have suppressive properties (Sharma *et al*., 2000). Serotype 2 strains do not replicate in lymphoid cells but grow in chicken embryo fibroblasts (CEFs) as do the tissue culture-adapted serotype l strains (Nieper and Muller, 1996, 1998). IBDV infection also changes the potassium current properties of CEFs (Repp *et al*., 1998), thus affecting intracellular ion homeostasis and contributing to cytolysis and death of the infected cells (Muller *et al*., 2003). The application of double-labelling techniques (Nieper *et al*., 1999) showed that apoptosis is induced by IBDV replication in productively infected chicken embryo cells and cells of the bursa, as well as in antigen-negative cells in their vicinity (Jungmann *et al*., 2001). The proportion of apoptotic cells correlated with the efficiency of IBDV replications; UV-inactivated IBDV particles did not induce apoptosis. These observations suggest that both necrosis and apoptosis contribute to the rapid depletion of cells in the IBDV-infected bursa (Muller *et al*., 2003). Chickens may die during the acute phase of the disease, although IBDV-induced mortality is highly variable and depends, among other factors, upon the virulence of the virus strain. Chickens that survive the acute disease clear the virus and recover from its pathological effects, with the depleted bursal follicles being repopulated with IgM^+ B cells (Sharma *et al*., 2000). During the acute phase, different vvIBDV isolates have different efficiency of replication and produce various percentages of apoptotic cells in bursae (Kong *et al*., 2004a)

Studies on the role of T cells in IBDV-induced immunopathogenesis and tissue recovery showed that both $CD4^+$ and $CD8^+$ T cells infiltrate the bursa, reaching maximal levels at 7 DPI (Kim *et al*., 2000; Sharma *et al*., 2000). Intrabursal T cells limit viral replication in the bursa in the early phase of the disease but also promote bursal tissue damage and delay tissue recovery through the release of cytokines and cycotoxic effects (Rautenschlein *et al*., 2002b). Recent investigations on the role of cell-mediated immunity (CMI) (Yeh *et al*., 2002) and the significance of virus-specific antibodies (Rautenschlein *et al*., 2002a) indicated that antibody alone is not sufficient in inducing protection against IBDV and that T cell involvement is critical for protection. The role of macrophages and the significance of cytokine release in IBD pathogenesis have also been reviewed (Van den Berg, 2000). Peters and colleagues (2004), demonstrated that IBDV polyprotein induces suppression of the growth of bursal B cells and their capacity for proliferation with mitogenic stimulation.

Infection with IBDV causes a transient inhibition of *in vitro* proliferative response of T cells to mitogens and is mediated by macrophages, which are activated in virus-exposed chickens and exhibit marked enhancement of expression of a number of cytokine genes (Sharma *et al*., 2000). They speculated that T cell cytokines such as IFN-γ might stimulate macrophages to produce nitric oxide and other cytokines with anti-proliferative activity. Further studies are needed to identify the possible direct immunosuppressive effect of IBDV on T cells and their functions, and to examine the effects of the virus on innate immunity.

Sharma and colleagues (1994) reported that reoviruses and IBDV are among the naturally occurring viruses that cause immunosuppression in chickens with necrotic lesions in the bursa and destruction of B cells. The mechanisms of virus-induced suppression of CMI are not well understood. However, both viruses inhibit the mitogenic response of T cells, which may be mediated by inhibitory cytokines such as TGF-β and nitric oxide produced by activated macrophages (Sharma *et al*., 1994). Similarly, Martinez-Costas and colleagues (2000) demonstrated that extracts of avian reovirus-infected cells are able to relieve the translation-inhibitory activity of dsRNA in reticulocyte lysates by blocking the activation of the dsRNA-dependent enzymes. In addition, protein sigmaA, an S1133 core polypeptide, binds irreversibly to dsRNA and clearing of this protein from extracts of infected cells abolishes their protranslational capacity; possibly sigmaA antagonizes the IFN-induced cellular response against avian reovirus by blocking the intracellular activation of enzyme pathways dependent on dsRNA. Infection with IBDV at an early age significantly compromises the humoral and local immune responses of chickens. The CMI response is also compromised, but apparently to a lesser extent and for a shorter period. The immunosuppression seems to be a result of a direct effect (lysis) of B cells or their precursors. Other mechanisms of immunosuppression have been suggested, notably the development of suppressor cells (Saif, 1991). Inhibition of the humoral immunity is attributed to the destruction of immunoglobulin-producing cells by the virus. Other mechanisms such as altered antigen-presenting and helper T cell functions may also be involved (Sharma *et al*., 2000).

The strain and the amount of the infecting virus, the age and the breed of the bird, the route of inoculation, and the presence or absence of neutralizing antibodies largely determine the outcome of an IBDV infection. The pathogenic IBDV serotype 1 field isolates can be grouped into classical virulent (cv) or vv pathotypes and antigenic variant strains. *In vivo* studies, sequencing and pathogenetic analyses led to the conclusion that some VP2 residues might be the molecular determinants for the virulence, cell tropism and pathogenic phenotype of vvIBDV (Yamaguchi *et al*., 1996; Brandt *et al*., 2001). However, Boot and colleagues (2000) reported that VP2 is not the sole determinant of virulence by demonstrating the exchange of VP2 between a cv and a vv phenotype. Repeated passages of cvIBDV in tissue culture at high multiplicity of infection led to the formation of a small plaque phenotype, which is highly attenuated (Müller *et al*., 1986; Lange *et al*., 1987) and has been used as a live vaccine for many years. Wild-type IBDV strains, particularly vvIBDV, normally do not grow in cell cultures. From sequence comparisons, specific amino acids in VP2 were identified to allow adaptation of vvIBDV to cell culture (Yamaguchi *et al*., 1996; Lim *et al*., 1999). VP2 and VP5 are involved in the induction of apoptosis in the chicken B-lymphocyte cell line RP9 as well as in CEF (Yao and Vakharia, 2001). Phylogenetic analysis of segment B nuclestide sequences of the emerging vvIBDV strains showed they formed a district cluster (Islam *et al*., 2001a) and it has been suggested that these strains might have derived segment B from a

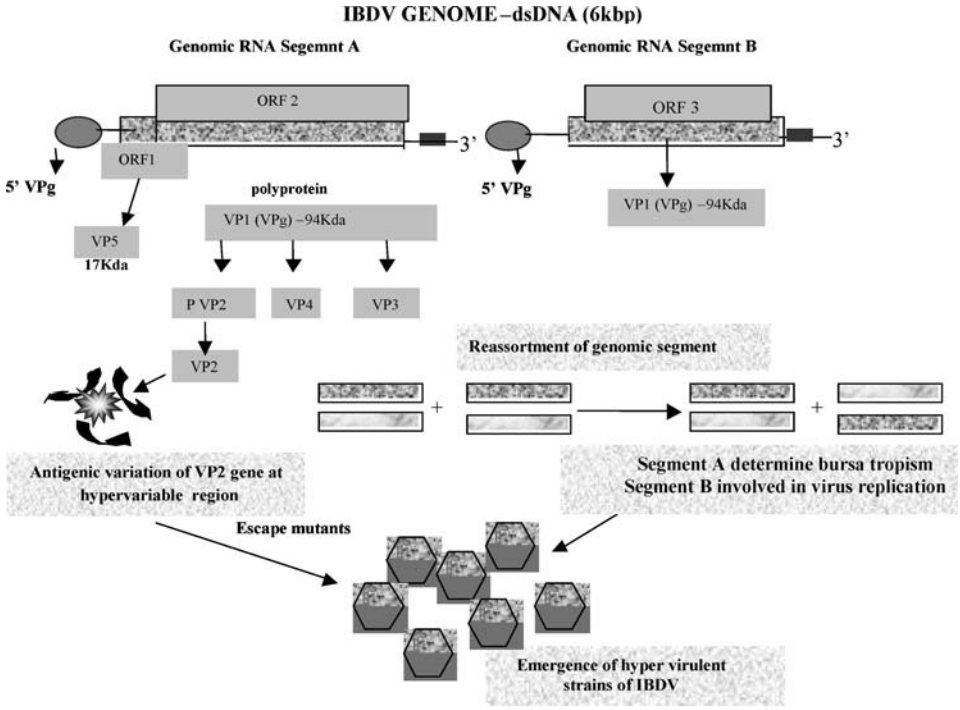

Figure 1. IBD virus genomic structure

hitherto unidentified source, possibly by segment reassortment. IBD viral genomic structure with coding proteins and the event of reassortment are schematically illustrated in Figure 1. Reassortant serotype1/serotype 2 IBDV showed that genome segment A determines bursa tropism, whereas segment B is involved in the efficiency of virus replication (Zierenberg *et al*., 2004). The molecular basis of antigenic variation in IBDV using the large genome segment A, encoding the structural proteins of the US variants, GLS, DS326, E/Del and the vaccine strain D78, was also determined (Vakharia *et al*., 1994). By comparison of the amino acid sequences of these variant viruses and their reactivities with IBDV-specific MAbs, the putative amino acids involved in the formation of virus-neutralizing epitopes were identified. Recently, *in vivo* studies showed that vvIBDV that had been adapted to chicken embryo cell cultures by using site-directed mutagenesis and the reverse genetics approach were partially attenuated for SPF chickens (Van Loon *et al*., 2002) and commercial chickens (Raue *et al*., 2004). However, reversion to wild type limits their application as potential live vaccine (Raue *et al*., 2004).

Diagnosis and confirmation of an IBDV infection are based on characteristic pathological changes in the bursa of Fabricius (Figure 2) and histopathological investigations combined with the demonstration of viral antigens by immunohistochemistry. Viral antigens can be demonstrated by AGPT, ELISA (Manoharan *et al*., 2004; Saravanan *et al*., 2004a,b) or AC-ELISA, with some limitations. AC-ELISA allows the identification of vvIBDV

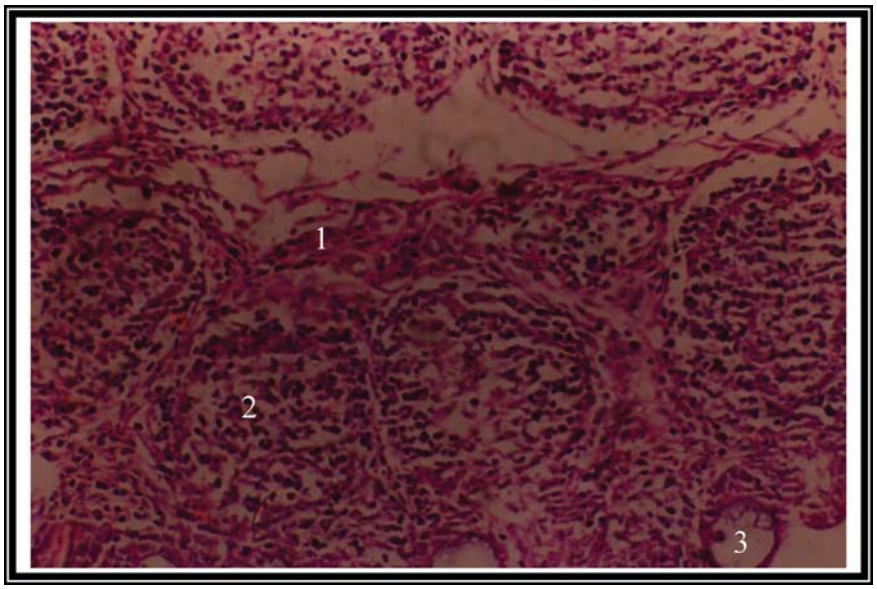

Figure 2. Broiler chicken infected with IBDV at 1-week of age. Bursa of Fabricius showing necrosis (1), CTL proliferation (2) and cyst formation (3) in the epithelial lining at 10 DPI (H&E, ×160)

(Eterradossi *et al*., 1998; Islam *et al*., 2001b), while VNT can reliably differentiate IBDV isolates into antigenic serotypes and subtypes (Jackwood and Saif, 1987). For differentiation of virulence strains of IBDV, restriction analysis of RT-PCR products of VP2 sequences (Kataria *et al*., 1999; Toroghi *et al*., 2003) and dot blot hybridization (Kataria *et al*., 2000) have also been employed. RT-PCR in combination with restriction enzyme analysis allows the rapid identifications of vvIBDV (Jackwood and Jackwood, 1994; Zierenberg *et al*., 200l; Toroghi *et al*., 2003). At present, RT-PCR based on VP2 and VP1 gene sequences is frequently applied in IBDV diagnosis (Kataria *et al*., 1998; Raue and Mazaheri, 2003; Tiwari *et al*., 2003;). Real-time RT-PCR has been demonstrated recently (Moody *et al*., 2000; Raue and Mazaheri, 2003; Jackwood *et al*., 2003), for identification of IBDV quasispecies using fluorescence resonance energy transfer (FRET) in a two-probe system (Jackwood and Sommer, 2002). An *in-situ* RT-PCR was also developed to identify early stages of infection in the IBDV-infected bursa (Zhang *et al*., 2002). Recently, a multiplex PCR for detection of avian adenovirus, avian reovirus, IBDV and CAV was also developed (Caterina *et al*., 2004).

Even with strict hygienic measures, vaccination is inevitable under high infectious pressure and is mandatory to protect chickens against infection during the first weeks of life, due to the highly infectious nature of disease and the high resistance of virus to inactivation. Layers are vaccinated with inactivated oil-emulsified vaccine, while chicks are immunized with live vaccine to induce high titres of antibodies. The vaccination time is crucial because persistent maternally derived antibodies might neutralize the vaccine (Tsukamoto *et al*., 1994). It has also to taken into consideration that vvIBDV will break through immunity

provided by highly attenuated vaccine strains. However, it is well known that less attenuated strains may cause lesions in the bursal follicles and, thus, immunosuppression will occur even in vaccinated birds. A 'trimming complex' vaccine has been developed, in which the vaccine virus is complexed *in vitro* with an optimum amount of antibodies (Whitfill *et al*., 1995) and is used for *in ovo* vaccination (Negash *et al*., 2004).

Although the exact mechanism of action of the immune complex vaccine is not yet clear, it has been suggested that the immune complex is taken up by follicular dendritic cells, where the virus resides until the maternal antibody subsides (Jeurissen *et al*., 1998). Experimental recombinant IBD vaccines have been developed using heterologous virus as vector, viz. FPV (Shaw and Davison, 2000; Butter *et al*., 2003), NDV (Huang *et al*., 2004), herpesvirus of turkey (Darteil *et al*., 1995), FAV (Sheppard *et al*., 1998; Francois *et al*., 2001, 2004), MDV (Tsukamoto *et al*., 2000, 2002) and Semliki Forest virus (Phenix *et al*., 2001). *In vitro* expressed VP2 (Dybing and Jackwood, 1998; Wang *et al*., 2000; Yehuda *et al*., 2000) or *in vitro* generated virus-like particles (VLP) of IBDV (Hu *et al*., 1999; Kibenge *et al*., 1999) have been found to be immunologically active. DNA vaccines have also been developed for IBDV (Fodors *et al*., 1999; Chang *et al*., 2001, 2003; Wang *et al*., 2003; Hulse and Romero, 2004; Kim *et al*., 2004); however, these vaccines have not yet been commercialized. Even though specific and sensitive diagnostic tools for IBDV infections and effective vaccines for prophylaxis are available, mutations in the IBDV genome resulting the emergence of antigenic variant strains in vaccinated flocks, as discussed earlier for vvIBDV, may constantly threaten the poultry industry. Furthermore, the identification of IBDV virulence markers will allow elucidation of the mechanisms of IBDV pathogenicity. It may be expected that continuous research efforts and the application of the techniques of molecular biology will provide inexpensive, effective and safe vaccines in the future.

HYDROPERICARDIUM SYNDROME

IBH-HPS is an important recently emerged immunosuppressive viral disease of poultry, particularly of 3 to 6-week-old broiler chicks, characterized by sudden onset, with high mortality ranging from 20% to 70%, typical hydropericardium and enlarged mottled and friable livers, with intranuclear inclusion bodies (INIBs) in the hepatocytes (Ahmad *et al*., 1989; Gowda and Satyanarayana, 1994). The disease is caused by FAV serotype 4, a non-enveloped, icosahedral virus belonging to the *adenovirus* C species of the *Adenovirus* genus of the *Adenoviridae* family (Balamurugan, *et al*., 2001; Ganesh *et al*., 2001). The first epidemic of HPS in broiler chicks was reported from Angara Goth near Karachi, Pakistan in late 1987 (Jaffery, 1988). The disease has subsequently been recorded in Iraq (Abdul-Aziz and Al-Attar, 1991), India (Gowda and Satyanarayana, 1994), Mexico, Ecuador, Peru, Chile (Voss *et al*., 1996), South and Central America (Shane, 1996), Slovakia (Jantosovic *et al*., 1991), Russia (Borisov *et al*., 1997) and Japan (Abe *et al*., 1998).

The course of the disease studied under natural conditions or following experimental oral inoculation ranged from 7 to 15 days (Akhtar, 1995). IBH is also seen in young broilers, but a high rate of mortality of 60–70% in Pakistan and of 10–60% in India is the only characteristic of HPS. The presence of aflatoxins in the feed at higher concentrations than 20 ppb is commonly associated with a large outbreaks of IBH causing heavy mortality

among 3- to 5-week-old broiler chicks, which displayed typical lesions of IBH in addition to hydropericardium (Singh *et al*., 1996). The mortality rate in various outbreaks on broiler farms in Pakistan ranged from 20% to 75% (Cheema *et al*., 1989), in Iraq from 10% to 30% (Abe *et al*., 1998), and in India from 30% to 80%, with an average of 61.62% (Kumar *et al*., 1997; Singh *et al*., 1997). The HPS agent can be isolated or propagated in primary cell cultures of chicken kidney (Khawaja *et al*., 1988) or chicken embryo liver (CEL) cells (Kataria *et al*., 1995, 1996, 1997a; Kumar *et al*., 2003). The virus can also be passaged or isolated in embryonated chicken eggs, in which it causes stunted growth, haemorrhages and death of the embryo (Naeem *et al*., 1995a; Jadhao, 1998). HPS is a contagious disease and is transmitted horizontally among broilers by mechanical means (Akhtar *et al*., 1992) and by contamination with infected faeces (Shafique and Shakoori, 1994). The HPS agent is highly pathogenic, spreading rapidly from flock to flock and farm to farm (Cowen, 1992). The bird-to-bird transmission of the virus in a flock occurs horizontally (Akhtar, 1995) by the oral–faecal route (Abdul-Aziz and Hasan, 1995). Toro and colleagues (2001) found that an association of FAV and CAV is necessary for the successful induction of the IBH-HPS in chicken when transmitted vertically.

The HPS agent seems to have a special affinity towards hepatic, endothelial and lymphatic cells. The incubation period varies from 5 to 18 days (Akhtar, 1992). There are reports showing the simultaneous presence of IBD and CIA viruses in areas where HPS occurs frequently (Shane and Jaffery, 1997). IBD and CIA are known for their immunosuppressive effects (Todd, 2000; Shivachandra *et al*., 2003) and FAVs require impairment of the immune response to produce their pathogenic potential (Monreal, 1996). However, Deepak and colleagues (1998) studied the putative immunosuppressive effects of FAV-4 isolated from outbreaks of HPS in day-old chicks and showed that FAV-4 caused immunosuppression by damaging lymphoid tissues. They observed that the HI antibody titres as well as the CMI response of chicks vaccinated only with NDV F strain at day one were significantly higher ($p = 0.01$) than those of chicks infected with FAV-4 and vaccinated with NDV F strain. They also observed significant reduction in the ratios of bursa and spleen weight to body weight in infected chicks compared to those in control chicks. The HPS agent also has a predilection for lymphoid tissues, which can result in immunosuppression (Naeem *et al*., 1995a; Deepak, 1998). Therefore, the presence of IBD and CIA viruses may predispose to HPS, or HPS may predispose to other viral infections. The role of IBD in precipitating HPS in layer flocks has been well documented by Shukla and colleagues (1997). Studies on the pathogenicity of FAV isolates have also suggested that there is a synergism with CIA or other viruses or that prior immunosuppression is necessary to produce IBH-HPS in chickens (Toro *et al*., 2000; Deepak, 1998). The susceptibility of chickens to oral infection with FAV, resulting in IBH-HPS, varies throughout the course of CIA infection (Toro *et al*., 2000). Nakamura and colleagues (2003) reported that the pathogenic characteristics of IBH strains and HPS strains in chickens were essentially the same. Deepak (1998) also observed, a CMI response shown by the lymphocyte transformation assay from 7 days following vaccination with an inactivated cell culture vaccine in 2-week-old broilers. However, further work is needed to determine the role of CMI in affording protection against HPS. The PARC-1 isolate of HPS caused immunosuppression, resulting in a reduced serological response to Newcastle disease vaccination compared to that in controls (Naeem *et al*., 1995a).

In natural outbreaks of HPS, the affected birds may not exhibit any signs (Jaffery, 1988) other than a heavy mortality, up to 75%, of sudden onset in well-grown healthy broiler flocks 3 to 5 weeks of age (Voss *et al*., 1996; Asrani *et al*., 1997). However, in the terminal stages, the individual birds may become dull and depressed, huddle in corners and have ruffled feathers, showing a characteristic posture, with their chest and beak resting on the ground and with closed eyelids (Asrani *et al*., 1997). The predominant and most consistent gross lesion is hydropericardium (Gowda and Satyanarayana, 1994), characterized by the accumulation of clear or amber green-coloured, watery or jelly-like fluid in the pericardial sac (Asrani *et al*., 1997; Kumar *et al*., 1997). Other changes observed were a discoloured, pale yellow, swollen, friable and mottled liver with large areas of focal necrotic patches and petechial and ecchymotic haemorrhages; oedematous and congested lungs; and pale yellow, swollen and friable kidneys containing deposits of urates in the tubules and ureters (Kumar *et al*., 1997; Nakamura *et al*., 1999). The liver showed histological changes, such as small multifocal areas of coagulative necrosis, mononuclear cell infiltration and the presence of basophilic INIBs in the hepatocytes surrounded by a clear halo or filling the entire enlarged nucleus (Kumar *et al*., 1997; Nakamura *et al*., 1999). These changes were confirmed by transmission electron-microscopic observation of the hepatocytes (Chandra *et al*., 1997). Other changes observed were lymphocytolysis and cyst formation in the bursa of Fabricius, thymus and spleen (Gowda and Satyanarayana, 1994; Asrani *et al*., 1997), which leads to depletion of lymphocytes in the medullae of the follicles in the bursa (Abdul-Aziz and Hasan, 1995; Kumar *et al*., 1997). Deepak (1998) also observed similar histopathological changes in various organs. Microscopically there was severe depletion of lymphocytes in the medullae of the follicles in the bursa (Figure 3), thymus and spleen (Deepak *et al*., 1998). Severe anaemia and alterations in the heterophil and lymphocyte counts due to infection and the lymphopenia may be due to lymphocytolysis, as reported by Gowda and Satyanarayana (1994) and Abdul-Aziz and Hasan (1995).

The sudden occurrence of high mortality among broiler chicks 3–6 weeks of age with hydropericardium and the demonstration of basophilic INIBs in hepatocytes are pathognomonic. Diagnosis of IBH-HPS infection has been made on the basis of gross lesions, histopathological lesions, particularly INIBs in hepatocytes (Gowda and Satyanarayana 1994; Kumar *et al*., 1997), demonstration of adenovirus particles in the nucleus of infected liver cells by transmission electron microscopy (Cheema *et al*., 1989; Chandra *et al*., 1997; Ganesh *et al*., 2001) or isolation of virus either in cell culture or in embryonated eggs (Kataria *et al*., 1996, 1997a) and confirmation by VNT using serotype specific sera (Jadhao *et al*., 1997). Immunodiagnosis can be made by various serological tests; the indirect haemagglutination test (Rahman *et al*., 1989), AGPT (Kumar *et al*., 1997) counter-immunoelectrophoresis (Oberoi *et al*., 1996; Kumar *et al*., 1997), the fluorescent antibody technique (Deepak, 1998; Balamurugan, 1999) and various modifications of ELISA (Oberoi *et al*., 1996; Balamurugan *et al*., 1999, 2001), the dot immunobinding assay (Naeem *et al*., 1995a; Rabbani *et al*., 1998) and molecular techniques such as polymerase chain reaction (Toro *et al*., 1999; Ganesh *et al*., 2002; Dahiya *et al*., 2002) are considered specific and reliable diagnostic methods.

Epidemiological safeguards associated with the development and spread of HPS (Akhtar *et al*., 1992), such as proper disinfection of premises and equipment, restricted entry of

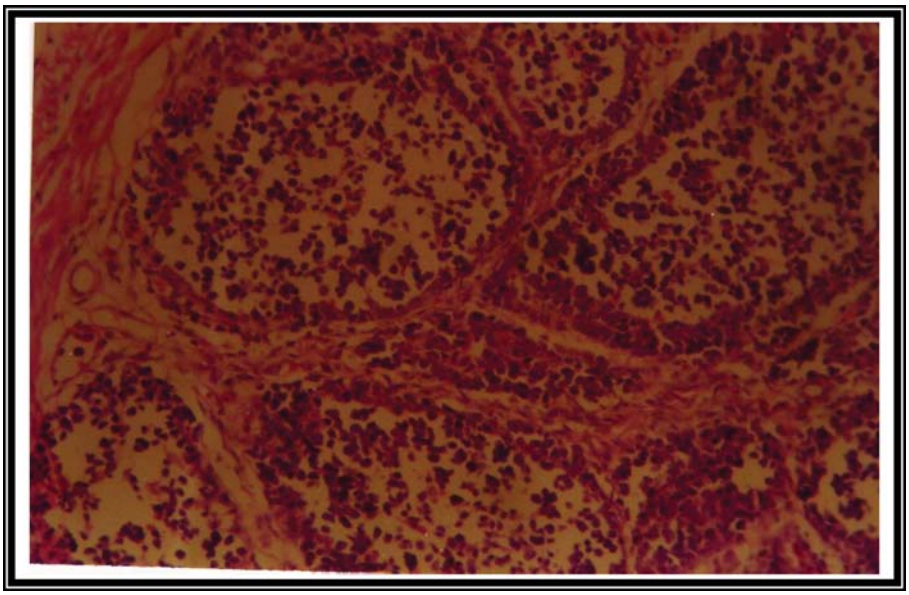

Figure 3. Broiler chicken infected with FAV-4 at 2 weeks of age. Bursa of Fabricius showing depletion of lymphocytes in the follicles at 10 DPI (H&E, ×400)

visitors and vaccination crews, ventilation and proper lighting in the poultry houses, play a significant role in prevention of the disease (Balamurugan and Kataria, 2004). The disease has been brought under control by formalin-inactivated vaccine prepared from infected liver homogenate or by oil-emulsified inactivated cell culture (Shane, 1996; Kumar *et al*., 1997). A dose of 0.25 ml/bird at 10–15 days of age provided 100% protection (Kumar *et al*., 1997). In India, a killed, oil-emulsified vaccine was prepared using HPS virus (FAV-4) in cell culture. Vaccination of 3-week-old chicks with 0.5 ml doses of vaccine ($10^{5.5}$ $TCID_{50}$/0.1 ml) provided 100% protection against challenge with HPS virus at 1, 2, 3, 4 or 6 weeks post vaccination (Kataria *et al*., 1997b). Owing to the contagious nature of the disease, the development of a suitable vaccine in SPF chicken and cell culture systems seems to be the best answer apart from strict biosecurity and high standards of hygiene and management (Balamurugan and Kataria, 2004). Inactivated chicken liver cell culture and embryonated egg-propagated vaccine used subcutaneously at $10^{3.5}$ LD_{50}/dose/bird provided protection against challenge with 1 ml of a 20% liver homogenate at a biological titre of 2×10^5 LD_{50}/0.5 ml (Naeem *et al*., 1995a,b). Toro and colleagues (2002) reported that effective protection of the progeny of chickens against IBH-HPS could be achieved by dual vaccination of breeders with FAV-4 and CAV. The vaccines are effective in the face of natural outbreaks or experimental challenge and significantly reduce the mortality. However, the development of a safe vaccine that will transmit a strong passive immunity, and could protect broiler chicks throughout the whole growing period may be established in the future.

CONCLUDING REMARKS

Immunosuppressive diseases have historically cost the poultry industry in terms of increased mortality and in performance factors during rearing, in addition to a negative impact on the ability of the poultry industry to process chickens owing to associated health problems. Strategies for controlling immunosuppressive diseases are not consistent between poultry companies. However, based on research and field observations, broiler industries are refining their strategies for controlling immunosuppression, which are largely based on the vaccination programmes for breeders and their progeny, and good management practices to minimize stress. There is a strong likelihood that these viral infections will be recognized in additional avian species. Should this prove to be the case, poultry veterinarians and avian disease experts will be challenged with new and prevailing viral infections or diseases in general, and immunosuppressive viral diseases in particular. As these agents are highly pathogenic, they spread rapidly among birds horizontally either by the oral–faecal route or by mechanical means, which leads to potential spread to other geographical area. Nevertheless, there is much ongoing research in this field and it is expected that many exciting developments will arise in the next decade. Recombinant DNA-based methodologies will have major roles in both the diagnosis of viral infections and the development of vaccines for the control of these diseases.

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