Journal of Applied Veterinary Sciences, 9 (4): 26 - 33 (October, 2024).
ISSN: Online: 2090-3308, Print: 1687-4072
Journal homepage : https://javs.journals.ekb.eg

Serological Detection of Newcastle Disease Virus Antibody in Vaccinated and Non-Vaccinated Indigenous Chickens and Guinea Fowls in Atacora and Donga, Northern Benin

Edmond Onidje[1*], Oluwole Oyetunde Oni[2], Benjamin Obukowho Emikpe[3], Vitus Burimuah[3], Amponsah Patrick Mensah[4], Derrick Adu Asare[3]

[1]Pan African University Life and Earth Sciences Institute (including Health and Agriculture), Ibadan, Nigeria
[2]School of Veterinary Medicine, University of Surrey, Guildford, Uk
[3]School of Veterinary Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[4]Kumasi Veterinary Laboratory, Kumasi, Ghana
***Corresponding Author:** Edmond Onidje, **E-Mail:** eonimond@gmail.com

ABSTRACT

Poultry farming is crucial for the livelihoods of small-scale producers in the Northen Benin, yet the industry faces challenges from diseases like ND, which threaten both poultry health and economic stability. This study investigates the seroprevalence of Newcastle Disease Virus (NDV) among indigenous chickens and guinea fowl in the Atacora and Donga regions of Northern Benin, addressing a significant gap in regional poultry health data. A cross-sectional study was designed, sampling a total of 300 birds, including 191 indigenous chickens and 109 guinea fowl, from six districts. Systematic random sampling was employed to select smallholder farms, and blood samples were collected for Hemagglutination inhibition (HI) tests to detect NDV antibodies. Statistical analyses, including chi-square tests, determined associations and differences in seroprevalence between regions and species. Results revealed notable variations in NDV seroprevalence between the two regions. In Atacora, 59.22% of chickens tested positive for NDV antibodies, with 52.33% of non-vaccinated and 94.12% of vaccinated chickens showing positive results. In Donga, the overall seroprevalence was 46.59%, with 27.78% of non-vaccinated and 59.62% of vaccinated chickens testing positive. A chi-square test indicated a significant difference in NDV seroprevalence between the two regions for chickens (x^2 = 12.901; P = 0.024). For guinea fowls, seroprevalence was 63.53% in Atacora and 62.50% in Donga, with no significant difference observed (x^2 = 1.102; P = 0.954). This study provides the first serological data on NDV prevalence in northern Benin, highlighting the endemic nature of NDV and the critical epidemiological role of guinea fowls due to frequent exposure and field infections, especially in the Atacora region. It recommends enhancing vaccination coverage, improving biosecurity measures, and conducting further research to isolate and characterize virus strains to develop more effective control strategies.

Keywords: Benin, Guinea Fowl, Indigenous Chickens, Newcastle Disease Virus (NDV), Seroprevalence.

Original Article:
DOI:https://dx.doi.org/10.21608/javs.2024.300310.1363
Received : 29 June, 2024.
Accepted: 24 August, 2024.
Published in October, 2024.

J. Appl. Vet. Sci., 9(4): 26-33.

INTRODUCTION

Poultry farming has long been a critical component of the agricultural economy in many developing regions, including the Republic of Benin, where it ranks as the second most important livestock sector after cattle **(Toko, 2008; Hailemichael *et al.*, 2017).** This sector significantly contributes to the livelihoods and nutritional needs of rural communities. For many small-scale producers who often face financial constraints, poultry farming has historically met essential domestic and economic needs, providing a vital source of cash income through the sale of poultry **(Camus *et al.* 2020).**

In recent years, the poultry sector in Benin has been predominantly composed of indigenous species, with guinea fowl being the third most common domestic bird after ducks and chickens **(DSA, 2024).** Recent data from Benin's Agricultural Statistics Department (DSA) indicates that the indigenous chicken population reached approximately 16.431 million heads in 2023 **(DSA, 2024).** This growth underscores the sector's

critical role in sustaining the livelihoods of many Beninese, particularly in rural areas.

Despite this growth, the indigenous poultry industry in Benin has faced several challenges. Issues such as low productivity, inadequate biosecurity measures, and limited access to inputs have been prevalent, especially among traditional smallholders who dominate domestic production. These farms have been particularly vulnerable to diseases like Newcastle Disease (ND), which pose significant threats to poultry health and economic stability in the region **(Samuel *et al.*, 2013).**

Newcastle disease (ND) is a contagious and complex disease caused by the Newcastle disease virus (NDV), a single-stranded, negative-sense RNA virus belonging to the genus *Avulavirus* within the family *Paramyxoviridae* **(Brown and Bevins, 2017).** Due to the virus's high genetic variability, several serotypes and pathotypes have been identified, each differing in virulence and clinical manifestations, as described by the Canadian Food Inspection **Agency (2014).**

NDV affects various bird species, including both domestic and wild birds **(Shekaili *et al.*, 2015; Brown and Bevins, 2017; Wodajo *et al.*, 2023).** Chickens are particularly susceptible, with the severity of the disease depending on the infecting pathotype. The virus can also infect other poultry, such as turkeys, ducks, geese, and guinea fowl, with varying clinical outcomes. Wild birds, particularly waterfowl and migratory birds, are significant carriers and play a crucial role in spreading the pathogen across regions and continents **(Dimitrov *et al.*, 2017).**

Given the unique farming practices and environmental conditions in Northern Benin, there is a critical need for region-specific data on NDV infection. Despite the importance of indigenous chicken and Guinea fowl in these regions, no studies have been conducted to assess the seroprevalence of NDV among these poultry species in the Atacora and Donga regions. This study aims to fill this gap by assessing the seroprevalence of NDV among indigenous chickens and guinea fowl in the Atacora and Donga regions of Northern Benin. Understanding the prevalence and antibody levels in these specific species will inform targeted vaccination and biosecurity programs, ultimately improving poultry health and productivity in the region.

MATERIALS AND METHODS

Description of Study Sites

This study was conducted in the northern regions of Benin, notably in the Atacora and Donga regions. As indicated in **Fig.1**, Atacora is a northern region bordering Togo to the west and Burkina Faso to the north. The Atacora region has nine communes, namely Boukoumbé, Cobly, Kérou, Kouandé, Matéri, Natitingou as the prefecture, and Pehunko. As of the last count, it covered an area of 20,499 km² and was made up of approximately 517 villages and town districts. Donga region was previously a division of Atacora region and covers 11,126 km² with the communes of Bassila, Copargo, Djougou, the prefecture, and Ouaké. Ecologically, the Atacora consists of different land types, comprising both the highlands and the lowlands, which give way to different climates and vegetation. Atacora is home to Pendjari National Park, which is part of the W-Arly-Pendjari (WAP) Complex, a UNESCO World Heritage Site, and one of the largest and most important national parks in West Africa **(WASF, 2019).** Its climate is tropical savanna, with two distinct seasons: the dry season and the rainy season. The same goes for the Donga region, which has rolling plains and shares a tropical savanna climate, thereby creating homogenous environmental conditions that can have an impact on poultry farming **(Agbani *et al.*, 2018).** In Atacora and Donga, poultry farming is mainly traditional and extensive. Farmers largely raise indigenous chickens and guinea fowl under free-range systems, in which birds are allowed to search for their food and roam about freely. These traditional practices involve minimal inputs, limited access to veterinary services, and sporadic vaccination programs **(Ayssiwede *et al.*, 2013).**

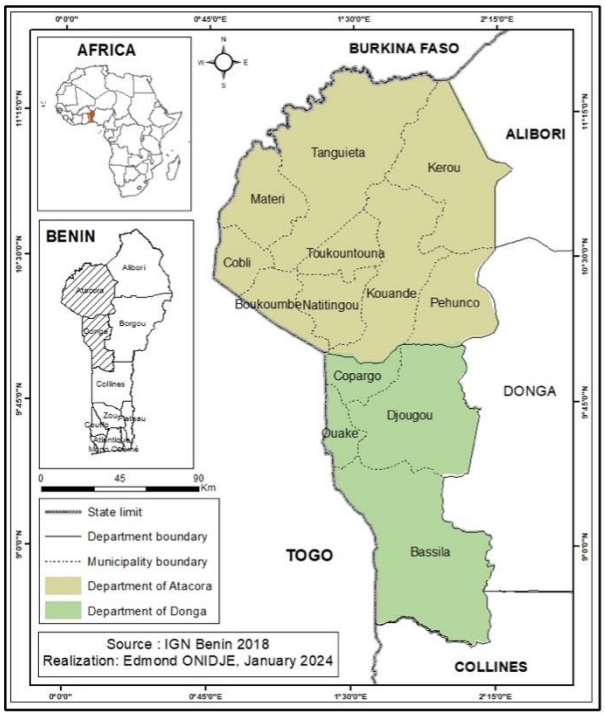

Fig.1: Map of the study area

Ethical Considerations

The data collection procedure for this study on the seroprevalence of NDV in indigenous chickens and guinea fowl was conducted in accordance with rigorous ethical standards. Before collecting blood samples from

their birds, informed consent was obtained from farm owners. The purpose and procedures of the study were completely disclosed to the participants, and their participation was entirely voluntary. The identities of the respondents were safeguarded, and all data collected, including biological samples, was treated with confidentiality. The collection of blood samples was conducted in a humane manner, ensuring that animals experienced minimal stress and distress according to the Ethical Guidelines for Animal Welfare **(Marr, 2015).**

Study Design

This cross-sectional study was designed to determine the seroprevalence of the Newcastle disease virus in Atacora and Donga regions of northern Benin. Six districts in northern Benin: Bassila, Boukoumbé, Djougou, Kouandé, Natitingou, and Pehunco, were purposefully selected due to their large populations of guinea fowl and indigenous chickens, which provide a comprehensive representation of the region's poultry. Smallholder farms within the selected communes were chosen using a systematic random sampling technique. A list of smallholder farms was obtained from local agricultural offices, and every fifth smallholder farm on the list was selected, ensuring a random and unbiased selection process. The vaccination status of the bird was accessed by farmers, and farms were categorized accordingly. Within each farm, 2 to 3 birds were randomly selected to include both vaccinated and non-vaccinated individuals, ensuring a representative sample of vaccination practices in the study area. Blood samples were taken from the birds for serological analysis to determine the presence of NDV antibodies.

Sample Size Determination

The sample size was determined by using Cochran's formula, taking into account the level of confidence desired (Z score at 95% confidence interval), proportion (p), and margin of error (e) (Bolarinwa 2020).

For this study, the following were assumed:

- Level of significance: 95% (Z = 1.96).
- Margin of error (d) = 0.05 (±5%)
- Proportion expected to be covered (p) = 0.728 derived from a study by Anzaku et *al.* (2017) who estimated the prevalence of Newcastle Disease Virus (NDV) in a neighboring country, Nigeria, which has similar ecological and farming contexts due to the lack of information about the prevalence of ND in the Atacora and Donga regions of Benin.

Using these, a sample size of 296 was estimated. The birds were thereafter selected using simple random sampling.

Sample Collection

Blood samples (2 ml) were collected aseptically from the brachial veins of indigenous chickens and guinea fowl using disposable needles and syringes. The blood was then transferred into labeled sample tubes and left in a slanted position at room temperature for clotting. Following clotting, samples were immediately placed in a cooler with ice packs to maintain a temperature between 2 and 8 °C during transportation to the laboratory. Upon arrival at the laboratory, samples were centrifuged at 2,500 revolutions per minute for 10 minutes to obtain the serum. Subsequently, the serum was harvested from each tube and stored in 2-mL cryovial tubes at −20 °C until testing. These conditions ensured the integrity of the samples and the reliability of the test results.

Laboratory procedures

Blood collection for HA and HI tests

Blood was withdrawn from a healthy chicken in an anticoagulant solution (Alsever's solution) to prevent clotting. All procedures followed approved biosafety and ethical guidelines to include measures guaranteeing the health and welfare of the animal.

Red blood cell preparation

The collected blood was washed three times in PBS by centrifugation at 1,500 x g for 5 minutes. After each centrifugation, the supernatant was discarded while the cells were resuspended in PBS. After the last wash, RBCs were re-suspended in a volume that provided a 1% RBC (PCV of 37%) suspension in PBS, which would be used in both HA and HI assays.

Source of the antigen

The hemagglutination (HA) test and the hemagglutination inhibition (HI) test were conducted using the I-2 ND vaccine sourced from the Accra Veterinary Laboratory as the antigen.

Standardization of the Antigen (I-2 ND Vaccine)

Standardization of the antigen involved the preparation of the 4HA units of the I-2 ND vaccine. The HA titre of the I-2 ND vaccine was determined by following the FAO protocol and proved to be 64. The dilution factor required to obtain 4 HA units was obtained by dividing the HA titre by 4, which equated to a dilution factor of 16. The antigen (I-2 vaccine) was diluted in phosphate-buffered saline (PBS) in a volume sufficient to provide 2.5 mL of a 4HA antigen per microwell plate. To obtain the diluted antigen to screen 10 microwell plates, the total volume needed was 25 ml. Accounting for the dilution factor, 25 mL was divided by 16 to arrive at 1.562 mL. This translates to a mixture of 1562 µL of the antigen suspension with 23.438 mL of PBS.

Hemagglutination Inhibition Test

The HI test was conducted to detect and quantify antibodies against NDV on the basis of their ability to prevent hemagglutination by this virus, according to FAO (2002) guidelines. For the assay procedure, 25 µL of PBS was added to each well of a V-bottomed microtiter plate. 25 µL of the serum sample was then added in the first well, and serial two-fold dilutions of the serum were carried out across the plate until column 11 to obtain developing concentrations. Column 12 was considered a control well. Subsequently, 25 µL of the standardized I-2 ND vaccine was added to the wells, gently mixed and incubated at room temperature for 30 minutes. After incubation, 25 µL of the 1% RBC suspension was added to each well, and the plate was incubated again at room temperature for 45 minutes.

The hemagglutination inhibition was assessed by tilting the plates and observing the red blood cells, with the results read by comparing the sample wells to the control wells. An HI titter was determined by using the highest dilution of serum that had inhibited hemagglutination, showing the existence and number of antibodies to NDV in the serum sample.

Data Management and Analysis

The data were entered into Microsoft Excel® 2019 and analyzed using STATA software. Descriptive statistics tables, graphs, and charts were generated. Chi-square tests, among others, were conducted as tests of association to see if any relationship existed between the seroprevalence of ND within the regions. Similarly, the seroprevalences in guinea fowl and chickens were also compared for any significant differences or trends.

RESULTS

This study presents an analysis of species-specific antibody titters and NDV seroprevalence across different regions, focusing on indigenous chickens and guinea fowls in the Atacora and Donga regions. **Fig. 2** shows the distribution of Newcastle Disease Virus (NDV) antibody titters in local indigenous chickens and guinea fowl across the Atacora and Donga regions.

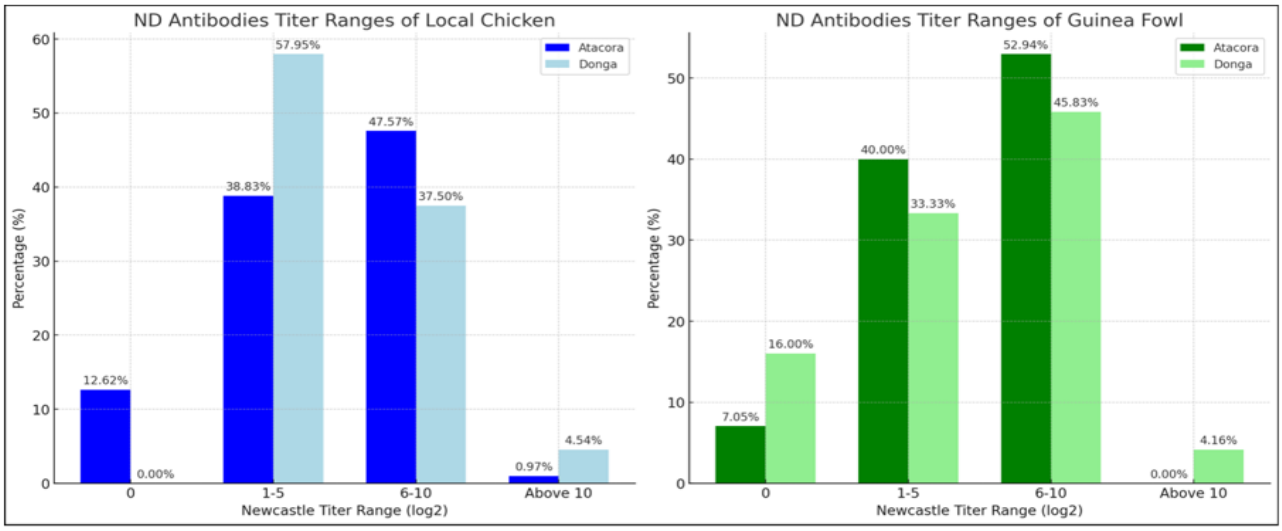

Fig. 2: Distribution of Newcastle Disease Virus (NDV) Antibody Titters in Local Chickens and Guinea Fowls Across Atacora and Donga Regions

The antibody titter ranges are expressed on a log2 scale and categorized as 0, 1, 5, 6, 10, and above 10. In the Atacora region, no chickens were found with a titter level of 0. The majority (57.95%) exhibited titters in the 6–10 log2 range, followed by 38.83% in the 1–5 log2 range, and a small fraction (3.22%) exceeded a titter level of 10. Conversely, the Donga region showed a higher prevalence of chickens with no detectable antibodies (12.62%) and a greater proportion in the 1–5 log2 range (47.57%). Fewer chickens in Donga had titters in the 6–10 log2 range (37.50%), and only 2.31% exceeded the titter level of 10.

Comparatively, in the Atacora region, Guinea fowl displayed a similar pattern to chickens, with a low percentage (4.54%) showing no detectable ND antibodies and the majority (52.94%) presenting titters in the 6–10 log2 range. About 40.00% of guinea fowls had titters in the 1–5 log2 range, and 2.52% exhibited titters above 10. In Donga, the percentage of guinea fowl without detectable antibodies was slightly higher (7.05%). The distributions for the 1–5 and 6–10 log2 titter ranges were nearly identical (45.83% each), and a minimal number

(1.29%) had titters exceeding 10. Furthermore, **Fig. 3** illustrates the log2 titter levels of both chickens and guinea fowl in the Atacora and Donga regions utilizing the Hellinger distance metric.

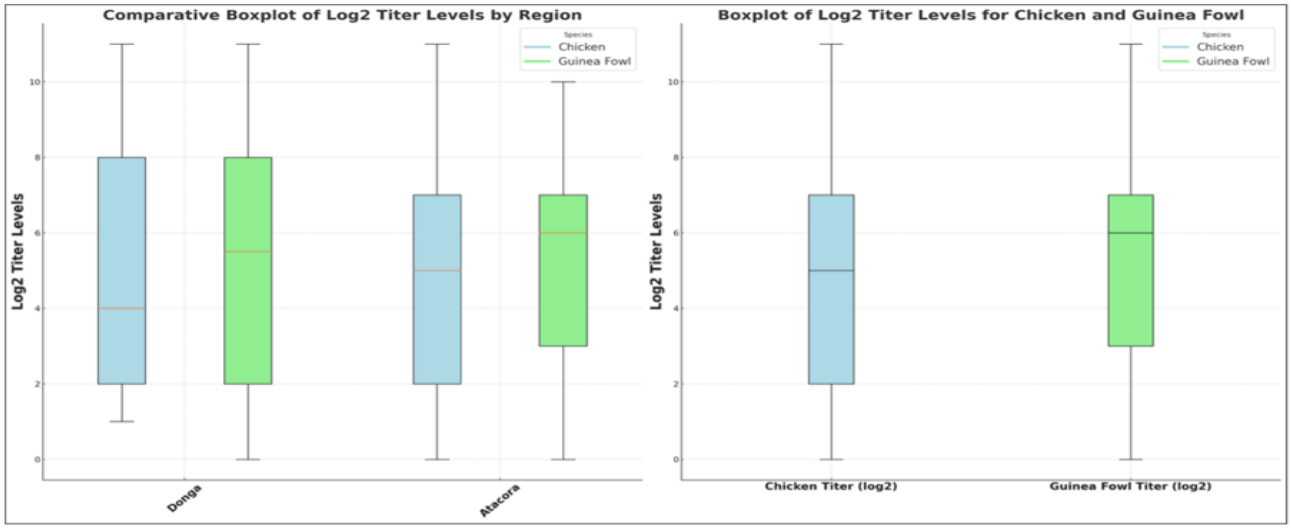

Fig. 3: Boxplot of Antibody Titters in Chicken and Guinea Fowl Samples by Region and by Species

The comparative boxplot of titter levels by region highlighted noticeable differences in the median levels for both species. It was consistently shown that the Atacora region had higher median titter levels for chickens (5.0) and guinea fowls (6.0) compared to the Donga region, where chickens and guinea fowls had median titters of 4.0 and 5.5, respectively. Additionally, a broader interquartile range and more outliers were observed in the Atacora region, indicating greater variability and the presence of extreme titter values. The overall boxplot comparing the log2 titters of chickens and guinea fowls revealed that their median titter levels were fairly close, with chickens at 5.0 and guinea fowls at 6.0, suggesting a similar central tendency. However, it was noted that the spread of titter levels for guinea fowl was slightly narrower compared to that of chickens. **Table 1** presents the seroprevalence of Newcastle Disease (ND) in indigenous chickens and guinea fowl across the Atacora and Donga regions. In Atacora, 52.33% of the non-vaccinated chickens tested positive for NDV antibodies, with 45 individuals affected. Among vaccinated chickens, 94.12% tested positive, involving 16 individuals. Overall, 59.22% of chickens in this region had NDV antibodies. In Donga, 27.78% of non-vaccinated chickens tested positive (10 individuals), while 59.62% of vaccinated chickens were positive (31 individuals). The overall NDV seroprevalence in Donga was 46.59%. The chi-square test revealed a significant difference ($c^2 = 12.901$; P-value of 0.024) in NDV seroprevalence between the Atacora and Donga regions for chickens.

However, in Atacora, 63.53% tested positive for antibodies, with 54 individuals affected. In Donga, the seroprevalence was 62.50%, with 15 guinea fowl testing positive. All guinea fowl sampled in both regions had never been vaccinated against NDV. Interestingly, there was no significant difference ($c^2 = 1.102$; P-value of 0.954) in NDV seroprevalence for guinea fowls between the two regions.

Table 1: Seroprevalence of NDV in Indigenous Chickens and Guinea Fowls by Region and Vaccination Status

Region	Species	Vaccination Status	Positive Cases	Region Seroprevalence (%)	X^2-value	P-value
Atacora	Indigenous Chickens	Non-vaccinated (n=86)	45 (52.33%)	59.22%	12.901	0.024*
		Vaccinated (n=17)	16 (94.12%)			
Donga	Indigenous Chickens	Non-vaccinated (n=36)	10 (27.78%)	46.59%		
		Vaccinated (n= 52)	31(59.62%)			
Atacora	Guinea Fowls	Non-vaccinated (n=85)	54 (63.53%)	63.53%	1.102	0.954NS
Donga	Guinea Fowls	Non-vaccinated (n=24)	15 (62.50%)	62.50%		

n= Number of samples tested; NS- Non significant at p-value < 0.05; *- Significant at p-value < 0.05;

DISCUSSION

The present study was aimed at determining the seroprevalence of the Newcastle Disease Virus in indigenous chickens and guinea fowl across Atacora and Dongs regions in Northern Benin, hence revealing regional differences and species-specific trends in NDV prevalence and antibody levels. The current research is the first to provide serological evidence of natural NDV infection in these species in this region and gives an idea about the susceptibility of these birds to NDV infection and their level of protection against this infectious disease.

In Atacora, the higher prevalence of mid-range antibody titers (6–10 log2) in chicken suggests moderate to high levels of antibodies, likely due to frequent NDV exposure attributed to low vaccination rates and poor biosecurity. **Ameh *et al*., (2016)** emphasized that improved vaccination practices could lead to higher titers, indicating better protection. Similarly, **Daodu *et al.,* (2019)** observed that 98.8% of indigenous chicken farmers never vaccinated their flocks, resulting in high mortality and frequent outbreaks, thereby increasing exposure and antibody titers. On the other hand, the Donga region displayed a higher percentage of chickens with low or undetectable antibodies (12.62%) and a greater proportion in the 1–5 log2 range (47.57%), suggesting low to moderate protection, less frequent NDV exposure, or improved biosecurity. **Toroghi *et al*., (2020)** found that enhanced biosecurity practices significantly reduced NDV exposure, suggesting better management in Donga.

In Atacora, 4.54% of guinea fowl have no detectable antibodies, indicating high susceptibility to NDV infections. In contrast, 52.94% of birds have titers in the 6–10 log2 range, suggesting previous exposure and moderate to good protection against NDV. In Donga, 7.05% of guinea fowl lack detectable antibodies, indicating high susceptibility, while 45.83% have titers in the 1–5 log2 range, reflecting some exposure but a continued risk of infection. The remaining 45.83% in Donga with titers in the 6–10 log2 range demonstrate a good immune response, providing moderate to high protection. These variations imply that Atacora experiences more frequent NDV exposure, leading to higher protection levels, whereas Donga has a mix of susceptibility and protection, likely due to better control measures. The species-specific immune response differences, as noted in this study, align with what **Walugembe *et al*., (2020)** highlighted: genetic factors could influence NDV resistance. This genetic predisposition might explain why chickens in Atacora show slightly higher antibody levels compared to guinea fowl, as chickens may have a stronger immune response to NDV exposure. **Boakye *et al.,* (2016) and Owoya *et al.,* (2020)** highlight that repeated or frequent exposure to NDV increases antibody levels in chickens.

The study identified a significantly higher seroprevalence of Newcastle Disease Virus (NDV) antibodies in indigenous chickens from the Atacora region (59.22%) compared to the Donga region (46.59%), with vaccinated chickens showing a greater prevalence than non-vaccinated ones, highlighting the importance of vaccination in controlling NDV. These findings align with previous studies on NDV seroprevalence in various regions. For instance, **Boakye *et al.,* (2016)** reported a high NDV seroprevalence of 81.8% in indigenous chickens in Kumasi, Ghana, indicating widespread exposure. Notably, certain non-vaccinated chickens also tested positive for NDV antibodies, suggesting natural exposure to the virus and indicating its endemic nature in the study areas. The study highlights the effectiveness of vaccination in controlling Newcastle Disease Virus (NDV) in chickens, as evidenced by **Wungak *et al.,* (2022) and Olorunshola *et al.,* (2022).** Moreover, this finding aligns with the results reported by **Ameh *et al.,* (2016),** who found a higher seroprevalence in vaccinated local chickens.

The significant difference in seroprevalence of chickens between the Atacora and Donga regions suggests variations in NDV exposure, vaccination coverage, and biosecurity measures. The presence of numerous veterinary clinics in the Donga region, compared to the Atacora region, might significantly increase the accessibility of vaccination for local farmers. Similar regional variations have been documented by **Owoya *et al.,* (2020)** and **Jagboro (2014),** highlighting the need for tailored, region-specific interventions to effectively manage and control NDV outbreaks.

The high prevalence of NDV antibodies in guinea fowl, despite the lack of vaccination (63.53% in Atacora and 62.50% in Donga), indicates widespread exposure to the field virus. This could be attributed to the scavenging behavior of guinea fowls, which increases their contact with NDV in the environment, as noted by **Boakye *et al.,* (2016).** The proximity of Atacora to Benin's largest forest park likely increases contact between guinea fowl and wild birds. The uniform seroprevalence across regions suggests consistent environmental factors influencing NDV exposure, as discussed by **Daodu *et al.,* (2019).** Most farmers in the study areas keep chickens and guinea fowl together **(Boko *et al.,* 2012)** which could increase the risk of cross-species transmission. Additionally, guinea fowl might act as carriers, maintaining and spreading the virus within the poultry population. **Wungak *et al.*, (2022)** noted that local chickens in live bird markets could serve as mixing points for infected

and susceptible birds, implying that guinea fowls could be important reservoirs for NDV, contributing to its persistence and dissemination in the environment.

Limitations of the study

Geographically, investigations were only done on parts of Benin and thus could not apply to other areas with varying environmental conditions and farming practices. It was a purely sectional study in design; therefore, it considers data at one point, which did not explain the seasonal variation or trend in NDV prevalence. This is further complicated by the fact that both vaccinated and non-vaccinated birds with incomplete vaccination histories have been involved, while vaccination has a significant influence on seroprevalence and antibody titters.

CONCLUSIONS

This study provides valuable insights into the prevalence and management of Newcastle Disease Virus (NDV) among indigenous chickens and guinea fowl in Northern Benin. It highlights the endemic nature of NDV and underscores the critical role that guinea fowl play in the virus's epidemiology in the area. The findings reveal higher NDV antibody levels in the Atacora region, attributed to frequent exposure and field infection, particularly among guinea fowl, probably due to lower vaccination rates and poor biosecurity measures. In contrast, a high proportion of birds in Donga region exhibited moderate to high protection against NDV, suggesting better management in Donga.

In both Atacora and Donga, enhancing vaccination coverage and improving biosecurity measures are critical to managing NDV outbreaks effectively. In Atacora, where high NDV exposure and field infections are prevalent, policymakers should focus on establishing mobile vaccination units and providing subsidies to increase vaccine accessibility and affordability. Educational campaigns should highlight the importance of vaccinating both chickens and guinea fowl, while training programs can equip farmers with biosecurity farming practices, such as controlling access to poultry areas and separating species to limit cross-infection. To effectively interrupt the epidemic cycle of Newcastle Disease Virus (NDV) and control the disease, it is essential to focus on guinea fowl as potential reservoirs. Further research should be conducted to isolate and characterize the virus strains circulating in the region, providing crucial information for developing and implementing more effective control strategies.

Acknowledgements

This research publication is part of the MSc thesis of the first author. Funding for the research is provided by the African Union through the Pan African University Life and Earth Sciences Institute (including health and agriculture), PAULESI.

Conflicts of Interest

The authors declare no conflict of interest. The sponsors had no role in the design, execution, interpretation, or writing of the study.

Authors' contributions

The research concept was developed by Edmond Onidje, Vitus Burimuah, Oluwole Oyetunde Oni, and Benjamin Obukowho Emikpe. Sample collection was carried out by Edmond Onidje. Laboratory analyses were conducted by Amponsah Patrick Mensah and Edmond Onidje. Statistical analysis was done by Derrick Adu Asare and Edmond Onidje. The draft of the manuscript was prepared by Edmond Onidje. All authors read and approved the final version of the manuscript and contributed equally to its content.

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How to cite this article:
Edmond Onidje, Oluwole Oyetunde Oni, Benjamin Obukowho Emikpe, Vitus Burimuah, Amponsah Patrick Mensah and Derrick Adu Asare, 2024. Serological Detection of Newcastle Disease Virus Antibody in Vaccinated and Non-Vaccinated Indigenous Chickens and Guinea Fowls in Atacora and Donga, Northern Benin. Journal of Applied Veterinary Sciences, 9 (4): 26-33. **https://dx.doi.org/10.21608/javs.2024.300310.1363**