



## Vaccines and Vaccination against Infectious Bursal Disease of Chickens: Prospects and Challenges

Pit Sze, LIEW,<sup>a</sup> Mat Isa, NURULFIZA,<sup>b,c</sup> Abdul Rahman, OMAR,<sup>a,c</sup>, Ideris, AINI,<sup>a,c</sup> Mohd, HAIR-BEJO<sup>a,c,\*</sup>

<sup>a</sup>Faculty of Veterinary Medicine, <sup>b</sup>Faculty of Biotechnology and Biomolecular Sciences, <sup>c</sup>Institute of Biosciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Malaysia

\*[mdhair@upm.edu.my](mailto:mdhair@upm.edu.my)

**Abstract** – Infectious bursal disease (IBD), also known as the Gumboro disease, has been a great concern for poultry industry worldwide. The first outbreak of IBD due to very virulent (vv) IBD virus (IBDV) infection in Malaysia was reported in 1991. The major economic impact of the disease is high mortality and poor performance. The virus causes immunosuppression where if the infected chicken recovered from the acute disease, they become more susceptible to infections of other pathogens and fail to respond to vaccines. Therefore, prevention is important and vaccination has become the principal control measure of IBDV infection in chickens. The conventional attenuated live and killed vaccines are the most commonly used vaccines. With the advancement of knowledge and technology, new generation of genetically-engineered vaccines like viral vector and immune complex vaccines have been commercialised. Moreover, hatchery vaccination is becoming a common practise, in addition to farm vaccination. Currently, the disease is considerably under controlled with the introduction of vaccination. However, occasional field outbreaks are still commonly reported. The demand for vaccines that could suit the field situation continues to exist. The endemicity of disease, presence of challenge in the farm and maternally derived antibody in chicks are affecting the choice vaccine as well as the vaccine development and vaccination strategies. In this review, advances made in various vaccines that have been commercialised or under development, and challenges that they face, are outlined. Furthermore, how the emergence of vvIBDV affect the progress of vaccine development and influence its vaccination strategy are discussed.

**Keywords:** Infectious bursal disease, infectious bursal disease virus, poultry, vaccination, vaccines.

---

### Introduction

Infectious bursal disease (IBD) also known as the Gumboro disease was first reported from broiler flocks in the area of Gumboro, Delaware in 1957 (Etteradossi & Saif, 2008). The causative agent of the disease was identified as the IBD virus (IBDV), which is the prototype member of the genus *Avibirnavirus*. Together with infectious pancreatic necrosis virus from genus *Aquabirnavirus*, Drosophila X virus from genus *Entomobirnavirus*, blotched snakehead virus and rotifer birnavirus, they form the family *Birnaviridae* (Delmas *et al.*, 2005). The members of the family possess two genome segments of double stranded RNA, within a single-shelled, non-enveloped capsid of icosahedral symmetry and a diameter of about 60 nm (Nick, Cursiefen, & Becht, 1976; Müller, Scholtissek, & Becht, 1979; Azad, Barrett, & Fahey, 1985). The IBDV genome segments are named A and B. Segment A encodes for two partially overlapping open reading frames (ORFs), where the large ORF produces a precursor polyprotein that is proteolytically cleaved to yield precursor VP2

(pVP2), VP4 and VP3 (Hudson, McKern, Power, & Azad 1986; Sánchez & Rodriguez, 1999). The pVP2 undergoes a series of cleavage to become mature VP2 (Müller & Becht, 1982; Irigoyen *et al.*, 2009; Irigoyen, Castón, & Rodríguez, 2012). The smaller ORF preceding and partially overlapping the large ORF encodes for the fifth identified IBDV protein VP5 (Mundt, Beyer, & Müller, 1995). The genome segment B encodes for polypeptide VP1 (Morgan, Macreadie, Harley, Hudson, & Azad, 1988). Two serotypes of IBDV are identified namely the serotypes 1 and 2. The serotype 1 is pathogenic in chickens and consists of three viral strains namely the classical (ca), very virulent (vv) and variant (va) IBDV. The serotype 2 is non-pathogenic in chickens.

The clinical acute outbreaks of caIBD in susceptible flocks are generally shown by sudden onset of high mortality. The birds often appear prostrated and unwilling to move with ruffled feathers. They frequently pick at their own vents and have soiled vent feathers from the watery or whitish diarrhoea (Etteradossi & Saif, 2008). Under commercial set up, the disease can cause up to 20% mortality in susceptible hybrid Leghorn replacement pullets (Lasher & Shane, 1994). Experimental infections in specific-pathogen-free (SPF) chickens resulted in a range a mortality rates, from 0 to 30% by the US standard challenge IBDV strain STC (Stoute *et al.*, 2013). The IBDV such as the British strain F52/70 could induce up to 36% mortality while that of German strain Cu-1WT could cause up to 54% mortality in SPF chickens (van den Berg *et al.*, 2004).

The mortality rate from the disease in broiler chickens seldom exceeded 2% (van den Berg, 2000). Furthermore, the infection may be less severe and could frequently go undetected due to the presence of maternally derived antibody (MDA) in endemic farms. However, increased carcasses condemnation rate in processing plants due to haemorrhages in the thigh and pectoral muscles from the clinical disease resulted in economic losses (Kibenge, Dhillon, & Russell, 1988). In fact, the economic significance was mainly due to immunosuppressive effect of the disease (Sharma, Dohms, & Metz, 2000). In certain circumstances, such as subclinical infection in chickens less than 3 weeks of age, it may result in immunosuppression (Lasher & Shane, 1994). The recovered chickens become more susceptible to infections of other pathogens such as viruses and bacteria, and fail to respond to vaccines. Depending on the nature of the disease, the immunosuppressed chickens may suffer from the secondary infection at later age at around 4- to 5-week-old in broiler or later in layer flocks. In view of this, a live vaccine using a mild or attenuated isolate of the virus was produced in SPF embryonated chicken eggs (Snedeker, Wills, & Moulthrop, 1967). Since then, the disease was satisfactorily controlled by vaccination.

### **Emergence of the Variant and Very Virulent IBDV Strains**

In the 1980's, vaccination failures and outbreak of the disease were reported from different parts of the world. The caIBDVs were experiencing a pathotypic shift and breaking through MDA in vaccinated chickens (Snyder, 1990). In the U.S., the control was compromised by the appearance of new IBDV variants (Jackwood & Saif, 1987). Although the new variants were genetically and phylogenetically related to the serotype 1 caIBDV, they demonstrated a distinct antigenicity pattern (Heine, Haritou, Failla, Fahey, & Azad, 1991). The variant strains were reported to induce little if any clinical signs and mortality, but could cause rapid bursal atrophy (Sharma, Dohms, & Metz, 1989). Although the variant strains are mainly confined to the U.S., the appearance of vaIBDV strains has later been reported in Australia as well (Sapats & Ignjatovic, 2000). These viruses were not only antigenically different from the variant strains in the U.S., but also demonstrated distinct genetic and phylogenetic relationships to that of the US variants (Sapats & Ignjatovic, 2000).

At the same time, in the 1980's the IBDVs were undergoing a pathotypic shift in Europe in at a practically opposite direction to that of the variant strains in the U.S. and identified as vvIBDV (van

den Berg, Gonze, & Meulemans, 1991). The new virus isolates demonstrated an increased virulence that could experimentally reproduce up to 100% mortality in SPF chickens. The disease spread worldwide; in the early 1990's, the appearance of vvIBDV was reported in Japan (Nunoya, Otaki, Tajima, Hiraga, & Saito, 1992; Lin *et al.*, 1993). Since then, vvIBDV has quickly spread all over Asia (van den Berg, 2000), including Malaysia in 1991 (Hair-Bejo, 1992). The virus has also been isolated in other major parts of the world like Central Europe, Russia, the Middle East and South America (van den Berg, 2000), and in December 2008, the first case of vvIBDV outbreak was reported from pullet flocks in California, U.S. (Stoute *et al.*, 2009).

The vvIBDV infection produces clinical disease signs similar to that of the caIBDV, except with an aggravated clinical signs in susceptible flocks (van den Berg, 2000). Under natural set up in endemic infection, the virus could induce up to 30% mortality in broilers and 60% in layers (Etteradossi *et al.*, 1999). While under experimental conditions, the vvIBDV can induce up to 100% mortality in SPF chickens (van den Berg, 2000). The disease pattern of the flocks shows mortality peaks with a sharp death curve that is followed by rapid recovery. The clinical disease is characterised by higher mortality rates and extended age of susceptibility.

With the emergence of vaIBDV and vvIBDV strains, rapid identification and characterisation of new IBDV isolates become important and three criteria have been used to assess and characterise the IBDV strains. In addition to the pathogenicity characteristics in SPF chickens, IBDV strains are also being grouped by their antigenicity and genetic relatedness profiles (van den Berg *et al.*, 2004). Although cross neutralization tests and sequence analyses to characterise IBDV are established, almost all of them are based on the VP2 region of IBDV (Lana, Beisel, & Silva,; van den Berg *et al.*, 1996; Sapats & Ignjatovic, 2000; Remorini *et al.*, 2006). Furthermore, recent progress in the IBDV research have revealed the occurrence of natural IBDV reassortant strains (Wei *et al.*, 2006; Le Nouën *et al.*, 2006; Jackwood *et al.*, 2011) or mosaic virus from homologous recombination within segments (Hon *et al.*, 2008; He *et al.*, 2009; Jackwood, 2012a), which demonstrated the insufficiencies of using VP2 alone for grouping of IBDV isolates. Experimental studies showed that the viral segment B does influence the virulence and disease outcome (Le Nouën *et al.*, 2012; Escaffre *et al.*, 2013; Yu *et al.*, 2013). Certainly, this has called for characterisation of IBDV to be based on both genome segments A and B in order to achieve better control of the disease (Jackwood *et al.*, 2011; Escaffre *et al.*, 2013; Liew, Omar, Ideris, & Hair-Bejo, 2013).

### **Vaccination against IBD**

As the IBD virus is stable in nature and resistant to many physical and chemical disinfectants, IBDV infections continue to occur and spread widely despite a properly done cleaning and disinfection procedures (van den Berg, 2000). Therefore, vaccination becomes the principal control measure of IBDV infection in chickens (Etteradossi & Saif, 2008). In the early days, protection was sufficiently achieved by active immunisation of breeding hens with combination use of live and inactivated vaccines (Lasher & Shane, 1994; Nagarajan & Kibenge, 1997). In replacement pullets that have been primed with live vaccine at 4 to 8 weeks of age, inactivated oil-emulsion vaccine is being administered before lay to boost antibody production that are transferable to chicks. In layer strain parent stocks, the booster dose is administered between 16 and 18 weeks of age while in broiler breeders it is performed at 20 weeks of age (Lasher & Shane, 1994). Later, a mid-cycle booster is done at around 40 to 42 weeks of age to induce a high MDA and long-lasting serum titer.

Compared to live attenuated embryo-propagated virus, booster vaccination with inactivated IBDV in oil-emulsion demonstrated a far more superior immunogenicity in hens, and a longer persistence of MDA in chicks (Lasher & Shane, 1994). The MDA was able to provide passive immunity to the

chicks (Wyeth & Cullen, 1976) for 4 to 5 weeks of life (Lucio & Hitchner, 1979; Naqi, Marquez, & Sahin, 1983). It was shown that the higher the MDA in chicks, the longer the protection could last (Al-Natour *et al.*, 2004). The MDA protect them from subclinical IBD and therefore prevent the detrimental immunosuppression effects associated with early age infection (Lucio & Hitchner, 1980). However, the level of protection afforded by MDA depends not only on strain present in the inactivated vaccine, but also the degree of virulence of the IBDV challenge strain (Maas *et al.*, 2001). They require a higher level of MDA against more virulent challenge virus in order to achieve complete protection against clinical disease (Maas *et al.*, 2001).

The emergence of vvIBDV, however, has brought the conventional vaccination programme into question (van den Berg, 2000). The vvIBDVs were able to break through the MDA in chicks (van den Berg & Meulemans, 1991; Maas *et al.*, 2001; Zorman-Rojs, Barlič-Maganja, Mitevski, Lübke, & Mundt, 2003; Jackwood *et al.*, 2009). It was shown that maximum mortalities of 60% in layers and 20% in broilers were recorded from chicks with MDA when challenged at the age of 38 days old (van den Berg & Meulemans, 1991). Although the chicks were protected against mortality at the age earlier than 38 days old, they nevertheless suffered extensive damage in the bursa of Fabricius. Similarly in the U.S., progenys from hens vaccinated with the serotype 1 caIBDV and vaIBDV were shown to succumb to clinical disease and infection when challenged with high dose of vvIBDV (Jackwood *et al.*, 2009). The chicks demonstrated bursal lesions and positive for IBDV viral genome upon RT-PCR although no mortality was detected. In an experiment mimicking the field situation in the U.S. involving competition from the endemic vaIBDV, it was shown that co-infection of both vvIBDV and vaIBDV in SPF chickens at the same time resulted in clinical disease typical of vvIBDV infection (Jackwood, 2011). However, the mortality rates were comparatively lower at between 30% and 40% than the positive control birds where they all died following infection by vvIBDV alone. And when the variant virus was inoculated at 2 days earlier, the occurrence of clinical signs, macroscopic and microscopic bursal lesions, as well as the viral load were reduced (Jackwood, 2011). Interestingly, infection at 10 days apart excluded the vvIBDV from establishing an infection as the variant viruses have destroyed most of the susceptible B lymphocytes (Jackwood, 2011).

### **Conventional IBD Vaccines**

In view of the changing situation in the field, immunisation with live vaccines become necessary to protect the chicks from vvIBDV infection and disease during the growing period (van den Berg & Meulemans, 1991). Although inactivated IBDV has been tested for oral immunisation in 3-week-old SPF chicks and was shown to be protective (Hoshi, Nakamura, Nunoya, & Ueda, 1995), the use of killed vaccines is mainly practised in breeder flocks to boost and prolong humoral immunity (van den Berg, 2000).

There are many choices of live vaccines and could be subjectively classified from mild, mild to intermediate, intermediate, intermediate plus or 'hot' (Etteradossi & Saif, 2008). Mild vaccine strains such as PBG98 and Lukert, as well as cell-culture adapted strain break through virus-neutralising (VN) titer of less than 1:100 and could be used in chicks with MDA titer less than 1:1000 (Skeeles, Lukert, Fletcher, & Leonard, 1979; Etteradossi & Saif, 2008). As the MDA in chicks decay over time with a half-life of about 4 days, high level of MDA during the early life may neutralise the mild vaccine viruses (Winterfield & Thacker, 1978; van den Berg *et al.*, 1991; Tsukamoto *et al.*, 1995) and they are therefore not advisable to be used in the first vaccination of the progeny (van den Berg, 2000).

Intermediate vaccines like D78 and SAL and 'hot' vaccine strains on the other hand could break through a VN antibody titers of 1:250 and 1:500, respectively (Etteradossi & Saif, 2008). They could neutralise the MDA and induce the development of active immunity in chickens, thus provide

protection to the chicks upon field virus challenge (Winterfield & Thacker, 1978; Rautenschlein *et al.*, 2005). It was shown that the 'hot' vaccine stimulated antibody production as early as 14 days post-vaccination in the presence of maternal VN antibodies while the intermediate vaccines took longer to induce the production of circulating IBD antibodies at 21 days post-vaccination (Rautenschlein, Kraemer, Vanmarcke, & Montiel, 2005). Nevertheless, intermediate vaccine was ineffective and failed to induce IBD antibody when administered via oral or eye-drops to chicks of one-day-old with high MDA (Hair-Bejo, M., Ng, & Ng, 2004).

Although the use of intermediate and intermediate plus vaccines do not induce mortality, they could produce severe bursal lesions and possibly resulted in immunosuppression in vaccinated chickens (Winterfield & Thacker, 1978; Rautenschlein, Yeh, & Sharma, 2003; Hair-Bejo *et al.*, 2004; Rautenschlein *et al.*, 2005; Etteradossi & Saif, 2008). In addition, Bursine 2 IBDV intermediate strain has been shown to cause lesions in non-bursal tissues like the spleen (Rautenschlein *et al.*, 2003). It was shown that viral antigens were detected in spleens from up to two thirds of the chickens vaccinated with intermediate vaccine strains, whereas the intermediate plus vaccine practically induced a severe bursal lesion resembling that of bursectomy (Rautenschlein *et al.*, 2005). It was speculated that the 'hot' vaccine viruses cause a severe destruction to the target lymphoid B cells, and thus able to reduce the impact of disease upon challenge with wild type virus. The use of 'hot' vaccines, although providing protection, is not safe as they carry higher inherent risk of reversion to virulence and may result in immunosuppression in chickens (van den Berg, 2000). Therefore, in an effort to develop appropriate vaccination programme for the farm, interference of MDA have become a critical factor in choosing the right vaccines (van den Berg, 2000). Flock profiling to determine the MDA levels in chicks and thereby predict the appropriate age for first vaccination is important (Etteradossi & Saif, 2008).

### **New Generation or Genetically-Engineered IBD Vaccines**

In addition to the conventional live and inactivated vaccines, genetically engineered IBD vaccines have also been developed as a result of improved understanding on the molecular structure and immunology of IBDV (Nagarajan & Kibenge, 1997). Generally, these could be divided into two main categories, reflective of their replicative nature upon delivery into the chicken.

#### *Non-replicative IBD vaccines*

Immunisation by DNA or subunit vaccines involve the use of non-replicating IBDV for induction of immune response in birds. DNA vaccination is based on direct inoculation of plasmid DNA encoding a target immunogen gene into subjects of study (Oshop, Elankumaran, & Heckert, 2002). Under the influence of a mammalian promoter, the target genes were expressed to produce proteins *in vivo* that are able to induce immune responses in the injected host. Repeated injections of DNA vaccines carrying the IBDV genes, either the polyprotein genes or gene of VP2 alone were shown to protect the chickens from challenge virus (Chang, Lin, & Wu, 2002; Chang, Lin, & Wu, 2003; Kim, Sung, Han, Jackwood, & Kwon, 2004; Hsieh, Wu, & Lin, 2010; Chen *et al.*, 2011). However, the presence of MDA could affect the efficacy of DNA vaccines and a high dose of DNA vaccines was required to overcome the interference of MDA and induce immune response in chickens (Hsieh *et al.*, 2010). In contrast to conventional live attenuated vaccines, DNA vaccines induce a low and nearly undetectable anti-IBDV antibody titers in ELISA (Chang *et al.*, 2002; Chang *et al.*, 2003). Even after virus challenge, it was reported that ELISA titres were low and some remained below the cut-off titer of 396 as given by the ELISA kit (Chang *et al.*, 2002; Chang *et al.*, 2003) although a separate study demonstrated otherwise (Kim *et al.*, 2004). A closer look into these two studies revealed the difference in the virulence of the challenge viruses used that might influence the post-challenge ELISA readings. In one of the trial from earlier study, the challenge virus used was a caIBDV STC

that induce from 0 to 20% mortality in the unvaccinated chickens (Chang *et al.*, 2002). In another study, the SH/92 vvIBDV was used and demonstrated to cause 90% mortality in unvaccinated and challenged control chickens (Kim *et al.*, 2004). The two studies nevertheless observed that almost all chickens vaccinated with DNA vaccines demonstrated antibody level not significantly different from the unvaccinated and challenged control.

In fact, the working mechanisms of DNA vaccines have not been fully elucidated and the work is still in its infancy (Oshop, Elankumaran, & Heckert, 2002). Nevertheless, many efforts have been made to improve the efficacy of DNA vaccines. For example, simultaneous administration of plasmid DNA carrying chicken cytokines like IL-2 (Hulse & Romero, 2004; Li *et al.*, 2004), IL-6 (Sun, Yan, Jiang, & Lu, 2005), or IFN- $\gamma$  (Hsieh *et al.*, 2006) were attempted to enhance the immune responses and thereby increase the level of protection. Furthermore, studies taking on the advantage of adjuvants such as CpG oligodeoxynucleotides (Wang *et al.*, 2003), cationic liposome (Li, Huang, Liang, Lu, Li, Yu, & Deng, 2003), or defensins (Zhang *et al.*, 2010) for DNA vaccines have also been reported. As DNA vaccination is still in trial stage, in addition to adjuvant, other factors such as the sequence of the target genes, choice of eukaryotic expression vector, route of administration, immunisation dosage and frequencies for proper optimization (Li *et al.*, 2003). Moreover, it was shown that a booster vaccination with inactivated IBD vaccine after priming with DNA vaccine provided better and higher protection to the chickens compared to injection with DNA vaccines alone, demonstrating the importance in designing appropriate vaccination strategy when using different types of vaccines (Hsieh *et al.*, 2007).

Subunit vaccination, on the other hand, involves immunisation with IBDV proteins expressed from *in vitro* systems like the baculovirus (Vakharia *et al.*, 1993; Pitcovski *et al.*, 1996; Dybing & Jackwood, 1997; Wang *et al.*, 2000; Martinez-Torrecuadrada *et al.*, 2003) or yeast (Fahey *et al.*, 1991; Pitcovski *et al.*, 2003; Villegas, Hamoud, Purvis, & Perozo, 2008). The generated recombinant protein, mainly the VP2 protein emulsified in oil adjuvants, has been shown to provide protection against the disease when given to the chickens (Vakharia *et al.*, 1993; Pitcovski *et al.*, 1996; Dybing & Jackwood, 1998; Pitcovski *et al.*, 2003; Villegas, Hamoud, Purvis, & Perozo, 2008) and progenies from the vaccinated hens were also shown to be passively protected (Fahey *et al.*, 1991; Vakharia *et al.*, 1994; Yehuda *et al.*, 2000). Such findings have highlighted the potential of recombinant vaccines in replacing the conventional inactivated vaccines and functioning as the booster vaccine in vaccination programme of the breeding hens.

Furthermore, the virus-like particles (VLPs) of IBDV have been produced through the baculovirus expression system (Kibenge, Qian, Nagy, Cleghorn, & Wadowska, 1999; Hu, Bentley, Edwards, & Vakharia, 1999; Jackwood, 2012b), and when used in immunisation trials, it was shown that the crude VLPs elicited immune responses in vaccinated chickens (Jackwood, 2012b). Compared to the chickens in the unvaccinated and challenged control group where all developed lesions in the bursa of Fabricius, up to 90% to 100% of the chickens vaccinated with crude VLPs were protected from bursal lesions upon challenge (Jackwood, 2012b). Nevertheless, the efficacy of the recombinant IBDV subunit vaccine was conformational dependent as chickens vaccinated with particulates containing VP2 capsids were shown to induce stronger neutralising antibodies than those vaccinated with pVP2 (VPX) tubules or polyprotein-derived mixture of VLPs and capped tubules (Martinez-Torrecuadrada *et al.*, 2003).

#### *Replication-competent IBD vaccines*

In addition, replication-competent viral vectors have been utilized to express and deliver immunogens of interest to chickens. In contrast to DNA and subunit vaccines, vaccination by live recombinant

virus vectors employed the use of live and replicating virus to produce IBDV antigen upon in vivo infection (Tsukamoto *et al.*, 2002). They have been shown to elicit both humoral and cell-mediated immune response in the chickens. As they could persistently infect the chickens, the potential of having a long-term protective immunity is high (Tsukamoto *et al.*, 2002). Besides, the recombinant viral vectors are less sensitive to MDA and could therefore evade the neutralisation by the maternal anti-IBDV antibody (van den Berg, 2000).

Several viruses have been experimented to express the VP2 protein of IBDV. This includes fowlpox virus (Bayliss *et al.*, 1991; Heine & Boyle, 1993), turkey herpesvirus (Darteil *et al.*, 1995; Tsukamoto *et al.*, 2002; Perozo, Villegas, Fernandez, Cruz, & Pritchard, 2009), fowl adenovirus (Sheppard *et al.*, 1998), Marek's disease virus (Tsukamoto *et al.*, 1999), Newcastle disease virus (Huang *et al.*, 2004), and avian adeno-associated virus (Perozo, Villegas, Estévez, Alvarado, & Purvis, 2007) among others. The VP2 protein expressed in vivo from these various studies have been shown to confer from partial to full protection to vaccinated chickens from mortality, although they do not prevent the damage to the bursa (Bayliss *et al.*, 1991; Heine & Boyle, 1993; Sheppard *et al.*, 1998; Tsukamoto *et al.*, 1999; Tsukamoto *et al.*, 2002; Huang, Elankumaran, Yunus, & Samal, 2004; Perozo *et al.*, 2008; Perozo *et al.*, 2009; Zhou *et al.*, 2010). Further, the commercially available recombinant herpesvirus of turkey vaccine expressing the IBDV VP2 antigen (Vaxxitek®; Merial) has been shown to induce immunity and confer protection to the chicks in the face of MDA (Bublout *et al.*, 2007; Lemiere *et al.*, 2011b). The vaccine has also been shown to be suitable for day-old chicks and compatible with vaccines like Marek's disease (Lemiere *et al.*, 2011a) and Newcastle disease for hatchery vaccination (Lemiere *et al.*, 2011b).

In addition, through the chimeric virus particles (CVPs) that employ epitope presentation strategy, insect baculovirus displaying IBDV VP2 gene (Xu *et al.*, 2011) or plant *Bamboo mosaic virus* presenting the VP2 P domain loop P<sub>BC</sub> of a vvIBDV (Chen *et al.*, 2012) has been produced. These studies showed that the recombinant viruses were able to induce protective immunity upon IBDV challenge, highlighting the feasibility of using CVPs for propagation of recombinant vaccine virus in non-animal system.

The reverse genetic system is another approach to produce live and replicating IBDV (Mundt & Vakharia, 1996; Boot, ter Huurne, Peeters, & Gielkens, 1999). Infectious virus could be rescued from the cell cultures transfected by synthetic RNA (Mundt & Vakharia, 1996) or cDNA (Boot *et al.*, 1999) sequences of IBDV. The system allows deliberate manipulation of the IBDV genes and thus produce attenuated strains with desired vaccine characteristics (van Loon, de Haas, Zeyda, & Mundt, 2002; Qin *et al.*, 2010; Gao *et al.*, 2011). Targeted mutagenesis at specific amino acids of VP2 have allowed direct adaptation of the vvIBDV to chicken embryo cell culture without going through extensive passages in cell culture, chorioallantoic membrane, or yolk sac of embryonated eggs (Lim, Cao, Yu, & Mo, 1999; van Loon *et al.*, 2002). The cell culture adapted vvIBDV was shown to be significantly attenuated in chickens and failed to induce mortality upon infection (van Loon *et al.*, 2002). In addition, IBDV with deleted VP5 gene (Qin *et al.*, 2010) or containing cell culture adapted VP2 gene of the vvIBDV (Gao *et al.*, 2011) were shown to confer protection to chickens upon challenge by the vvIBDV. Even though the use of reverse genetic system permits generation of new vaccines with desired characteristics, they have the risk of reversion (Raue *et al.*, 2004). It was shown that infection of the cell culture adapted IBDV in commercial chickens had seen the virus reverted to wild-type phenotype on 3 days post-inoculation. Besides, the use of rescued virus as vaccines will still need to face the interference of MDA (van den Berg, 2000).

Although the high titer of MDA has been linked to neutralisation of IBDV vaccines when given to day-old commercial chicks, it was speculated that the vaccine virus actually formed complexes with maternal antibodies (Rautenschlein *et al.*, 2005). This is highly possible because IBD immune complex formulated by mixing live IBDV with anti-IBDV antibodies has been shown to be efficacious when used as vaccine (Whitfill *et al.*, 1995). Day-old SPF chicks inoculated with the immune complexes were shown to have delayed appearance of lesions in the bursa until after 6 to 8 days of age and demonstrated active antibody production at 22 days old, which coincided with the induction of bursa lesions (Whitfill *et al.*, 1995). Likewise, the use of immune complex for day-old IBD vaccination in commercial chicks with MDA has seen the development of active immunity and protection from challenge in these chicks (Haddad *et al.*, 1997). Interestingly, such phenomenon has been observed with the use of the less attenuated 'hot' vaccines, where they induced the production of antibody in the presence of MDA that was correlated with the appearance of lesions and detection of IBDV antigen in the bursa of Fabricius (Rautenschlein *et al.*, 2005). Indeed, it was proposed that the immune complex vaccine was initially trapped within the follicular dendritic cells in both the spleen and bursa (Jeurissen *et al.*, 1998). The virus were then released from the antibody possibly through dissociation, degradation, or a mechanism yet to be known, and caused infection to chickens. Nevertheless, how the type of MDA present within the chicks and the degree of virulence of challenge virus in the field can influence the protective efficacy of the immune complex IBD vaccine would require further studies.

### ***In ovo* Vaccination**

In contrast to at-hatch or post-hatch vaccination using either conventional or newer generation molecular-based IBD vaccines that have been described earlier, *in ovo* vaccination generally deliver vaccines to the chicken embryo at around 18 days of incubation by automated injection through the eggshells (Johnston *et al.*, 1997). This reduces the handling cost of post-hatch or on-farm vaccination. The approach of using immune complex IBD vaccine has been tested in *in ovo* vaccination and shown to confer protection to chicks upon challenge on day 21 or 28 post-hatch (Johnston *et al.*, 1997). The 18-day old chicken embryo inoculated *in ovo* did not develop bursal lesions until 5 days after hatching and showed increased antibody production between 21 to 28 days of age concomitant with diminished MDA levels (Johnston *et al.*, 1997). Depending on the level of MDA in commercial chicks, the immune complex vaccine when given *in ovo* could protect up to 83% of the broilers from bursal lesions upon challenge (Corley, Giambrone, & Dormitorio, 2002), although the percentage is lower in SPF chicks (Corley, Giambrone, & Dormitorio, 2001).

In addition to immune complex vaccines, live attenuated IBD vaccines (Sharma, 1986; Coletti *et al.*, 2001; Giambrone, Dormitorio, & Brown, 2001), DNA vaccine carrying IBDV VP2 gene (Haygreen, Kaiser, Burgess, & Davison, 2006), or recombinant Newcastle disease virus expressing VP2 gene of a vvIBDV (Ge *et al.*, 2014) have also been tested *in ovo*. Compared to post-hatch vaccination, *in ovo* injection of a live intermediate vaccine allowed faster recovery from bursa lesions although both methods exhibited similar protection against challenge (Rautenschlein & Haase, 2005). Experiments showed that the *in ovo* vaccination with live intermediate vaccines provided complete protection to the SPF birds against challenge (Coletti *et al.*, 2001; Rautenschlein & Haase, 2005) while the protection was partial in the commercial broilers (Coletti *et al.*, 2001; Giambrone *et al.*, 2001). Depending on the virulence and dosage of the vaccine strains, vaccination *in ovo* may cause increased of post-hatch mortality (Giambrone *et al.*, 2001) and immunosuppression (Corley & Giambrone, 2002). Although *in ovo* delivery of vaccines is an attractive alternative to post-hatch vaccination, various factors including the dosage, virulence, and efficacy, among others must be properly optimized before pursuing large scale vaccinations.

### **Plant-Produced IBD Vaccines**

The plant-based expression system is a growing alternative platform for production and development of animal vaccines (Floss, Falkenburg, & Conrad, 2007; Liew & Hair-Bejo, 2015). Being one of the pathogenic agents of importance in poultry, plant-based expression system using the stable (Wu *et al.*, 2004; Wu *et al.*, 2007; Liew *et al.*, 2015), transient (Gómez *et al.*, 2013), or chimeric viral particles (Chen *et al.*, 2012) approach has been used to produce IBD vaccine containing VP2 capsid protein. Transgenic rice expressing the VP2 protein was shown to protect the chickens from challenge following oral immunisation (Wu *et al.*, 2007). Compared to chickens that received live attenuated vaccine, chickens fed orally with transgenic rice achieved better lesion score when evaluated based on lesion scoring of the bursa after challenge. Moreover, immunofluorescence assay revealed that the amount of antigen present in the bursal tissue was also lesser in the orally immunised chickens (Wu *et al.*, 2007).

Recently, the VP2 protein of IBDV has been transiently expressed in *Nicotiana benthamiana* leaves and extracted for subunit vaccination in chicken (Gómez *et al.*, 2013). The recombinant VP2 protein emulsified in oil adjuvant, injected intramuscularly to chicks at 18 days of age and followed by booster doses after 22 and 35 days, were shown to induce the production of anti-IBDV antibody with neutralising ability in these chicks. Further, replication-competent viral vector using plant virus has been studied for its feasibility for production of IBDV proteins in plant-based expression system. It was shown that the CVPs carrying an immunogenic domain of VP2 were viable and allowed for propagation in plants (Chen *et al.*, 2012). Moreover, the CVPs emulsified in oil adjuvant were able to induce IBDV-specific antibodies in 3-week-old SPF chickens and protected them against challenge from a vvIBDV at 28 days post-vaccination (Chen *et al.*, 2012). These studies have concluded that plants represent a promising expression system for the mass production of immunogenic proteins that can be conveniently used as vaccines for chickens.

### **Conclusion and Perspectives**

Thus far, four types of IBD vaccines are commercially available in Malaysia for prevention of IBD, which include the conventional attenuated live or killed vaccines, and new generation viral vector based vaccines using herpesvirus of turkey and IBD immune complex vaccine. Many IBD vaccines produced in the newer generation production systems have yet to make it to commercialisation, mainly due to issues like safety and protective efficacy. As animal experimental trials do not fully reflect the field condition, additional optimisation and testing in the field are required. On the other hand, how feasible a vaccine can be applied in the field depends not only on safety and efficacy, but also affordability and availability of the vaccine. The herpesvirus of turkey based viral vector and immune complex vaccines can be as much as 10 times more expensive than the conventional live or killed vaccines. Most poultry producers especially the small farm holders will therefore tend to shun away from the new generation IBD vaccines. The use of killed vaccines generally involve injections and could trigger tissue reactions, thus it is not suitable for use in broiler birds due to potential carcasses condemnation at slaughter house. Although the live attenuated vaccines represent the cheapest options of vaccine, interference of MDA is a critical factor in its implementation. The choice of live vaccines is also subjected to the endemicity of the disease; when the challenge is high, the use of intermediate and intermediate plus vaccines will be more practical and effective in preventing an outbreak. In addition, vaccination strategy, be it *in ovo*, at-hatch or on-farm vaccinations, determines the choice of vaccines used in the farm. Compared to farm vaccination, hatchery vaccination enables large scale operation with relatively lesser trained personnel and manpower. Most of the major poultry producers are more inclined to opt for hatchery vaccination as it could cut down the cost of production in the farm. It must be noted that no universal vaccination programme for IBD could be implemented in all farms due to factors influencing the maternal immunity, multifaceted nature of the disease, farm

conditions, as well as management decisions. Therefore, the feasibility in implementation of an IBD vaccine on the farm lies essentially on its vaccination programme in the farm, after taking into consideration on the safety, efficacy, affordability and availability of the vaccine.

Infectious bursal disease has been an endemic viral disease of poultry worldwide and was first reported in Malaysia in 1991. Despite the introduction of vaccination, occasional outbreaks are still reported from the field. A growing body of evidence revealed that both genome segments A and B of IBDV contribute to the viral virulence and pathogenicity. It thus becomes crucial to carry out the examination on both genome segments A and B of IBDV for molecular diagnosis and genetic assessment. Moreover, this has also signified the importance to include IBDV genome segments A and B in the vaccine preparation as vaccination is the primary control of IBD in poultry. The current selection of IBD vaccines are insufficient under the ever changing disease situation in the field and the industry always thirst for a better vaccine to control the disease. Therefore, the needs for IBD vaccines that could be tailored and revised according to the field situation persist. Such incessant needs signify not only good prospects for continuous development towards ideal IBD vaccines, but also represent challenges to the researchers and scientists in designing effective vaccine that can be delivered to the field on a timely basis to quench the thirst. Likewise, appropriate and effective vaccination programmes must be established. The programme will require revision from time to time in pace with the changes in the field to protect the chickens from unintended outbreak or immunosuppression, and to allow for establishment of cost-effective vaccination schedules. With better and continuous advancement of knowledge on the molecular and immunological biology of IBDV, more safe and effective IBD vaccines that are affordable and readily available can be developed in a near future as a part of the initiative to curb the disease.

## References

- Al-Natour, M. Q., Ward, L. A., Saif, Y. M., Stewart-Brown, B., & Keck, L. D. (2004). Effect of different levels of maternally derived antibodies on protection against infectious bursal disease virus. *Avian Diseases*, 48(1), 177-182.
- Azad, A. A., Barrett, S. A., & Fahey, K. J. (1985). The characterization and molecular cloning of the double-stranded RNA genome of an Australian strain of infectious bursal disease virus. *Virology*, 143(1), 35-44.
- Bayliss, C. D., Peters, R. W., Cook, J. K., Reece, R. L., Howes, K., Binns, M. M., & Boursnell, M. E. G. (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(3-4), 193-205.
- Boot, H. J., ter Huurne, A. A. H., Peeters, B. P., & Gielkens, A. L. (1999). Efficient rescue of infectious bursal disease virus from cloned cDNA: evidence for involvement of the 3'-terminal sequence in genome replication. *Virology*, 265(2), 330-341.
- Bublott, M., Pritchard, N., Le Gros, F. X., & Goutebroze, S. (2007). Use of a vectored vaccine against infectious bursal disease of chickens in the face of high-titred maternally derived antibody. *Journal of Comparative Pathology*, 137, S81-S84.
- Chang, H. C., Lin, T. L., & Wu, C. C. (2002). DNA-mediated vaccination against infectious bursal disease in chickens. *Vaccine*, 20(3), 328-335.
- Chang, H. C., Lin, T. L., & Wu, C. C. (2003). DNA vaccination with plasmids containing various fragments of large segment genome of infectious bursal disease virus. *Vaccine*, 21(5), 507-513.
- Chen, Y. Y., Hsieh, M. K., Tung, C. Y., Wu, C. C., & Lin, T. L. (2011). Infectious bursal disease DNA vaccination conferring protection by delayed appearance and rapid clearance of invading viruses. *Archives of Virology*, 156(12), 2241-2250.
- Chen, T. H., Chen, T. H., Hu, C. C., Liao, J. T., Lee, C. W., Liao, J. W., Lin, M. Y., Liu, H. J., Wang, M. Y., Lin, N. S., & Hsu, Y. H. (2012). Induction of protective immunity in chickens immunized

- with plant-made chimeric bamboo mosaic virus particles expressing very virulent infectious bursal disease virus antigen. *Virus Research*, 166(1), 109-115.
- Coletti, M., Del Rossi, E., Franciosini, M. P., Passamonti, F., Tacconi, G., & Marini, C. (2001). Efficacy and safety of an infectious bursal disease virus intermediate vaccine *in ovo*. *Avian Diseases*, 1036-1043.
- Corley, M. M., Giambrone, J. J., & Dormitorio, T. V. (2001). Detection of infectious bursal disease vaccine viruses in lymphoid tissues after *in ovo* vaccination of specific-pathogen-free embryos. *Avian Diseases*, 45(4), 897-905.
- Corley, M. M., Giambrone, J. J., & Dormitorio, T. V. (2002). Evaluation of the immune response and detection of infectious bursal disease viruses by reverse transcriptase-polymerase chain reaction and enzyme-linked immunosorbent assay after *in ovo* vaccination of commercial broilers. *Avian Diseases*, 46(4), 803-809.
- Corley, M. M., & Giambrone, J. J. (2002). Immunosuppression in specific-pathogen-free broilers administered infectious bursal disease virus vaccines by *in ovo* route. *Avian Diseases*, 46(4), 810-815.
- Darteil, R., Bublot, M., Laplace, E., Bouquet, J. F., Audonnet, J. C., & Rivière, M. (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(2), 481-490.
- Delmas, B., Kibenge, F. S. B., Leong, J. C., Mundt, E., Vakharia, V. N., & Wu, J. L. (2005). *Birnaviridae*. In C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, & L. A. Ball (Eds.), *Virus taxonomy: VIIIth report of the International Committee on Taxonomy of Viruses* (pp. 570-578). Academic Press.
- Dybing, J. K., & Jackwood, D. J. (1997). Expression of MD infectious bursal disease viral proteins in baculovirus. *Avian Diseases*, (41)3, 617-626.
- Dybing, J. K., & Jackwood, D. J. (1998). Antigenic and immunogenic properties of baculovirus-expressed infectious bursal disease viral proteins. *Avian diseases*, (42)1, 80-91.
- Escaffre, O., Le Nouën, C., Amelot, M., Ambroggio, X., Ogden, K. M., Guionie, O., Toquin, D., Müller, H., Islam, M. R., & Eterradossi, N. (2013). Both genome segments contribute to the pathogenicity of very virulent infectious bursal disease virus. *Journal of Virology*, 87(5), 2767-2780.
- Eterradossi, N., Arnauld, C., Tekaiia, F., Toquin, D., Le Coq, H., Rivallan, G., Guittet, M., Domenech, J., van den Berg, T. P. & Skinner, M. A. (1999). Antigenic and genetic relationships between European very virulent infectious bursal disease viruses and an early West African isolate. *Avian Pathology*, 28(1), 36-46.
- Eterradossi, N., & Saif, Y. M. (2008). Infectious bursal disease. In Y. M. Saif, A. M. Fadly, J. R. Glisson, L. R. McDougald, L. K. Nolan, & D. E. Swayne (Eds.), *Diseases of Poultry* (pp. 185-208). Ames, IA: Blackwell Publishing Professional.
- Fahey, K. J., Chapman, A. J., Macreadie, I. G., Vaughan, P. R., McKern, N. M., Skicko, J. I., Ward, C. W., & Azad, A. A. (1991). A recombinant subunit vaccine that protects progeny chickens from infectious bursal disease. *Avian Pathology*, 20(3), 447-460.
- Floss, D. M., Falkenburg, D., & Conrad, U. (2007). Production of vaccines and therapeutic antibodies for veterinary applications in transgenic plants: an overview. *Transgenic Research*, 16(3), 315-332.
- Gao, L., Qi, X., Li, K., Gao, H., Gao, Y., Qin, L., Wang, Y., & Wang, X. (2011). Development of a tailored vaccine against challenge with very virulent infectious bursal disease virus of chickens using reverse genetics. *Vaccine*, 29(33), 5550-5557.
- Ge, J., Wang, X., Tian, M., Wen, Z., Feng, Q., Qi, X., Gao, H., Wang, X., & Bu, Z. (2014). Novel *in-ovo* chimeric recombinant Newcastle disease vaccine protects against both Newcastle disease and infectious bursal disease. *Vaccine*, 32(13), 1514-1521.
- Giambrone, J. J., Dormitorio, T., & Brown, T. (2001). Safety and efficacy of *in ovo* administration of infectious bursal disease viral vaccines. *Avian Diseases*, 144-148.
- Gómez, E., Lucero, M. S., Zoth, S. C., Carballeda, J. M., Gravisaco, M. J., & Berinstein, A. (2013). Transient expression of VP2 in *Nicotiana benthamiana* and its use as a plant-based vaccine against infectious bursal disease virus. *Vaccine*, 31(23), 2623-2627.

- Haddad, E. E., Whitfill, C. E., Avakian, A. P., Ricks, C. A., Andrews, P. D., Thoma, J. A., & Wakenell, P. S. (1997). Efficacy of a novel infectious bursal disease virus immune complex vaccine in broiler chickens. *Avian Diseases*, *41*(4), 882-889.
- Hair-Bejo, M. (1992). An outbreak of infectious bursal disease in broilers. *Jurnal Veterinar Malaysia*, *4*(168), 124-128.
- Hair-Bejo, M., Ng, M. K., & Ng, H. Y. (2004). Day old vaccination against infectious bursal disease in broiler chickens. *International Journal of Poultry Science*, *8*, 124-128.
- Haygreen, E. A., Kaiser, P., Burgess, S. C., & Davison, T. F. (2006). *In ovo* DNA immunisation followed by a recombinant fowlpox boost is fully protective to challenge with virulent IBDV. *Vaccine*, *24*(23), 4951-4961.
- He, C. Q., Ma, L. Y., Wang, D., Li, G. R., & Ding, N. Z. (2009). Homologous recombination is apparent in infectious bursal disease virus. *Virology*, *384*(1), 51-58.
- Heine, H. G., Haritou, M., Failla, P., Fahey, K., & Azad, A. (1991). Sequence analysis and expression of the host-protective immunogen VP2 of a variant strain of infectious bursal disease virus which can circumvent vaccination with standard type I strains. *Journal of General Virology*, *72*, 1835-1843.
- Heine, H. G., & Boyle, D. B. (1993). Infectious bursal disease virus structural protein VP 2 expressed by a fowlpox virus recombinant confers protection against disease in chickens. *Archives of Virology*, *131*(3-4), 277-292.
- Hon, C. C., Lam, T. T. Y., Yip, C. W., Wong, R. T. Y., Shi, M., Jiang, J., Zeng, F., & Leung, F. C. C. (2008). Phylogenetic evidence for homologous recombination within the family Birnaviridae. *Journal of General Virology*, *89*(12), 3156-3164.
- Hoshi, S., Nakamura, T., Nunoya, T., & Ueda, S. (1995). Induction of protective immunity in chickens orally immunized with inactivated infectious bursal disease virus. *Vaccine*, *13*(3), 245-252.
- Hsieh, M. K., Wu, C. C., & Lin, T. L. (2006). The effect of co-administration of DNA carrying chicken interferon- $\gamma$  gene on protection of chickens against infectious bursal disease by DNA-mediated vaccination. *Vaccine*, *24*(47), 6955-6965.
- Hsieh, M. K., Wu, C. C., & Lin, T. L. (2007). Priming with DNA vaccine and boosting with killed vaccine conferring protection of chickens against infectious bursal disease. *Vaccine*, *25*(29), 5417-5427.
- Hsieh, M. K., Wu, C. C., & Lin, T. L. (2010). DNA-mediated vaccination conferring protection against infectious bursal disease in broiler chickens in the presence of maternal antibody. *Vaccine*, *28*(23), 3936-3943.
- Hu, Y. C., Bentley, W. E., Edwards, G. H., & Vakharia, V. N. (1999). Chimeric infectious bursal disease virus-like particles expressed in insect cells and purified by immobilized metal affinity chromatography. *Biotechnology and Bioengineering*, *63*(6), 721-729.
- Huang, Z., Elankumaran, S., Yunus, A. S., & Samal, S. K. (2004). A recombinant Newcastle disease virus (NDV) expressing VP2 protein of infectious bursal disease virus (IBDV) protects against NDV and IBDV. *Journal of Virology*, *78*, 10054-10063.
- Hudson, P. J., McKern, N. M., Power, B. E., & Azad, A. A. (1986). Genomic structure of the large RNA segment of infectious bursal disease virus. *Nucleic Acids Research*, *14*(12), 5001-5012.
- Hulse, D. J., & Romero, C. H. (2004). Partial protection against infectious bursal disease virus through DNA-mediated vaccination with the VP2 capsid protein and chicken IL-2 genes. *Vaccine*, *22*(9), 1249-1259.
- Irigoyen, N., Garriga, D., Navarro, A., Verdaguier, N., Rodríguez, J. F., & Castón, J. R. (2009). Autoproteolytic activity derived from the infectious bursal disease virus capsid protein. *Journal of Biological Chemistry*, *284*(12), 8064-8072.
- Irigoyen, N., Castón, J. R., & Rodríguez, J. F. (2012). Host proteolytic activity is necessary for infectious bursal disease virus capsid protein assembly. *Journal of Biological Chemistry*, *287*(29), 24473-24482.
- Jackwood, D. H., & Saif, Y. M. (1987). Antigenic diversity of infectious bursal disease viruses. *Avian Diseases*, *31*(4), 766-770.

- Jackwood, D. J., Sommer-Wagner, S. E., Stoute, S. T., Woolcock, P. R., Crossley, B. M., Hietala, S. K., & Charlton, B. R. (2009). Characteristics of a very virulent infectious bursal disease virus from California. *Avian Diseases*, 53(4), 592-600.
- Jackwood, D. J. (2011). Viral competition and maternal immunity influence the clinical disease caused by very virulent infectious bursal disease virus. *Avian diseases*, 55(3), 398-406.
- Jackwood, D. J., Sommer-Wagner, S. E., Crossley, B. M., Stoute, S. T., Woolcock, P. R., & Charlton, B. R. (2011). Identification and pathogenicity of a natural reassortant between a very virulent serotype 1 infectious bursal disease virus (IBDV) and a serotype 2 IBDV. *Virology*, 420(2), 98-105.
- Jackwood, D. J. (2012a). Molecular epidemiologic evidence of homologous recombination in infectious bursal disease viruses. *Avian Diseases*, 56(3), 574-577.
- Jackwood, D. J. (2012b). Multivalent virus-like-particle vaccine protects against classic and variant infectious bursal disease viruses. *Avian Diseases*, 57(1), 41-50.
- Jeurissen, S. H., Janse, E. M., Lehrbach, P. R., Haddad, E. E., Avakian, A., & Whitfill, C. E. (1998). The working mechanism of an immune complex vaccine that protects chickens against infectious bursal disease. *Immunology*, 95(3), 494-500.
- Johnston, P. A., Liu, H., O'Connell, T., Phelps, P., Bland, M., Tyczkowski, J., Kemper, A., Harding, T., Avakian, A., Haddad, E., Whitfill, C., Gildersleeve, R. & Ricks, C. A. (1997). Applications in *in ovo* technology. *Poultry Science*, 76, 165-178.
- Kibenge, F. S. B., Dhillon, A. S., & Russell, R. G. (1988). Biochemistry and Immunology of Infectious Bursal Disease Virus. *Journal of General Virology*, 69(8), 1757-1775.
- Kibenge, F. S., Qian, B., Nagy, E., Cleghorn, J. R., & Wadowska, D. (1999). Formation of virus-like particles when the polyprotein gene (segment A) of infectious bursal disease virus is expressed in insect cells. *Canadian Journal of Veterinary Research*, 63(1), 49-55.
- Kim, S. J., Sung, H. W., Han, J. H., Jackwood, D., & Kwon, H. M. (2004). Protection against very virulent infectious bursal disease virus in chickens immunized with DNA vaccines. *Veterinary Microbiology*, 101(1), 39-51.
- Lana, D. P., Beisel, C. E., & Silva, R. F. (1992). Genetic mechanisms of antigenic variation in infectious bursal disease virus: analysis of a naturally occurring variant virus. *Virus Genes*, 6(3), 247-259.
- Lasher, H. N., & Shane, S. (1994). Infectious bursal disease. *World's Poultry Science Journal*, 50(02), 133-166.
- Le Nouën, C., Rivallan, G., Toquin, D., Darlu, P., Morin, Y., Beven, V., de Boisseson, C., Cazaban, C., Comte, S., Gardin, Y., & Etteradossi, N. (2006). Very virulent infectious bursal disease virus: reduced pathogenicity in a rare natural segment-B-reassorted isolate. *Journal of General Virology*, 87(1), 209-216.
- Le Nouën, C., Toquin, D., Müller, H., Raue, R., Kean, K. M., Langlois, P., Cherbonnel, M., & Etteradossi, N. (2012). Different domains of the RNA polymerase of infectious bursal disease virus contribute to virulence. *PloS One*, 7(1), e28064.
- Lemiere, S., Wong, S. Y., Saint-Gerand, A. L., Goutebroze, S., & Le Gros, F. X. (2011a). Compatibility of turkey herpesvirus-infectious bursal disease vector vaccine with Marek's disease rispens vaccine injected into day-old pullets. *Avian diseases*, 55(1), 113-118.
- Lemiere, S., Fernández, R., Pritchard, N., Cruz-Coy, J., Rojo, F., Wong, S. Y., Saint-Gerand, A. L., Gauthier, J. C., & Perozo, F. (2011b). Concomitant turkey herpesvirus-infectious bursal disease vector vaccine and oil-adjuvanted inactivated Newcastle disease vaccine administration: consequences for vaccine intake and protection. *Avian diseases*, 55(4), 642-649.
- Li, J., Huang, Y., Liang, X., Lu, M., Li, L., Yu, L., & Deng, R. (2003). Plasmid DNA encoding antigens of infectious bursal disease viruses induce protective immune responses in chickens: factors influencing efficacy. *Virus Research*, 98(1), 63-74.
- Li, J., Liang, X., Huang, Y., Meng, S., Xie, R., Deng, R., & Yu, L. (2004). Enhancement of the immunogenicity of DNA vaccine against infectious bursal disease virus by co-delivery with plasmid encoding chicken interleukin 2. *Virology*, 329(1), 89-100.
- Liew, P.S., Omar, A.R., Ideris, A., & Hair-Bejo, M. (2013). Complete coding sequence and phylogenetic analyses of very virulent infectious bursal disease virus isolate of Malaysia. In *Proceeding of WPSA (Malaysia Branch) and WVPA (Malaysia Branch) Scientific Conference-*

- Advancing Poultry Production for Food Security*, 30<sup>th</sup> November- 1<sup>st</sup> December 2013, Faculty of Veterinary Medicine, Selangor, pp. 99-100.
- Liew, P.S., Nurulfiza, M. I., Omar, A.R., Ideris, A., & Hair-Bejo, M. (2015). Expression of Segment A Gene of UPM04/190 Infectious Bursal Disease Virus in *Arabidopsis thaliana*. In *Proceeding of WPSA (Malaysia Branch) and WVPA (Malaysia Branch) Scientific Conference- Enhancing Innovation in Poultry Health and Production*, 21<sup>st</sup>- 22<sup>nd</sup> September 2015, Kuala Lumpur Convention Centre, Malaysia, pp. 175-177.
- Liew, P. S., & Hair-Bejo, M. (2015). Farming of plant-based veterinary vaccines and their applications for disease prevention in animals. *Advances in Virology*. <http://dx.doi.org/10.1155/2015/936940>, 12 pages.
- Lim, B. L., Cao, Y., Yu, T., & Mo, C. W. (1999). Adaptation of very virulent infectious bursal disease virus to chicken embryonic fibroblasts by site-directed mutagenesis of residues 279 and 284 of viral coat protein VP2. *Journal of Virology*, 73(4), 2854-2862.
- Lin, Z., Kato, A., Otaki, Y., Nakamura, T., Sasmaz, E., & Ueda, S. (1993). Sequence comparisons of a highly virulent infectious bursal disease virus prevalent in Japan. *Avian Diseases*, 37(2), 315-323.
- Lucio, B., & Hitchner, S. B. (1979). Infectious bursal disease emulsified vaccine: effect upon neutralizing-antibody levels in the dam and subsequent protection of the progeny. *Avian Diseases*, 466-478.
- Lucio, B., & Hitchner, S. B. (1980). Immunosuppression and active response induced by infectious bursal disease virus in chickens with passive antibodies. *Avian Diseases*, 24(1), 189-196.
- Maas, R. A., Venema, S., Oei, H. L., Pol, J. M., Claassen, I. J., & ter Huurne, A. A. (2001). Efficacy of inactivated infectious bursal disease (IBD) vaccines: comparison of serology with protection of progeny chickens against IBD virus strains of varying virulence. *Avian Pathology*, 30(4), 345-354.
- Martinez-Torrecuadrada, J. L., Saubi, N., Pagès-Manté, A., Castón, J. R., Espuña, E., & Casal, J. I. (2003). Structure-dependent efficacy of infectious bursal disease virus (IBDV) recombinant vaccines. *Vaccine*, 21(23), 3342-3350.
- Morgan, M. M., Macreadie, I. G., Harley, V. R., Hudson, P. J., & Azad, A. A. (1988). Sequence of the small double-stranded RNA genomic segment of infectious bursal disease virus and its deduced 90-kDa product. *Virology*, 163(1), 240-242.
- Müller, H., Scholtissek, C., & Becht, H. (1979). The genome of infectious bursal disease virus consists of two segments of double-stranded RNA. *Journal of Virology*, 31(3), 584-589.
- Müller, H., & Becht, H. (1982). Biosynthesis of virus-specific proteins in cells infected with infectious bursal disease virus and their significance as structural elements for infectious virus and incomplete particles. *Journal of Virology*, 44(1), 384-392.
- Mundt, E., Beyer, J., & Müller, H. (1995). Identification of a novel viral protein in infectious bursal disease virus-infected cells. *Journal of General Virology*, 76, 437-443.
- Mundt, E., & Vakharia, V. N. (1996). Synthetic transcripts of double-stranded Birnavirus genome are infectious. *PNAS*, 93(20), 11131-11136.
- Nagarajan, M. M., & Kibenge, F. S. (1997). Infectious bursal disease virus: a review of molecular basis for variations in antigenicity and virulence. *Canadian Journal of Veterinary Research*, 61(2), 81-88.
- Naqi, S. A., Marquez, B., & Sahin, N. (1983). Maternal antibody and its effect on infectious bursal disease immunization. *Avian Diseases*, 27(3), 623-631.
- Nick, H., Cursiefen, D., & Becht, H. (1976). Structural and growth characteristics of infectious bursal disease virus. *Journal of Virology*, 18(1), 227-234.
- Nunoya, T., Otaki, Y., Tajima, M., Hiraga, M., & Saito, T. (1992). Occurrence of acute infectious bursal disease with high mortality in Japan and pathogenicity of field isolates in specific-pathogen-free chickens. *Avian Diseases*, 36(3), 597-609.
- Oshop, G. L., Elankumaran, S., & Heckert, R. A. (2002). DNA vaccination in the avian. *Veterinary Immunology and Immunopathology*, 89(1), 1-12.
- Perozo, F., Villegas, P., Estévez, C., Alvarado, I., & Purvis, L. (2007). A recombinant avian adeno-associated virus as a vector for infectious bursal disease vaccination. *Revista Científica*, 17(6).

- Perozo, F., Villegas, P., Estévez, C., Alvarado, I. R., Purvis, L. B., & Williams, S. (2008). Protection against infectious bursal disease virulent challenge conferred by a recombinant avian adeno-associated virus vaccine. *Avian Diseases*, 52(2), 315-319.
- Perozo, F., Villegas, P., Fernandez, R., Cruz, J., & Pritchard, N. (2009). Efficacy of single dose recombinant HVT-IBDV vaccination against a variant IBDV strain. *Avian Diseases*, 53(4), 624-628.
- Pitcovski, J., Di-Castro, D., Shaaltiel, Y., Azriel, A., Gutter, B., Yarkoni, E., Michael, A., Krispel, S., & Levi, B. Z. (1996). Insect cell-derived VP2 of infectious bursal disease virus confers protection against the disease in chickens. *Avian Diseases*, 40(4), 753-761.
- Pitcovski, J., Gutter, B., Gallili, G., Goldway, M., Perelman, B., Gross, G., Krispel, S., Barbakov, M., & Michael, A. (2003). Development and large-scale use of recombinant VP2 vaccine for the prevention of infectious bursal disease of chickens. *Vaccine*, 21(32), 4736-4743.
- Qin, L., Qi, X., Gao, Y., Gao, H., Lu, X., Wang, Y., Bu, Z., & Wang, X. (2010). VP5-deficient mutant virus induced protection against challenge with very virulent infectious bursal disease virus of chickens. *Vaccine*, 28(21), 3735-3740.
- Raue, R., Islam, M. R., Islam, M. N., Islam, K. M., Badhy, S. C., Das, P. M., & Müller, H. (2004). Reversion of molecularly engineered, partially attenuated, very virulent infectious bursal disease virus during infection of commercial chickens. *Avian Pathology*, 33(2), 181-189.
- Rautenschlein, S., Yeh, H. Y., & Sharma, J. M. (2003). Comparative immunopathogenesis of mild, intermediate, and virulent strains of classic infectious bursal disease virus. *Avian Diseases*, 47(1), 66-78.
- Rautenschlein, S., & Haase, C. (2005). Differences in the immunopathogenesis of infectious bursal disease virus (IBDV) following *in ovo* and post-hatch vaccination of chickens. *Veterinary Immunology and Immunopathology*, 106(1), 139-150.
- Rautenschlein, S., Kraemer, C., Vanmarcke, J., & Montiel, E. (2005). Protective efficacy of intermediate and intermediate plus infectious bursal disease virus (IBDV) vaccines against very virulent IBDV in commercial broilers. *Avian Diseases*, 49(2), 231-237.
- Remorini, P., Calderón, M. G., Aguirre, S., Periolo, O., Torre, J. L., & Mattion, N. (2006). Characterization of infectious bursal disease viruses from Argentina. *Avian Diseases*, 50(2), 245-251.
- Sánchez, A. B., & Rodriguez, J. F. (1999). Proteolytic processing in infectious bursal disease virus: identification of the polyprotein cleavage sites by site-directed mutagenesis. *Virology*, 262(1), 190-199.
- Sapats, S. I., & Ignjatovic, J. (2000). Antigenic and sequence heterogeneity of infectious bursal disease virus strains isolated in Australia. *Archives of Virology*, 145(4), 773-785.
- Sharma, J. M. (1986). Embryo vaccination of specific-pathogen-free chickens with infectious bursal disease virus: tissue distribution of the vaccine virus and protection of hatched chickens against disease. *Avian Diseases*, 30(4), 776-780.
- Sharma, J. M., Dohms, J. E., & Metz, A. L. (1989). Comparative pathogenesis of serotype 1 and variant serotype 1 isolates of infectious bursal disease virus and their effect on humoral and cellular immune competence of specific-pathogen-free chickens. *Avian Diseases*, 33(1), 112-124.
- Sharma, J. M., Kim, I. J., Rautenschlein, S., & Yeh, H. Y. (2000). Infectious bursal disease virus of chickens: pathogenesis and immunosuppression. *Developmental & Comparative Immunology*, 24(2), 223-235.
- Sheppard, M., Werner, W., Tsatas, E., McCoy, R., Prowse, S., & Johnson, M. (1998). Fowl adenovirus recombinant expressing VP2 of infectious bursal disease virus induces protective immunity against bursal disease. *Archives of Virology*, 143(5), 915-930.
- Skeeles, J. K., Lukert, P. D., Fletcher, O. J., & Leonard, J. D. (1979). Immunization studies with a cell-culture-adapted infectious bursal disease virus. *Avian Diseases*, (23)2, 456-465.
- Snedeker, C., Wills, F. K., & Moulthrop, I. M. (1967). Some studies on the infectious bursal agent. *Avian Diseases*, 11(4), 519-528.
- Snyder, D. B. (1990). Changes in the field status of infectious bursal disease virus. *Avian Pathology*, 19(3), 419-423.

- Stoute, S. T., Jackwood, D. J., Sommer-Wagner, S. E., Cooper, G. L., Anderson, M. L., Woolcock, P. R., Bickford, A. A., Senties-Cué, C. G., & Charlton, B. R. (2009). The diagnosis of very virulent infectious bursal disease in California pullets. *Avian Diseases*, 53(2), 321-326.
- Stoute, S. T., Jackwood, D. J., Sommer-Wagner, S. E., Crossley, B. M., Woolcock, P. R., & Charlton, B. R. (2013). Pathogenicity associated with coinfection with very virulent infectious bursal disease and Infectious bursal disease virus strains endemic in the United States. *Journal of Veterinary Diagnostic Investigation*, 25(3), 352-358.
- Sun, J. H., Yan, Y. X., Jiang, J., & Lu, P. (2005). DNA immunization against very virulent infectious bursal disease virus with VP2-4-3 gene and chicken IL-6 gene. *Journal of Veterinary Medicine, Series B*, 52(1), 1-7.
- Tsukamoto, K., Tanimura, N., Kakita, S., Ota, K., Mase, M., Imai, K., & Hihara, H. (1995). Efficacy of three live vaccines against highly virulent infectious bursal disease virus in chickens with or without maternal antibodies. *Avian Diseases*, 39(2), 218-229.
- Tsukamoto, K., Kojima, C., Komori, Y., Tanimura, N., Mase, M., & Yamaguchi, S. (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(2), 352-362.
- Tsukamoto, K., Saito, S., Saeki, S., Sato, T., Tanimura, N., Isobe, T., Mase, M., Imada, T., Yuasa, N., & Yamaguchi, S. (2002). Complete, long-lasting protection against lethal infectious bursal disease virus challenge by a single vaccination with an avian herpesvirus vector expressing VP2 antigens. *Journal of Virology*, 76(11), 5637-5645.
- Vakharia, V. N., Snyder, D. B., He, J., Edwards, G. H., Savage, P. K., & Mengel-Whereat, S. A. (1993). Infectious bursal disease virus structural proteins expressed in a baculovirus recombinant confer protection in chickens. *Journal of General Virology*, 74(6), 1201-1206.
- Vakharia, V. N., Snyder, D. B., Lütticken, D., Mengel-Whereat, S. A., Savage, P. K., Edwards, G. H., & Goodwin, M. A. (1994). Active and passive protection against variant and classic infectious bursal disease virus strains induced by baculovirus-expressed structural proteins. *Vaccine*, 12(5), 452-456.
- van den Berg, T. P., Gonze, M., & Meulemans, G. (1991). Acute infectious bursal disease in poultry: isolation and characterisation of a highly virulent strain. *Avian Pathology*, 20(1), 133-143.
- van den Berg, T. P., & Meulemans, G. (1991). Acute infectious bursal disease in poultry: protection afforded by maternally derived antibodies and interference with live vaccination. *Avian Pathology*, 20(3), 409-421.
- van den Berg, T. P., Gonze, M., Morales, D., & Meulemans, G. (1996). Acute infectious bursal disease in poultry: immunological and molecular basis of antigenicity of a highly virulent strain. *Avian Pathology*, 25(4), 751-768.
- van den Berg, T. P. (2000). Acute infectious bursal disease in poultry: a review. *Avian Pathology*, 29(3), 175-194.
- van den Berg, T. P., Morales, D., Eterradossi, N., Rivallan, G., Toquin, D., Raue, R., Zierenberg, K., Zhang, M. F., Zhu, Y. P., Wang, C. Q., Zheng, H. J., Wang, X., Chen, G. C., Lim, B. L., & Müller, H. (2004). Assessment of genetic, antigenic and pathotypic criteria for the characterization of IBDV strains. *Avian Pathology*, 33(5), 470-476.
- van Loon, A. A., de Haas, N., Zeyda, I., & Mundt, E. (2002). Alteration of amino acids in VP2 of very virulent infectious bursal disease virus results in tissue culture adaptation and attenuation in chickens. *Journal of General Virology*, 83(1), 121-129.
- Villegas, P., Hamoud, M., Purvis, L. B., & Perozo, F. (2008). Infectious bursal disease subunit vaccination. *Avian Diseases*, 52(4), 670-674.
- Wang, M. Y., Kuo, Y. Y., Lee, M. S., Doong, S. R., Ho, J. Y., & Lee, L. H. (2000). Self-assembly of the infectious bursal disease virus capsid protein, rVP2, expressed in insect cells and purification of immunogenic chimeric rVP2H particles by immobilized metal-ion affinity chromatography. *Biotechnology and Bioengineering*, 67(1), 104-111.
- Wang, X., Jiang, P., Deen, S., Wu, J., Liu, X., & Xu, J. (2003). Efficacy of DNA vaccines against infectious bursal disease virus in chickens enhanced by coadministration with CpG oligodeoxynucleotide. *Avian Diseases*, 47(4), 1305-1312.
- Wei, Y., Li, J., Zheng, J., Xu, H., Li, L., & Yu, L. (2006). Genetic reassortment of infectious bursal disease virus in nature. *Biochemical and Biophysical Research Communications*, 350(2), 277-287.

- Whitfill, C. E., Haddad, E. E., Ricks, C. A., Skeeles, J. K., Newberry, L. A., Beasley, J. N., Andrews, P. D., Thoma, J. A., & Wakenell, P. S. (1995). Determination of optimum formulation of a novel infectious bursal disease virus (IBDV) vaccine constructed by mixing bursal disease antibody with IBDV. *Avian Diseases*, 39(4), 687-699.
- Winterfield, R. W., & Thacker, H. L. (1978). Immune response and pathogenicity of different strains of infectious bursal disease virus applied as vaccines. *Avian Diseases*, 22(4), 721-731.
- Wu, H., Singh, N. K., Locy, R. D., Scissum-Gunn, K., & Giambrone, J. J. (2004). Expression of immunogenic VP2 protein of infectious bursal disease virus in *Arabidopsis thaliana*. *Biotechnology Letters*, 26(10), 787-792.
- Wu, J., Yu, L., Li, L., Hu, J., Zhou, J., & Zhou, X. (2007). Oral immunization with transgenic rice seeds expressing VP2 protein of infectious bursal disease virus induces protective immune responses in chickens. *Plant Biotechnology Journal*, 5(5), 570-578.
- Wyeth, P. J., & Cullen, G. A. (1976). Maternally derived antibody-effect on susceptibility of chicks to infectious bursal disease. *Avian Pathology*, 5(4), 253-260.
- Xu, X. G., Tong, D. W., Wang, Z. S., Zhang, Q., Li, Z. C., Zhang, K., Li, W., & Liu, H. J. (2011). Baculovirus virions displaying infectious bursal disease virus VP2 protein protect chickens against infectious bursal disease virus infection. *Avian Diseases*, 55(2), 223-229.
- Yehuda, H., Goldway, M., Gutter, B., Michael, A., Godfried, Y., Shaaltiel, Y., Levi, B. Z., & Pitcovski, J. (2000). Transfer of antibodies elicited by baculovirus-derived VP2 of a very virulent bursal disease virus strain to progeny of commercial breeder chickens. *Avian Pathology*, 29(1), 13-19.
- Yu, F., Ren, X., Wang, Y., Qi, X., Song, J., Gao, Y., Qin, L., Gao, H., & Wang, X. (2013). A single amino acid V4I substitution in VP1 attenuates virulence of very virulent infectious bursal disease virus (vvIBDV) in SPF chickens and increases replication in CEF cells. *Virology*, 440(2), 204-209.
- Zhang, H. H., Yang, X. M., Xie, Q. M., Ma, J. Y., Luo, Y. N., Cao, Y. C., Chen, F., & Bi, Y. Z. (2010). The potent adjuvant effects of chicken  $\beta$ -defensin-1 when genetically fused with infectious bursal disease virus VP2 gene. *Veterinary Immunology and Immunopathology*, 136(1), 92-97.
- Zhou, X., Wang, D., Xiong, J., Zhang, P., Li, Y., & She, R. (2010). Protection of chickens, with or without maternal antibodies, against IBDV infection by a recombinant IBDV-VP2 protein. *Vaccine*, 28(23), 3990-3996.
- Zorman-Rojs, O., Barlič-Maganja, D., Mitevski, D., Lübke, W., & Mundt, E. (2003). Very virulent infectious bursal disease virus in southeastern Europe. *Avian Diseases*, 47(1), 186-192.