Infectious bursal disease (Gumboro disease)

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Summary
Infectious bursal disease (IBD) (Gumboro disease) has been described throughout the world, and the socio-economic significance of the disease is considerable worldwide. 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 which is often difficult to measure. Finally, the control measures used are subject to variations, and seldom follow a specific or standardised plan. In the context of expanding international trade, the authors provide an overview of existing knowledge on the subject to enhance available information on the epidemiology of IBD, the identification of reliable viral markers for diagnosis, and the implementation of specific control measures to ensure a global and co-ordinated approach to the disease.

Keywords

Introduction
For many years, infectious bursal disease (IBD) (Gumboro disease) has constituted a serious problem for the poultry industry, and the recent ‘re-emergence’ of the infectious bursal disease virus (IBDV) in the form of antigenic variants and hypervirulent strains has been the cause of significant losses. Direct losses are linked to specific mortality, and depend on the dose and virulence of the strain, the age and breed of the animals, and the presence or absence of passive immunity. The indirect economic impact of the disease is also considerable, due to virus-induced immunosuppression and/or potential interactions between IBDV and other viruses, bacteria or parasites. These indirect losses are due to secondary infections, growth retardation and condemnation of carcasses at the slaughterhouse. Moreover, the increased use of antibiotics against secondary infections constitutes a growing public health concern.

The most recent survey of international poultry specialists, conducted by World Poultry, highlighted continuing concern in the sector over the sanitary status of poultry. Gumboro disease topped the list of the most serious poultry diseases (165). The aim of this paper is to present an up-to-date review of the different forms of the disease and of control to provide the reader with an overview of this complex problem.

The disease

Definition
Infectious bursal disease is a viral infection, affecting the immune system of poultry. The disease is highly contagious, affects young chickens, and is characterised by the destruction of the lymphoid organs, and in particular the bursa of Fabricius, where B lymphocytes mature and differentiate. The target cell of the virus is the B lymphocyte in an immature stage, and the infection, when not fatal, causes an immunosuppression, in most cases temporary, the degree of which is often difficult to determine.
Incidence and distribution

The first report of a specific disease affecting the bursa of Fabricius in chickens was made by Cosgrove in 1962 (21). The first cases were observed in the area of Gumboro, in Delaware (United States of America [USA]), which is the origin of the name, although the terms 'IBD' or 'infectious bursitis' are more accurate descriptions. Between 1960 and 1964, the disease affected most regions of the USA (85), and reached Europe in the years 1962 to 1971 (34). From 1966 to 1974, the disease was identified in the Middle East, southern and western Africa, India, the Far East and Australia (34, 36, 72, 84, 126, 159, 165). Infectious bursal disease is currently an international problem: 95% of the 65 countries that responded to a survey conducted by the Office International des Epizooties (OIE) in 1995 declared cases of infection (28), including New Zealand which had been free of disease until 1993 (72). These findings led to the adoption of a specific resolution of the International Committee of the OIE during the 63rd General Session in May 1995 (117).

Morbidity and mortality

Infectious bursal disease is extremely contagious. In infected flocks, morbidity is high, with up to 100% serological conversion after infection, whilst mortality is variable. Until 1987, the field strains isolated were of low virulence and caused only 1% to 2% of specific mortality. However, since 1987 an increase in specific mortality has been described in different parts of the world. In the USA, new strains responsible for up to 5% of specific mortality were described (131). At the same time, in Europe and subsequently in Japan, high mortality rates of 50% to 60% in laying hens and 25% to 30% in broilers were observed. These hypervirulent field strains caused up to 100% mortality in specific-pathogen-free (SPF) chickens (116, 160).

Clinical signs

The incubation period is very short: two to three days. In acute cases, the animals are exhausted, prostrated, dehydrated, suffer from watery diarrhoea, and feathers are ruffled. Mortality commences on the third day of infection, reaches a peak by day four, then drops rapidly, and the surviving chickens recover a state of apparent health after five to seven days. Disease severity depends on the age and breed sensitivity of the infected birds, the virulence of the strain, and the degree of passive immunity. Initial infection on a given farm is generally very acute, with very high mortality rates if a very virulent strain is involved. If the virus persists on the farm and is transmitted to successive flocks, the clinical forms of the disease appear earlier and are gradually replaced by subclinical forms. Nonetheless, acute episodes may still occur. Moreover, a primary infection may also be apparent when the viral strain is of low pathogenicity or if maternal antibodies are present.

The clinical signs of IBD vary considerably from one farm, region, country or even continent to another. Schematically, the global situation can be divided into three principal clinical forms, as follows:

a) the classical form, as described since the early 1960s, is caused by the classical virulent strains of IBDV. Specific mortality is relatively low, and the disease is most often subclinical, occurring after a decline in the level of passive antibodies (34)

b) the immunosuppressive form, principally described in the USA, is caused by low-pathogenicity strains of IBDV, as well as by variant strains, such as the Delaware variant E or GLS strains, which partially resist neutralisation by antibodies against the so-called 'classical' viruses (67, 140)

c) the acute form, first described in Europe, and then in Asia, is caused by hypervirulent strains of IBDV, and is characterised by an acute progressive clinical disease, leading to high mortality rates on affected farms (17, 145, 160).

Pathology and lesions

Although the other lymphoid organs are affected (135, 148, 149), the principal target of the virus is the bursa of Fabricius (73), which is the reservoir of B lymphocytes in birds. Indeed, the target cell is the B lymphocyte in active division, for which the infection is cytoplastic (14). Cell sorting studies have demonstrated that the B lymphocyte is susceptible in the immature stage, during which immunoglobulin M is carried on the surface of the lymphocyte (55, 112). This accounts for the paradoxical immune response to IBDV, in which immunosuppression co-exists with high anti-IBDV antibody titres. The mature and competent lymphocytes will expand as a result of stimulation by the virus whereas the immature lymphocytes will be destroyed.

Macroscopic lesions are observed principally in the bursa which presents all stages of inflammation following acute infection (96, 166). Autopsies performed on birds that died during the acute phase (three to four days following infection) reveal hypertrophic, haemorrhagic and oedematous bursas. The most severe cases are characterised by a major infection of the mucous membrane and a serous transudate, giving the bursal surface a yellowish colour. This appearance is often accompanied by petechiae and haemorrhages. By the fifth day, the bursa reverts to normal size and by the eighth day becomes atrophied to less than a third of the normal size. The affected animals are severely dehydrated, and many birds have hypertrophic and whitish kidneys containing deposits of urate crystals and cell debris. Haemorrhages in the pectoral muscles and thighs are frequently observed, probably due to a coagulation disorder (139). Certain variants from the USA are reported to cause rapid atrophy of the bursa without a previous inflammatory phase (94). Moreover, in the acute form of the disease caused by hypervirulent strains, macroscopic lesions may also be observed in other lymphoid organs (thymus, spleen, caecal tonsils, Harderian glands, Peyer's patches and bone marrow) (51, 59, 60, 155).
Henry et al. have developed a system for evaluating microscopic lesions of the affected organs, with a score ranging from one to five according to severity (50). The B lymphocytes are destroyed in the follicles of the bursa as well as in the germinal centres and the perivascular cuff of the spleen. The bursa is infiltrated by heterophils and undergoes hyperplasia of the reticulo-endothelial cells and of the interfollicular tissue. As the disease evolves, the surface epithelium disappears and cystic cavities develop in the follicles. Severe panleukopenia is also observed. These microscopic lesions are exacerbated in the acute forms of the disease.

**Distribution and persistence of the virus**

A kinetic study using immunofluorescence (109) has shown that, 4 h after oral inoculation, the virus is found in the lymphoid tissues associated with the digestive tract, where the first cycle of viral replication occurs. The virus subsequently enters the general circulation via the hepatic portal vein. A phase of primary viraemia ensues, during which the virus reaches the bursa, 11 h after infection, and a major secondary replication cycle occurs. A phase of secondary viraemia then occurs, and the other lymphoid organs become massively infected.

**Immunosuppression**

The destruction of immature B lymphocytes in the bursa creates an immunosuppression, which will be more severe in younger birds (35). In addition to the impact on production and role in the development of secondary infections, this will affect the immune response of the chicken to subsequent vaccinations which are essential in all types of intensive animal production (39).

The most severe and longest-lasting immunosuppression occurs when day-old chicks are infected by IBDV (4, 35, 134, 136). In field conditions, this rarely occurs since chickens tend to become infected at approximately two to three weeks, when maternal antibodies decline. Evidence suggests that the virus has an immunosuppressive effect at least up to the age of six weeks (38, 92, 175).

Immunosuppression is most often demonstrated using experimental models based on the measurement of humoral responses induced by different antigens such as *Brucella abortus* (57), sheep red blood cells, or Newcastle disease vaccines (4, 35, 39) (Table I). The best assessment is clearly the measurement of vaccinal protection against a challenge infection by the Newcastle disease virus, as described in the OIE Manual of Standards for Diagnostic Tests and Vaccines (119), since this constitutes a measurement of both humoral and cellular immunity. Unfortunately, these techniques are time-consuming, tedious, costly, and require the use of animals. Thus, they are usually confined to IBD vaccine registration procedures.

**Economic impact**

The economic impact of IBD is difficult to assess due to the multi-factorial nature of the losses involved. In addition to direct losses related to specific mortality (which in turn depends on the dose and virulence of the strain, the age and breed of the animals and the presence or absence of passive immunity), indirect losses also occur, due to acquired immunodeficiency or potential interactions between IBDV and other viruses, bacteria or parasites. Further losses may occur as a result of growth retardation or the rejection of carcasses showing signs of haemorrhages.

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**Table I**

Experimental models for the detection of immunosuppression induced by infectious bursal disease virus in specific-pathogen-free chickens

<table>
<thead>
<tr>
<th>Age</th>
<th>Treatment</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>Inoculation of IBDV</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>21 days</td>
<td>Injection of an inactivated Newcastle disease vaccine</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>42 days</td>
<td>Challenge with a virulent Newcastle disease virus</td>
<td>100%*</td>
<td>8%</td>
<td>84%</td>
<td>100%</td>
<td>57</td>
</tr>
<tr>
<td>1 day</td>
<td>Inoculation of IBDV</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>14 days</td>
<td>Intramuscular injection of 10^9 CFU of <em>Brucella abortus</em> per subject</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>57</td>
</tr>
<tr>
<td>Up to 7 weeks</td>
<td>Kinetic monitoring of <em>B. abortus</em> agglutinating antibodies</td>
<td>Maximum batch average ≥ 300 IU per ml</td>
<td>Maximum batch average &lt; 21 IU per ml</td>
<td>57</td>
<td>57</td>
<td>57</td>
</tr>
</tbody>
</table>

* Percentage of mortality observed after testing 25 subjects
  - : no treatment
  + : treatment
  IBDV : infectious bursal disease virus
  CFU : colony-forming unit
  IU : international units
Surveys conducted in Northern Ireland revealed a 14% reduction in income from broiler flocks affected by subclinical IBD when compared with uninfected flocks (98, 99). An 11% reduction in yield was reported for flocks with evidence of IBD over an average grow-out period of forty-two days, in comparison to non-exposed flocks. A 10% loss in profit in the 991 IBDV-infected flocks surveyed was due to weight loss and a reduction in feed conversion efficiency in comparison to non-infected flocks.

Two simulations were performed at a ten-year interval. The first estimated a loss of US$10 million per year in the event of introduction of classical strains into New Zealand (20). In the second, Shane et al., simulating two broiler operations in North America under the same conditions, one infected by IBDV, the other uninfected, estimated that the introduction of IBD would cause a 10% rise in production costs (133).

The emergence of hypervirulent strains across the world has further increased the financial impact of the disease.

Public health issues
No evidence exists of transmission of IBDV to humans (125); the disease thus has no direct impact on public health.

Viral structure
Two viral proteins are of importance in the context of this review. These are the VP2 and VP3 structural proteins which form the viral capsid. The epitopes responsible for the induction of neutralising and protective antibodies are located on the VP2 protein (5, 6, 122, 158), and several groups in Europe, the USA and Australia have prepared neutralising monoclonal antibodies against the VP2 protein (29, 30, 128, 141, 162, 164). Neutralising monoclonal antibodies are serotype-specific. The non-neutralising monoclonal antibodies are directed against either VP2 or VP3; some are group-specific, others are type-specific (66, 123).

Protection
Humoral immunity plays a decisive role in protection against IBD. A very close correlation exists between titres of neutralising antibodies and protection (71, 114, 161, 163). This is borne out by the excellent passive protection provided by maternal antibodies against immunosuppression, bursal lesions, or mortality. The half-life of the passive antibodies, depending on blood volume, varies between three days (for broilers) and five days (for laying hens) (27, 138). Thus, if the antibody titre of a chick at hatch is known, then the time of maximum flock susceptibility to the wild or vaccinal virus can be determined. This information is very important when establishing the timing of vaccination programmes (27, 91).

Evolution
The evolution of the virus since 1984 has been marked by two major events. The first was the discovery of an antigenic drift in serotype 1 viruses. Commencing in 1984, several strains of this serotype were isolated in the USA from broiler flocks that had been properly vaccinated (131). The new viruses did not cause the characteristic clinical signs of the infection, but had a major immunosuppressive potential. These strains were termed 'variant' since they were capable of infecting chicks that possessed an antibody titre considered protective in normal circumstances. The variant viruses have since been found to carry modified neutralising epitopes, and several successive generations of these viruses, which gradually have accumulated antigenic mutations, have been found in the USA. Thus, six sub-groups have been described among thirteen strains tested by serum neutralisation (SN) (67). These results were confirmed using neutralising monoclonal antibodies (141, 143). Nonetheless, only one of these sub-types was considered to be a 'true' variant in cross-protection tests (131). Vaccinal protection against the infections caused by these sub-groups has required the development of specific vaccines (40, 47, 62, 108).

The second major epidemiological event was the emergence, in 1987, of 'hypervirulent' viruses (vHIBDV) in Europe, particularly on farms that were well managed and on which all hygiene and sanitary control measures had been implemented (17, 29, 145, 154, 160). These viruses are significantly more pathogenic than the classical strains, and are also capable of infecting chicks with normally protective

Infectious bursal disease virus

Description of the aetiological agent
The virus responsible for IBD belongs to the genus Avibirnavirus, in the family Birnaviridae, characterised by a genome composed of two segments of double-stranded ribonucleic acid (RNA). These viruses have no envelope, a icosahedral capsid structure, and a diameter of 58 nm-60 nm (75, 159). This relatively simple structure renders the virus very resistant to the outside environment. Two serotypes of IBDV exist, namely: serotype 1 which is pathogenic for poultry, and serotype 2, which is apathogenic and has been isolated from chickens and turkeys. The two serotypes are differentiated in vitro by the absence of cross-neutralisation, and in vivo, by the absence of cross-protection (8, 61, 65, 66, 97).

In addition to serological classification, the viral strains may be classified according to virulence (mortality and bursal lesions). Thus, strains of IBDV may be considered apathogenic, attenuated (vaccines), classical virulent, variant, or hypervirulent (vHIBDV). Serotype 2 strains cause neither mortality nor bursal lesions in SPF chickens and are thus apathogenic for chicks. Within serotype 1, a great deal of confusion can be found in the descriptions of virus virulence. In particular, the term 'hypervirulent' has been used to describe hypervirulent strains from Europe as well as the variant strains from the USA, although the latter are responsible for less than 5% specific mortality.
antibody titres (161). As no antigenic mutation characteristic of the vIIBDV was detected, these viruses are generally considered to be pathotypic variants (160, 164). In the absence of specific virulence markers, the only valid criteria for classifying IBDV strains into ‘pathotypes’ is virulence (mortality, lesions) in SPF chickens. Moreover, increases in virulence are apparently unrelated to antigenic variation, and research is currently underway to determine virulence markers.

Culture systems
Culture of IBDV may be performed in embryonated SPF eggs from nine to eleven days of age. Inoculation by the chorio-allantoic membrane (CAM) or the yolk sac route is preferable to the classical allantoic route, as the latter provides a greater yield of virus (56, 130, 147). Embryo death occurs three to seven days following inoculation. The affected embryos are oedematous, congested, with a gelatinous appearance of the skin, and haemorrhages are often present in the toes or the encephalon. The embryonic membranes are not modified. The variants from the USA cause less embryonic mortality, splenomegaly and no marked lesions of hepatic necrosis. Among the different compartments of the inoculated egg, the embryo is the place where the highest titres of virus occur. The liver shows scattered petechiae and foci of necrosis, and is the organ which is the richest in viral particles (96).

After adaptation, some IBDV strains can be cultured to relatively high titres in primary chicken embryo cell cultures or continuous cell lines of mammalian origin (22, 47, 54, 63, 76, 77, 93, 155, 181).

However, most of the strains isolated in the field, and in particular, the hypervirulent strains, cannot be multiplied in cell cultures, since they require either previous passages on embryonated eggs or several blind passages in cell cultures before a cytopathogenic effect is obtained. This adaptation is accompanied by an attenuation of the strain. For this reason, no satisfactory alternative to the use of three- to six-week-old SPF chickens has been found for the preparation of challenge viruses or the characterisation of these strains according to virulence. However, the continuous LSCC-BK3 line has been used for propagation of hypervirulent strains without any visible cytopathic effect (155). Primary bursa cells could also be useful, although to date, use has been limited (132).

Epidemiology

Host range
Only chickens (Gallus gallus) develop IBD after infection by serotype 1 viruses. Turkeys (Meleagris gallopavo) may be asymptomatic carriers of serotype 2 (61, 65, 97), and at times, of serotype 1 viruses whose pathogenicity for turkeys is ill-defined (124, 127). The Pekin duck (Cairina moschata) can also be an asymptomatic carrier of serotype 1 viruses (97). Anti-IBDV antibodies have been detected in guinea-fowl (Numida meleagris) (1), common pheasants (Phasianus colchicus) (89) and ostriches (Struthio camelus) (15), which have also been demonstrated to carry serotype 2 viruses (41). Neutralising or precipitating antibodies have been detected, inter alia, in various species of wild duck, goose, tern, puffin, crow and penguin, which may mean that wild birds act as reservoirs or vectors (37, 120, 169).

Susceptibility factors
The age of maximum susceptibility is between three and six weeks, corresponding to the period of maximum bursa development, during which the acute clinical signs are observed. Infections occurring prior to the age of three weeks are generally subclinical and immunosuppressive. Clinical cases may be observed up to the age of fifteen to twenty weeks (86, 121). Light strains of laying stock are more susceptible to disease than the heavy broiler strains (13, 45, 161).

Transmission
Only horizontal transmission has been described, with healthy subjects being infected by the oral or respiratory pathway. Infected subjects excrete the virus in faeces as early as 48 h after infection, and may transmit the disease by contact over a sixteen-day period (167). The possibility of persistent infection in recovered animals has not been researched. The disease is transmitted by direct contact with excreting subjects, or by indirect contact with any inanimate or animate (farm staff, animals) contaminated vectors. Some researchers have suggested that insects may also act as vectors (58). The extreme resistance of the virus to the outside environment enhances the potential for indirect transmission. The virus can survive for four months in contaminated bedding and premises (9), and up to fifty-six days in lesser mealworms (Alphitobius sp.) taken from a contaminated environment (95). In the absence of effective cleaning, disinfection and insect control, the resistance of the virus leads to perennial contamination of infected farm buildings.

Resistance to disinfectants
The virus is sensitive to sodium hydroxide (it is totally inactivated when pH exceeds 12), but it is not affected at pH 2 (9). The iodinated and chlorinated derivatives, as well as the aldehydes (formaldehyde, glutaraldehyde) are also active (83, 106, 137).

Potential risk of spreading infectious bursal disease virus through trade
Vertical transmission of the disease has not been reported. Horizontal transmission due to external contamination of eggshells has not been documented (but fertile eggs to be incubated can be disinfected by fumigation). As a result, the most likely sources of contamination during commercial trade of poultry products are live animals and poultry meat. Infectious bursal disease is an OIE List B disease, and countries importing live poultry may refer to Chapter 3.6.1. of the International Animal Health Code (118).
The IBD-free status of imported live animals can only be established by a negative serological test, repeated after a quarantine sufficiently long to allow for the eventualty of seroconversion (at least three weeks).

Although imported meat has not been demonstrated to be responsible for the spread of IBDV, this remains a theoretical possibility. Contaminated meat may be produced, either by the slaughter of viraemic asymptomatic chickens (167, 170), or by the slaughter of convalescent chickens which, ten to sixteen days after infection, are no longer symptomatic, but continue to carry pathogenic virus in the digestive tract, and thus may constitute a viral source of cross-contamination along the slaughter line. The resistance of infectivity of IBDV to temperatures below freezing (at least three years at -20°C) (19), and to heat (2, 9), is another factor in the spread of IBDV through trade in poultry meat derivatives.

Aside from these theoretical possibilities, it should be noted that current scientific data are in many respects insufficient to quantify precisely the risk under discussion. In particular, more precise data are required on the prevalence, the tropism of the different strains (in particular for muscle tissue), the risk of the spread of an imported virus to an IBD-free population, and the preferred technique(s) for detecting IBDV in meat.

Diagnosis

**Clinical and differential diagnosis**

The clinical diagnosis of the acute forms of IBD is based on disease evolution (a mortality peak followed by recovery in five to seven days), and relies on the observation of the symptoms and post-mortem examination of the pathognomonic lesions, in particular of the bursa of Fabricius. The conditions most liable to be clinically mistaken for IBD are avian coccidiosis, Newcastle disease in some visceral bronchitis. In all acute cases, the presence of bursal lesions allows for a diagnosis of IBD. In subclinical cases, an atrophy of the bursa may be confused with other diseases such as Marek's disease or infectious anaemia. Histological examination of the bursa will allow differentiation between these diseases (94).

**Histological diagnosis**

Histological diagnosis is based on the detection of modifications occurring in the bursa (see the sub-section entitled ‘Clinical signs’). The ability to cause histological lesions in the non-bursal lymphoid organs, such as the thymus (59), the spleen or bone marrow (60), has been reported as a potential characteristic of hypervirulent IBDV strains. The histological approach has the advantage of allowing for diagnosis of both the acute and chronic or subclinical forms of the disease.

**Serological diagnosis**

In areas contaminated by IBDV, most broiler flocks have anti-IBDV antibodies when leaving the farm. Current serological tests cannot distinguish between the antibodies induced by pathogenic IBDV and those induced by attenuated vaccine viruses, so serological diagnosis is of little interest in endemic zones. Nonetheless, the quantification of IBDV-induced antibodies is important for the medical prophylaxis of the disease in young animals, in order to measure the titre of passive antibodies and determine the appropriate date for vaccination (27, 78, 110) or in laying hens to verify success of vaccination (90, 107). Serology is likewise essential to confirm the disease-free status of SPF flocks. Each serological analysis must include a sufficient number (at least twenty) of individual serum samples representative of the flock under study. A kinetic study requires at least two serological analyses separated by an interval of three weeks (paired sera).

The most widely used quantitative tests are the detection of precipitating antibodies by agar gel immunodiffusion (AGID) (23, 52), enzyme-linked immunosorbent assay (ELISA) (102, 107), and SN in cell culture (168).

Agar gel immunodiffusion is the simplest, but least sensitive technique. Results are obtained after an incubation period of 48 h. Variability in results may be due to the investigator, as well as the nature of the viral strain used as an antigen (115, 160, 168, 171, 172).

Serum neutralisation presents the disadvantages that specialised equipment and five days incubation are required. The technique is much more sensitive than AGID and correlates better with the level of protection of the subjects tested (67, 129, 168).

The ELISA is the most rapid and sensitive method, and presents the fewest variations due to the viral strain used as an antigen (129). Considerable inter- and intra-laboratory variability can occur with certain commercial kits (79, 80). Although the correlation between results obtained using SN and ELISA is high, ELISA remains less sensitive, and does not detect low neutralising titres which are sufficient to block vaccine administration (residual maternal antibodies). Enzyme-linked immunosorbent assays which use a recombinant VP2 protein as the sole antigen may be better correlated with protection (71, 163).

**Virological diagnosis**

Infectious bursal disease virus may be detected in the bursa of Fabricius of chicks in the acute phase of infection, ideally within the first three days following the appearance of clinical signs.
Isolation
A filtered homogenate of the bursa of Fabricius is inoculated in nine- to eleven-day-old embryonated eggs originating from hens free of anti-IBDV antibodies. The most sensitive route of inoculation is the CAM; the yolk sac route is also practicable, and the intra-allantoic route is the least sensitive. The specificity of the lesions observed must be demonstrated by neutralising the effect of the virus with a monospecific anti-IBDV serum. Isolation in embryonated eggs does not require adaptation of the virus by serial passages, and is suitable for vvIBDVs. In the absence of lesions, the embryos from the first passage should be homogenised in sterile conditions and clarified, and two additional serial passages should be performed (56, 94, 130).

Detection of viral antigens
Thin sections of the bursa of Fabricius
The viral antigens specific to IBDV may be detected by direct and indirect immunofluorescence (3, 105) or by immunoperoxidase staining (18) in the bursal follicles of infected chickens between the fourth and sixth day after inoculation. No viral antigen is detectable from the tenth day. However, the virus can be isolated from bursae sampled from the second to the tenth day, with a maximum infectious titre after four days (167, 170). The use of monoclonal antibodies for detection of the virus enhances the specificity of the test (18).

Suspensions of the bursa of Fabricius
The AGID technique is based on a comparison between the suspension to be tested and a specific antiserum or a monoclonal antibody. The appearance of precipitation lines signals the presence of viral antigens (53, 142, 146).

Agglutination tests, using latex beads coated with an anti-IBDV monoclonal antibody (113) or sheep red blood cells coupled with anti-IBDV immunoglobulins are also possible (111).

Antigenic capture as revealed by ELISA (AC-ELISA) consists of capturing the viral antigens present in the suspensions, using anti-IBDV antibodies and a polystyrene support. The viral antigens captured are detected through a sandwich ELISA with an anti-IBDV antibody conjugated with peroxidase (154), or with an anti-IBDV antibody followed by an adapted anti-species conjugate (30, 46, 81, 82, 141, 164). The use of a polyclonal serum for capture enhances the sensitivity of the test. The use of monoclonal antibodies in the capture or detection stages allows for more precise antigenic characterisation of the captured viruses. Different batteries of monoclonal antibodies enable a tentative identification of the variants from the USA (141) or vvIBDVs (30, 31, 32).

Detection of the viral genome
Deoxyribonucleic acid probes
Deoxyribonucleic acid (DNA) probes labelled with $^{32}$P (26, 64, 74), biotin (68) or digoxigenin (48) have been used on prints of infected tissues to detect the multiple virus strains of serotypes 1 and 2. No genomic probe enabling differentiation between variant viruses or vvIBDVs has yet been described, undoubtedly owing to the very high degree of genetic resemblance between serotype 1 strains of the virus.

Reverse transcription and genetic amplification by polymerase chain reaction
Reverse transcription-polymerase chain reaction (RT-PCR) allows the detection of viral RNA in homogenates of infected organs or embryos, as well as in cell cultures, irrespective of the visibility of the virus present. The choice of amplified genomic zones depends on the objective. When the only objective is to detect multiple strains of the virus, primers are selected in the highly preserved zones (144, 150, 173, 174). When the characterisation of the amplified fragment is to allow for identification of the virus strains, the central, so-called variable portion of VP2 is generally chosen (87, 88). The amplified fragment may then be characterised by direct sequencing (87), and the analysis of the coded aminopeptide sequence (Fig. 1). The simultaneous presence of four amino acids (alanine 222, isoleucine 256, isoleucine 294 and serine 299) is considered as indicative of vvIBDV (11, 16, 32, 182). The electrophoretic profile of the amplified fragment may also be studied after digestion with different restriction endonucleases (RT-PCR/RE) (70, 88). The value of the results

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**Fig. 1**
Schematic view of existing genetic relationship between different strains of infectious bursal disease virus (IBDV) (based on the analysis of the VP2 variable domain)

The OH strain of serotype 2 is used as an 'outgroup'. The same type of tree can be obtained by neighbour-joining and maximum parsimony methods.

Source: Eterradossi et al. (32) with amendments
obtained will depend on the choice of endonucleases. In a
given virus, the absence of restriction sites for enzymes BstNI
and Styl, located respectively at codons 222 and 253 of
the gene coding for VP2, has been correlated with an atypical
antigenicity, such as that found in the variant viruses from the
USA (69, 70).

**Evaluation of pathogenicity**

Most of the highly pathogenic IBDVs isolated since 1987 have
common genetic and antigenic characteristics (see the
sub-sections above entitled 'Detection of viral antigens' and
'Detection of the viral genome'). These characteristics have not
yet been demonstrated to be factors in pathogenicity, and thus
at present, these are regarded as hypothetical markers
(Table II). Formal proof of the pathogenicity of an IBDV
isolate requires experimental inoculation in susceptible
chickens. However, no standardised test has yet been defined,
and research on the subject is generally confined to a few well
equipped laboratories, such as the OIE Reference Laboratories.

**Methods of prevention and control**

**Exclusion/eradication**

The very high resistance of IBDV to physical and chemical
agents (9) accounts for persistence of the virus in the outside
environment, particularly on contaminated farms, despite
disinfection. Eradication in the affected countries therefore
seems unrealistic. Prevention of IBD necessitates hygiene
measures and medical prophylaxis. No vaccine can solve the
problem if major sanitary precautions are not taken. These
precautions include 'all-in/all-out' farming methods, cleaning
and disinfection of premises, and observance of a 'down time'
(a period of rest between depopulation and restocking) (101).
Given the very contagious nature of the disease and the
resistance of the virus, certain essential steps in the
cleaning/disinfection process should be adhered to. Prior to
cleaning, all insects and pests (e.g. rats and mice) must be
eliminated as soon as the farm premises are empty. Old
bedding and dung must be eliminated and composted. All
farm equipment must be disassembled and stored in cleaning
rooms located outside the farm buildings. The buildings,
immediate surroundings and farm equipment must be
dry-cleaned first, in order to eliminate all dust, and then
washed using hot water (60°C) with a detergent, at a pressure
of 80 bar to 150 bar. A second disinfection of the full premises
must be performed before the introduction of the chicks. Feed
silos must be emptied completely and cleaned inside and
outside. Under no circumstances may feed remains from
previous flocks be reused. Disinfection is to be undertaken
only after all the buildings have been cleaned. All disinfectants
are more active at a temperature above 20°C; however,
chlorinated and iodinated disinfectants cannot be heated
above 43°C. The quantity of disinfectant solution to be used is
approximately 4 litres per 15 m² (104).

**Genetic selection for resistance**

Different consanguineous lines of poultry show highly
variable susceptibility to experimental infection with the same
strain of IBDV. The results of crosses between resistant and
susceptible lines show that resistance is a dominant hereditary
characteristic. However, the genes responsible for resistance
have not yet been identified, and genetic selection for resistance
has not yet been practised (12, 13).

**Vaccination**

In addition to strict compliance with rules of hygiene and
disinfection, the success of vaccination depends on the choice
of the vaccine strain and on the vaccination schedule. These
must take account of the existence of certain pathotypes and
the presence of antigenic variants in certain regions.

**Types of vaccines used**

Attenuated live virus vaccines and oil-emulsion inactivated
virus vaccines are used against IBDV (151). The general
principles governing the choice and the use of these vaccines

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**Table II**

Information provided by different characterisation tests applicable to infectious bursal disease virus

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Type of test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degree of antigenic similarity</td>
<td>Cross-neutralisation in embryonated eggs or cell culture</td>
</tr>
<tr>
<td>between different viral strains</td>
<td>Cross-protection: administration of the first strain to SPF chickens, followed 15 days later by challenge with the second strain</td>
</tr>
<tr>
<td>Evaluation of pathogenicity</td>
<td>Study of the different viruses using batteries of monoclonal antibodies (antigen capture, AGID, IIF)</td>
</tr>
<tr>
<td>Assessment of genetic similarity</td>
<td>Inoculation of susceptible SPF chickens (with standardised age and strain of subjects, viral dose and inoculation route)</td>
</tr>
<tr>
<td></td>
<td>RT-PCR of the genomic region concerned (most often the variable domain of VP2 or the A segment)</td>
</tr>
</tbody>
</table>

**Legend**

- SPF: specific-pathogen-free
- AGID: agar gel immunodiffusion test
- IIF: indirect immunofluorescence on section of infected organs
- RT-PCR: reverse transcription-polymerase chain reaction
- RE: digestion with different restriction enzymes. Positive or negative result (presence or absence of restriction site)
- RFLP: restriction fragment length polymorphism. Digestion by different restriction enzymes and viral characterisation based on the size of the digested fragments obtained
Vaccines may be administered at day-old by nebulisation in pullets (103). These are also administered to chicks in breeder inactivated vaccine before lay. Maternal antibodies, and are administered when these antibodies have disappeared, i.e. between the fourth and eighth week of age, depending on whether the grandparent flocks have or have not been vaccinated with an oil-emulsion inactivated vaccine before lay.

The mild strains are used chiefly for the vaccination of breeder flocks. These are very sensitive to interference by homologous maternal antibodies, and are administered when these antibodies have disappeared, i.e. between the fourth and eighth week of age, depending on whether the grandparent flocks have or have not been vaccinated with an oil-emulsion inactivated vaccine before lay.

Intermediate vaccines are used for vaccinating broilers and pullets (103). These are also administered to chicks in breeder flocks which are at risk of challenge by highly pathogenic strains at an early age. Although intermediate vaccines are also sensitive to neutralisation by passive antibodies, these vaccines may be administered at day-old by nebulisation in order to protect a chick that may not have a sufficient level of specific antibodies. Another reason for such early vaccination is to bring about replication of the vaccine virus in the chicks, and the dissemination of the virus within the farm; this would, at least partially, provide indirect vaccination to the other chicks at a time when they become sensitive to the infection. In high-risk farms, two vaccinations are generally performed. The age at vaccination depends on the maternal antibody titres present in the chicks at hatch. Vaccines are usually administered through drinking water, although nebulisation is also possible.

Live IBDV vaccines are compatible with other avian vaccines. However, the strains that cause serious lesions to the bursa of Fabricius may also provoke immunosuppression, exacerbate the pathogenicity of other immunosuppressive viruses (Marek's disease virus [MDV] and chicken anaemia virus [CAV]) and jeopardise the immunisation of poultry against other diseases. Registration procedures for these vaccines must include tests to verify the absence of interference with other vaccinations as well as the absence of reversion to virulence in the course of serial passages in three- to six-week-old SPF chickens.

A vaccine for in ovo vaccination of embryos has recently been developed. The vaccine is a mixture of virus and specific antibody, and is injected into eighteen-day-old embryos. Broiler chicks hatched from these eggs are immunised against IBDV throughout the growing period. This method avoids interference by parental antibodies (44).

Various vaccines using recombinant viruses expressing the VP2 protein of IBDV have been described, and have proven efficacy in laboratory tests. The advantages of these vaccines are the absence of residual pathogenicity, sensitivity to maternal antibodies and risk of selection of mutants, as well as the possibility of use in ovo and of differentiation between infected and vaccinated animals (7, 25, 49, 151, 156). No commercial version of these vaccines is currently available.

Inactivated vaccines are essentially used to produce high, uniform and persistent antibody titres in hens prior to lay that have been vaccinated with a live virus or have been naturally infected through exposure to the virus on the farm (24, 42, 177, 178). These vaccines are administered by the subcutaneous or intramuscular route at the age of sixteen to twenty weeks.

Progeny of hens that have been vaccinated in this way have protective antibodies until the age of approximately thirty days (10, 161, 176, 179, 180). The chicks are thus protected during the period of susceptibility to the IBDV strains that only provoke immunosuppression. However, the chicks are not protected from other highly pathogenic strains that may infect high mortality rates at later stages (161, 178). The decision to use an inactivated vaccine will thus depend on the epidemiological context, namely: presence or absence of highly pathogenic strains requiring vaccination of broilers with live virus vaccines. Where no risk of infection with vvIBDVs exists, boosting of laying hens with an inactivated vaccine just before lay is fully justified. However, the duration and uniformity of the immunity thus conferred upon chicks will, to a great extent, depend on the concentration and the antigenic specificity of the virus present in the vaccine. These vaccines are obtained either from bursal homogenates of infected chicks, or from viral cultures on embryonated eggs or fibroblasts, which are then inactivated by formaldehyde and presented as oil emulsions. Sub-unit vaccines produced in yeast (33, 100) or insect cell cultures (157) have also been described, but are not currently in use.

Vaccination failure: potential causes

The causes of failure of live-virus vaccinations are numerous. The most trivial causes are non-observance of the expiry date, inappropriate storage, non-observance of recommended doses, and incorrect or deficient vaccination techniques. Freeze-dried live vaccines must be rehydrated immediately before use in distilled water. The use of distilled water to dilute the vaccines is compulsory when the spray technique is used. When the vaccine is administered in drinking water, it...
is particularly important to deprive the birds of water for two to three hours before distributing the vaccine solution. Only fresh water, with no organic matter, chlorine or heavy metals, may be used. Adding powdered milk at a concentration of 2 g per litre helps to stabilise the vaccinal virus.

Interference from parental antibodies is one of the most frequent causes of failure. The date of vaccination of the offspring must therefore be determined on the basis of the immune status of the chicks, and thus of the vaccination protocol used for the parents.

Vaccination failure with inactivated vaccines is rare, but may occur, either due to the absence of previous contact of some of the birds with a live virus (a vaccine virus or otherwise), or to the existence of antigenic variants not present in the vaccine. All suspected cases of antigenic variation in the field should be tested in isolation units on SPF birds after vaccination with classical strains.

Differentiation between vaccine and wild virus strains

No markers are currently available to differentiate between vaccine and wild virus strains. A simple discrimination method (although only practicable in certain well-equipped laboratories) might be to cultivate field strains on chicken embryo fibroblasts. The vaccine strains, with the exception of the hot strains, are cytopathic, whereas the highly pathogenic strains are not. Moreover, the amplification and sequencing of the gene coding for the VP2 protein after reverse transcription would allow differentiation between all the vaccine strains, and may be a preferred method.

No serological test is able to distinguish between the response to a pathogenic or a vaccine strain at the flock level, due to the high degree of cross-reactivity.

Conclusion

Infectious bursal disease virus presents a certain number of characteristics that are of importance in the diagnosis and control of IBD. The disease is caused by a small, non-enveloped virus, highly resistant to the outside environment. Substantial economic losses result from both the clinical and subclinical (or immunosuppressive) forms of the disease, but also from interactions between IBDV and other viruses (CAV and MDV). Infectious bursal disease virus has a high mutation rate and may thus give rise to viruses of modified antigenicity or increased virulence. Although satisfactory protection may be provided by the induction of high neutralising antibody titres, interference of parental antibodies in vaccination has become the most important obstacle in the establishment of control programmes.

This situation requires heightened vigilance and the development of techniques to monitor the field situation on an ongoing basis. In particular, as recommended by the OIE, the incidence and prevalence of the clinical and immunosuppressive forms must be evaluated more precisely, for example, through the identification of reliable viral markers to characterise the different pathotypes (117). Likewise, more appropriate control measures should be developed to afford a higher degree of protection to young birds that carry a maternal immunity. To achieve this, research should be supported and promoted, preferably on an international level. An example of such work in Europe is Concerted Action COST 839 on immunosuppressive viral diseases of poultry.

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References


