

Infectious Bursal Disease and Hemorrhagic Enteritis¹

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ABSTRACT Infectious bursal disease (IBD) of chickens and hemorrhagic enteritis (HE) of turkeys are caused by infectious bursal disease virus (IBDV) and hemorrhagic enteritis virus (HEV), respectively. Both diseases have common features, including an acute stage followed by immunosuppression, resulting in lowered resistance to a variety of infectious agents and poor response to commonly used vaccines.

The IBDV and HEV infections are widespread in commercial chicken and turkey flocks, respectively. The acute stage of the disease, the immunosuppression that follows, and the widespread distribution of both dis-

eases, are major factors contributing to the economic significance of both diseases. The mechanism of immunosuppression for both infections has similarities, both affect lymphocytes and macrophages, and both are lymphocidal.

In this report, an overview of both diseases with emphasis on some of the recent findings will be presented. There has been greater research activity on IBD than on HE, reflecting the relative economic importance of the species affected and the recent changes in the antigenic make up and pathogenicity of the IBDV.

(*Key words:* Infectious Bursal Disease, Hemorrhagic Enteritis Disease, Immunosuppression, lymphocytes, macrophages)

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INFECTIOUS BURSAL DISEASE

History

Infectious bursal disease (IBD) was described in 1962 by Cosgrove (Cosgrove, 1962) and the first outbreaks occurred around Gumboro, Delaware, hence the name Gumboro disease, which was used extensively in the past. Winterfield (Winterfield *et al.*, 1962) isolated the causative agent of the disease, infectious bursal disease virus (IBDV). The immunosuppressive nature of IBDV was first reported by Allan *et al.* (1972). The discovery of antigenic diversity among IBDV and identification of a serotype 2 in 1980 by McFerran (McFerran *et al.*, 1980), the recognition of major antigenic variants in 1984 (Saif, 1984), and the emergence of highly pathogenic strains of the virus in the old world are important historical events (Chettle *et al.*, 1989).

Etiology

The virus, IBDV, is a member of the *Birnaviridae* family, whose genome is made of two segments of double-

stranded RNA. The virus has five proteins recognized as VP1 to 5. The small segment B of the genome encodes for VP1 and the large segment A encodes for VP2, 3, 4, and 5. The VP2 and VP3 are the major proteins constituting 51 and 40% respectively of the total proteins and contain the major neutralizing epitopes. The VP2 has the serotype specific epitope and VP3 has a group specific antigen.

Epidemiology

The IBDV virus has a worldwide distribution. It is resistant to a variety of disinfectants and is environmentally very stable, which accounts for its persistence in poultry houses. There is no evidence of egg transmission of the virus and no carrier state has been detected in chickens. The lesser meal worm is recognized as a carrier and the virus has been isolated from mosquitos and evidence of infection in rats has been reported but there is no indication that either species is a reservoir for the virus.

Serologic evidence of natural infection with the virus has been reported in chickens, turkeys, ducks, guinea fowl, and ostriches. Nonetheless, the disease is recognized in chickens only, although microscopic lesions were

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Abbreviation Key: AGP = agar gel precipitation; BF = bursa of Fabricius; FA = Fluorescence antibody; HE = hemorrhagic enteritis; HEV = hemorrhagic enteritis virus; IBD = Infectious bursal disease; IBDV = Infectious bursal disease virus; PCR = polymerase chain reaction; RE = restriction enzyme; RT = reverse transcriptase; VN = virus neutralization.

reported in association with the infection in some of the other avian species. Disease in chickens is caused by Serotype 1 only, but they are susceptible to infection with Serotype 2 viruses. Turkeys are also susceptible to both serotypes of the virus, but disease is not reported.

Clinical Signs

The IBD has a sudden onset with a short incubation period (2 to 3 d). Viral antigen is detected in the bursa of Fabricius (BF) within 12 h after oral inoculation. There is usually 100% morbidity, but the mortality varies depending on the virus strain. The clinical signs are not specific and include: diarrhea, anorexia, depression, and ruffled feathers, the severity of clinical signs is dependent on the virus strain.

Lesions

Changes in lymphoid organs are typical of the disease. The BF, which is the main target of the virus, undergoes major changes beginning at 3 d postinfection (PI) when it increases in size reaching twice the normal size by 4 d PI followed by atrophy, reaching one third of its original weight by 8 d PI. The increase in size is accompanied by a red coloration. The highly virulent strains of the virus cause a decrease in thymic weight and severe lesions in other lymphoid tissues. Lymphocyte necrosis is the most common histologic lesion and is accompanied by edema, hyperemia, and accumulation of heterophils. Cystic cavities replace lymphocytes in follicles and later there is some regeneration of lymphocytes. Other lesions include dehydration and hemorrhages in the leg, thigh, and breast muscles.

Diagnosis

Clinical signs alone are not sufficient to make a diagnosis, but when combined with gross lesions, it is possible to arrive at a preliminary diagnosis.

Virus Isolation. Confirmation of diagnosis should be accomplished by virus isolation or detection of viral antigens in tissues (BF) from suspect cases. Isolation is commonly done in embryonating chicken eggs inoculated via the chorioallantoic membrane route. A variety of primary and established cell lines have been used for isolation and propagation of the virus. Once the virus is isolated, it could be identified by reacting it with a known anti-IBDV serum using any of a number of antigen-antibody tests such as virus neutralization (VN), fluorescence antibody (FA) test, ELISA, or agar gel precipitation (AGP).

The viral antigens can be detected in tissues by a variety of tests including FA, antigen-capture ELISA, AGP, nucleic acid probes and polymerase chain reaction (PCR) and its derivatives.

Serology. Acute and convalescent sera can be tested by VN, ELISA, or AGP tests. With the exception of the VN

test, all the commonly used test procedures for viral antigens or antibodies detect group specific antigens without differentiating serotypes. The lack of serotype specificity should be considered when results of antibody and virus assays are interpreted.

Virus Types

There are two recognized serotypes of IBDV, designated 1 and 2. Viruses of both serotypes naturally infect chickens and turkeys, but the disease is recognized only in chickens and only Serotype 1 viruses are pathogenic. A variety of antigenic types are detected within serotypes as indicated by the VN test.

In the USA, antigenic variants of Serotype 1 were first recognized in 1984 (Saif, 1984) and these variants induced disease in chickens carrying antibodies to commonly used Serotype 1 vaccine strains (classic). The antigenic diversity of strains within serotypes was demonstrated by the VN test (Jackwood and Saif, 1987; McNulty and Saif, 1988) and the relevance of determining antigenic differences to protection was illustrated in cross-protection studies (Ismail and Saif, 1991), which indicated the usefulness of the VN test in predicting the immunogenicity of virus strains.

Studies in our laboratory using the VN test have indicated that there are currently two major antigenic types of IBDV circulating in the US. These are Serotype 1 classic and variants, and a variety of subtypes of both the antigenic types are commonly encountered. These two antigenic types have also been shown by cross-protection studies to be the major immunogenic types of the virus.

Infection by viruses of Serotype 2 do not provide any protection against challenge by viruses of Serotype 1. Classic Serotype 1 viruses will provide partial protection against variant Serotype 1 viruses. The magnitude of that partial protection is dependent on the strain and titer of the challenge and vaccine viruses. Variant viruses provide complete protection against variants and classic viruses (Ismail and Saif, 1991). Classic and variant strains of the virus share minor antigen(s) that elicits some protection.

Highly virulent strains of IBDV emerged in the late eighties and were first recognized in Europe (Chettle *et al.*, 1989) and spread throughout the Old World causing substantial economic losses. The antigenic nature of these strains is not completely clear, but they are recognized as Serotype 1 viruses.

Considerations in Virus Typing

With the advent of the highly sensitive molecular techniques, such as the reverse transcriptase (RT)/PCR-restriction enzyme (RE) and restriction fragment length polymorphism, it became possible to differentiate virus strains and utilizing such information in studying the molecular epidemiology of the virus. These tests detect major and minor changes in the makeup of the virus. Unfortunately, there has been a tendency to refer to some

IBDV showing minor sequence differences as variants that has resulted in terminology confusion with strains previously termed antigenic variants based on antigenicity studies. It should be emphasized that differences shown by molecular tests are not necessarily a result of antigenic differences. Most of the sequence differences do not correlate with antigenicity or immunogenicity of the virus.

Immunosuppression

Early description of IBD indicated involvement of lymphoid tissues (Cheville, 1967). Later it was shown that lymphoid cells and macrophages in the intestine are infected first and these cells carry the virus to the BF and other sites (Muller *et al.*, 1979). The IBDV has a predilection for actively proliferating cells "immature or precursor" lymphocytes (Muller, 1986). The BF is the primary site of virus replication, but lesions are also detected in the spleen, thymus, cecal tonsils, and gland of Harder.

The humoral immune response is clearly depressed, but a transient depression occurs in the cellular immune response (Confer *et al.*, 1981). The peripheral blood B cells are usually decreased, but the T cells are not changed (Harkness *et al.*, 1975; Sivanandan and Maheswaran, 1980). In established cell lines, it was shown that the virus replicates in B cells, but not in T cells (Hirai and Calnek, 1979). Infections at an early age results in the greatest level of immunosuppression evident as complete lack of IgG and the presence of only a monomeric IgM (Ivanyi, 1975; Ivanyi and Morris, 1976).

There are numerous reports in the literature on the detrimental effect of IBD on resistance of chickens to a multitude of bacterial, viral, and parasitic diseases. There are also several reports on the depressed response to vaccines following the infection.

Prevention

Vaccination is the most practical procedure for prevention of the disease. A variety of attenuated vaccines made of classic and variant viruses, and propagated in embryos or tissue culture are commercially available. Live viruses vary in the degree of attenuation and are recognized as mild, intermediate, or hot. Also available are inactivated vaccines made of classic and variant viruses and propagated in either chicken embryos, tissue culture or BF. The inactivated vaccines are commonly used for secondary vaccination in breeders. As indicated earlier, vaccines made of variant viruses are probably sufficient to elicit protection against classic and variant virus challenge.

Vaccination of breeder hens is commonly practiced to ensure the passage of high levels of maternal antibodies to the chicks. Vaccination of chicks at hatch with live vaccines is a controversial practice. Recent unpublished studies from our laboratory cast doubt on the usefulness of this practice.

In an early study (Wyeth and Cullen, 1979), inactivated vaccine made from bursal tissues of infected chickens was more potent than vaccine made from tissue culture. Conversely, in our studies it was shown that inactivated vaccines derived from tissue culture or BF that contained similar antigen masses, elicited similar titers of virus neutralizing antibodies (Hassan and Saif, 1996).

Vaccination regimens vary, but the principles are similar. Briefly, all regimes strive to provide passive protection to the hatching chick, followed by active immunization and a series of boosts in layers and breeder flocks. Monitoring the response to vaccination is indeed a useful practice.

Research Needs

The following are thoughts on some of the current research needs: delineation of the molecular basis of antigenicity, immunogenicity, pathogenicity, and immunosuppression; persistence of immunosuppression; the value of early vaccination; development of practical *in vitro* tests to differentiate virus types; and correlation between virus types detected by different tests and the antigenicity and the immunogenicity of these viruses.

HEMORRHAGIC ENTERITIS

The proceedings of this symposium contain an article by James S. Guy on virus infections of the gastrointestinal tract with a description of various aspects of hemorrhagic enteritis (HE) and the causative hemorrhagic enteritis virus (HEV). Hence, only a brief description of immunosuppression caused by HEV will be presented here.

Birds affected with HE develop lesions in the gastrointestinal tract in conjunction with characteristic gross lesions in the spleen that usually include enlargement and mottled appearance. The spleen is usually hyperplastic with lymphocyte necrosis as a common lesion. Lymphocyte depletion is also detected in the thymus and BF.

The IBDV affects lymphocytes and macrophages. Depletion of IgM bearing cells in the spleen and blood has been reported during the acute phase of the disease (Suresh and Sharma, 1995). The resulting B cell depletion impairs viral replication (Fadly and Nazerian, 1982; Fitzgerald and Reed, 1991; Suresh and Sharma, 1995). B cells and macrophages may serve as the primary virus targets (Suresh and Sharma, 1995). The degree of immunosuppression is dependent on the strain of the virus (Larsen *et al.*, 1985; Pierson, 1993).

Infection with HEV singly or in combination with other agents was shown to predispose birds to infection with *Escherichia coli* in natural and experimental settings (Larsen *et al.*, 1985; Newberry *et al.*, 1993; Kwaga *et al.*, 1994; Pierson *et al.*, 1996a, 1996b). It has been suspected that HEV infection depresses the response to vaccines.

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