DIAGNOSIS AND CONTROL OF INFECTIOUS Bursal Disease
INFECTION OF POULTRY

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THE DISEASE

Infectious bursal disease (IBD) is endemic throughout the world. First identified at Gumboro, the USA (COSGROVE 1962), it has been detected in all countries with intensive poultry industry. In recent time IBDV have undergone significant degree of changes, with emergence of pathogenic and antigenic variants. IBDV strains prevalent today can be classified into three major categories: classical, variant and very virulent (vv) strains. Classical IBDV predominated until mid 1980's in all countries; their major feature is the ability to induce immunosuppression and only a few are able to induce low levels of mortality (Lasher and Shane, 1994). Antigenic variants have emerged in the USA in mid 1980's where they have almost entirely replaced classical IBDV strains. Antigenic variants have also been detected in Australia, however, they distribution is limited and Australia is one of the few countries where classical strains still predominate (Sapats and Ignjatovic, 2000). VvIBDV have emerged in Europe in 1987 and have since spread to most countries, with the exception of North America, Australia & New Zealand.

IBDV DISTRIBUTION IN INDONESIA

In Asia, vvIBDV were first detected in 1991 (Japan). Virus is now endemic in all Asian countries, where it has replaced classical IBDV. Antigenic variants have not been detected in Asia, with the possible exception of China. In Indonesia, studies by Balitvet, Bogor have shown that vvIBDV entered the country in 1991; the first isolate was made in the region around West Java and Medan. Since then vvIBDV have been detected in Java, Sumatra, Sulawesi, Kalimantan and Bali. Survey from different parts of Indonesia between 1991 and 2001 showed that more than 20 individual farms were vvIBDV positive, whereas only on two farms classical IBDV strains were present. Genetic analysis showed that the majority of Indonesian isolates are genetically identical with vvIBDV from other countries and that these also induce high levels of mortality typical of vvIBDV. Some Indonesian vvIBDV isolates have one unique amino acid change not found previously in other vvIBDV strains. These strains however also induce high mortality in chicks.

INDONESIAN VVIBDV SITUATION

Mortality rates in flocks from which vvIBDV were isolated during 1991 & 1994 were 25 - 30% in broilers and 30 – 60% in layers. Also farms were confronted with subclinical form of vvIBD in 2-3 weeks old chicks due to immunosuppression. Since vvNDV is the main cause of economic losses in local poultry, Newcastle disease vaccination failure, due to immunosuppression cause d by vvIBDV, represents the main concern. Today, mortality due to vvIBDV is lower, between 5 – 25%, likely the result of more uniform breeder vaccination and higher level of immunity in progeny chicks. However, vaccination of broilers has also been introduced. While all large commercial companies vaccinate against vvIBDV, smaller producers do not, and vvIBDV outbreaks on such farms are not uncommon. This lack of uniform control allows for a large source of virus to be generated and maintained in the environment and thus poses a risk for other poultry holders. Other birds (such as ducks & quails), insects and rodents may also be the host for IBDV and might be involved in transmission of virus from non-vaccinated to clean (vaccinated) farms. Comprehensive vaccination in areas with high concentration of poultry is therefore necessary for effective control of vvIBDV.

DIAGNOSIS

Differential diagnosis

Until recently differentiation of vvIBDV from other types of IBDV relied only on clinical signs and mortality. However in countries were vvIBDV is endemic differential diagnosis has become more difficult. Most countries now routinely vaccinate with intermediate and hot IBDV vaccines and clinical disease due to field vvIBDV challenge is difficult to clearly differentiate from that induced by adverse vaccine reaction. Virus isolation, pathogenicity testing in specific pathogen-fee (SPF) chicks and genetic analysis using nucleotide sequencing are tests that will...
unambiguous differentiate vvIBDV. However all of these tests are time consuming and require specialised laboratory set-up.

**Virus isolation**

For positive diagnosis isolation of virus is necessary. Isolation of vvIBDV in cell culture is not possible since vvIBDV do not replicate in cell culture at first inoculation. If propagated in cell culture vvIBDV will undergo change, both in nucleotide sequence and pathogenicity. Thus, isolation of vvIBDV is best done by inoculation of bursal homogenate to 3-week-old SPF chickens.

**Pathogenicity testing**

Mortality induced in SPF chickens remains the only definitive method for typing of vvIBDV. Chick of between 3 and 6 weeks are susceptible to mortalities, whereas little or no mortality will be induced in younger chicks. Inoculated material must contain sufficient amount of virus (≥100 egg infective doses). Cumulative mortality at 4 or 5 days after infection is expected to be 25% – 75%.

**Histopathology**

Histopathological examination of bursa alone, although used frequently, is not able to differentiate vvIBDV as some classical pathogenic strains will induce identical bursal lesions.

**Molecular diagnostic tests**

Nucleotide sequencing of the portion of viral protein 2 (VP2) gene is also a method of choice for conformation of vvIBDV. The amino acids alignment of suspected isolate with other IBDV strain will show if virus contains four amino acids [222(Ala), 256(Ile), 294(Ile) and 299(Ser)] typical of vvIBDV. Method known as RT-PCR/restriction enzyme analysis is also able to differentiate vvIBDV from other types of IBDV strains. The method is faster than DNA sequencing, however it also requires specialised laboratory set-up.

**Antigen detection tests**

Antigen capture ELISA tests are now available for differentiation of vvIBDV. One ELISA is based on a panel of seven mouse monoclonal antibodies and has been in use at French Food Safety Agency, Ploufragan, France (Eterradossi et al.1998). This test however is not commercially available. CSIRO Australian Animal Health Laboratory, Geelong, Australia, has developed another antigen ELISA that differentiates vvIBDV from other types of IBDV. This test, although not commercially available, has however been transferred to Balitvet, Bogor where it is available for analysis of samples from farms suspected to have had vvIBDV outbreaks.

**CONTROL OF VVIBDV**

Following appearance of vvIBDV several changes in conventional IBDV vaccination were necessary in order to achieve some degree of control. Breeder vaccination was improved by using inactivated vaccines in order to arm progeny chicks with high and uniform levels of maternal antibodies. Relevant to this is that various inactivated vaccines are available; the majority are tissue culture propagated strains whereas some are derived from infected bursa. Currently it is uncertain if bursa derived inactivated vaccines provide superior immunity. Soon after appearance of vvIBDV it has become apparent that maternal antibodies, even at the highest levels, could not last throughout the life of the chick; boilers becoming infected at a critical stage, just before processing. For that reason vaccination of young birds was necessary. However, effective vvIBDV control has been difficult to achieve in most circumstances. Various management strategies were implemented; upgrading disinfection, use of intermediate vaccines on a variety of different programs, such as day old application, multiple vaccination, and vaccination at predetermined time based on ELISA antibody titre. These management strategies met with little success and today there is no universally adopted vaccine or vaccination regime for control of vvIBDV. vvIBDV is probably best controlled in Europe, where overt outbreaks are rare, however incidences of low flock mortality and inferior performance do occur.

**MATERNAL ANTIBODY STATUS**

Level of maternal antibody and its uniformity play important role. Vaccine “take” is dependent on antibody level and if applied too early, vaccine is neutralised. If vaccine is applied too late, the field virus has the opportunity to infect chicks first. For that reason timing of vaccination must be determined before hand; at least one week before vaccination, usually by measuring ELISA antibody levels at day old (KOUWENHOVEN and VAN DEN BOS, 1994). In broilers, maternal antibody decay by 50% in 3-5 days. Since there are individual differences in antibody levels, multiple applications of (usually) intermediate vaccines have been practiced on
some occasions to achieve vaccination of all chicks. Such costly vaccination however is not always successful due to other factors such as for example, strain of vaccine used; e.g. some vaccines inability to spread from vaccinated to unvaccinated chicks. Solution to this was to use invasive vaccines, which have ability to spread from vaccinated to unvaccinated chicks and thus maintain continuing vaccine presence in the population. However even with the use of such vaccines timing of vaccination seems also critical to insure quick and effective protection. It has been thus generally accepted that flocks with mean ELISA titres of 500 (IDEXX 1:500 dilution; Elisa group 5, TropBio Ltd) are susceptible to vaccination and resistant to field challenge. This means that in a commercial flock there will be individual chicks with antibody levels below 500 that are susceptible to initial vaccination and from which other chicks will become infected. Thus more homogenous antibody titres will result in a more uniform vaccine take. For that reason breeders are re-vaccinated (booster) with inactivated vaccines regularly (every 2 or 3 months).

Some have argued that vaccination of breeders with inactivated vaccine should be discontinued to allow for vaccination of broilers at an early age using less pathogenic vaccines. It has been noticed in the early years following vvIBDV outbreaks that progeny of breeders that have not been given inactivated vaccine contracted disease at about 2 weeks of age with mortality of 3-5%, instead of up to 25% at 30 days of age. Overall economic losses were less due to less value of chicks, and also because immunosuppression did not occur at that age. However, value of this type of approach might differ for different breed of chicks or might be compromised where other diseases are present, such as NDV in Indonesia; so far it has not been accepted and applied by intensive commercial poultry industry.

VACCINES

Vaccine type is also important in control of vvIBDV. Mild, intermediate and hot vaccine strains are available. Mild and intermediate vaccines were developed and used mostly for vaccination of replacement pullets and for priming birds for the inactivated vaccines. Their use for control of vvIBDV, initially in Europe, had limited impact of vvIBDV. Mild vaccines are not invasive enough and are not able to induce protective immunity in time to prevent vvIBDV challenge. Intermediate vaccines take in the presence of moderate levels of maternal antibodies and break through about one weeks after vvIBDV are able to do so (CHETTLE et al., 1994). This means that these vaccines do not work consistently and are for use on sites where vvIBDV challenge is not heavy and with optimum husbandry conditions. There are number of intermediate vaccines; strains include CU-1M, D78, CK37, 228TC, Bursine2 etc, and choice of vaccine seems to depend on an individual site/practices. There are claims that some of these vaccines are not able to spread from chick to chicks.

Hot vaccines were developed as it become apparent that intermediate vaccines were not effective on some sites. Use of hot vaccines is usually restricted to the severely effected areas where other measures have failed. They are also used for a limited time only. Hot vaccines give the best window of opportunity for vaccine immunity to develop before vvIBDV challenge could occur; however they are more pathogenic and immunosuppressive than intermediate vaccines and might affect flock performance. They are usually used on heavily contaminated sites and exchanged with intermediate vaccines. Strain 228E and TAD75 (Gumboro Vac Forte) are examples. Both produce bursal lesions and no mortality. In Europe use of 228E requires permission from the authorities. Tad Forte is considered less virulent and its wide application in Germany does not require official approval. In Indonesia, use of intermediate hot vaccine also requires official approval following laboratory trials of such vaccines using a local isolate of vvIBDV.

NEW GENERATION OF IBDV VACCINES

Since conventional live IBDV vaccines do not consistently provide protection against vvIBDV, a number of alternative approaches were attempted in recent years. Chettle et al., (1994) reported that vaccination of day-old broilers and 7-day-old commercial layers chicks with oil emulsion vaccine protected well against vvIBDV challenge. Subunit vaccines have also been developed, although aimed at breeder vaccination. Unlike conventional oil-inactivated vaccines that contain the whole IBD virus, subunit vaccines contain only the protective antigen of VP2, which is expressed most commonly in baculovirus. These vaccines provide protection against clinical signs, however, bursa damaged in not always controlled. Use of killed, or subunit vaccines, particularly in broilers is neither practical nor economically viable.

Since inactivated vaccines offer effective protection of broilers against vvIBDV, but the delivery of such vaccines is expensive, immunization of chicks with VP2 delivered in another virus (vector) has also been attempted. Marek’s disease virus, herpes virus of turkey, fowlpox and adenovirus were all genetically modified to carry the VP2 of IBDV. In most instances, however, these vectored vaccines provided protection against morality and clinical IBDV but not against bursal damage.
More recently the reverse genetic system has been used to generate new vaccines for IBDV. These vaccines, although not specifically made to provide protection against vvIBDV, nevertheless are an important development since the system enables for a recombinant virus to be modified as desired, for example to reduce its virulence and immunosuppressive effect.

*In ovo* vaccination, that uses an live intermediate vaccine which is complexed with IBDV antibody, seems to afford the highest degree of protection (Chetlet *et al.*, 1994). Such vaccination seems to enable each chick to be vaccinated at the time when its antibody drops to a level that allows vaccine to break trough. Thus chicks are vaccinated without delay, leaving little or no window of opportunity for field challenge. Such vaccines were available in Europe, until recently.

**FARM HYGIENE**

An effort should be made to reduce amount of virus present, or even eliminate virus, from chicken farms. The reduced amount of challenge virus will give an intermediate vaccine time to infect chicks and induce immunity. Entry to farm premises should be restricted. Disinfection between batches would significantly reduced virus load; it is best achieved in closed shed with concrete floor; dirt floors are difficult to disinfect thoroughly. Infected litter should be removed from farms and not stored in the vicinity. At least, the standard biosecurity system should be applied as best as possible. Biosecurity is commonly practiced at large commercial farms in Indonesia, while less stringent biosecurity is common on small scale (up 100 birds) and medium size (up to 10000 birds) farms.

**VACCINES AVAILABLE IN INDONESIA**

There are at least 17 different sources of IBDV vaccines that are used in Indonesia for control of vvIBDV in approximately 1 billion chicks/year (mostly broilers). Many vaccines are based on the same virus strain and the majority are tissue culture adapted strains. Some are classified as intermediate or intermediate plus. There are only two local vaccine factories producing IBD vaccines using overseas vaccine seeds.

It is important to notice that in Indonesia, vaccination program to control vvIBDV has to be closely allied with control of NDV, especially in broiler. These vaccination programmes should also take into the account existence of new genetic breeds. Today broilers are reared until 5 weeks of age, instead of previous practice of 6 to 7 weeks of age. Most vaccination programs however, have not changed to accommodate this new practice.

In conclusion, a considerable challenge and scope exists for Indonesian governmental agencies in agricultural research to foster and support innovations and advances in diagnosis and control of poultry diseases.

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