Oral Presentation (MP-15)

# Characterization of Infectious Bursal Disease Isolate with Propagation in Chicken Embryonated Eggs and Molecular Biology

Syamsidar[1], Arini Nurhandayani[1], Steffi Ong[1], Aprilia Kusumastuti[1], Ardi Budi Prakoso[1,]
Gusti Ayu yuniati Kencana[2]

[1]PT. Sanbio Laboratories Research & Development, Bogor, Indonesia
[2]Laboratorium Virologi Veteriner, Fakultas Kedokteran Hewan Universitas Udayana, Denpasar, Indonesia

**Keywords:** chicken embryonated eggs, infectious bursal disease, molecular biology.

## INTRODUCTION

Infectious Bursal Disease (IBD), also known as Gumboro disease is one that has an adverse economic effect. This disease is a threat to the poultry industry (Mueller, 2003). Deaths in chickens caused by IBD can even reach 100%. Outbreaks remain widespread despite vaccination programmes (Soedoedono, 2001). In Indonesia, the IBD disease is the cause of high death rates in poultry from the *very virulent* IBD (vvIBD) throughout the year 2014. Prevention of the disease can be done through vaccination. However, a tight vaccination programme so not ensure the safety of poultry from IBD. Therefore, there is a need for a vaccine strain to be suitable with those in the field. This can be accomplished with collecting virus isolates from the field and identifying the suitable vaccine strain. The purpose of this research is to characterize virus IBD isolates through propagation in embryonated chicken eggs. In addition, molecular methods will be used from PCR to identification with sequencing.

## MATERIALS AND METHODS

### Isolation of the Infectious Bursal Disease Virus.

Isolates from this study were obtained from East, West, and Central Java. They were taken from chickens with symptoms of its anatomical pathology resembling those of IBD. The bursa fabricius of the infected chicken was used for this research.

### Propagation of the IBD virus.

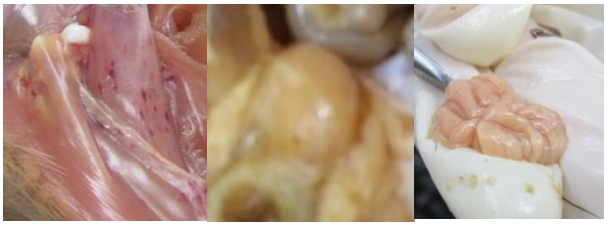
The bursa fabricius was made into 20% suspension with Phosphate Buffer Saline (PBS) containing 1% antibiotic. The suspension is inoculated into 10-year-old Specific Pathogen Free (SPF) embryonated eggs via the Chorioallantoic Cavity (CAC) as much as 0.2ml per egg. The eggs were incubated for 5 days at a temperature of 37°C and checked everyday during incubation. Dead embryoes were transferred into a cool room with a room temperature of 2-8°C. After 5 days, all eggs were taken out of the incubator and observed. The eggs were candled and the physique of the embryo observed.

### Molekuler Biology Test.

All isolates identified by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) method using primers IBDF (5'-TCA CCG TCC TCT GCT TAC -3') and IBDR (5'-TCA GGA TTT GGG ATC AGC-3'). Positive PCR results were tested by sequencing.

## RESULTS AND DISCUSSION

For this study, isolates were taken from a farm in the Java area where there were cases of chickens diagnosed with IBD. Clinical symptoms shown were atrophy of the bursa fabricius, presence of thick yellow mucus in the bursa, and redness in the muscles of a few infected chickens **(Figure 1)**.



Haemorrhaging in muscles | Atrophy of bursa | Yellow mucus in bursa

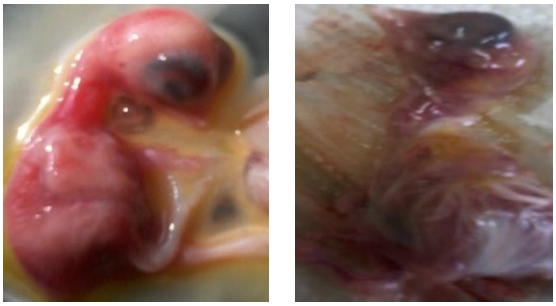
Figure 1. Anatomical pathology of chickens infected by IBD.

Changes in the bursa is mainly caused by an infection of the bursa by the IBD virus, inducing peripheral cytolytic lymphocytes. This causes bursal atrophy, lymphoid depletion, and immunosuppression due to the depletion of the sIgM gene, which is a precursor lymphocyte (Rodrigues et al, 2005). According to Harris (2010), the case of IBD is caused by classic IBD virus that causes inflammation and hypertrophy of the bursa 3 days post infection. This causes a double in weight of the bursa from the normal size in 4 days post vaccinatino dur to oedema and hyperemia. After 5 days, the size of the bursa went back to normal and starts to atrophy, shrinking to 1/3 of its normal size on the 8th day. In the case of a variang IBD viral infection, inflammation did not

occur. The virus causes bursal atrophy rapidly, mucosal oedema, and softening of the bursa fabricius. Necrotic lesions foci and ecchymotic haemorrhaging can be seen on the surface of the infected bursa (Harris, 2010)

**Results of virus propagation**

Propation of the IBD field isolate virus on SPF embryonated eggs tested positive. This is proven with the rapid death of the eggs that were injected with the isolate. Several embryos died 24 hours after inoculation and others 48 hours after. Upon observation, it is clear the embryo experienced redness, dwarfism, organs were red in colour and were swollen (**Figure 2**).



Positive IBD Negatif Control

Figure 2. Changes in the embryo after injection of IBD virus and the negative control

According to Rahadjo and Suwarno (2005), Specific changes towards embryonated chicken eggs caused by IBD virus infection are dwarfism and bleeding of the embryo. Changes in the embryo will be more visible in the third passage compared to the first. This difference is significant to the classic IBD virus and the variant described by Harris (2010). The embryo of the SPF egg inoclated with classic IBD virus throught the CAM died after 3-5 days post inoculation. In addition, other anatomic symptoms were cutaneous congestion, abdomen distension (oedema), petechiae haemorrhaging in the cerebral and finger joints, heart necrosis, pale heart, congestion and necrosis of the kidneys, extreme congestion in the lungs, and paleness in the spleen followed by necrotic foci. In contrast, the IBD variant virus do not cause death in embryos and haemorrhaging and congestion is seldom detected. Embryo lesions are characterised by cerebral and abdominal oedema, dwarfism, pale or cream-coloured skin and feathers, necrosis and *bile stasis* in the liver and splenomegaly. However, according to Takaseet al (1996), the variant strain is able to cause high death rates and also lesions in embryos at a low dose.

**PCR analysis**

Diagnosis through molecular methods show that the RNA sample of the West Java isolate was amplified using the RT-PCR (reverse transcription – polymerase chain reaction) method. 700bp of IBD sample was amplified (**Figure 3)**. According to Dharmayanti et al (2004), the sensitivity and specificity of an RT-PCR primer sequence choice is crucial. Specific primers were designed to amplify a conserved region of a gene matrix for *single tube reverse transcription*-PCR to identify virus target. In this test, the primer was designed to amplify at the gene of protein VP2 which plays a huge role in the antigenicity of the IBD virus (Rahardjo & Suwarno, 2005). The VP2 protein is part of the antigenic site of IBDV and plays a huge role in the stimulation of specific antibody production (Nagarajan & Kibenge, 1997). Besides that, the sequence of the VP2 protein is sufficiently sensitive to changes appearing in new variants (Cao et al, 1998).

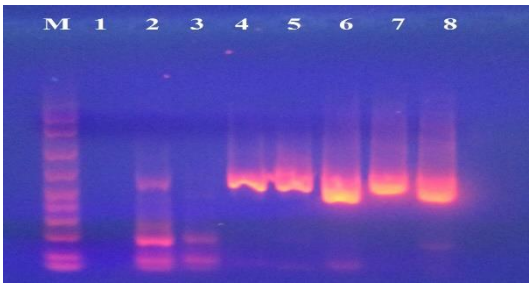



Figure 3. Amplification results of RT-PCR IBD virus isolate seen in wells 4 – 8.

Results of the RT-PCR amplification can be seen in Figure 4. It can be seen that the samples (wells 4-8) produces bands what is the same as the positive control. The sample was sequenced to identify the genetic sequence of the isolate.

**Sequencing results**

The sequencing results of the IBD isolates were analysed and presented with a phylogenetic tree. The sequences were compared with other sequences found in GenBank to identify the genetic relationship (**Figure 4**). All samples that were sequenced were found to represent the sequence of that found in *very virulent* IBD (vvIBD). This phylogenetic information has been published before by Mahardika (2008) comparing the pylogenetic relationship with IBD isolates from bali with isolates worldwide.

The analysis of the phylogenetic tree shows that the IBD isolate belongs to the group of vvIBD (**Figure 3**). The virus isolate is antigenically different than the several serotypes already known before such as the classic serotype. The classic IBD virus is one that can be found across Indonesia as a vaccine strain such as the Lukert and W2512 strain. These strains are used widely in poultry farms. The level of difference between the IBD viral isolate and that of the vaccines strain causes vaccination programs to be ineffective. Marhadika (2008) stated that the field IBD isolate that has spread across Indonesia is that of the vvIBD. This

virus originated from the same viral origin and are related to one other to form a genetic group. However, vvIBD also known as variant, experienced a change in antigenicity. The most change occurred in the sequence of protein VP2 at the residue of 206 and 350 (Nagaraja & Kibenge, 1997). For example, the variant E/DEL, substitutions of amino acids in the second hydrophilic region enables the virus to evade neutralization of antibodies induced by the virus vaccine containing the IBD virus classic type (Heine et al, 1991 in Nagaraja and Kibenge, 1997). This is the cause of non-protectivity of classic strain IBD vaccines in the field.

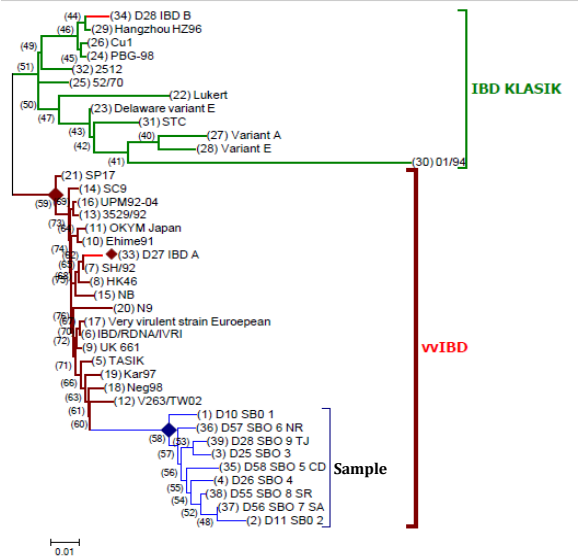



Figure 4. Genetic relationship of IBD isolates compared to data from Genbank.

## CONCLUSION

Propagation of IBD field-isolated virus in SPF-embryonated eggs showed positive results evidenced by rapid embryonic mortality in eggs infected by IBD field isolate virus. IBD virus that is widely circulating in Indonesia especially in west java, central java and east java is a very virulent IBD virus which can cause 50% outbeak of chicken information.

## REFERENCES


[1] Dharmayanti NLP, Damayanti R, Wiyono, Inriani, Darminto. 2004. *Identification avian influenza virus isolates Indonesia with reverse-transcriptase-polymerase chain reaction (RT-PCR)* Balai Penelitian Veteriner. Bogor

[2] Harris, Jacqueline Marie. 2010. *Characterization of infectious bursal disease* viruses *isolated from commercial chickens*. Thesis. Magister Science in Animal and Food Sciences. Faculty of University of Delaware.

[3] Mahardika IGNK, Lies Parede. 2008. *Phylogenetic analysis of nucleotide sequence hypervariable VP2 protein gumboro virus isolates Indonesia*. Balai Besar Penelitian veteriner. Bogor

[4] Nagarajan Malliga M, Kibenge FSB. 1997. Infectious bursal disease virus; a review of molecular basis for variations in antigenicity and virulence. *Can J. Vet. Res.* 61:81-88

[5] Rahardjo, Adi Prijo dan Suwarno. 2005. Immuogenitas protein VP2 bursal virus infection local isolates as the basis for the development of sub-unit vaccine. *Media Kedokteran Hewan* Vol 21 (1): 1-6

[6] Soedoedono, Retno Damajanti. 2001. Challenge test field isolates with ibd virus in chicken getting active and inactive ibd vaccine commercial. Bogor Agricultural University.

[7] Takase K, GM Baba, R Ariyoshi, H Fujikawa. 1996. Susceptibility of chicken embryos to highly virulent infectious bursal disease virus. *J. Vet. Med. Scie.* 58:1129-1131.

[8] Cao YC, WS Yeung, M Law YZ, Bi FC, Leung B L Lim. 1998. Molecular characterization of seven Chinese isolates of infectious bursal disease virus: classical very virulent and variant strains. *Avian Diseases* 42:340-351.