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Efficacy and Safety of an Inactivated Novel Variant Infectious Bursal Disease Virus in Broiler Chickens

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ABSTRACT

The infectious bursal disease virus (IBDV) is severe and highly contagious, causing high mortality and immunosuppression in chickens worldwide. A new novel variant, IBDV (nVarIBDV), has recently emerged in Asian countries, including Malaysia, highlighting the need to develop a new vaccine against this strain due to the inadequacy of existing commercial vaccines in protecting chickens from nVarIBDV infection. Therefore, the current study aimed to evaluate the efficacy and safety of inactivated nVarIBDV as a potential vaccine candidate in broiler chickens. A total of 65 one-day-old Arbo Acres broiler chickens were randomly divided into three groups (five animals in each group with four replications) before the challenge, namely A, B, and C. Groups A and B were immunized subcutaneously at day old with inactivated nVarIBDV (10^7 EID_{50}/0.2 ml), and Group B was boosted at day 14. Group C was an unimmunized control. The experimental animals were divided into three subgroups and were challenged with pathogenic nVarIBDV (105 EID50/1.0ml) on day 28 post-inoculation through ocular and oral routes. The challenge sub-groups were named ACH, BCH, and CCH, respectively. The live body weight, bursa weight, and blood samples of the chickens were recorded. Gross lesions were examined, and samples of the bursa of Fabricius were collected from all the groups for histological evaluation. All the chickens appeared healthy and normal throughout the trial. Body weight increased in all groups without significant differences. The bursa weight and the bursa-to-body weight ratio of the booster group (Group B) were significantly higher than the non-booster and control groups. Gross lesions were not observed in the investigated groups. The challenged control group had higher bursa lesion scoring than the vaccinated groups. The IBDV antibody titer of challenged chickens in ACH, BCH, and CCH groups was higher than those of unchallenged groups A, B, and C at 35 days post-inoculation. The IBDV antibody titer of challenged chickens in group B was higher than challenged chickens in groups A and C (ACH and CCH). In conclusion, the inactivated nVarIBDV demonstrated safety and efficacy, with the booster Group (B) showing elevated humoral immune responses compared to the non-booster group.

Keywords: Antibody, Chicken, Efficacy, Inactivated vaccine, Novel variant infectious bursal disease virus

INTRODUCTION

Infectious bursal disease virus (IBDV) belongs to the genus *Avibirnavirus* and family *Birnaviridae*, causing a severe, highly infectious disease in chickens (Delmas et al., 2019). The IBDV is classified into serotypes 1 (pathogenic in chicken) and 2 (non-pathogenic in chicken). Serotype 1 is made up of very virulent, classical, antigenic variants and artificially attenuated subtypes (Müller et al., 2003). The IBDV primarily affects young chickens, targeting the lymphoid organs, especially the bursa of Fabricius where it causes severe atrophy (Müller et al., 2003). However, the severity of the disease differs from strain to strain. However, all the strains have a heavy economic impact on the poultry industry worldwide. Some virus strains are highly virulent and may cause up to 20% or more mortality in chickens aged 3 weeks and even older, while other strains can cause a severe, prolonged immunosuppressive reaction in chickens infected at an early age (Eterradosi and Saif, 2013).

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The IBDV is a non-enveloped virus indicating an icosahedral symmetry with a diameter of about 55-65 nm. It has a double-stranded RNA genome with two segments A and genome B. The IBDV segments encode five viral proteins, namely VP1, VP2, VP3, VP4, and VP5 (Qin and Zheng, 2017). The VP2 and VP3 are the major structural proteins of IBDV, identified in western-blotting experiments with convalescent sera as important IBDV-derived antigens (Cheggag et al., 2020). In addition to these findings, recent advancements in classification methods based on VP1 and VP2 characteristics have been proposed (Wang et al., 2021). Based on the improved scheme, cIBDV, varIBDV, vvIBDV, and aIBDV are classified as Genotype A1B1 made up of serotypes A2aB1, A2bB1, and A2cB1 and A2B2 made up of A3B2 and A8B1.

A new variant called novel variant IBDV (nVarIBDV) which is genetically different from the earlier reported variant IBDV, has been circulating in China since 2017 (Wang et al., 2021). It was first reported in Malaysia in 2019 (Aliyu et al., 2021). Although this variant does not immediately result in mortality, it has high morbidity, causing severe atrophy of the bursa of Fabricius. This, in turn, results in immunosuppression, loss in production performance, and subsequently, severe economic losses (Fan et al., 2019; Babazadeh and Asasi, 2021). The nVarIBDV is classified under Genotype A2dB1 and was also reported in Japan (Myint et al., 2021) and South Korea (Thai et al., 2021). Recently, it was also described that the Chinese nVarIBDV and the early variant IBDV originally found in America belong to the same branch of variant IBDV although they are still divided to form two distinct sub-branches discrete from one another (Aliyu et al., 2021).

Given the adverse economic consequences of nVarIBDV on infected chickens, it is important to protect chicks against immunosuppression and production loss. However, nearly all commercial vaccines currently in use target vvIBDV, and do not mount a sufficient immune response against nVarIBDV (Wang et al., 2021). Thus, the development of vaccines that have the same antigenicity as the nVarIBDV circulating in Malaysia is essential for the prevention and control of nVarIBDV in the Malaysian poultry industry. A viral particle resembling a vaccine candidate named SHG19-VLP produced neutralizing antibodies, which provided 100% protection against the nVarIBDV (Wang et al., 2021). In another instance, an attenuated nVarIBDV strain termed Gt was used to develop a reassortment virus strain rGtVarVP2, which when used, completely protected chickens against nVarIBDV (Fan et al., 2020a). The aim of this study was to inactivate nVarIBDV and use it as a potential vaccine candidate to evaluate its safety, efficacy, viral load, and viral shedding on broiler chickens after challenge with a pathogenic field isolate of nVarIBDV.

MATERIALS AND METHODS

Ethical approval

The guidelines and ethics of the University Putra Malaysia (UPM) Institutional Animal Care and Use Committee (IACUC) on handling animals for experiments approved with reference number UPM/IACUC/ AUP-U014/2022 were followed in this study.

Virus

A nVarIBDV isolated from a 23-day-old broiler chicken from a commercial farm in Selangor, Malaysia, named UPM1432/2019 with accession number MT431217 (Aliyu et al., 2021), obtained from the Institute of Bioscience, UPM, and confirmed as a novel variant by PCR was used for this experiment.

Inactivation, preparation, and sterility test

This process was conducted in a Biosafety level 2 Virology laboratory in the Faculty of Veterinary Medicine, UPM, following standard biosafety and biosecurity measures for handling viruses (Artika and Ma'roef, 2017). In this regard, 6 ml of nVarIBDV was measured into a centrifuge tube, and 120 µl of Binary Ethylene Imine (BEI, Sigma-Aldrich, St. Louis, MO, USA) was also added and incubated at 37^{o}C. The mixture was vortexed every 30 minutes for 36 hours, after which 12 µl of sodium thiosulfate was added and mixed thoroughly by vortexing at 37^{o}C for an hour. The inactivated nVarIBDV isolate was then filtered through a 0.22 µm syringe filter and mixed with Montanide 71 VG adjuvant at a ratio of 30:70 (inactivated nVArIBDV: Montanide 71 VG) by vortexing for 2 hours and stored at 4^{o}C until use as vaccines for the study. Safety and sterility test was conducted by inoculating 0.1ml of the inactivated nVarIBDV with Montanide 71 VG into the specific-pathogen-free embryonated chicken egg through the chorioallantoic membrane route and incubated at 37^{o}C. The eggs were observed for mortality for 7 days (Habib et al., 2006).

Design and study animals

A total of 65 one-day-old Arbo Acres commercial broiler chicks with an average weight of 56.6 ± 1.57 were randomly divided into three groups (denoted as A, B, and

C, with five chicks in each group and four replications. On day 28 of the experiment, five chickens from each group were selected for the challenge and named ACH, BCH, and CCH (Okura et al., 2021). Commercial broiler feed and water were provided *ad libitum*. Light was constantly provided, and the temperature was kept at 24^{o}C throughout the trial. When they were day old, chickens from Groups A and B were inoculated (0.2 ml) with 10^7 EID_{50}/0.2 ml of inactivated nVarIBDV via the subcutaneous route. Chicks from Group C were not inoculated. Five chicks from Group C were sacrificed on the day of inoculation by cervical dislocation. At 14 days old, 0.2 ml of 10^7 EID_{50}/0.2 ml inactivated nVarIBDV were inoculated to chickens from Group B through the subcutaneous route. On days 14, 28, and 35, five chickens from each of Groups A, B, and C were humanely sacrificed through cervical dislocation. Throughout the 35-day trial period, all chickens were subjected to daily observation for clinical signs. When the chickens reached 28 days of age, those designated for the challenge in Groups A, B, and C were exposed to a pathogenic field strain of nVarIBDV, administered at 105 EID 50/1.0 ml through the ocular (0.2 ml) and oral (0.8 ml) routes (Mutinda et al., 2015). Subsequently, the chickens were monitored for 7 days post-challenge. In the case of chicken mortality, necropsy procedures were conducted, and for those that survived, euthanasia and necropsy were performed by a veterinarian.

Sample collection

For sampling, each chicken was placed on a digital scale, and its weight in grams was recorded. After sacrifice, the bursa from each chicken was removed aseptically and weighed on a digital scale. Live body weight and bursa weights were recorded for each sampled chicken. About 5 ml of blood samples were also collected for each chicken before sacrifice, and about 1 ml of serum was extracted for the detection of IBD antibodies using the ELISA technique (Orakpoghenor et al., 2020). Chickens (post-mortem) were examined for gross lesions in the bursa, spleen, muscles, proventriculus, and thymus, which were recorded accordingly for each chicken. Aseptically collected samples of the bursa and cloacal swabs were stored at -20^{o}C prior to the molecular detection of the challenge virus by RT-qPCR technique (Aliyu et al., 2021). Five bursa samples from each replicate in a group on each sampling day were fixed in 10% buffered formalin for histological examination and lesion scoring.

Enzyme-linked immunosorbent assay analysis

The serum was harvested within 24 hours from each sampled chicken, centrifuged at 240 × g for 5 minutes, and kept at -20^{o}C prior to use. A serum sample from each chicken was analyzed for IBDV antibodies using the ELISA technique with a commercial kit (BioCheck IBD ELISA, Hounslow, UK) according to the manufacturer's recommendation. The results were read at 405 nm with an ELISA reader (Dynatech MR7000, Rauw et al., 2009).

Copy number of nVarIBD challenge virus in the bursa and cloaca of challenged chickens

Quantitative real-time PCR was conducted targeting the VP2 protein of nVarIBDV with samples of bursa and cloaca swabs. RNA was extracted and purified using Kylt® RNA/DNA purification kit (SAN Group Biotech, Germany) according to the manufacturer's recommended procedures. The purified RNA was checked for purity and concentration with a spectrophotometer (Eppendorf, Germany) at a 260 nm wavelength. The purified RNA was used to conduct an RT-qPCR assay with specific nVarIBDV primers (F1432 – CCAACAAGGGAGTACACCGA and R1432 – CCAAATGCTCCTGCAATCTT) and probe (Probe2 – AGTACTTCATGGAGGTGGCCGACCTCAA) to quantify the viral genome copies in the samples (Aliyu et al., 2021).

Histopathology and lesion scoring

Bursa of Fabricius samples obtained from each chicken were first checked for gross lesions, and a portion was fixed in 10% buffered formalin solution for 48 hours (Zhao., 2015). After fixing, the bursa was processed into transparent glass slides, and the slides were stained using hematoxylin and eosin. The slide was allowed to air dry before examination under the light microscope (Leica ASP 300, Germany) for histopathological changes on a scale of 0 to 5, ranging from normal to severe (Elawad et al., 2020).

Statistical analysis

The data analysis was conducted using Analysis of Variance (ANOVA) in SPSS version 28.0 (Chicago, USA). To discern differences, the Turkey Honestly Significant Difference (HSD) posthoc test was applied at a significance level of 5% ($p < 0.05$).

RESULTS

Clinical signs

The findings indicated no abnormal clinical signs and no mortality in any of the chickens in the vaccinated groups and the non-vaccinated unchallenged group

throughout the 35 days of the trial. However, the non-vaccinated challenged group recorded mild depression and ruffled feathers 2 days post-challenge but recovered.

Body weight

The body weight of chickens in each group progressively increased until day 35 and was not significantly different across groups. There was no significant difference in the body weight of chickens between groups at days 14 and 35 post-inoculation (dpi; $p > 0.05$). However, at 28 dpi, the body weight of chickens in the non-booster group was significantly higher than those of the other groups ($p < 0.05$). At 35 dpi, corresponding to 7 days post-challenge, the challenged chickens in Group B had the highest body weight than Groups A and C, but they were not statistically significant ($p > 0.05$, Figure 1). The body weight of challenged chickens in Group B was significantly higher than their non-challenged counterpart ($p < 0.05$).

Bursa weight

No significant difference was observed in the bursa weight between different groups on days 14, 28, and 35 among the non-challenged chickens. However, bursa weight of the challenged chickens in Group B was significantly higher than the other groups at 35 dpi corresponding to 7-day post-challenge ($p < 0.05$, Figure 2).

Bursa to body weight ratio

There was no significant difference in the bursa-to-body weight ratio of the chickens among unchallenged throughout the trial ($p > 0.05$). However, the bursa-to-body weight ratio of challenged chickens in Group B was significantly higher than Groups A and C at day 35 post-inoculation or day 7 post-challenge ($p < 0.05$, Figure 3).

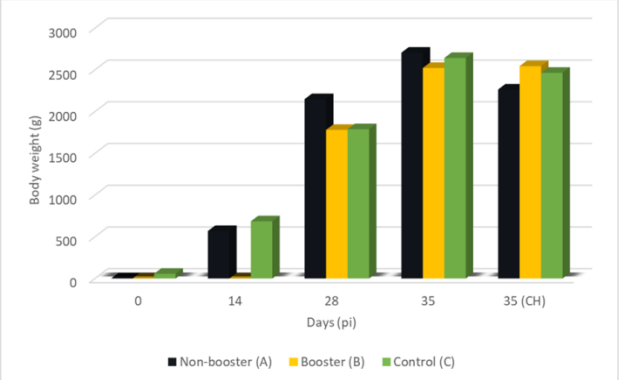

Figure 1. Body weight of Arbo Acres chickens inoculated with inactivated novel variant infectious bursal disease virus at day old for 35 days. CH: Challenged; pi: Post-inoculation

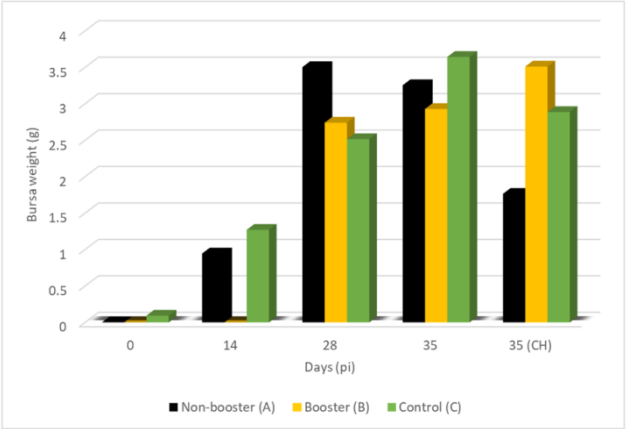

Figure 2. Bursa weight of Arbo Acres broiler chickens inoculated with inactivated novel variant infectious bursal disease virus at day old. CH: Challenged; pi: Post-inoculation

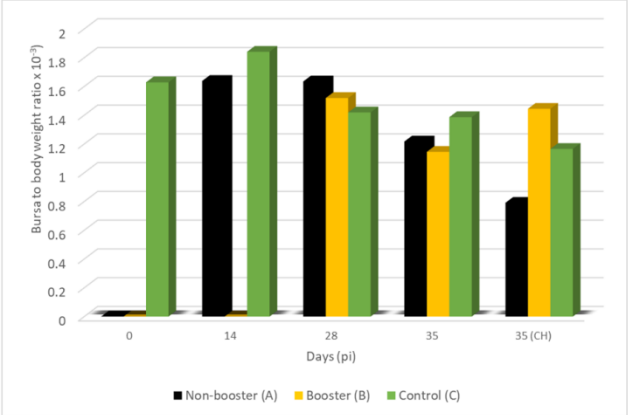

Figure 3. The ratio of bursa to body weight of Arbo Acres broiler chickens inoculated with inactivated novel variant Infectious Bursal Disease Virus at day old. CH: Challenged; pi: Post-inoculation

Gross lesions

Incubation time and 14 post-inoculation

The bursa of day-old chicks was normal, with no gross lesions on incubation time and 14 dpi (Figure 4).

Day 28 post-inoculation

The bursa of Fabricius from Groups A, B, and C was normal, with no gross lesions at 28 dpi (Figure 5).

Day 35 post-inoculation

The bursa samples from Groups A, B, and C were normal, with no gross lesions at 35 dpi (Figure 6).

Day 7 post-challenged

The bursa of Fabricius from Groups A, B, and C were normal, with no gross lesions (Figure 7) at day 35 pi among the challenged chickens in each group.

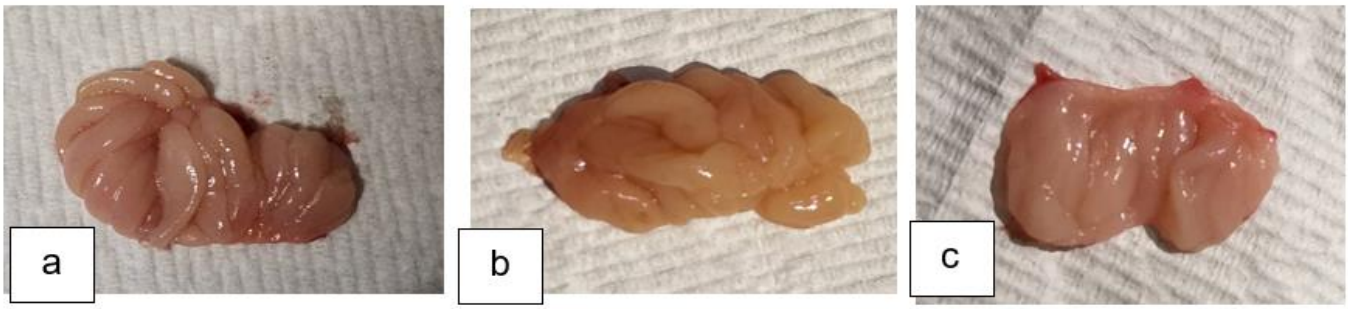

Figure 4. Normal bursa of Fabricius of Arbo Acres broiler chickens inoculated with inactivated novel variant infectious bursal disease virus at day old. Group C (a, 14 dpi), Group A (b, 14 dpi), and Group B (c, 14 dpi).

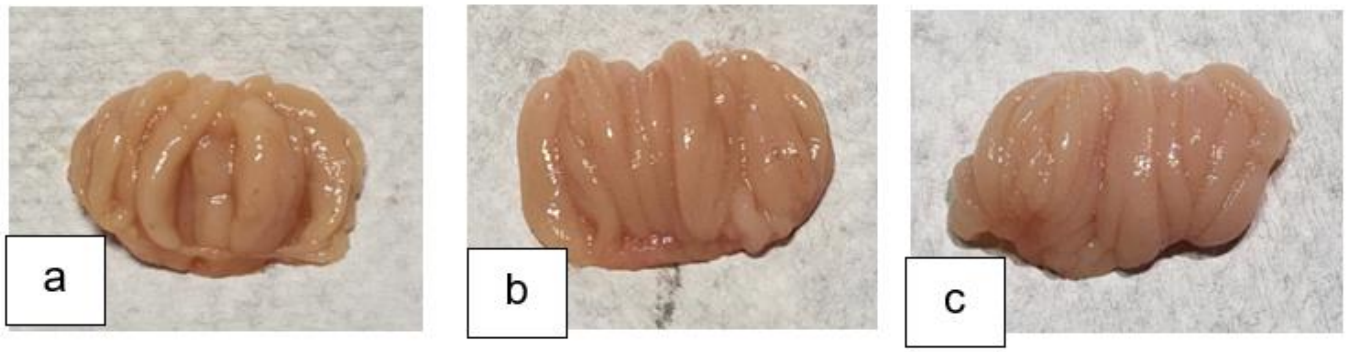

Figure 5. Normal bursa of Fabricius of Arbo Acres broiler chickens inoculated with inactivated novel variant infectious bursal disease virus at day old observed on 28 dpi. Group A (**a**), Group B (**b**), and Group C (**c**).

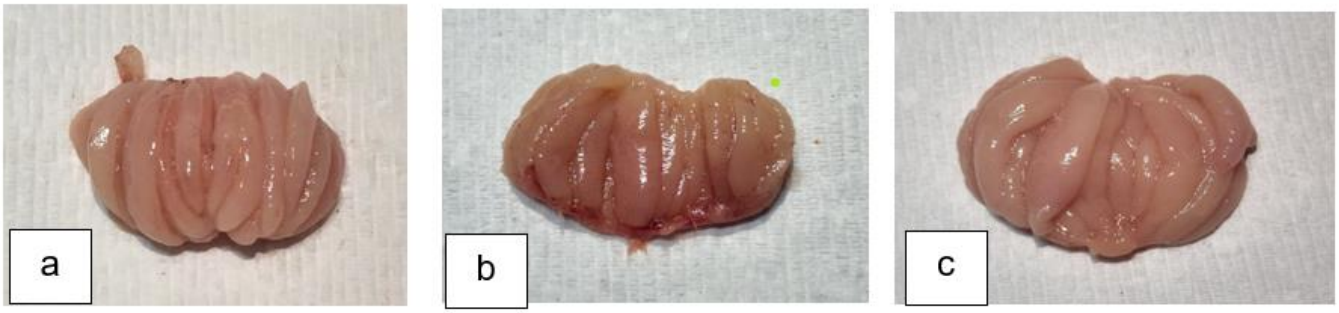

Figure 6. Normal bursa of Fabricius of Arbo Acres broiler chickens inoculated with inactivated novel variant infectious bursal disease virus at day old on 35 dpi. Group A (**a**), Group B (**b**), and Group C (**c**).

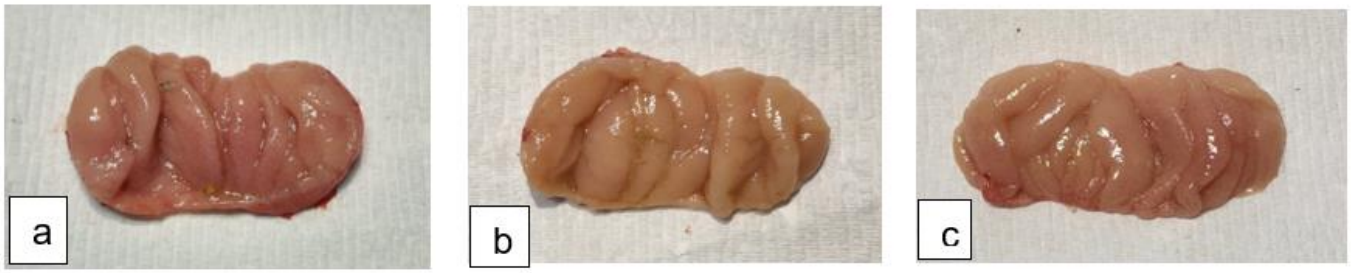

Figure 7. Normal bursa of Fabricius of challenged Arbo Acres broiler chickens inoculated with inactivated novel variant infectious bursal disease virus at day old on 35 dpi and 7-day post-challenge. Group A (**a**), Group B (**b**), and Group C (**c**).

Histological lesions

Incubation time and 14 post-inoculation

Mild degeneration, especially at the medullary region of the lymphoid follicles, was observed among the non-booster Group A chickens (white arrow). No necrosis and heterophils were present (Figure 8a). No histological lesions were observed from Groups A and C at 14 dpi (Figures 8b and 8c).

Days 28 and 35 post-inoculation(non-challenged chickens)

No histological lesions were observed for Group B. For Groups A (white arrow) and C (black arrow), mild degeneration, especially at the medullary region of the lymphoid follicles, was observed (Figure 9).

Day 35 post-inoculation (Challenged chickens)

For Groups A and B, there were mild degenerations, especially in the medullary region of the lymphoid follicles (white arrows). However, Group C chickens had mild to moderate degeneration, necrosis in the medulla, and infiltration of inflammatory cells (black arrow, Figure 10).

Bursa lesion score

The bursa lesion score was not significantly different throughout the trial for all the groups. The bursa lesion score of challenged Group C (CCH) was higher than Groups A (ACH) and B (BCH) but not statistically significant ($p > 0.05$, Figure 11).

Virus loading and shedding (*RT-qPCR)*

The virus copies in the bursa and cloacal swab samples were higher in challenged chickens in Group C compared to those in Groups A and B on 35 dpi or day 7 post-challenged (Figure 12).

Infectious bursal disease antibody titer

The IBD antibody titer of day-old chicks was 3546 ± 555.89 ELISA unit. The antibody titer of Group B was significantly higher ($p < 0.05$) than Groups A and C at 28 and 35 dpi (Figure 13).

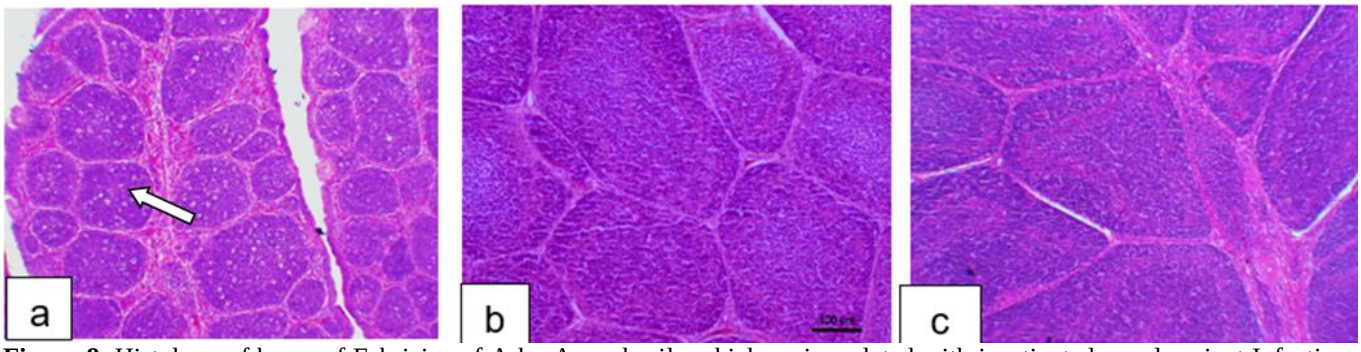

Figure 8. Histology of bursa of Fabricius of Arbo Acres broiler chickens inoculated with inactivated novel variant Infectious Bursal Disease Virus at day old on the day of inoculation and 14 post-inoculation (**a**), showing mild degeneration of the lymphoid follicles among Group A chickens (white arrow, Lesion scoring of 1); (**b**) and (**c**) on day 14 pi, showing normal bursa (Lesion scoring of 0). HE, Bar = 100µm.

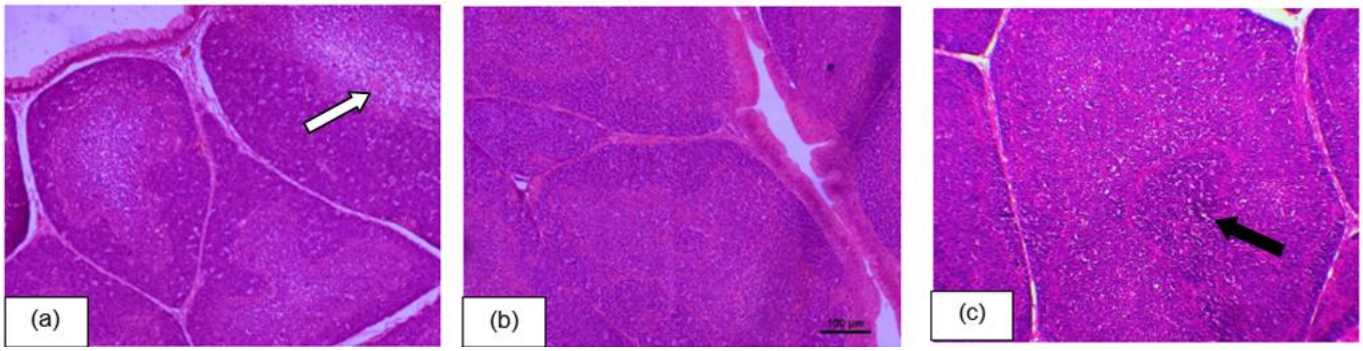

Figure 9. Histology of bursa of Fabricius of Arbo Acres broiler chickens inoculated with inactivated novel variant Infectious Bursal Disease Virus at day old on 28 dpi (CH) and day 35 post-inoculation. **a:** Mild degeneration (white arrow) Group A (Lesion scoring of 1), **b:** Group B (Lesion scoring of 0), and **c:** mild degeneration (white arrow) Group C (Lesion scoring of 1). HE, Bar = 100µm.

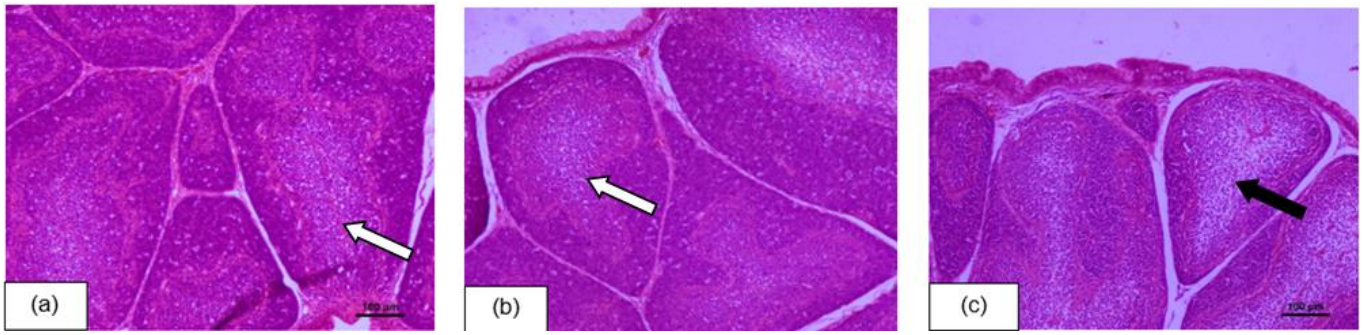

Figure10. Histology of bursa of Fabricius Arbo Acres broiler chickens inoculated with inactivated novel variant Infectious Bursal Disease Virus at day old on 35 dpi (CH) and day 7 post-challenge. **a:** Mild degeneration (white arrow), Group A (Lesion scoring 1), **b:** Mild degeneration (white arrow), Group B (Lesion scoring of 1), and **c:** Mild to moderate degeneration (black arrow), Group C (Lesion scoring of 2). HE, Bar = 100µm.

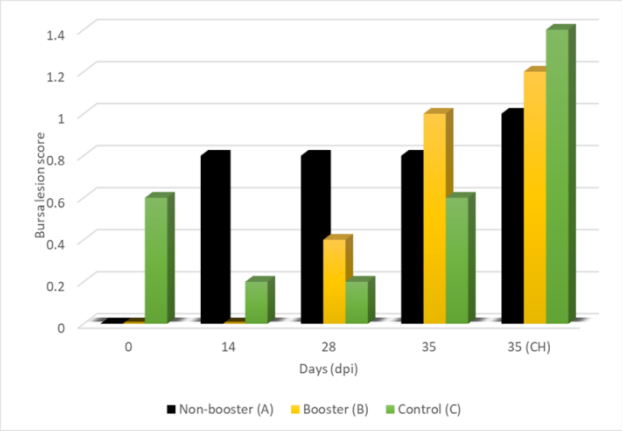

Figure 11. Bursa lesion score of Arbo Acres broiler chickens inoculated with inactivated novel variant infectious bursal disease virus at day old and monitored for 35 days . CH: Challenged; pi: Post-inoculation

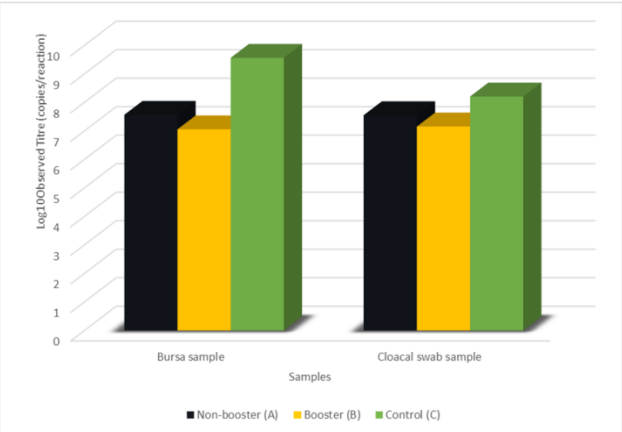

Figure 12. Virus load and shedding of Arbo Acres broiler chickens inoculated with inactivated novel variant infectious bursal disease virus at day old and challenged with the pathotype (35 dpi or day 7 post-challenged).

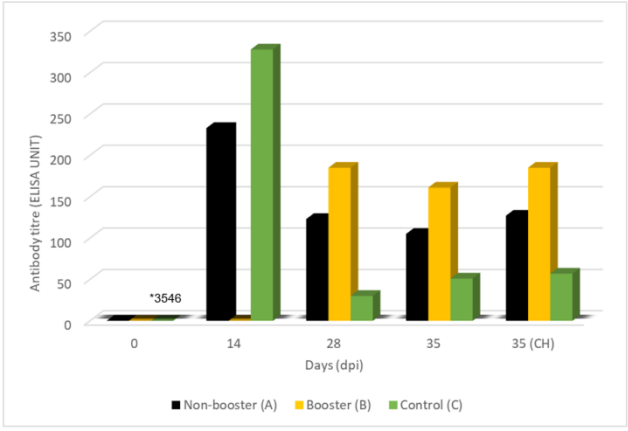

Figure 13. Infectious bursal disease virus antibody titer of Arbo Acres broiler chickens inoculated with inactivated novel variant Infectious Bursal Disease Virus at day old and monitored for 35 days. CH: Challenged; pi: post-inoculation

DISCUSSION

The novel variant IBDV isolate was successfully inactivated with binary ethylene imine (BEI), which does not interfere with the antigenicity of viruses (Delrue et al., 2012). Given that Montanide 71VG, employed as the adjuvant, has been documented to support the induction of long-lasting immunity (Tehrani et al., 2016), it is anticipated that the vaccine could be highly immunogenic and prove valuable in preventing IBD in chickens.

Throughout the trial, no abnormal clinical signs were observed. The body weight of chickens in all the vaccinated groups increased steadily until day 35, highlighting the safety of the inactivated nVarIBDV and the adjuvant on the broiler chickens. Since chickens

infected with the pathogenic nVarIBDV typically experience lower body weight (Fan et al., 2019), it is evident that the inactivated nVarIBDV did not impede the growth performance of the chickens as a pathogenic field isolate would have. Among the challenged chickens, booster Group B had the highest body weight than the other groups. The challenged chickens in the booster Group B had significantly higher body weight than their corresponding non-challenged chickens. This result indicated that the booster dose provided better protection for the chickens against challenges by maintaining better growth performance among the booster chickens.

The nVarIBDV causes lesions and severe atrophy in the bursa of Fabricius (Fan et al., 2020b; Myint et al., 2021; Thai et al., 2021). In this study, the bursa weight did not exhibit significant differences among all groups, except on day 35 among the challenged chickens, where the bursal weight of chickens in the booster Group B was significantly higher than that of Groups A and C. This suggests that the booster dose provided the most effective protection to the bursa against the nVarIBDV field isolate. The bursa-to-body weight ratio, which is a more accurate parameter to determine bursa atrophy caused by nVarIBDV was not significantly different for all the groups except on day 35 among challenged chickens. The bursa-to-body weight ratio challenged chickens in Group B was significantly higher than that of chickens in Groups A and C. This was similar to the report by Wang et al. (2020), in which the bursa-to-body weight index of the control chickens was significantly lower than that of the trial chickens vaccinated with live nVarIBDV vaccines. This shows that the inactivated nVarIBDV with Montanide 71VG adjuvant has the potential to protect chickens against pathogenic nVarIBDV.

According to a study by Fan et al. (2019), the bursa of chickens with nVarIBDV appeared to be atrophied, with hemorrhages, and yellowish with inflammatory exudation at 3-5 dpi. Similar gross lesions in the bursa of 24-38-day-old chickens were identified in studies conducted by Thai et al. (2021) and Myint et al. (2021) although congestion of the bursa appeared to be an additional finding. In this study, no gross lesions were present in the bursa of chickens inoculated with inactivated nVarIBDV at inoculation day, 14, 28, and 35 dpi. This indicates that the inactivated nVarIBDV with Montanide 71VG adjuvant does not cause gross pathological changes in the bursa and can be safely used. The bursa of the challenged chickens in all groups also showed no gross lesions. The absence of lesions in the challenged Group C might be attributed to the duration between the challenge virus inoculation and the bursa sampling, which may not have been sufficiently long enough to enable the obvious manifestation of clinical signs or lesions of nVarIBDV in the bursa of Group C chickens.

In the current study, no significant histopathological changes were recorded in chickens inoculated with inactivated nVarIBDV at 0, 14, 28, and 35 (non-challenged) dpi. This finding suggests that the inactivated nVarIBDV with Montanide 71VG did not cause histopathological changes in the bursa of chickens and is, therefore, safe to be used. The challenged chickens in Groups A and B showed no significant histopathological changes in the bursa. This is in line with the findings of Wang et al. (2021), where no microscopic lesions were observed in the vaccinated groups. However, chickens in Group C had mild to moderate degeneration and necrosis of lymphoid cells in the bursa follicles. Infiltration of inflammatory cells was also observed in the bursa of Group C-challenged chickens. Similar changes were previously reported (Fan et al., 2020b). Some histopathological changes that are typical of nVarIBDV include severe follicular lymphoid necrosis and depletion and multifocal follicular lymphoid infiltration. There may be minimal to no inflammatory response. Additionally, there could be reticular and macrophage infiltration in lymphoid follicles, cystic cavities in lymphoid follicles, proliferation of fibrous tissues, severe follicle atrophy, and infolding epithelium into damaged follicles in broilers aged 24-38 days infected with nVarIBDV (Fan et al., 2019; Myint et al., 2021; Thai et al., 2021). The results of this study suggest that the inactivated nVarIBDV elicited a sufficient humoral immune response, which prevented tissue damage in the bursa of the challenged chickens.

The histological lesions of the bursa were scored to provide a better understanding and statistical picture of the bursa lesions. The bursa lesion score was not significantly different throughout the trial for all groups. However, the bursa lesion score of challenged chickens in Group C was higher than Groups A and B although these findings were not statistically significant. This is consistent with the finding that nVarIBDV can cause lesions in the bursa of the Fabricus (Fan et al., 2020b). Together with the results of the above histological lesions, the current findings confirm the efficacy of inactivated nVarIBDV in providing an immunoprotective effect on infected chickens.

For both sample types, the virus copies of Group C were higher than Groups A and B. Given the lack of previous studies pertaining to evaluating virus loading and

shedding among inoculated chickens for nVarIBDV, it remains unclear to which degree the viral copies are attributed to the improved efficacy of the trial vaccine. It has been emphasized in previous reports that the evaluation of vaccine efficacy should address measuring the ability of the vaccine to limit the shedding of the pathogenic virus (Miller et al., 2009). This is crucial for preventing the dissemination of the virus in the environment and breaking the chain of transmission (Ugwu et al., 2022). However, the results of the RT-qPCR showed clear evidence of the ability of the inactivated nVarIBDV to elicit the production of sufficient neutralizing antibodies that reduce the viral load in the bursa and induced blocking immunity responsible for reduced virus shedding among the infected chickens.

The study showed that the IBDV antibody titer in Group B was significantly higher than in Groups A and C at 28 and 35 dpi, indicating a booster dose of inactivated nVarIBDV may be more desirable in the prevention of novel variant IBDV infection in chickens. The role of humoral immunity in protecting chickens against IBDV has been previously documented (Yang et al., 2020). Neutralizing antibodies are of utmost importance in preventing and controlling IBDV infection (Van Den Berg, 2000). The study revealed a significantly higher copy number of nVarIBDV challenge virus in the bursa of challenged control chickens than in the vaccinated chickens. This suggests that the inactivated nVarIBDV with Montanide 71VG induced the production of neutralizing antibodies, resulting in the effective clearance of the challenge virus from the bursa of vaccinated chickens. These findings are promising and position the inactivated nVarIBDV as a potential vaccine candidate. There is no significant difference in the antibody titer of the challenged and non-challenged groups. Two limitations may account for the observed results. Firstly, the shortened duration between the inoculation of the challenge virus and the sampling may not have allowed sufficient time for a robust immune response to develop. Ebrahimi et al. (2020) reported that the antibody titer of IBDV was higher at day 42 of the age of chickens in their trial compared to days 28 and 35. However, the titer recorded in this trial was comparable to that noted by Habib et al. (2006) in their trial with BEI-inactivated IBDV. Secondly, the limited understanding of the true pathogenicity and virulence status of nVarIBDV in existing studies may influence the extent of the immune response.

CONCLUSION

In conclusion, nVarIBDV inactivated with BEI and mixed with Montanide 71VG adjuvant was safe, immunogenic, and efficacious against pathogenic field strains of nVarIBDV in Arbo Acres broiler chickens in Malaysia. It is, however, recommended that the vaccine should be evaluated further with specific pathogen-free chickens to avoid maternal antibody interferences and that the duration of the trial should be increased especially after the challenge to better study the efficacy of inactivated nVarIBDV in chickens.

DECLARATIONS

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Availability of data and materials

The Data and materials are available upon demand.

Competing interests

The authors hereby declare that there is no competing interest.

Ethical considerations

The authors have avoided plagiarism, misconduct, data fabrication/falsification, and double submission/publication and have given consent to publish this article.

Authors' contributions

Mohd Hair Bejo acquired the funds, conceptualized and supervised the work, and read the manuscript. Lathasha Gauthaman, Mazlina Mazlan, Norfitriah Mohamed Sohaimi, and Chidozie Clifford Ugwu conducted the experiments, collected and analyzed the data, and prepared the manuscript. All authors read and approved that last manuscript version.

REFERENCES

Aliyu HB, Hair-Bejo M, Omar AR, and Ideris A (2021). Genetic diversity of recent infectious bursal disease viruses isolated from vaccinated poultry flocks in Malaysia. Frontiers in Veterinary Science, 8: 643976. DOI: https://www.doi.org/10.3389/fvets.2021.643976

Artika IM and Ma'roef CN (2017). Laboratory biosafety for handling emerging viruses. Asian Pacific Journal of Tropical Biomedicine, 7(5): 483-491. DOI: https://www.doi.org/10.1016/j.apjtb.2017.01.020

Babazadeh D and Asasi K (2021). Effects of *in ovo* synbiotic injection on growth performance, intestinal bacterial load and antibody titres in broiler chickens vaccinated against infectious Bursal disease. Bulgarian Journal of Veterinary Medicine, 24(4): 520-532. Available at: http://tru.uni-sz.bg/bjvm/BJVM%20December%202021%20p.520-532.pdf

Van Den Berg TP (2000). Acute infectious Bursal disease in poultry: A review. Avian Patholology, 29(3): 175-194. DOI: https://www.doi.org/10.1080/03079450050045431

Cheggag M, Zro K, Terta M, Fellahi S, Mouahid M, El Houadfi M, Sebbar G, and Kichou F (2020). Isolation, molecular, and pathological characterization of infectious Bursal disease virus among broiler chickens in Morocco. Journal of World's Poultry Research, 10(3): 493-506. DOI: https://www.doi.org/10.36380/jwpr.2020.57

Delmas B, Attoui H, Ghosh S, Malik YS, Mundt E, Vakharia VN, and ICTV Report Consortium (2019). ICTV virus taxonomy profile: Birnaviridae. Journal of General Virology, 100(1): 5-6. DOI: https://www.doi.org/10.1099/jgv.0.001185

Delrue I, Verzele D, Madder A, and Nauwynck HJ (2012) Inactivated virus vaccines from chemistry to prophylaxis: Merits, risks and challenges. Expert Review of Vaccines, 11(6): 695-719. DOI: https://www.doi.org/10.1586/erv.12.38

Ebrahimi MM, Yousefi AR, Shahsavadi S, Zaghari M, and Bassami MR (2020). Comparison of the immunogenicity of four infectious Bursal disease intermediate vaccines in commercial broiler flocks in Iran: A field trial study. Archives of Razi Institute, 75(2): 205-212. DOI: https://www.doi.org/10.22092/ari.2019.124890.1292

Elawad AH, Hair-Bejo M, Omar AR, Liew PS, Saeed MI, Arshad SS, Awad EA, and Aini I (2020). Scoring system for lesions induced by different strains of infectious Bursal disease virus in chicken. International Journal of Poultry Science, 19(4): 153-160. DOI: https://www.doi.org/10.3923/ijps.2020.153.160

Eterradosi N and Saif Y (2013). Infectious Bursal disease. In: J. R. Glisson, L. R. McDougald, L. K. Nolan, D.L. Suarez, V. L. Nair (Editors), Diseases of poultry. Wiley-Blackwell., Oxford, UK, pp. 219-236.

Fan L, Wu T, Hussain A, Gao Y, Zeng X, Wang Y, Gao L, Li K, Wang Y, Liu C et al. (2019). Novel variant strains of infectious bursal disease virus isolated in China. Veterinary Microbiology, 230: 212-220. DOI: https://www.doi.org/10.1016/j.vetmic.2019.01.023

Fan L, Wang Y, Jiang N, Gao L, Li K, Gao Y, Cui H, Pan Q, Liu C, Zhang Y et al. (2020a). A reassortment vaccine candidate of the novel variant infectious bursal disease virus. Veterinary Microbiology, 251: 108905. DOI: https://www.doi.org/10.1016/j.vetmic.2020.108905

Fan L, Wu T, Wang Y, Hussain A, Jiang N, Gao L, Kai L, Gao Y, Liu C, Cui H et al. (2020b). Novel variants of infectious bursal disease virus can severely damage the bursa of fabricius of immunized chickens. Veterinary Microbiology, 240: 108507. DOI: https://www.doi.org/10.1016/j.vetmic.2019.108507

Habib M, Hussain I, Fang WH, Rajput ZI, Yang ZZ, and Irshad H (2006). Inactivation of infectious bursal disease virus by binary ethylenimine and formalin. Journal of Zhejiang University Science B, 7(4): 320-323. DOI: https://www.doi.org/10.1631/jzus.2006.B0320

Miller PJ, Estevez C, Yu Q, Suarez DL, and King DJ (2009). Comparison of viral shedding following vaccination with inactivated and live Newcastle disease vaccines formulated with wild-type and recombinant viruses. Avian Diseases, 53(1): 39-49. DOI: https://www.doi.org/10.1637/8407-071208-Reg.1

Müller H, Islam MR, and Raue R (2003). Research on infectious bursal disease – the past, the present and the future. Veterinary Microbiology, 97(1-2): 153-165. DOI: https://www.doi.org/10.1016/j.vetmic.2003.08.005

Mutinda WU, Njagi LW, Nyaga PN, Bebora LC, Mbuthia PG, Kemboi D, Githinji JW, and Muriuki A (2015). Isolation of infectious Bursal disease virus using indigenous chicken embryos in Kenya. International Scholarly Research Notices, 2015: 464376. https://www.doi.org/10.1155/2015/464376

Myint O, Suwanruengsri M, Araki K, Izzati UZ, Pornthummawat A, Nueangphue P, Fuke N, Hirai T, Jackwood DJ, and Yamaguchi R (2021). The bursa atrophy at 28 days old by the variant infectious Bursal disease virus makes a negative economic impact on broiler farms in Japan. Avian Pathology, 50(1): 6-17. https://www.doi.org/10.1080/03079457.2020.1822989

Okura T, Otomo H, Suzuki S, Ono Y, Taneno A, and Oishi E (2021). Efficacy of a novel *in ovo*-attenuated live vaccine and recombinant vaccine against a very virulent infectious bursal disease virus in chickens. Journal of Veterinary Medical Science, 83(11): 1686-1693. DOI: https://www.doi.org/10.1292/jvms.21-0319

Orakpoghenor O, Oladele SB, and Abdu PA (2020). Research note: Detection of infectious bursal disease virus antibodies in free-living wild birds in Zaria, Nigeria. Poultry Science, 99(4): 1975-1977. DOI: https://www.doi.org/10.1016/j.psj.2019.11.036

Qin Y and Zheng SJ (2017). Infectious bursal disease virus-host interactions: Multifunctional viral proteins that perform multiple and differing jobs. International Journal of Molecular Sciences, 18(1): 161. https://www.doi.org/10.3390/ijms18010161

Rauw F, Gardin Y, Palya V, van Borm S, Gonze M, Lemaire S, van den Berg T, and Lambrecht B (2009). Humoral, cell-mediated and mucosal immunity induced by oculo-nasal vaccination of one-day-old SPF and conventional layer chicks with two different live Newcastle disease vaccines. Vaccine, 27(27): 3631-3642. DOI: https://www.doi.org/10.1016/j.vaccine.2009.03.068

Tehrani NK, Mahdavi M, Maleki F, Zarrati S, and Tabatabaie F (2016). The role of montanide ISA 70 as an adjuvant in immune responses against Leishmania major induced by thiol-specific antioxidant-based protein vaccine. Journal of Parasitic Diseases, 40(3): 760-776. DOI: https://www.doi.org/10.1007/s12639-014-0574-8

Thai TN, Jang I, Kim HA, Kim HS, Kwon YK, and Kim HR (2021). Characterization of antigenic variant infectious bursal disease virus strains identified in South Korea. Avian

Pathology, 50(2): 174-181. https://www.doi.org/10.1080/03079457.2020.1869698

Ugwu CC, Hair-Bejo M, Nurulfiza MI, Omar AR, and Ideris A (2022). Efficacy, humoral, and cell-mediated immune response of inactivated fowl adenovirus 8b propagated in chicken embryo liver cells using bioreactor in broiler chickens. Veterinary World, 15(11): 2681-2692. DOI: www.doi.org/10.14202/vetworld.2022.2681-2692

Wang Y, JiangN, Fan L, Gao L, Li K, Gao Y, Niu X, Zhang W, Cui H, Liu A et al. (2021). Development of a viral-like particle candidate vaccine against novel variant infectious Bursal disease virus. Vaccines, 9(2): 142. DOI: https://www.doi.org/10.3390/vaccines9020142

Wang Z, Mi J, Wang Y, Wang T, Qi X, Li K, Pan Q, Gao Y, Gao L, Liu C et al. (2020). Recombinant lactococcus expressing a novel variant of infectious bursal disease virus VP2 protein can induce unique specific neutralizing antibodies in chickens and provide complete protection. Viruses, 12(12): 1350. https://www.doi.org/10.3390/v12121350

Yang H, Wang Y, and Ye C (2020). Rapid generation of attenuated infectious Bursal disease virus from dual-promoter plasmids by reduction of viral ribonucleoprotein activity. Journal of Virology, 94(7): e01569-19. DOI: https://www.doi.org/10.1128/JVI.01569-19

Zhao J, Zhong Q, Zhao Y, Hu YX, and Zhang GZ (2015). Pathogenicity and complete genome characterization of fowl adenoviruses isolated from chickens associated with inclusion body hepatitis and hydropericardium syndrome in China. PLoS One, 10(7): e0161744. DOI: https://www.doi.org/10.1371%2Fjournal.pone.0133073