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Research Article

Pathogenicity and Immunogenicity Study of Infectious Bursal Disease 098 Bogor '19 Virus Isolate as a Master Seed Candidate for Live IBD Vaccine

I Wayan Wisaksana Yasa[1], I Wayan Teguh Wibawan[2], Okti Nadia Poetri[2]*

[1]Post Graduate Student of Medical Microbiology, Animal Biomedicine Program, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Jawa Barat, Indonesia; [2]Medical Microbiology Division, Animal Disease and Veterinary Public Health Department, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Jawa Barat, Indonesia.

Abstract | Infectious Bursal Disease (IBD) remains a significant threat to the poultry industry, cause 40-50% mortality rate among the flock. Vaccination remains an effective strategy for preventing infection, thus making safe and potent IBD vaccines essential. This study aims to evaluate the pathogenicity and immunogenicity of the IBD virus 098 Bogor '19 as a candidate for IBD live vaccine master seed through the bursal-body-weight ratio (BBWR), index of bursal-body-weight ratio (IBBWR), bursal lesion scoring (BLS), and IBD antibody titre evaluation. The IBD master seed candidate should be safe and have good immunogenicity responses. Thirty Specific Pathogen Free (SPF) chickens were divided into three groups of 10 chickens each. Group 1 was inoculated with the IBD virus 098 Bogor '19, Group 2 was inoculated with the very virulent (vv) IBD virus 078 Lampung '18, and Group 3 served as the control group. The challenge virus titre was $10^{2,7}$ EID50/ dose (0.3 ml) administered via oral route. Clinical signs were observed for 21 days post-inoculation . Serum samples were collected on days 0, 7, 14, and 21 post-inoculation , and antibody titres were measured using an enzyme-linked immunosorbent assay (ELISA). The BBWR, IBBWR, and BLS values were evaluated at 21 post-inoculation . None of the chickens in Group 1 showed clinical symptoms or mortality during the observation period, while Group 2 exhibited a 30% mortality rate. The average antibody titre in Group 1 was significantly higher ($P<0.001$) than in Group 2. BBWR, IBBWR, and BLS values in Groups 1 and 2 indicated infection leading to bursa atrophy. In conclusion, the IBD 098 Bogor '19 is safe and immunogenic which make it a promising candidate for an IBD intermediate-plus vaccine master seed.

Keywords | Infectious bursal disease (IBD), Pathogenicity, Immunogenicity, Live vaccine, Poultry vaccination, Intermediate-plus

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***Correspondence** | Okti Nadia Poetri, Medical Microbiology Division, Animal Disease and Veterinary Public Health Department, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Jawa Barat, Indonesia; **Email:** dia_poetri@apps.ipb.ac.id
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INTRODUCTION

Infectious Bursal Disease (IBD) is caused by the IBD virus and primarily affects chickens aged 3 to 6 weeks. Infection with this virus can lead to decreased production, increased mortality, and immunosuppression. Despite being first identified 60 years ago the disease is responsible for major economic losses in the poultry industry worldwide (Dey *et al*., 2019; Myint *et al*., 2021). The IBD virus specifically targets and attacks the lymphoid cells of the bursa of fabricius. Clinical symptoms of IBD infection include lethargy, dull plumage, drooping wings, and a dirty cloaca due to diarrheal. On the third day post-infection, swelling of the bursa of fabricius is observed, often accompanied by the accumulation of yellowish gelatinous fluid or bleeding within the bursa. By seven days post-infection, the bursa of Fabricius typically shrinks and undergoes atrophy (Damairia, 2023). The IBD virus, which belongs to the Birnaviridae family, is a double-stranded RNA virus with two segments: segment A and segment B. Segment A encodes structural proteins VP2, VP3, VP4, and the non-structural protein VP5, while segment B encodes the VP1 protein. In studies of pathogenicity and immunogenicity, the genes encoding VP1 and VP2 are crucial for assessing the virulence of IBD (Wang *et al*., 2019; Xu *et al*., 2020). The VP2 protein is essential for determining the antigenicity and immunogenicity of the IBD virus (Alfonso-Morales *et al*., 2013), while the VP1 protein is important for evaluating the pathogenicity of IBD virus isolates.

The IBD virus is classified into two serotypes: serotype 1 and serotype 2. Serotype 1 IBD virus strains are highly virulent in chickens, while serotype 2 strains primarily infect turkeys and are non-pathogenic to chickens. Within serotype 1, IBD virus strains are further classified into seven genogroups based on their antigenic, genetic traits, and pathogenicity: G1 - classic IBD (cIBD), G2 - variant IBD (varIBD), G3 - very virulent IBD (vvIBD), G4 - distinct IBD (dIBD), G5 - recombinant variant/classic IBD, G6 - ITA IBD, and G7 - Australian IBD (Jackwood *et al*., 2018). Each genogroup exhibits varying levels of pathogenicity. In vivo studies have demonstrated that vvIBD strains have the highest pathogenicity, causing up to 70% mortality and severe bursal damage, compared to variant and classic IBD strains, which, while generally subclinical, cause extensive damage to the bursa of fabricius and severe immunosuppressive effects (Jayasundara *et al*., 2017; Hussein *et al*., 2019; Xu *et al*., 2020).

Prevention of IBD on farms is primarily achieved through strict vaccination and biosecurity programs. Despite these measures, IBD cases continue to occur, with most instances attributed to the vvIBD virus strain. First identified in 1962 by Cosgrove, this strain is becoming prevalent worldwide and exhibits significant antigenic variation (Nishizawa *et al*., 2007). Thus, give this IBD strain a significant economic impact caused by high mortality or immunosuppressive effects it produces. In Indonesia, biomolecular studies conducted by Parede *et al*. (2003) have shown that the majority of IBD virus isolates from outbreak cases are vvIBD strains. The protection and efficacy of the IBD vaccine are significantly influenced by the type and strain of the virus used as the master seed. Generally, IBD live vaccines are classified into three types based on the pathogenicity of the master seed strain: mild IBD vaccines, intermediate IBD vaccines, and intermediate-plus (hot strain) IBD vaccines (Chowdhury *et al*., 2018; Müller *et al*., 2012). These vaccine types vary in their attenuation and pathogenicity characteristics. Mild IBD vaccines are typically safe and do not cause clinical symptoms, while intermediate IBD vaccines may cause a few post-vaccination clinical symptoms. However, both types have limited efficacy in penetrating maternal antibodies (MAb) that are often high in 1-day-old chickens (DOC). In contrast, intermediate plus IBD vaccines, which use master seed from virulent IBD virus strains, are more effective at penetrating MAb but may cause immunosuppression due to lymphoid cell damage and result in bursal atrophy. Additionally, due to the characteristics of the master seed strain and the vaccine application procedures, some vaccines may offer only partial immunity against the vvIBD virus or antigenic variations of the IBD virus that have emerged in the field over the past 30 years (Müller *et al*., 2012). This partial immunity can extend the duration of IBD virus shedding in the environment, contribute to the horizontal spread of the virus, and lead to viral escape through antigenic changes such as gene shifting, gene drifting, and gene reassortment (Orakpoghenor *et al*., 2020; Wang *et al*., 2019). Consequently, there is an urgent need to develop an IBD vaccine that is safe, provides robust protection, and minimizes post-vaccination immunosuppressive side effects.

This study aims to evaluate the pathogenicity and immunogenicity of the IBD 098 Bogor '19 virus isolate as a live vaccine master seed candidate, which originates from naturally attenuated field IBD virus. The study will provide insights into the pathogenicity of the master seed candidate and its ability to induce IBD-specific antibody titres, ensuring that the live vaccine produced is both safe and effective against the circulating field IBD viruses.

MATERIALS AND METHODS

Ethical Approval

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) of the School of Veterinary Medicine and Biomedicine, IPB University under approval number 024/KEH/SKE/IX/2022. All experimental chicks were handled according to the IPB University Animal Handling and Research Ethics Guidelines.

Place and Time of Research

This research was conducted at PT Vaksindo Satwa Nusantara in Gunung Putri, Bogor Regency, from May 2023 to March 2024, utilizing the ABSL-2 trial facility and the Serology Laboratory of the Product Development and Clinical Research Sub Department. Histopathology testing was performed at the Agrilab Pathology Laboratory, Agrinusa Jaya Sentosa, Sentul.

Virus

The viral isolates used in this study are IBD 098 Bogor '19 and IBD 078 Lampung '18. These isolates have been purified, identified, and molecularly characterized using PCR and sanger sequencing method by the Research and Diagnostic Sub Department of PT Vaksindo Satwa Nusantara as vvIBD virus isolates and classified into genogroup III. The results of the Koch's postulate test revealed that the IBD 078 Lampung '18 isolate was highly virulent, with a mortality rate of approximately 40%. In contrast, the IBD 098 Bogor '19 virus, while genotypically classified in genogroup III (vvIBD), did not cause any clinical symptoms or mortality during the observation period of the Koch's postulate test. The IBD 098 Bogor '19 and IBD 078 Lampung '18 isolate each were subsequently passaged three times using embryonated SPF eggs and prepared as a viral inoculum in the experiment.

Animals

The experimental animals for this study were thirty 14-day-old Specific Pathogenic Free (SPF) chickens obtained from the SPF farm owned by PT Vaksindo Satwa Nusantara. These SPF chickens were housed in BSL-2 experimental animal facilities and provided with ad libitum feed and water.

Clinical Trial Design

IBD virus titre used is $10^{2.7}$ EID50/ dose (0.3 ml), and administered via the oral route. Group 3 receive the same volume of PBS solution via the same route. Clinical symptoms and mortality were monitored daily for 21 days post-inoculation, with all observations and mortality were recorded. At the end of the clinical trial period, 21 days post-inoculation, all remaining SPF chickens from each treatment group will be humanely euthanized using manual cervical dislocation method. Post-mortem observations will be recorded and documented. The bursa of fabricius will be collected and assessed for BBWR and IBBWR. The collected bursa of fabricius will be preserved in 10% neutral buffered formalin (NBF) for further histopathological analysis. Bursal damage was be evaluated using the BSL method.

Blood samples will be collected at four time points: pre-vaccination (day 0), and at 7, 14, and 21 days post-inoculation. These samples will be tested for IBD antibody titres using a commercial IBD ELISA Kit (IDEXX Flockchek® IBD, IDEXX Laboratories, USA).

Determination of IBD Virus Pathogenicity

During the 21-day post-infection period, clinical symptoms and mortality caused by the IBD virus were recorded. Birds that died during the observation period were necropsied, and pathological findings were documented. Birds that survived until the end of the observation period were humanely sacrificed in accordance with animal ethics guidelines. These birds were then necropsied, and their bursa of Fabricius was collected for further analysis.

The pathogenicity of the IBD master seed was determined both macroscopically and microscopically. Macroscopic evaluation involved calculating the BBWR (Bursal-Body-Weight Ratio) and IBBWR (Index of Bursal-Body-Weight Ratio) values, while microscopic evaluation focused on calculating the BLS (Bursal Lesion Scoring) value (Aliyu *et al.*, 2022).

The BBWR and IBBWR values were calculated using the following formulas (Sharma *et al.*, 1989):

$$BBWR = \frac{Weight\ of\ Bursa\ of\ Fabricius\ (g)}{Weight\ of\ Individual\ Chicken\ (g)} \times 100$$

$$IBBWR = \frac{BBWR\ of\ Infected\ Individual\ Chicken}{BBWR\ of\ Normal\ Individual\ Chicken} \times 1000$$

Chickens with an IBBWR value of less than 0.7 were considered to have bursal atrophy.

Table 1: Bursal lesion scoring (BLS) assessment parameter.

No	Skoring	Criterion
1	0	Normal & no abnormalities in the fibrous bursa lymphoid follicles.
2	1	Lymphoid follicles depletion is minimal (<25% of lymphoid follicles are damaged).
3	2	Moderate follicle depletion (25 -50% of lymphoid follicles are damaged).
4	3	Severe follicle depletion (> 50 -75% of lymphoid follicles are depleted).
5	4	Very severe follicle depletion (>75 – 100% of lymphoid follicles are depleted) which is characterized by follicular atrophy accompanied by the presence or absence or absence of cystics.

The BLS scoring was based on microscopic observation of the degree of necrosis and lymphoid cell depletion in the bursa of Fabricius following IBD virus infection. The BLS scoring assessment parameters were based on the standards set by Hair-Bejo *et al.* (2000) and Chowdhury *et al.* (2018) (Table 1).

IBD Antibody Measurement

The serum samples collected will be analysed for antibody titres against IBD using the Enzyme-Linked Immunosorbent Assay (ELISA) method, employing a commercial IBD ELISA kits (IDEXX IBD FlockChek®, IDEXX Laboratories, USA). The ELISA test will be conducted following the manufacturer's protocol. A serum sample will be considered positive if it has an IBD antibody titre greater than 396.

Data Analysis

The results from the pathogenicity and immunogenicity tests in this study were statistically analysed using the JAMOVI program version 2.2. Clinical symptom and mortality data were analysed with Microsoft Excel and presented as survival rate graphs. A one-way ANOVA analysis with the Kruskal-Wallis post-hoc test was performed to determine the significance of differences between groups for BBWR, IBBWR, and BLS data. A P-value of <0.05 was considered statistically significant.

Statistical analysis of IBD antibody titre data was conducted using a two-way ANOVA with Tukey's post-hoc test. A P-value of <0.05 was considered significant, while a P-value of <0.01 was considered highly significant.

RESULTS AND DISCUSSION

Pathogenicity of IBD 098 Bogor '19

The pathogenicity of the IBD 098 Bogor '19 master seed isolate was compared to that of the vvIBD 078 Lampung '18 isolate. No clinical symptoms of IBD were observed in the treatment group inoculated with the IBD 098 Bogor '19 master seed isolate during the 21-day observation period. Similarly, the negative control group, inoculated with PBS solution, also showed no clinical symptoms. In contrast, the group inoculated with the vvIBD 078 Lampung '18 virus isolate exhibited clear clinical symptoms of IBD, including decreased feed intake, lethargy, watery diarrhoea, dull feathers, and mortality. Clinical symptoms and mortality in this group began to appear on day 8 and progressively increased, reaching 30% by the end of the observation period (Figure 1). These findings are consistent with the research of Xu *et al.* (2020), which reported that vvIBD isolates from 2013 and 2018 in China caused high mortality rates and severe damage to the lymphoid organs of infected chickens.

Petechiae in the thigh and chest muscles, bursal atrophy of the Fabricius, and renal swelling accompanied by uric acid accumulation were observed in individuals from the vvIBD 078 Lampung '18 group that experienced post-inoculation death (Figure 2). These findings are pathognomonic for IBD virus infection (Orakpoghenor *et al.*, 2020). Infected bursae of Fabricius initially exhibit hypertrophy, often accompanied by the accumulation of yellowish gelatinous fluid on their surface. Additionally, petechiae may develop due to the inflammatory process (Tanimura and Sharma, 1998; Lukert and Saif, 2003). By the 7th or 8th day post-infection, the bursa typically undergoes atrophy due to lymphoid cell depletion (Cheville, 1967). Pathological changes are also observed in the kidneys and spleen of infected chickens. The kidneys undergo hypertrophy, with uric acid accumulating in the tubules and ureters. In some cases, the spleen becomes enlarged and exhibits greyish spots distributed uniformly across the organ.

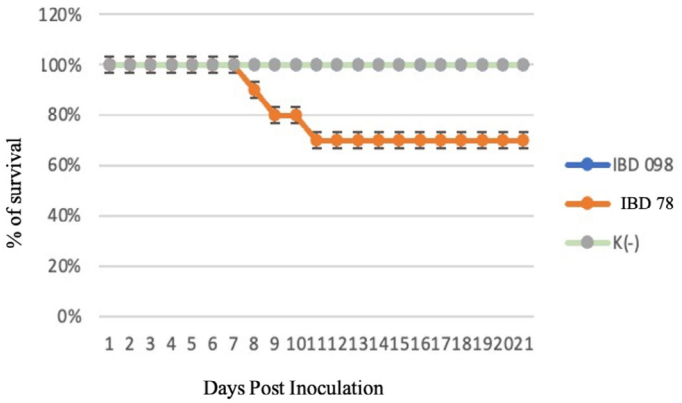

Figure 1: Survival rate graph of IBD 098 Bogor '19 and vvIBD 078 Lampung '18 inoculation group.

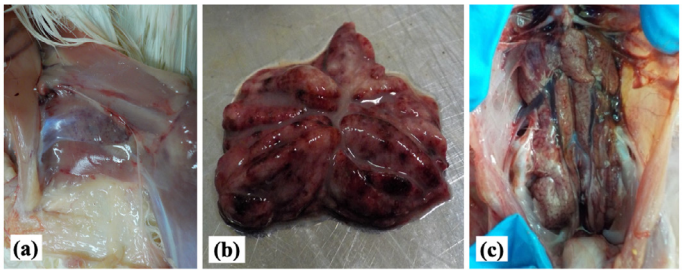

Figure 2: Pathology anatomy observe of the vvIBD 078 Lampung '18 group that experienced post-inoculation mortality. (a) Petechiae haemorrhage on the thigh muscle ; (b) Hypertrophy of bursa fabricious with accompanied by yellowish gelatinous accumulation and haemorrhage and (c) Renal hypertrophy accompanied by accumulation of uric acid.

The BBWR (Bursal-Body-Weight Ratio) test results revealed a significant difference (P<0.05) (Figure 3). The BBWR values for the IBD 098 Bogor '19 group, the vvIBD 078 Lampung '18 group, and the negative control group were 1.89 ± 0.392, 1.00 ± 0.462, and 5.17 ± 0.574, respectively. The lowest BBWR value was observed in the group inoculated with the vvIBD 078 Lampung '18 virus isolate. Although the BBWR value for the group inoculated with the IBD 098 Bogor '19 virus master seed was higher than that of the vvIBD 078 Lampung '18 group, it was still significantly lower compared to the BBWR value of the negative control group. This indicates that the size

and weight of the bursa of Fabricius decreased in the IBD virus-inoculated groups following virus exposure.

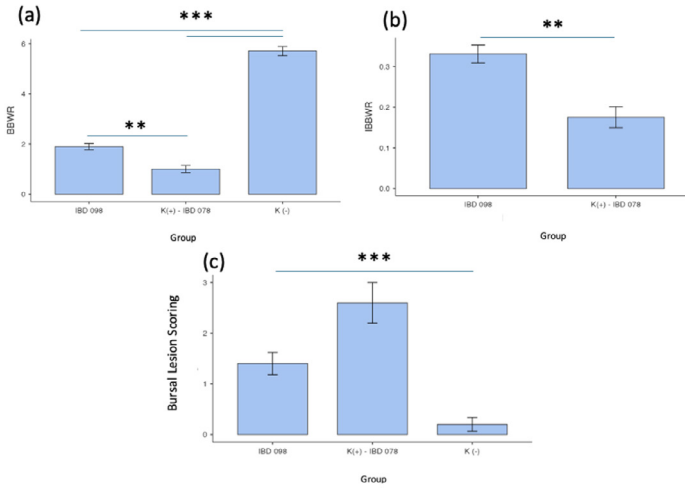

Figure 3: Graph result of (a) Bursal body weight ratio (BBWR); (b) Index of Bursal body weight ratio (IBBWR); (c) Bursal lesion scoring (BLS). The value is expressed in mean (± standard deviation). The difference in the significance value of P<0.05 is considered significant.

Similar results were observed in the IBBWR (Index of Bursal-Body-Weight Ratio) measurements. The IBBWR values for the IBD 098 Bogor '19 and vvIBD 078 Lampung '18 inoculation groups were significantly different (P<0.05) (Figure 3). Specifically, the IBBWR values were 0.331 ± 0.069 and 0.175 ± 0.081, respectively. Both values are below the normal standard of IBBWR, which is >0.7. This indicates that the bursa of Fabricius in both inoculation groups is damaged and undergoing atrophy.

The BLS (Bursal Lesion Scoring) results corroborated the findings from BBWR and IBBWR measurements. Both groups inoculated with the master seed isolates IBD 098 Bogor '19 and vvIBD 078 Lampung '18 exhibited significant bursal damage compared to the negative control group (Figure 3). The most severe damage to lymphoid organs was observed in the vvIBD 078 Lampung '18 inoculated group, with a BLS value of 2.60 ± 1.27. The group inoculated with the IBD 098 Bogor '19 master seed had relatively lower lymphoid organ damage, with a BLS value of 1.40 ± 1.23, compared to the vvIBD 078 Lampung '18 group. The negative control group showed minimal or no significant bursal lesions. The BBWR, IBBWR, and BLS result findings are similar with the result conduct by Alliyu *et al.* (2022), which found that vvIBD virus tend to have lower BBWR and IBBWR and higher BLS scoring.

Histopathological examination of the bursa of Fabricius from the IBD 098 Bogor '19 and vvIBD 078 Lampung '18 inoculation groups revealed similar lesions (Figure 4). The observed histopathological changes included lymphoid cell depletion in lymphoid follicles, cystic follicular formation, and fibrosis in the lymphoid follicles and bursal epithelium. These lesions are characteristic of bursal atrophy resulting from IBD virus infection. Additionally, such histopathological features are frequently observed in chickens vaccinated with intermediate or intermediate-plus IBD vaccines (Geerligs *et al.*, 2015). In contrast, the negative control group exhibited normal lymphoid tissue morphology with no specific histopathological lesions. These findings are consistent with those reported by Wang *et al.* (2019) and Lian *et al.* (2022), who noted that inoculation with virulent strains of the IBD virus leads to bursal atrophy, lymphoid cell depletion, cystic follicles, and fibrosis, as confirmed by histopathological examination.

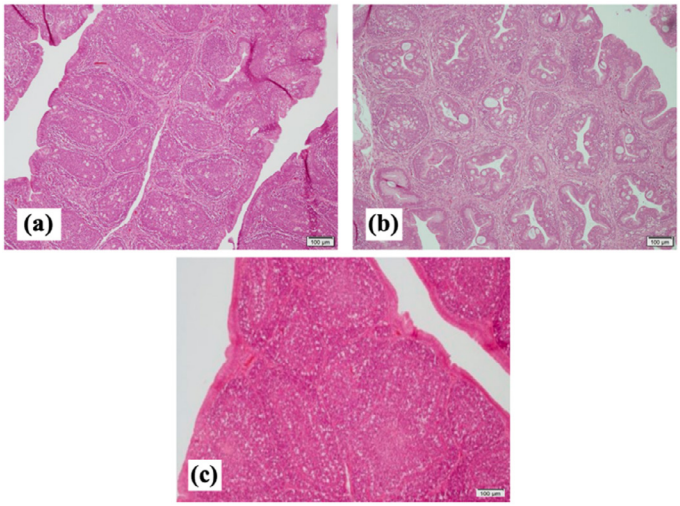

Figure 4: Comparison of bursa fabricious histopathological observation of bursa fabricious in group (a) IBD 098 Bogor '19, (b) IBD 078 Lampung '18; (c) K (-).100× microscope magnification.

The low BBWR and IBBWR values, combined with high BSL scores in the two inoculation groups, indicate that the bursa of Fabricius underwent significant atrophy. The bursa of Fabricius is the primary target organ for IBD virus infection, which initially causes damage to lymphoid cells and is accompanied by a severe inflammatory response (Orakpoghenor *et al.*, 2020). This results in swelling of the bursa and the presence of yellowish gelatinous exudate, which may be associated with petechiae in the bursal mucosa.

The extent of bursal damage varies depending on the virulence of the IBD virus strain. The vvIBD strains are more virulent, often resulting in high mortality rates, while IBD variant viruses generally cause sub-clinical infections without leading to death (Lian *et al.*, 2022). Infections with IBD variant viruses lead to depletion of lymphoid cells in the bursa, which are then replaced by connective tissue, resulting in bursal atrophy. This atrophy ultimately impairs the bird's ability to produce antibodies, leading to immunosuppression (Lupini *et al.*, 2019). This immunosuppressive condition will prone the bird to other diseases, since

the bursal lost its ability to develop antibody against those diseases.

The results from the BBWR, IBBWR, and BSL tests indicate that the IBD 098 Bogor '19 master seed isolate is a virulent IBD virus capable of causing bursal atrophy. Despite of the pathogenicity result, the IBD 098 Bogor '19 master seed isolate is safe and did not cause any mortality in the inoculated group. Phylogenetic analysis of the VP2 protein, conducted by the RandD department of PT Vaksindo Satwa Nusantara, identifies IBD 098 Bogor '19 as a vvIBD strain within genogroup III, which is generally highly virulent. Further study on the biomolecular level should be addressed to investigated the pathogenicity or antigenic variation occurs, which lead this isolate become attenuated. The study of VP2 hypervariable region could identified the genetic shift occurs on VP2 protein, since the VP2 protein plays an important role on determining the antigenicity and virulency of the IBD virus.

The pathogenicity test results suggest that the IBD 098 Bogor '19 isolate is a promising candidate for an intermediate-plus live IBD vaccine. This vaccine is designed to effectively penetrate high maternal antibody titres. It mimics the natural viral infection process by replicating directly in the bursa of Fabricius and targeting bursal lymphoid tissue (Müller *et al*., 2012). This replication strategy limits further viral replication in the bursa during field challenges and enhances protection against IBD infection (Rautenschlein *et al*., 2005).

The balance between safety and immunogenicity is crucial when evaluating a live IBD vaccine master seed candidate (Thomrongsuwannakij *et al*., 2021). The vaccine should induce a strong immune response while minimizing or eliminating post-vaccination immunosuppression. At the same time, it must have a high ability to penetrate maternal antibodies. Further research is necessary to assess the safety and potential immunosuppressive effects of the IBD 098 Bogor '19 master seed candidate before its use as a master seed for an active IBD vaccine.

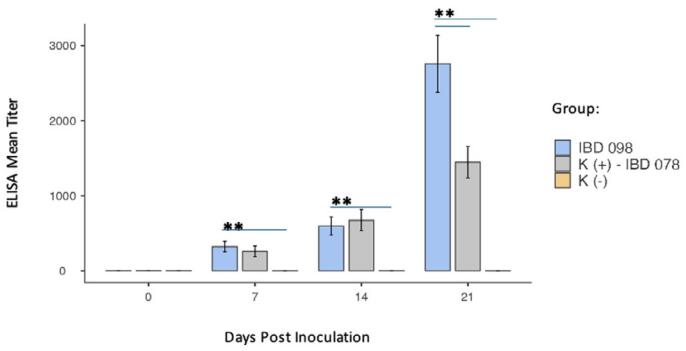

Figure 5: IBD ELISA mean titer (± standard deviation). The value of P<0.05 is significantly different and the value of P<0.01 is considered very significantly different.

Antibody Response

IBD antibody titres in both the IBD 098 Bogor '19 and vvIBD 078 Lampung '18 isolates began to be detectable on day 7 and continued to rise until day 21 post-inoculation (Figure 5). On days 7 and 14 post-inoculation, there were no significant differences in antibody titres between the two groups. However, by day 21, the antibody titres in the groups inoculated with the IBD 098 Bogor '19 and vvIBD 078 Lampung '18 master seed isolates differed significantly (P<0.001) (Table 2).

Table 2: IBD antibody titers determined in the experimental groups on day 0, 7, 14 and 21 post inoculation by ELISA. Data expressed as mean ± standard deviation.

		ELISA Mean Titer (±SD)			
No	Group	0	7	14	21
1	IBD 098	3,40 ± 6,33	324± 219	599 ± 380	2757 ± 1198
2	K (+) – IBD 078	5,60 ± 7,66	261 ± 231	675 ± 372	1447 ± 552*
3	K (-)	4,10 ± 6,84	1,80 ±5,03**	3,20 ±6,75**	1,30 ± 3.47**

Means in the same column with different superscript differ significantly (*, P<0.05) or highly significant (**, P<0.01).

The IBD antibody titres observed in the inoculated groups demonstrated that the IBD 098 Bogor '19 isolate elicited a better humoral antibody response compared to the vvIBD 078 Lampung '18 isolate. The timing and level of antibody production are crucial factors in determining the efficacy of live IBD vaccines in the field. The vaccine's ability to overcome maternal antibody barriers impacts the bursa of Fabricius' infection process and, consequently, the development of IBD humoral immunity. Maternal antibodies can persist in chicks up to 14 days of age, and live intermediate-plus IBD vaccines have shown a greater ability to induce a robust humoral response compared to other live IBD vaccine types (Meher *et al*., 2022).

The live intermediate-plus type IBD vaccine, derived from a virulent IBD virus that has undergone attenuation, is capable of inducing higher levels of IFN-γ, which in turn significantly enhances humoral antibody production (Jakka *et al*., 2014). Additionally, research by Camilotti *et al*. (2015) found that the efficacy of a live IBD vaccine correlates with its ability to induce robust antibody titres and provide significant protection within 14 to 28 days post-vaccination. Although the IBD 098 Bogor '19 master seed causes bursal atrophy, its ability to rapidly induce humoral antibody formation is a key factor in its selection as a live intermediate-plus IBD vaccine master seed. However, since the IBD 098 Bogor '19 also cause bursal atrophy, further study on the immunosuppression effects should be done. The efficacy of the master seed candidate against field IBD virus infections should also be addressed.

CONCLUSIONS AND RECOMMENDATIONS

The IBD 098 Bogor '19 master seed candidate exhibits favourable pathogenicity as a live intermediate Plus IBD vaccine. It effectively infects the bursa of Fabricius without causing clinical symptoms or mortality. The ability of IBD 098 Bogor '19 to induce high levels of humoral antibody titres early in the infection process indicates its strong immunogenicity. Therefore, the IBD 098 Bogor '19 master seed is a promising candidate for use in live intermediate-plus IBD vaccines against circulating IBD virus on field, as it demonstrates potentially generating higher antibody titres. However, further investigation is needed to evaluate potential immunosuppressive effects, the vaccine's efficacy, and duration of immunity in providing protection against field IBD virus infections.

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NOVELTY STATEMENTS

The IBD 098 Bogor '19 is a vvIBD virus isolate exhibits favourable pathogenicity as a live intermediate Plus IBD vaccine master seed candidate, which not cause any clinical sign nor mortality and could establish strong immunogenicity by induce high levels of humoral antibody titres early after inoculation.

AUTHOR'S CONTRIBUTIONS

I Wayan Wisaksana Yasa, I Wayan Teguh Wibawan and Okti Nadia Poetri: Equal authors, conducted the study, conceptualized the study, data analysis, and finalized the manuscript. The authors have read, reviewed, and approved the final content of the manuscript and agree to the conditions outlined in the copyright assignment form.

Conflict of Interest

The authors declare no conflict of interest regarding the publication of this article.

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