

Velogenic newcastle disease lesions suppress antibody response to infectious bronchitis vaccination in La Sota vaccinated and unvaccinated chickens

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Summary

Background: Velogenic Newcastle disease (vND) is a major disease of poultry worldwide. One of the common lesions of the disease is atrophy of the lymphoid organs. **Aim:** This project studied the ability of these lesions to suppress antibody response to infectious bronchitis (IB) vaccination in both chickens vaccinated and unvaccinated with the La Sota vaccine. **Methods:** One hundred fifty two-week-old cockerel chickens were divided into three groups, namely: a vaccinated challenged (VC) group, an unvaccinated challenged (UC) group, and an unvaccinated and unchallenged (UU) group. The VC group was given the La Sota vaccine at two weeks of age. At four weeks of age, all of the groups were challenged with the vND virus (vNDV). **Results:** Mortality was 82% in the UC group with lesions in the digestive and lymphoid organs. The VC group showed no clinical signs but there was severe atrophy and necrosis of the lymphoid organs. Fifteen chickens in the VC and UU groups and fifteen in the UC group were vaccinated with an infectious bronchitis vaccine at 14 and 28 days post challenge. After each vaccination, blood was collected from the chickens on days 7, 14, 21 and 28 post vaccination, and the sera were assayed for the IB antibody titers. The results showed that the titers in the UU chickens were significantly higher ($p < 0.05$) than those in both the VC and UC chickens on nearly all of the days tested. **Conclusions:** The findings indicate that vNDV infection suppresses the antibody response to infectious bronchitis vaccination in both vaccinated and unvaccinated chickens.

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INTRODUCTION

Newcastle disease (ND) is an important and worldwide disease of poultry. It affects a very wide range of avian species, causing varying degrees of clinical signs and lesions that can be mild, moderate or

severe depending on the species of the avian host, age, concurrent infections, level of immunity, the pathotype and dose of the infecting virus (Miller & Koch, 2020). Chickens are the most susceptible species, and mortality can reach up to 100%. ND is a pantropic disease, but most of the clinical signs are manifestations of lesions in the digestive, nervous, respiratory and reproductive systems. The aetiological agent is the pathogenic strains of the ND virus (NDV), which is an avian *orthoavulavirus* 1 species in the genus *Orthoavulavirus*, subfamily *Avularvirinae*, family *Paramyxoviridae* and order *Monogravirales* (Amarasinghe *et al.*, 2019; Walker *et al.*, 2019). The velogenic pathotype of the NDV (vNDV) causes the most devastating form of ND in the world, and it is enzootic in many countries of Asia, Africa, Middle East, Central and South America (Khorajiya *et al.*, 2015; Absalon *et al.*, 2019; Welch *et al.*, 2019).

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Outbreaks of vND cause huge economic losses annually for poultry producers due to high bird mortality rates and the loss of poultry products, especially eggs. Controlling ND has been challenging for many reasons. The disease is not easy to diagnose early in the field because it has no pathognomonic lesions and its clinical signs resemble those of many other avian diseases (Cattoli *et al.*, 2011). The virus affects some avian species that manifest little or no clinical signs but constitute a reservoir of infection for poultry (Igwe *et al.*, 2014). The emergence of many NDV strains with high genetic and antigenic differences has led to many ND outbreaks in vaccinated flocks in many parts of the world where vNDV is enzootic (Welch *et al.*, 2019). Out of the 21 genotypes discovered so far, seven have been isolated in Nigeria (Dimitrov *et al.*, 2019; Welch *et al.*, 2019).

One of the major lesions of vNDV infection, as reported by several workers in poultry, is atrophy of the bursa, spleen and thymus due to the necrosis and depletion of the lymphocytes in these organs. La Sota vaccines also do not protect chickens from histologic lesions in the same way that they protect them from overt clinical signs (Wakamatsu *et al.*, 2006; Cattoli *et al.*, 2011; Okpe *et al.*, 2015; Ezema *et al.*, 2016; Igwe *et al.*, 2019). In this experiment, we studied the ability of these lesions to decrease antibody response to infectious bronchitis vaccination in chickens.

MATERIALS AND METHODS

Experimental chickens

Experimental animals were raised in accordance with the recommended best practices, and animals were humanly handled throughout the experiment. This study was approved by the Institutional Animal Care and Use Committee of the Faculty Veterinary Medicine, University of Nigeria Nsukka, Nigeria (Approval Reference Number: FVM-UNN-IACUC-2019-11/201).

One hundred and fifty one-day-old cockerel chickens were purchased from a commercial hatchery. They were reared in isolation using deep litter. Feed and water were supplied *ad libitum*. At two weeks of age they were divided into three groups: (i) the Vaccinated challenged (VC) group contained 30 chickens; (ii) the Unvaccinated challenged (UC) group contained 90 chickens; and (iii) the Unvaccinated unchallenged (UU) group contained 30 chickens.

La Sota vaccination

Chickens in group VC were vaccinated with the La Sota vaccine at two weeks of age. The vaccine was manufactured by the National Veterinary Research Institute, Vom, Plateau State, Nigeria. It had a median chicken embryo infective dose (EID₅₀) of 10^{6.2} per ml. Vaccination was by oral drenching.

Challenge of the birds

The Newcastle disease virus inoculum used was a local velogenic viscerotropic strain, duck/Nigeria/903/KUDU-113/1992, which is a class II, genotype XVII NDV strain (Shittu *et al.*, 2016). At four weeks of age, all of the chickens in the VC and UC groups were each given 0.2 ml of the challenge strain intramuscularly using a median embryo infective dose (EID₅₀) of 10^{6.5} per ml.

Examination for clinical signs and lesions

All of the groups were monitored for clinical signs for a duration of 20 days post challenge (PC). Three dead or sacrificed chickens were examined for gross lesions on days 3, 6, 10, and 15 PC. The bursa, spleen and thymus were fixed in 10% formal saline for a minimum of 24 hr and processed for histopathology.

Post challenge vaccination with infectious bronchitis vaccine

At intervals of 14 and 28 days PC, 15 chickens in the VC group, 15 in the UU group, and 15 in the UC group were isolated and vaccinated with an infectious bronchitis (IB) vaccine (Izovac H120 live attenuated virus vaccine of IBH, strain Massachusetts H120).

Serology

After each vaccination against infectious bronchitis, 2 ml of blood was collected from 10 chickens in each of the three groups on days 7, 14, 21 and 28 post vaccination (PV). Sera samples were collected and used for antibody testing using an indirect enzyme-linked immunosorbent assay (ELISA) for infectious bronchitis (ID Screen®). These ELISA kits are commercially available from ID Vet Innovative Diagnostics (France). Specific antibodies directed against infectious bronchitis virus in the sera samples were tested using the manufacturer's recommended protocol.

Statistical analysis

The ELISA results were subjected to an independent T Test and one-way analysis of variance (ANOVA) to compare the mean antibody titers in the groups at each sampling time in the experiment. The results were expressed as the mean \pm standard error of the mean (mean \pm SE). Duncan's Multiple Range test (DMRT) was used to determine the significant differences in the mean titers among the three groups. The significance level was accepted at $p \leq 0.05$.

RESULTS

Clinical signs

Chickens in the VC and UU groups showed no clinical signs. Chickens in the UC group showed a decrease in feed and water consumption, and somnolence on day 3 PC. There were ruffled feathers, droopy wings, huddling, diarrhoea with greenish faeces, severe depression and later torticollis. Deaths occurred from days 4 to 7 PC. Total mortality was 82.0%.

Lesions

Chickens in the UC group showed congested skeletal muscles. Haemorrhages on the proventricular glands, haemorrhagic ulcers in the ileum, and swollen haemorrhagic caecal tonsils were observed. The kidneys were swollen and either congested or haemorrhagic. The bursa, spleen and thymus were atrophic (Figures 1A, B, C). The only gross lesion in chickens in the VC group was atrophy of the bursa, spleen and thymus. Histopathology sections showed necrosis and depletion of the lymphocytes in the bursa, spleen and thymus of the VC and UC chickens (Figures 2A, B, C).

Serology

In the chickens vaccinated on day 14 PC, the mean antibody titer of the UU group chickens was significantly higher ($p < 0.05$) than that of the UC group chickens on day 7 PV (Figure 3A). On days 14, 21 and 28 PV, the titers of UU chickens were significantly higher than those of UC and VC chickens. In the groups vaccinated on day 28 PC, on days 7 and 14 PV the titers of the UU chickens were significantly higher ($p < 0.05$) than those of the UC chickens and also significantly higher than those of the UC and VC chickens on days 21 and 28 PV (Figure 3B).

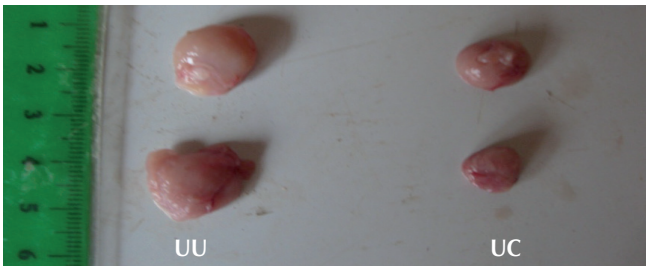


Figure 1A: Severe atrophy of the bursa of Fabricius in dead UC chickens on day 6 PC when compared with those of the UU group /// *Atrophie sévère de la bourse de Fabricius chez les poulets UC morts au 6^e jour après challenge par rapport à ceux du groupe UU*



Figure 1B: Severe atrophy of the spleen of the UC and VC chickens sacrificed on day-6 post challenge /// *Atrophie sévère de la rate des poulets UC et VC sacrifiés au 6^e jour après challenge*

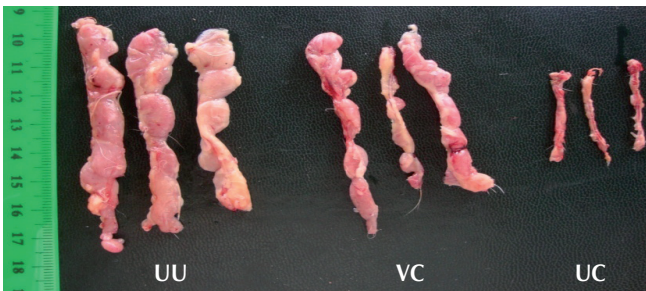


Figure 1C: Severe atrophy of the thymus of the UC and VC chickens sacrificed on day 15 PC /// *Atrophiesévère du thymus des poulets UC et VC sacrifiés au 15^e jour après challenge*

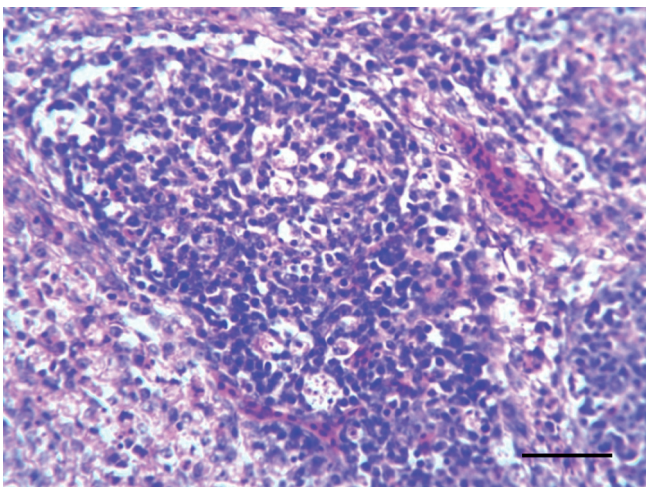


Figure 2A: Bursa of a VC chicken that was sacrificed on day 3 PC showing hyperemia, necrosis and depletion of lymphocytes. Haematoxylin and eosin. Bar=50 microns long /// *Bourse de Fabricius du poulet VC sacrifié au 3^e jour après challenge montrant une hyperémie, une nécrose et un appauvrissement en lymphocytes. Hématoxyline et éosine. Barre = 50 µm de long*

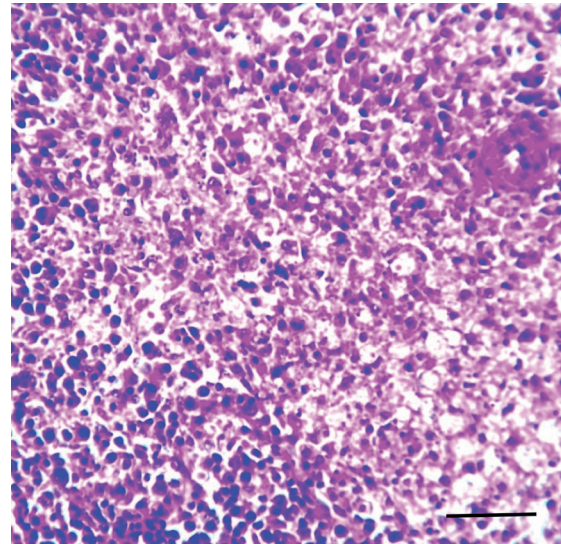


Figure 2B: Spleen of a UC chicken that died on day 6 PC showing necrosis and depletion of lymphocytes. Haematoxylin and eosin. Bar=50 microns long /// *Rate d'un poulet UC mort au 6^e jour après challenge montrant une nécrose et une déplétion des lymphocytes. Hématoxyline et éosine. Barre = 50 µm de long*

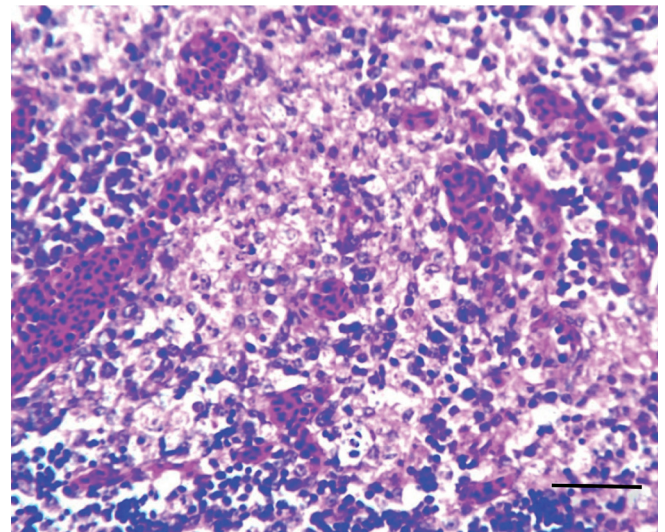


Figure 2C: Thymus of a VC chicken sacrificed on day 3 PC showing hyperemia, necrosis and depletion of lymphocytes. Haematoxylin and eosin. Bar=50 microns long /// *Thymus d'un poulet VC sacrifié au 3^e jour après challenge présentant une hyperémie, une nécrose et une raréfaction des lymphocytes. Hématoxyline et éosine. Barre = 50 microns de long*

DISCUSSION

In this study, we demonstrated that vNDV can induce immunosuppression even in ND-vaccinated animals, as evidenced by reduced antibody production levels against the IB vaccine. The ELISA test was used to monitor immune responses following IB vaccination, as it is increasingly replacing haemagglutination inhibition (HI) and serum neutralization (SN) tests for detecting NDV and IBV antibodies (Rautenschlein *et al.*, 2007; Bwala *et al.*, 2012; Cornax *et al.*, 2012; OIE, 2018). This shift is due to the ELISA test's automation, rapid processing time, and ability to efficiently analyze a large number of samples. Additionally, IBV and NDV ELISA kits are commercially available from a variety of manufacturers. According to the

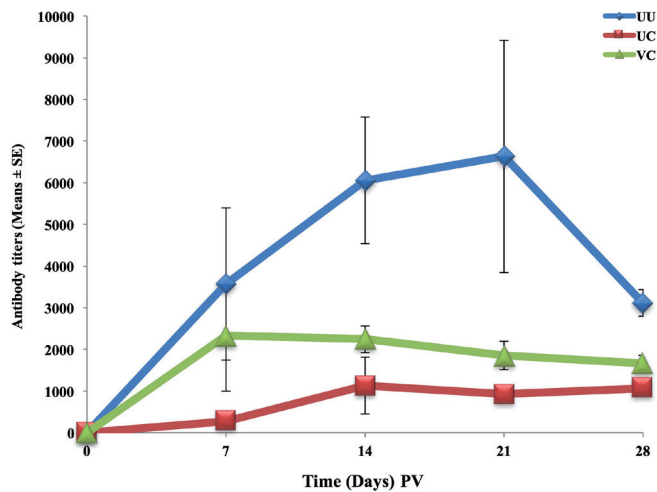


Figure 3A: Serum antibody titers of chickens vaccinated against IB on day 14 PC and monitored for 28 days PV /// Titres en anticorps sériques de poulets vaccinés contre la bronchite infectieuse au 14^e jour après challenge et suivis pendant 28 jours après vaccination

UU = unvaccinated unchallenged group /// groupe non vacciné et non challengé
 UC = unvaccinated challenged group /// groupe challengé non vacciné
 VC = vaccinated challenged group /// groupe challenge vacciné

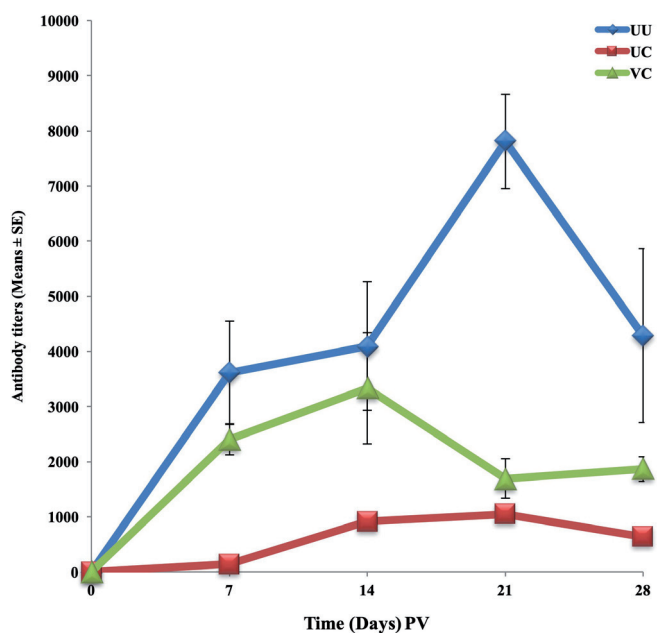


Figure 3B: Serum antibody titers of chickens vaccinated against IB on day 28 PC and monitored for 28 days PV /// Titres en anticorps sériques de poulets vaccinés contre la bronchite infectieuse au 28^e jour après challenge et suivis pendant 28 jours après vaccination

UU = unvaccinated unchallenged group /// groupe non vacciné et non challengé
 UC = unvaccinated challenged group /// groupe challengé non vacciné
 VC = vaccinated challenged group /// groupe challenge vacciné

OIE (2018), commercially available ELISA kits are widely used for monitoring serum antibody responses following infectious bronchitis vaccination. The OIE (2018) further stated that the antigens used in these kits exhibit broad cross-reactivity among serotypes, enabling comprehensive serological monitoring of both vaccine-induced immunity and field challenges.

Our results showed that the lesions of the vNDV infection in the lymphoid organs significantly suppressed antibody response to IB

vaccination both in unvaccinated chickens and in chickens vaccinated with the La Sota vaccine. This is due to the severe necrosis and depletion of the lymphocytes in the bursa, spleen and thymus which are the main antibody producing lymphoid organs of the avian immune system. The bursa produces the B lymphocytes which produce the humoral antibodies response, while the thymus produces the T lymphocytes that produce the cellular response. Our experiment did not determine the duration of immunosuppression; however, previous studies have shown that bursa destruction before two weeks of age leads to permanent immunosuppression in chickens, whereas destruction occurring after this period results in only transient suppression (Giambone, 1979). The macroscopic lesions of organs and the serology results of this experiment showed that the atrophy was lower and the antibody response against infectious bronchitis was better in the VC group than the UC group. Consequently, it is possible that the VC chickens will recover earlier from the immunosuppression than the UC chickens, showing that the vaccination was not completely useless. Ezema *et al.* (2016) provided what may be the first reported observation that vNDV infection suppressed antibody response to La Sota vaccination in chickens. Hitherto, vND has not been well recognized as an immunosuppressive disease because little or no convincing evidence has been produced to confirm this. In a review, Hoerr (2010) mentioned infectious bursal disease (IBD), chicken infectious anaemia (CIA), Marek's disease, retroviral tumour diseases, haemorrhagic enteritis and other adenoviral diseases, some enteric viral diseases and respiratory diseases including ND and pneumovirus infection, as the immunosuppressive diseases of poultry. The only evidence cited by Hoerr (2010) to describe ND as an immunosuppressive disease comes from the reports by Lam and Vasconcelos (1994) and Lam (1996), which indicated that NDV infection induces apoptosis in chicken peripheral blood lymphocytes. There was no evidence of immunosuppression in either publication. In their book on poultry diseases, Miller and Koch (2020) stated that NDV infections had the ability to cause immunosuppression. They cited Rautenschlein *et al.* (2007), who only studied the bilateral effects of vaccination against ND and infectious bursal disease (IBD) in commercial broiler chickens and specific-pathogen-free layers. Rautenschlein *et al.* (2007) never studied nor did they show any evidence that either ND vaccination or ND pathogenic virus infection caused immunosuppression in their research. The results of the present study showed that the chickens vaccinated with La Sota vaccine and challenged with virulent NDV (VC group) had no clinical signs, but they developed atrophy of the lymphoid organs which resulted in low antibody titers. The lower antibody titers in the VC chickens when compared with the UU chickens confirmed that the vNDV caused immunosuppression even in La Sota vaccinated animals. Our previous publications (Ezema *et al.*, 2016; Igwe *et al.*, 2019) reported that La Sota vaccination alone did not induce lymphoid organ atrophy, confirming that this effect is specifically caused by the vNDV challenge. Immunosuppression leads to vaccine failures and increased susceptibility to other infections. Indeed, we have also demonstrated that was immunosuppression following later vaccinations with the IB vaccine. This characteristic of vNDV infection presents a major challenge to the global poultry industry, particularly in countries where the virus is enzootic. It poses a significant problem for poultry producers, as it can lead to severe production issues that may be difficult to diagnose in vaccinated chickens. The La Sota vaccine is the most widely used ND vaccine because it is highly antigenic and easy to administer in drinking water. In recent years, several studies have shown that the La Sota vaccination can protect against the clinical signs of infection, but it does not prevent lymphoid organ atrophy, shedding of the vNDV in faeces and oropharyngeal secretions, or decreased egg production when poultry are challenged with vNDV (Miller *et al.*, 2007, 2013; Ezema *et al.*, 2009; Bwala *et al.*, 2012; Okwor *et al.*, 2016; Bastami *et al.*, 2018; Wajid *et al.*, 2018). A drop in egg production without

any other clinical sign is the most common indicator of vNDV infection in vaccinated layers in countries where vNDV is enzootic (Okechukwu *et al.*, 2020), and leads to huge economic losses annually. Currently, there is an urgent need to develop a vaccine or vaccination program that provides satisfactory protection against vND in chickens without making animals susceptible to other diseases. Until this is achieved, strict biosecurity measures will be very important in the control of vND and other diseases in the poultry industry. The results of this study indicate that the La Sota vaccine can impact the immune response to the IB vaccine. Therefore, farmers should be advised on the importance of spacing the vaccination periods for birds receiving different vaccines. It is recommended that further research should be conducted to ascertain the proper vaccination regime between the La Sota vaccination and the IB vaccination.

CONCLUSION

Velogenic NDV infection suppressed antibody response to infectious bronchitis vaccination in both vaccinated and unvaccinated chickens.

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Conflict of interest

The study was carried without any conflict of interest.

Author contributions

JOAO: Conceptualization, methodology, supervision. JNO, AOI and HIG: Investigation, DCE: Formal analysis and interpretation. JOAO: Writing original draft. All of the authors read and approved the manuscript for submission.

Ethics approval

The experimental animals were raised in accordance with the recommended best practices and animals were humanly handled throughout the experiment. This study was approved by the Institutional Animal Care and Use Committee of the Faculty Veterinary Medicine, University of Nigeria Nsukka, Nigeria (Approval Reference Number: FVM-UNN-IACUC- -2019-11/201)

Data availability

The data were not deposited in an official repository. The data that support the study findings are available from the authors upon request.

Declaration of Generative AI in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

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Résumé

Eze D. C., Omeke J. N., Igwe A. O., Gambo H., Okoye J. O. A. Les lésions vélogènes de la maladie de Newcastle suppriment la réponse des anticorps à la vaccination contre la bronchite infectieuse chez les poulets vaccinés et non vaccinés par la souche La Sota

Contexte : La maladie de Newcastle vélogène (vND) est une maladie majeure de la volaille dans le monde entier. L'atrophie des organes lymphoïdes est l'une des lésions courantes de la maladie. **Objectif** : Ce projet a étudié la capacité de ces lésions à supprimer la réponse des anticorps à la vaccination contre la bronchite infectieuse (BI) chez les poulets vaccinés et non vaccinés contre la maladie de Newcastle avec le vaccin La Sota. **Méthodes** : Cent cinquante coquelets âgés de 2 semaines ont été divisés en 3 groupes, à savoir : un groupe vacciné et challengé (VC), un groupe non vacciné et challengé (UC) et un groupe non vacciné et non challengé (UU). Le groupe VC a reçu le vaccin La Sota à l'âge de 2 semaines. À l'âge de 4 semaines, tous les groupes ont été exposés au virus vND. **Résultats** : La mortalité était de 82 % dans le groupe UC avec des lésions dans les organes digestifs et lymphoïdes. Le groupe VC n'a montré aucun signe clinique mais a présenté une atrophie sévère et une nécrose des organes lymphoïdes. Quinze poulets des groupes VC et UU et quinze poulets du groupe UC ont été vaccinés avec le vaccin contre la bronchite infectieuse à 14 et 28 jours après le test. Après chaque vaccination, du sang a été prélevé sur les poulets les 7^e, 14^e, 21^e et 28^e jours après la vaccination et les sérums ont été analysés pour déterminer les titres en anticorps BI. Les résultats ont montré que les titres des poulets UU étaient significativement plus élevés ($p < 0,05$) que ceux des poulets VC et UC pour presque tous les jours testés. **Conclusions** : On peut conclure que l'infection par le virus de la vND a supprimé la réponse des anticorps à la vaccination contre la bronchite infectieuse chez les poulets vaccinés et non vaccinés.

Mots-clés : Poulet, maladie de Newcastle, bronchite infectieuse aviaire, vaccin, immunodépression, Nigeria

Resumen

Eze D. C., Omeke J. N., Igwe A. O., Gambo H., Okoye J. O. A. Las lesiones velogénicas de la enfermedad de Newcastle suprimen la respuesta de los anticuerpos a la vacunación contra la bronquitis infecciosa en pollos vacunados y no vacunados con la cepa La Sota

Contexto: La enfermedad de Newcastle velogénica (vND) es una enfermedad grave que afecta a las aves de corral en todo el mundo. Una de las lesiones habituales de la enfermedad es la atrofia de los órganos linfoides. **Objetivo**: Este proyecto estudió la capacidad de tales lesiones para suprimir la respuesta de los anticuerpos ante la vacunación contra la bronquitis infecciosa (BI) en los pollos vacunados con la vacuna La Sota contra la enfermedad de Newcastle y en los pollos no vacunados con esta vacuna. **Métodos**: Ciento cincuenta gallos jóvenes de 2 semanas de edad se dividieron en 3 grupos: un grupo vacunado y con desafío (VC), un grupo no vacunado y con desafío (UC) y un grupo no vacunado sin desafío (UU). El grupo VC recibió la vacuna La Sota a la edad de 2 semanas. A la edad de 4 semanas, todos los grupos se expusieron al virus vND. **Resultados**: La mortalidad fue del 82 % en el grupo UC, con lesiones en los órganos digestivos y linfoides. El grupo VC no mostró ningún signo clínico, pero presentó una atrofia severa y una necrosis de los órganos linfoides. Quince pollos de los grupos VC y UU y quince pollos del grupo UC se vacunaron con la vacuna contra la bronquitis infecciosa 14 y 28 días después de la prueba. Se tomaron muestras de sangre de los pollos los días 7^o, 14^o, 21^o y 28^o posteriores a cada vacunación, y se analizaron los sueros para determinar los títulos de anticuerpos BI. Los resultados mostraron que los títulos en los pollos UU eran significativamente más elevados ($p < 0,05$) que en los pollos VC y UC para casi todos los días muestreados. **Conclusiones**: Se puede concluir que la infección por el virus de la vND suprime la respuesta de los anticuerpos ante la vacunación contra la bronquitis infecciosa en los pollos vacunados y no vacunados.

Palabras clave: Pollo, virus de la enfermedad de Newcastle, bronquitis infecciosa aviar, vacuna, inmunodepresión, Nigeria