CEVA HANDBOOK
OF POULTRY DISEASES

1 GUMBORO DISEASE

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DEFINITION

Infectious Bursal Disease (IBD; Gumboro) is an acute, highly contagious viral disease affecting chickens, characterized by inflammation and subsequent atrophy of the bursa of Fabricius, various extents of nephrosonephritis, marked haemorrhagic diathesis and immunosuppression.

HISTORY AND SYNONYMS

In 1957, A.S. Cosgrove observed a syndrome which was later described as avian nephrosis in a broiler chicken farm in the Gumboro community, Delaware (USA) (Cosgrove, 1962).

The syndrome, rapidly baptised "Gumboro disease" became more and more prevalent in Delaware. Gumboro is characterized by a 10% morbidity rate and a death rate of between 1% and 10% in affected flocks (Cover, 1960; 1961). The prevailing opinion at the time, considering the macroscopic changes in kidneys, was that the syndrome was caused by the Gray strain of the variant infectious bronchitis virus. In the early 1960s, the Gumboro disease was established in many other American states (Lasher & Davis, 1997). Winterfield & Hitchner (1962) managed to isolate two viruses – one from kidneys and another from the bursa of Fabricius of chickens displaying symptoms of the new disease. The virus isolated in the bursa of Fabricius has been linked to the effects observed on the birds' organs. In his prevention and control report, Edgar (1966) was the first to describe the syndrome as "infectious bursal disease" instead of "Gumboro disease". IBD rapidly spread beyond the USA and reached other regions in the world.

The appearance of the disease in a variant or highly virulent form in Europe during the second half of the 1980s entailed substantial economical losses (Van den Berg, 2000). The same author reports that until 1987 viral strains were not strongly virulent and resulted in a death rate of below 2%, allowing for satisfactory control of IBD by vaccination. But by 1987, post-vaccination occurrences had been observed in different parts of the world. First, outbreaks of acute IBD among adult broiler chickens were reported in Europe (Van den Berg, 2000). There after it became a pressing necessity to catalogue the various strains in circulation and adapt vaccination schedules to this new epidemiological reality.

In 1995, acute clinical cases were reported in 80% of countries. The 1990s saw the emergence of very virulent strains of IBDV (vvIBDV). At present, these strains are prevalent worldwide, however no cases have yet been reported in Australia or New Zealand (Eterradossi & Saif, 2008).

ETIOLOGY, CHARACTERISTICS AND NOMENCLATURE OF THE PATHOGEN

The infectious bursitis virus (IBDV) belongs to the family Birnaviridae, genus Avibirnavirus.

Other genera in the Birnaviridae family include Aquabirnavirus, a cause of necrotic pancreatitis in fish, and Entomobirnavirus, which affects insects. Before they were first differentiated in 1984, the members of the family Birnaviridae were considered part of the Reoviridae family (Zarkov, 2003).

This new family was defined on the basis of shared morphological and biological traits, and the family name is derived from its two RNA segments (bi = two) (Muller et al., 1979; McDonald, 1980).

Virions possess a double capsid with icosahedral symmetry, measuring 60 nm (Hirai & Shimakura, 1974).

The virus is composed of 5 proteins and RNA. Viral proteins are named VP1 ÷ VP5 respectively. Some of them are structural, and others are not. The VP3 protein is group-specific, whereas VP2 is type-specific and contains virus-neutralizing antigenic determinants (Saif, 1998).

The birnaviral genome consists of double-stranded RNA divided into 2 segments: A and B.

The virus is replicated in the cytoplasm of infected cells for about 18–22 hours. After activation of RNA-RNA polymerase, two mRNA are synthesized. The transcription and replication begin prior to capsid deproteinization and occur separately for each segment. The genomic segment A codes for the synthesis of a protein. It is then processed into pre-VP2 and VP3.

Pre-VP2 is subsequently cleaved to form VP2, which is assembled. Viral particles accumulate in the cytoplasm of infected cells and about half of them are released after cell lysis (Mandelli et al., 1967; Zarkov, 2003).

It is reported that VP5 and/or VP2 induce apoptosis in infected cells (Fernandez-Arias et al., 1997; Yao and Vakharia, 2001).

From antigenic point of view, two serotypes of IBDV are known, and only serotype 1 is pathogenic.

Clinical disease is observed only in chickens, although turkeys, guinea fowl, ducks and ostriches may also be infected. Serotype 2 is isolated from turkeys and geese, but it is not pathogenic. The virulence of different IBDV strains varies, from very

virulent (provoking acute disease) to limited virulence (resulting in asymptomatic infection or mild disease).

It was demonstrated that most vvIBDV strains were antigenically similar to viruses from the classic serotype 1 (Abdel-Alim & Saif, 2001). Further studies have however shown some extensive antigenic changes in an atypical vvIBDV isolate (Eterradossi et al., 2004).

At present, 3 antigenic types have been defined: classical/standard, variant serotype 1, and serotype 2 (Eterradossi & Saif, 2008).

Subtypes of the three antigenic types have also been identified. IBDV infections are characterized by immunosuppressive effects. The first reports were those of Allan et al. (1972) and Faragher et al. (1974). Infected birdsare unable to produce an adequate immune response when vaccinated against other diseases. In addition to the immune response suppression (antibody formation), chickens infected with IBDV at an early age become more susceptible to Marek's disease, chicken anaemia agent, infectious bronchitis, inclusion body hepatitis, gangrenous dermatitis etc. (Eterradossi & Saif, 2008).

Despite the immunosuppressive effects of IBDV infections with regard to a number of antigens, the immune response against IBDV is adequate (Skeeles et al., 1979).

IBDV is highly resistant and can be propagated in premises housing diseased chickens for up to 4 months. It remains viable for 1 hour at pH 2 but is completely inactivated at pH 12. The IBD virus is also highly thermostable. It is preserved for 3 weeks at 25°C, for 90 min at 37°C and for up to 3 years at –20°C. The virus of IBD is sensitive to quaternary ammonium salts, formalin, chloramine etc. (Girginov, 1984).

EPIDEMIOLOGY AND PATHOGENESIS

Nowadays, vvIBDV strains are prevalent in Europe, Asia, Africa and South America (Eterradossi and Saif, 2008). In Australia, the spread of classical and variant types of the virus is reported (Sapats & Ignjatovic, 2000). In natural conditions, chickens at 3 to 15 weeks of age are susceptible to IBD, but particularly those aged between 3 and 6 weeks.

In growing layers, IBD is generally encountered at a later age by reason of the longer half-life of maternal antibodies due to their slower growth rate. The expression of the disease at this age depends on the development of the bursa of Fabricius, which is the main target of the virus. Chickens under 3 weeks of age (protected by maternal antibodies) and over 16 weeks of age (involution of the bursa of Fabricius) do not manifest clinical signs of IBD. Chickens infected with IBDV after bursectomy also fail to develop clinical signs.

Although clinical signs of IBD are not observed in chickens younger than 3 weeks, a subclinical infection with immunosuppressive effects is possible, carrying significant economic consequences (Eterradossi & Saif, 2008).

IBDV primarily attacks the lymphocytes of the bursa of Fabricius, although the spleen, thymus and caecal lymphoid tonsils are also affected. Chickens possess two primary lymphoid organs: the thymus and the bursa of Fabricius. Attempts to investigate the pathogenesis of IBD have revealed that the first detected alterations occurred in the medullary zone of single bursal follicles as early as 36 hours after initial infection. By post infection days 3–4, all follicles are affected by severe degenerative necrobiotic and inflammatory changes. Such alterations are observed in the interfollicular connective tissue (Cheville, 1967). The depletion of lymphoid bursal cells after IBDV infection is due to apoptosis and necrosis. It has been observed that IBDV-induced immunosuppression can be at least partly attributed to apoptosis (Ojeda et al., 1997; Tanimura & Sharma, 1998; Nieper et al., 1999). The effect of immuno-suppression on cell-mediated immunity (CMI) has also been proven.

In the other lymphoid organs, the thymus and the spleen, moderate to severe necrobiotic lesions and hyperplasia of reticular components have been observed. In consequent studies of the pathogenesis, microscopic bursal lesions (haemorrhages, necrosis, polymorphonuclear leukocytes) were compared to Arthus reaction. The reaction is of the type of local immune damage and is due to antigen-antibody-complement complexes (Ivanyi & Morris, 1976).

The disease spreads through alimentary infection. Diseased chickens and chickens in the remission phase are the main source of infection. The virus is carried in the faeces, secretions and excreta of

infected birds. It is reported to keep its virulence in feed, water and litter obtained from premised 52 days after an outbreak (Benton et al., 1967). Due to the highly contagious nature of the virus, an outbreak of infection in one facility will result in rapid infection of the other facilities on the same farm. Thermostability and resistance to sanitizers contribute to IBDV's survival in the environment between outbreaks and to the stationary character of the disease.

Among IBDV vectors are some avian species, blood-feeding insects and mites. Snedeker et al. (1967) managed to induce IBD in susceptible chickens with a suspension derived from darkling beetle larvae (Alphitobiusdiaperinus).

Reservoir hosts and vector of IBDV include mosquitoes and rats (Howie & Thorsen, 1981; Okoye & Ushe, 1986). Humans may also transmit the infection via clothes, shoes and equipment. The epidemiological traits of vvIBDV strains are caused by a significant mutation of VP2 (Van den Berg, 2000).

CLINICAL SIGNS AND PATHOLOGY

The morbidity rate in affected flocks can be up to 100%. Death rates after infection with conventional IBDV strains generally vary between 1% and 10%, whereas vvIBDV infection can result in 20–30% fatalities.

The disease affects infected birds for 5–7 days, with peak mortality in the middle of this period. Recovery is rapid, but is always accompanied with stunted growth and thus substantial economic losses. The incubation period is short. Clinical signs appear 23 days after infection, almost simultaneously across the flock. Depression, anorexia, lying down, usually on the chest and abdomen with legs extended backward and ruffled feathers, especially in the head and neck region, are common signs *(Fig 1)*.

Another almost constant symptom is a grey-whitish watery diarrhoea with high urate contents, resulting in vent feather staining *(Fig 2)*.

Fig.1
Depression and ruffled feathers in the head and neck region.

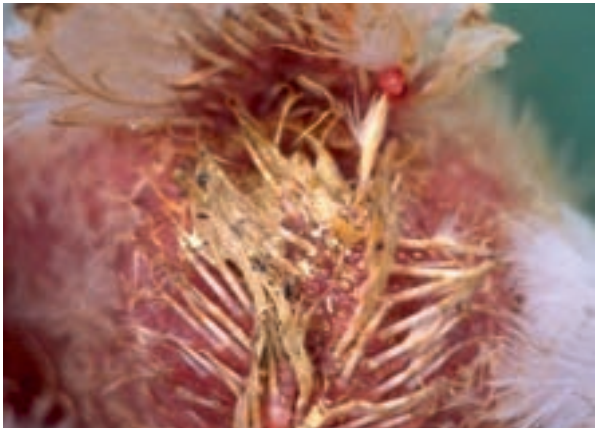

Fig.2
Feathers around the vent are stained by faeces containing plenty of urates.

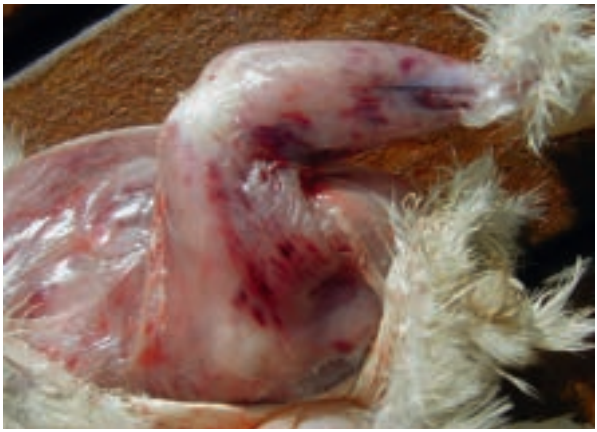

Fig.3

The dead bird carcasses are dehydrated. After skinning, typical intramuscular haemorrhages are observed in more than 80% of cases. Echimoses and linear haemorrhages are seen mainly in the thigh and drumstick muscles.

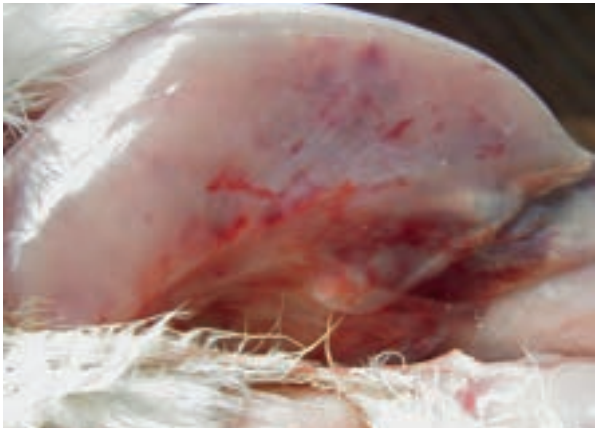

Fig.4

Linear haemorrhages in the pectoral muscles.

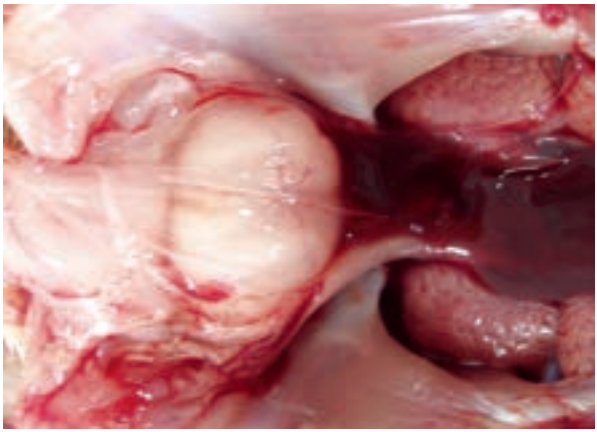

Fig.5

The lesions in the bursa of Fabricius are progressive. In the beginning, the bursa is enlarged, oedematous and covered with a gelatinous transudate.

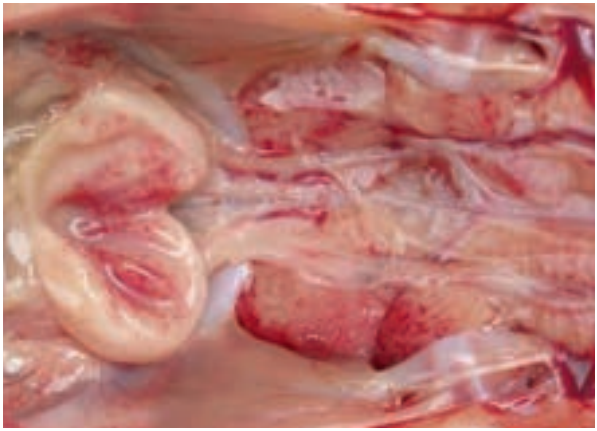

Fig.6

IBD begins as a serous bursitis.

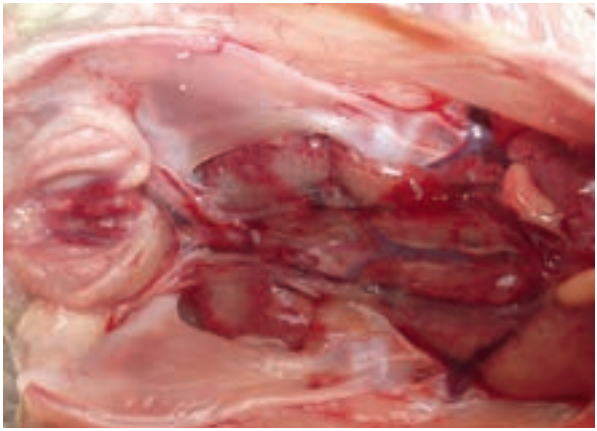

Fig.7
At a later stage, the inflammation of the bursa is serous and haemorrhagic.

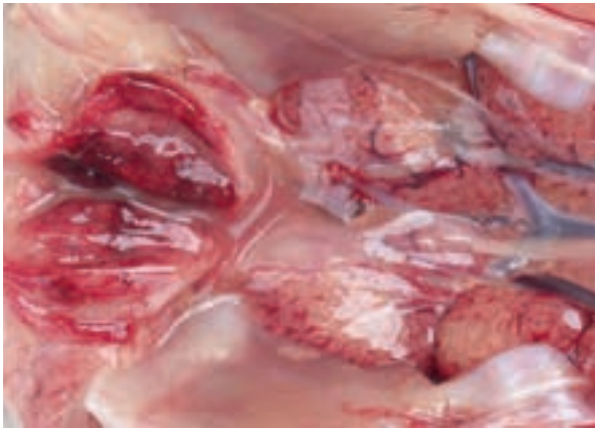

Fig.8
Haemorrhagic bursitis.

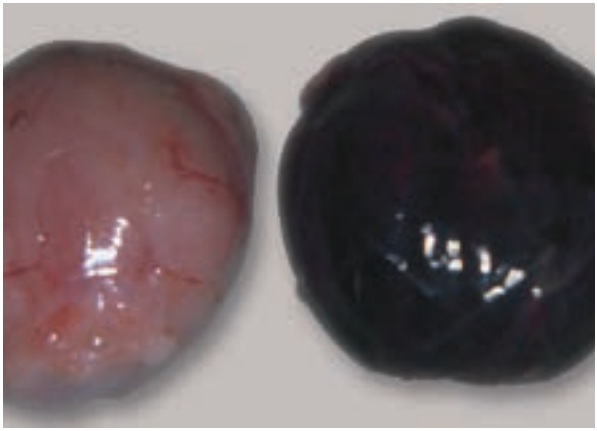

Fig.9

Left – serous; right – haemorrhagic bursitis.

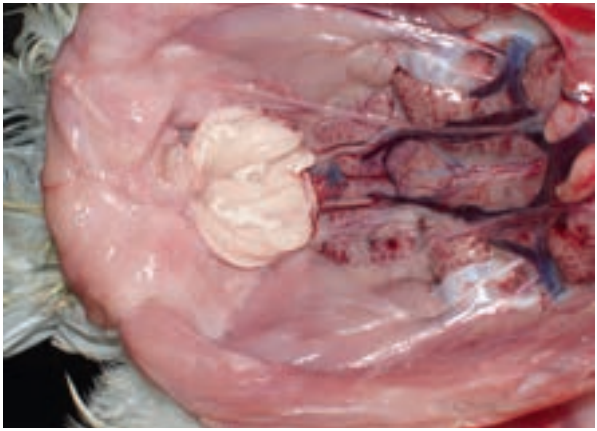

Fig.10

In some cases, the bursa is filled with coagulated fibrinous exudate that usually corresponds to the contours of the mucosal folds.

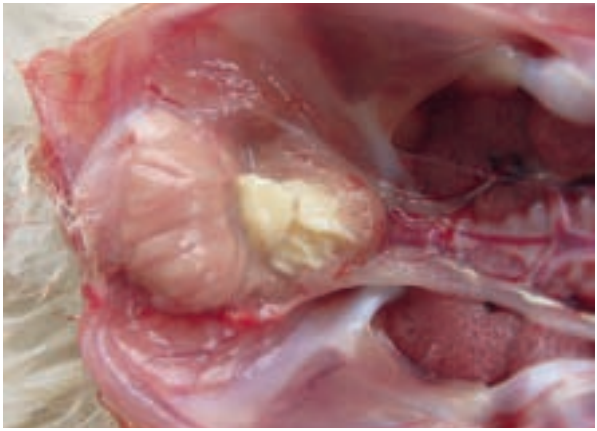

Fig.11
The bursa is completely filled with coagulated fibrinous caseous exudate.

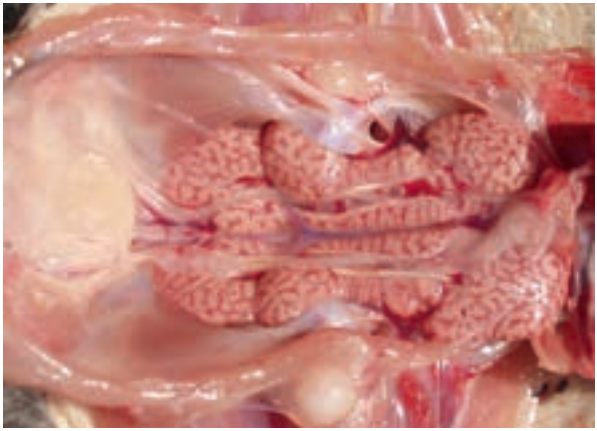

Fig.12
The kidneys are affected by a severe urate diathesis. The renal tubules are prominating due to overfilling with urates.

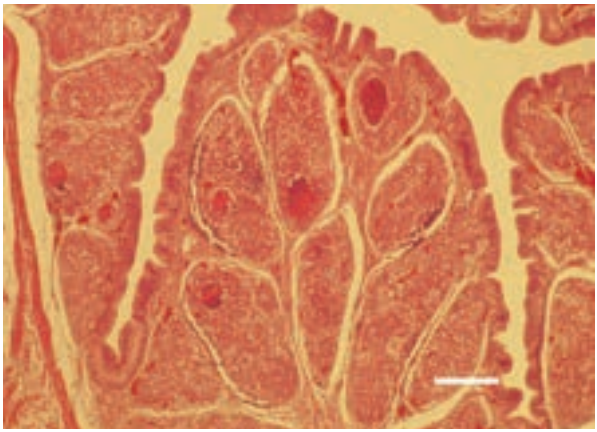

Fig.13

Marked interfollicular inflammatory oedema, haemorrhages and inflammatory necrotic lesions in the medullary zone of bursal follicles. H/E, Bar = 100 µm.

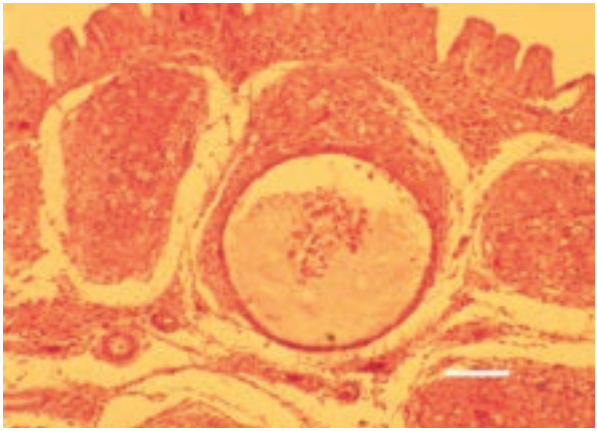

Fig.14

Sometimes, in the medullary zone of follicles, cystic cavities may form, containing exudate, inflammatory cells and detritus. H/E, Bar = 35 µm.

DIAGNOSIS

Diagnosing IBD is not difficult. The existing epidemiological data, clinical signs and gross lesions makes it possible to rapidly diagnose clinical IBD. Subclinical IBD is mostly diagnosed retrospectively, after gross or microscopic examination and detection of atrophied bursa of Fabricius. Infection with some of the variant IBDV strains may be detected only via a bursal histology or after isolation of the virus (Eterradossi & Saif, 2008).

When the disease is caused by vvIBDV, the signs are similar to those observed in the conventional type 1, but some epidemiological characteristics will differ. The acute phase is much more severe and generalized in the affected flock, and the age of susceptible birds is higher (older birds are also affected). A sharp peak followed by sharp decline in the death rate curve is observed (Van den Berg et al., 1991; Tsukamoto et al., 1992).

The severe lymphoid atrophy affects not only the bursa, but the thymus as well (Sharma et al., 1993).

Since it is acknowledged that the replication of the virus takes place in B lymphocytes, the most appropriate place to look is the bursa of Fabricius, where the viral concentration is the highest. For direct detection and differentiation of the different t IBDV strains (classical, variant and vvIBDV) from infected tissues, RT – PCR protocols are available (Kusk et al., 2005; Mickael & Jackwood, 2005).

Among serological assays, ELISA is most widely applied for detection of IBDV antibodies. To assess the effectiveness of the vaccination process, an antibody profile of the flock should be performed by assaying at least 20 serum samples. Sera for analysis should be collected from both one-day-old chickens and from the respective breeder flock.

DIFFERENTIAL DIAGNOSIS

In light of the haemorrhagic diathesis symptoms, IBD should be differentiated from inclusion body hepatitis (IBH), in which the localization and the type of intramuscular haemorrhages are indistinguishable. A distinction can, however, be made if there are IBH-specific lesions, particularly microscopic, in the liver and a lack of alterations in the bursa of Fabricius. Although rare, after detection of haemorrhages on the proventricular mucosa caused IBD, Newcastle disease and haemorrhagic syndrome should be considered as possibilities.

The nephrosis observed in almost all cases should be distinguished from the state provoked by some nephropathogenic strains of the infectious bronchitis virus. In this case, the bursal lesions and the lack of respiratory signs in IBD infection are taken into account.

PREVENTION AND CONTROL

Hygienic measures are not always sufficient because of the rapid spread and high resistance of IBDV in the environment. Furthermore, the cleansing of premises and even equipment in some farms between different batches of chickens is not thorough enough. In such circumstances, chickens are exposed to the virus from the earliest age. These conditions, as well as any previous history of the disease at a farm, should be taken into consideration when prevention programmes are developed.

All of which, coupled with the potential for economic losses caused by clinical or subclinical IBD, suggest that the key to disease prevention is vaccination. This implies the development, implementation and application of efficient vaccines. Young chicks are considered to be protected against the infection by the passive immunity from vaccination of the breeder flock with live and inactivated vaccines. A routine practice is to vaccinate broiler breeder flocks with an oil-emulsion vaccine soon before laying in order to induce a higher level of passive immunity in the offspring, protecting the chicks from immunosuppressive risk after birth (Box, 1989).

This method of prevention appeared to be generally satisfactory before the appearance of vvIBDVs (Van den Berg, 2000). The power of the maternal antibodies is crucial when drawing up the vaccination schedule. Serological monitoring is proposed in order to determine the optimal moment for vaccination (Van den Berg & Meulemans, 1991; Kouwenhoven & Van den Bos, 1994). That is why the implementation of tests allowing for a distinction between passive (antibody- positive, CMI-negative) and active immunity (antibody-positive, CMI-positive) immunity is essential (Lambrecht et al., 2000).

Live vaccines have the advantage of being excreted into the environment, where they compete with field strains of the virus.

Depending on the virulence and antigenic diversity, live vaccines are determined as mild, mild intermediate, intermediate, intermediate plus or hot (Eterradossi & Saif, 2008). Most intermediate vaccines are not capable of breaking through the maternal antibody levels.

Intermediate plus vaccines are today the best compromise since their invasiveness helps to break through a higher residual level of MDA, while retaining an acceptable safety.

Oil emulsion adjuvant vaccines are used to enhance and prolonging the immunity of breeder flocks. Their efficacy is higher after initial vaccination of chickens with a live vaccine. Such vaccines may contain standard and variant IBDV strains.

While new vaccines have been developed, focusing on genetic viral strain attenuation, interference with passive immunity is still encountered. As a result, recombinant viral vaccines expressing the VP2 protein of IBDV have been proposed, as they are less sensitive to neutralization by maternal antibodies fighting IBDV.

Examples are the Marek's disease virus (Tsukamoto et al., 1999), the fowl pox virus (Bayliss et al., 1991; Heyne & Boyle, 1993), the turkey herpes virus (HVT) (Darteil et al., 1995) and avian adenovirus (Sheppard et al., 1998). Jonson et al. (1997) demonstrated in ovo application of HVT vaccines. An in ovo virus-antibody complex vaccine has also been proposed. It is based upon the use of a specific hyperimmune neutralizing antiserum or virusneutralization factor. The vaccinal virus is not neutralized and at the same time, the pathological effects of the vaccine are delayed.

This allows for more efficient vaccination of young chickens with passive immunity (Haddad et al., 1997). Expectations regarding the potential benefit of this technology with regard to future IBDV control are high (Jeurissen, et al., 1998).

REFERENCES

- Abdel-Alim G.A. & Y.M. Saif, 2001. Immunogenicity and antigenicity of very virulent strains of infectious bursal disease viruses. Avian Dis., 45, 92-101.
- Allan W.H., J.T. Faragher & G.A. Cullen, 1972. Immunosuppression by the infectious bursal agent in chickens immunized against Newcastle disease. Vet. Rec., 90, 511-512.
- Bayliss C. D., R. W. Peters, J. K. A. Cook, R. L. Reece, K. Howes, M.M. Binns & M.E.G. Boursnell, 1991. A recombinant fowlpox virus that expresses the VP2 antigen of infectious bursal disease virus induces protection against mortality caused by the virus. Archives of Virology, 120, 193-205.
- Benton W.J., M.S. Cover, J.K. Rosenberger & R.S. Lake, 1967. Physicochemical properties of the infectious bursal agent (IBA). Avian Dis., 11, 438-445.
- Bolis D.A., F.J. Paganini, V.A. Simon, M.F. Zuanaze, Neto H. Scanavini, A.R.A. Correa & N.M.K. Ito, 2003. Gumboro Disease: Evaluation of Serological and Anatomopathological Responses in Vaccinated Broiler Chickens Challenged with Very Virulent Virus Strain, Brazilian Journal of Poultry Science /Revista Brasileira de Ciencia Avicola, May-Aug. 2003, v.5, n.2, 137-146.
- Box P., 1989. High maternal antibodies help chickens beat virulent virus. Wold Poultry, 53, 17-19.
- Cheville N.F., 1967. Studies on the pathogenesis of Gumboro disease in the bursa of Fabricius, spleen, and thymus of the chicken. Am.J. Pathol., 51, 4, 527-551.
- Cosgrove A.S., 1962. An apparently new disease of chickens-avian nephrosis. Avian Dis., 6, 385-389.
- Cover M.S., 1960. Poultry Pathology Letter. University of Delaware, 29, 1.
- Cover M.S., 1961. Poultry Pathology Letter. University of Delaware, 32.
- Darteil R., M. Bublot, E. Laplace, J.F. Bouquet, J.C. Audonnet & M. Riviere, 1995. Herpesvirus of turkey recombinant viruses expressing infectious bursal disease virus (IBDV) VP2 immunogen induce protection against an IBDV virulent challenge in chickens. Virology, 211, 481-490.
- Girginov, G., 1984. Bursitis infectiosa, in: Arsov, R., E. Iovtchev, G. Girginov & T. Iliev, Private epizootology and zooprophylaxis, Zemizdat, Sofia, pp. 422-425.
- Edgar S.A., 1966. Infectious bursal disease/Gumboro disease/ prevention and control. 10th Annual Poultry Health and Management Short Course, Clemcon, South Carolina, 93-98, 1966.
- Eterradossi N. & Y.M.Saif, 2008. Infectious Bursal Disease. in: Saif, Y.M., Fadly, A.M., Glisson, J.R., McDougald, L.R., Nolan, L.K., Swayne, D.E. (Eds), Diseases of poultry, 12th ed, Blackwell publishing, Ames, Iowa, USA, 185-208.
- Eterradossi N., C. Gauthier, I. Reda, S. Comte, G. Rivallan, D. Toquin, C. de Boisseson, J. Lamande, V. Jestin, Y. Morin, C. Cazaban & P.M. Borne, 2004. Extensive antigenic changes in anatypical isolate of very virulent infectious bursal disease virus and experimental clinic control of this virus an antigenically classical live vaccine. Avian Patol., 33, 423-431.
- Faragher, J.T., W.H. Allan & C.J. Wyeth, 1974. Immunosuppressive effect of infectious bursal agent on vaccination against Newcastle disease. Vet. Rec., 95, 385-388.
- Fernandez-Arias A., S. Martinez & J.F. Rodriguez, 1997. The major antigenic protein of infectious bursal disease virus, VP2, is an apoptotic inducer. J.Virol., 71, 8014-8018.
- Gagic M., C.St. Hill & J.M. Sharma, 1999. In ovo vaccination of specific-pathogen-free chickens with vaccines containing multiple antigens. Avian. Dis., 43, 293-301.
- Guittet M., H. Le Coq, J.P. Picault, N. Eterradossi & G. Bennejean, 1992. Safety of infectious bursal disease vaccines: assessment of an acceptability threshold. Developments of Biological Standards, 79, 147-152.
- Haddad E.E., C.E. Whitfill, A.P. Avakian, C.A. Ricks, P.D. Andrews, J.A. Thoma & P.S. Wakenell, 1997. Efficacy of a novel infectious bursal disease virus immune complex vaccine in broiler chickens. Avian Diseases, 41, 882-889.
- Hirai K. & S. Shimakura, 1974. Structure of infectious bursal disease virus. J. Virol., 14, 957-964.
- Hitchner S., 1970. Infectivity of infectious bursal disease virus for embryonating eggs. Poult. Sci., 49, 511-516.
- Howie R.I. & J. Thorsen, 1981. Identification of a strain of infectious bursal disease virus isolated from mosquitoes. Can. J. Comp.Med., 45, 315-320.
- Ivanyi J. & R. Morris, 1976. Immunodeficiency in the chicken. IV. An immunological study of infectious bursal disease. Clin. Exp. Immunol., 23, 154-165.
- Jeurissen S.H., E.M. Janse, P.R. Lehrbach, E.E. Haddad, A. Avakian & C.E. Whitfill, 1998. The working mechanism of an immune complex vaccine that protects chickens against infectious bursal disease. Immunology, 95, 494-500.
- Kouwenhoven, B & J. Van den Bos, 1994. Control of very virulent Infectious Bursal Disease (Gumboro Disease) in the Netherlands with more virulent vaccines. Proceedings of the International symposium on infectious bursal disease and chicken infectious anaemia (pp. 262-271). Rauischholzhausen, Germany.
- Kusk M., S. Kabell, P.H. Jorgensen & K.J. Handberg, 2005. Differentiation of five strains of infectious bursal disease virus: development of a strain-specific multiplex PCR. Vet. Microbiol., 109, 159-167.

- Lambrecht B., M. Gonze, G. Meulemans & T.P. Van den Berg, 2000. Production of antibodies against chicken interferon-y: demonstration of neutralizing activity and development of a quantitative ELISA. Veterinary Immunology and Immunopathology, 74, 137-144.
- Lasher H.N., V.S. Davis, 1997. History of Infectious Bursal Disease in the U.S.A.- The First Two Decades. Avian Diseases, 41, 11-19.
- MacDonald R.D., 1980. Immunofluorescent detection of double-stranded RNA in cells infected with reovirus, infectious pancreatic necrosis virus, and infectious bursal disease virus. Can. J. Microbiol., 26, 256-261.
- Mandelli G., A. Rinaldi, A. Cerioli & G. Cervio, 1967. Aspetti ultrastrutturali della borsa di Fabrizio nella malattia di Gumboro de pollo. Atti. Soc. Ital. Sci. Vet., 21, 615-619.
- Mickael C.S. & D.J. Jackwood, 2005. Real time RT-PCR analysis of two epitope regions encoded by the vP2 gene of infectious bursal disease viruses. J. Virol. Med., 128, 37-46.
- Muller H., C. Scholtissek & H. Becht, 1979. Genome of infectious bursal disease virus consists of two segments of doublestranded RNA. J. Virol., 31, 584-589.
- Nieper H., J.P. Teifke, A. Jungmann, C.V. Lohr & H. Muller, 1999. Infected and apoptotic cells in the IBDV-infected bursa of Fabricus, studied by double-labelling techniques. Avian Pathology, 28, 279-285.
- Ojeda F., I. Skardova, M.I. Guarda, J. Ulloa & H. Folch, 1997. Proliferation and apoptosis in Infection with Infectious Bursal Disease Virus: a flow cytometric study. Avian Diseases, 41, 312-316.
- Okoye J.O.A. & U.E.Uche, 1986. Serological evidence of infectious bursal disease virus infection in wild rats. Acta. Vet. Brno, 55, 207-209.
- Saif Y.M., 1998. Infectious Bursal Disease and Hemorrhagic Enteritis. Poultry Science, 77, 1186-1189.
- Sapats S.I. & J. Ignjatovic, 2000. Antigenic and sequence heterogeneity of infectious bursal disease virus strains isolated in Australia. Arch. Virol., 145, 773-785.
- Sharma J.M., J. Dohms, M. Walser & D.B. Snyder, 1993. Presence of lesions without virus replication in the thymus of chickens exposed to infectious bursal disease virus. Avian Diseases, 37, 741-748.
- Skeeles J.K., P.D. Lukert, E.V. De Buysscher, O.J. Fletcher & J. Brown, 1979. Infectious bursal disease virus infections. II. The relationship of age, complement levels, virus-neutralizing antibody, clotting and lesions. Avian. Dis., 23, 107-117.
- Snedeker C., F.K. Wills & I.M. Moulthrop, 1967. Sone studies on the infectious bursal agent. Avian. Dis., 11, 519-528.
- Tanimura N. & J.M. Sharma, 1998. In-situ apoptosis in chickens infected with infectious bursal disease virus. Journal of Comparative Pathology, 118, 15-27.
- Tsukamoto K., C. Kojima, Y. Komori, N. Tanimura, M. Mase & S. Yamaguchi, 1999. Protection of chickens against very virulent infectious bursal disease virus (IBDV) and Marek`s disease virus (MDV) with a recombinant MDV expressing IBDV VP2. Virology, 257, 352-362.
- Tsukamoto K., N. Tanimura, H. Hihara, L. Shirai, K. Imai, K. Nakamura & M. Maeda, 1992. Isolation of virulent infectious bursal disease virus from field outbreaks with high mortality in Japan. Journal of Veterinary and Medical Science, 54, 153-155.
- Van den Berg T.P., M.Gonze & G.Meulemans, 1991. Acute infectious bursal disease in poultry; isolation and characterisation of a highly virulent strain. Avian Pathology, 20, 133-143.
- Van den Berg T.P. & G.Meulemans, 1991. Acute infectious bursal disease in poultry; protection afforded by maternally derived antibodies and interference with live vaccination. Avian Pathology, 20, 409-421.
- Van den Berg, T.P., 2000. Acute infectious bursal disease in poultry: a review. Avian Pathology, 29, 175-194.
- Whitfill C.E., E.E. Haddad, C.A. Ricks, J.K. Skeeles, L.A. Newberry, J.N. Beasley, P.D. Andrews, J.A. Thoma & P.S. Wakenell, 1995. Determination of optimum formulation of a novel infectious bursal disease (IBD) vaccine constructed by mixing bursal disease antibody with IBDV. Avian. Dis., 39, 687-699.
- Winterfield R.W., S.B.Hitchner, 1962. Etiology of an infectious nephritis-nephrosis syndrome of chickens. Am.J. Vet. Res., 23, 1273-1279.
- Yao K. & V.N. Vakharia, 2001. Induction of apoptosis in vitro by the 17kDa non-structural protein of infectious bursal disease virus: possible role in viral pathogenesis. Virology, 285, 50-58.
- Zarkov, I. 2003. Veterinary virology, Infectious bursal disease virus, RIK "Iskra-M-I", Stara Zagora, p. 238.