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Full Length Research Article

DETERMINATION OF NEWCASTLE DISEASE VIRUS ANTIBODY TITRE IN VACCINATED AND UNVACCINATED POULTRY BIRDS WITHIN KADUNA SOUTH LOCAL GOVERNMENT AREA KADUNA STATE, NIGERIA

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ABSTRACT
Newcastle Disease Virus (NDV) is a major threat to poultry health worldwide, leading to significant economic losses. This study aims to determine and compare NDV antibody titres in vaccinated and unvaccinated poultry birds, assess biosecurity practices, and identify risk factors associated with the disease within Kaduna South L.G.A., Kaduna State, Nigeria. Two hundred serum samples were collected from vaccinated and unvaccinated birds from 20 poultry farms to determine their antibody titres using Haemagglutination Inhibition (HI) tests. The results showed that vaccinated birds had significantly higher mean antibody titres (512-4,096) compared to unvaccinated birds (64-2048), with some unvaccinated birds, particularly day-old chicks, showing transient protective titres due to maternally derived antibodies. Seropositivity rates were significantly higher in vaccinated birds (100% ± 5.37%, range: 20-100%) than in unvaccinated birds (64% ± 39.29%, range: 0-100%). However, the immune status of most unvaccinated birds was inadequate, highlighting the importance of vaccination in achieving long-term immunity. Bio-security and bio-safety information from structured questionnaires revealed that most farms adhered to certain practices, such as controlled access (95%) and proper feed storage (100%). In comparison, critical gaps were observed in low vaccination uptake (65% of farms did not vaccinate) and poor vehicle disinfection (10%). Risk factors such as poor vaccination compliance, inconsistent disease monitoring, and improper manure management were identified as major contributors to infection vulnerability, particularly in commercial layer birds. The study concludes that vaccination programs, identified risk factors, and enhanced bio-security measures are essential for effective NDV control.

Keywords: Newcastle, Haemagglutination, Antibodies, Vaccination, Immune Status.

INTRODUCTION

Poultry farming is an important source of livelihood and a major occupation. This has been known to combat malnutrition, unemployment and provide supplementary income (Jat and Yadav, 2012).
Poultry has a great economic importance to the entire human population because it provides meat and eggs for the growing human population. The bones are also used as bone meal for farm animals (Ibrahim, 2020). Worldwide, it is observed that the demand for poultry and poultry products is tremendously increasing as a result of population growth. Still, poultry production in Nigeria faces challenges from infectious diseases (Ibrahim, 2020).

Newcastle Disease (ND) is one of the most important infectious diseases of poultry. It is distributed worldwide and has the potential to cause large economic losses in the poultry industry (Dortmans *et al.,* 2011). Newcastle disease is one of the highly pathogenic viral diseases of avian species. It is economically significant due to the substantial mortality and morbidity associated with it (Ganar *et al.,* 2014).

Newcastle disease virus (NDV) is the causative agent of ND in poultry. The first report of a disease amongst chickens, according to the clinical signs retrospectively appears to have been Newcastle Disease, stems from the island of Java in 1926. The respiratory and neurological signs were notable, and the disease was first called pneumoencephalitis (Abdisa and Tagesu, 2017). The World Organisation for Animal Health defined ND as an infection of poultry with virulent strains of NDV. Lesions affecting the neurological, gastrointestinal, respiratory, and reproductive systems are most often observed in poultry birds (Dimitrov *et al*., 2019).
Some of the signs of NDV infection in chickens include restlessness, weakness, green diarrhoea, muscular tremors, paralysis of legs and wings, which leads to death, etc.
NDV can be spread mainly through the movement of live birds, fomites, personnel, and poultry products from infected premises to susceptible birds (Brown *et al*., 2021).

Newcastle disease is particularly devastating for small-scale poultry farmers who usually have limited means of protecting their flocks based on the level of their bio-security practices, and increased movement in poultry transportation has resulted in the exposure of chickens to the Newcastle disease virus from infected areas. Live bird markets are found in strategic locations around major road intersections in Kaduna South. They usually do not follow appropriate cleaning and disinfecting techniques, which allows for the possibility of environmental spread. These birds run the risk of disseminating NDV as they leave the markets, posing a health threat to household chickens (Adanu *et al.,* 2021).

Vaccination is the main method for controlling ND. Vaccination against ND in domestic poultry was first proposed in the early 1930s, shortly after the identification of NDV. Vaccine-induced immunity is short-lived and currently considered to last 10-12 weeks. To maintain adequate protection, repeated vaccinations are needed. Notably, parental immunity also interferes with vaccine effectiveness. Therefore, vaccination programs are often delayed until chicks reach the age of 1-2 weeks. Vaccination continues to

play a pivotal role in controlling and eradicating ND in Kaduna South. The most commonly used vaccine strains are LaSota and Hitchner B1 (Charlie *et al*., 2021).

Diagnosis of ND is done by isolation of the virus in embryonated chicken eggs, in addition to using serological tests such as various Enzyme-Linked Immunosorbent Assay (ELISA) and Haemagglutination Inhibition (HI) tests (Chaka *et al*., 2012). Because isolation of the virus in embryonated eggs takes a relatively long time to culture the virus and the use of molecular diagnostic assays is not within the capability of many laboratories in developing countries because of the equipment, reagents and expertise required, serological tests such as ELISA and HI are often relied upon for the laboratory diagnosis of ND (Reta *et al*., 2020).

The level of antibodies in poultry birds is important in controlling the clinical signs of the disease and reducing shedding of the virus. The minimum live vaccine titre required to control the clinical signs of the disease is 10^4; however, with the minimum titre, the reduction of virus shedding will be limited or non-existent. This justifies why the usage of higher titres is important (Dimitro *et al*., 2016). Understanding the immune status of both vaccinated and unvaccinated birds provides insights into the effectiveness of current vaccination programs. Additionally, assessing bio-security practices and identifying risk factors for NDV infection will guide interventions to reduce disease incidence, improve poultry health, and enhance economic productivity in the region.

MATERIALS AND METHODS

Study Area

The study area included twenty (20) farms within the Kaduna South Local Government Area. Kaduna South is a Local Government Area in the Kaduna Metropolis of Kaduna State, Nigeria. Its headquarters is located in Makera. Some of its wards are Barnawa, Tudun Wada, Narayi, Maigero, Television, Kakuri, Kurmin Mashi, etc. It has an area of about 46.2 km².

Sample Size

A total of 200 samples were collected from twenty (20) farms within the Kaduna South Local Government Area. One hundred (100) samples were collected from vaccinated commercial poultry birds from 10 farms, and 100 samples from unvaccinated commercial poultry birds. Before sample collection, a structured questionnaire was used to collect clinical information and data on risk factors likely to be associated with NDV.

Collection of Samples

Blood samples were collected using the wing vein puncture technique. The chicken's wing was extended and held in place. On identifying the wing vein, the puncture site was sterilized with methylated spirit (antiseptic). A needle (syringe) was inserted into the wing vein at a 20-30º angle. The needle was advanced slowly until blood flowed into the syringe. After 3-5ml of blood was collected, the needle was slowly and carefully withdrawn. Then pressure was applied gently to the puncture site with cotton wool soaked with methylated spirit. Blood was transferred into labelled Eppendorf tubes (chicken's identification and collection date). The sample was stored by freezing (serum) or refrigeration (blood).

Preparation of 1% Red Blood Cells (RBCs)

Approximately, 2ml of Alsever's (Sigma-Aldrich) solution was measured in a calibrated test tube. About 4mls of blood was measured into the tube containing Alsever's solution and evenly mixed. Then, 5ml of normal saline was poured into the tube and centrifuged at 1500rpm for 5 minutes. After centrifuging, the supernatant was discarded using a Pasteur pipette, leaving the blood sample in the tube. The tube was filled to 11ml with normal saline and then centrifuged at 1500rpm for 5 minutes. After centrifuging, the supernatant was discarded using a Pasteur pipette, leaving the blood sample in the tube. Haematocrit tubes were placed inside the tube to collect blood and were sealed by burning the tips using the Bunsen burner. Then the haematocrit tubes were centrifuged at 1500rpm for 5 minutes. After centrifuging, Haematocit tubes were read on the haematocrit reader.

Then calculate 10(RBC percentage) x 12 = 120

10mls (Contents in Tube) – 120mls = 110ml (Normal saline)

Blood was diluted with normal saline according to the results above to get 1% RBC for the Haemagglutination Test.

Haemagglutination Test (HA)

About 50µL of normal saline was added into the wells (F, G & H) in the micro-titre plates. Approximately 50µL of Newcastle Disease Virus was then added into the first set of wells, except row H, the last row (row H for the negative control). A two-fold serial dilution was carried out from the first two wells to the last wells, and then the remaining dilution was discarded. About 50µL of 1% RBC was added to the 3 rows of wells. The microtitre plate was incubated for 30 minutes at 27ºc-30ºc. The plate was observed at 90º for agglutination. Using the observation, the 4HA value was calculated to reconstitute the virus:

$4HA= 2^{n-2}$

$4HA= 2^{9-2} = 2^7 = 128$

127mls (normal saline) and 1ml (virus) to make the reconstituted virus

Haemagglutination Inhibition Test (HI)

About 50µL of normal saline was added to each well, including wells (G–H) for controls (positive and negative). About 50µL of sample (serum) was added into the first set of wells, except for (G-H). A two-fold serial dilution was performed in the remaining wells, and then the remaining dilution was discarded. Then 50µL of reconstituted virus was added into the wells, except the positive control well, and was incubated for 15 minutes at 27ºC - 30ºC. Approximately 50µL of 1% RBC was added into all wells, including positive and negative controls, and was incubated at 27ºc - 30ºc for 30 minutes. Plates were held at 90º, observed, and recorded.

RESULTS

Table 1 shows the vaccination history of birds and their antibody titre.

Farms with consistent vaccination practices displayed higher antibody titres (farms 3-8 and 10), while farms with irregular or incomplete vaccination schedules (1, 2, and 9) showed reduced immunity (Table 1).

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Table 1: Vaccination history of birds and their antibody titre in selected farms from Kaduna South LGA

Farm	Age (weeks)	Date of vaccination	Sampling date	Antibody titre range
1	48	30/09/24	14/10/24	32-256
2	6.4	14/09/24	14/10/24	16-256
3	46	05/10/24	17/10/24	512-4,096
4	21	17/09/24	30/09/24	1,024-4,096
5	36	15/08/24	02/09/24	512-4,096
6	37	15/08/24	10/09/24	1,024-4,096
7	109	15/08/24	12/09/24	512-4,096
8	21	30/09/24	12/10/24	128-4,096
9	40	18/09/23	10/10/23	256-1,024
10	109	12/09/24	10/10/24	1,024-4,096

Table 2 shows the antibody titre of unvaccinated birds. While most unvaccinated birds exhibited low antibody titres, some farms with day-old chicks showed higher titres (farms 18 -20) (Table 2).

Table 2: Antibody Titre of Unvaccinated Birds in selected farms from Kaduna South LGA

Farm	Age (weeks)	Sampling date	Antibody titre range
11	32	2/10/24	1-512
12	21	10/09/24	16-512
13	56	12/02/24	32-256
14	52	21/10/24	1-32
15	48	21/10/24	2-512
16	47	22/10/24	32-1,024
17	24	30/10/24	64-2048
18	0.1	19/09/24	32-1,024
19	0.1	08/10/24	256-4,096
20	0.1	19/09/24	128-4,096

Table 3 shows the protective levels and geometric mean titres (GMT) of Newcastle Disease Virus (NDV) antibodies in vaccinated poultry populations. In vaccinated birds, the antibody titres of vaccinated birds consistently exceed the protective level (100%) (Table 3).

Table 3: Protective Level of NDV Antibodies Titre in Vaccinated Poultry Birds Populations

Farm	Frequency	Protective level (%)		GMT	Standard deviation
		@1:16	@1:128		
1	2	-	20	5.9	2.5
2	10	100	-	5.5	2.3
3	10	-	100	11.4	4.0
4	10	-	100	11.2	3.9
5	10	-	100	10.9	3.9
6	10	-	100	11.8	4.2
7	10	-	100	11.5	4.1
8	10	-	100	9.4	3.3
9	10	-	100	8.8	3.1
10	10	-	100	11.4	4.0

Table 4 shows the Exposure Level and Geometric Mean Titres (GMT) of Newcastle Disease Virus (NDV) antibodies in unvaccinated poultry populations. Unvaccinated birds showed a lower antibody titre (below 100%) compared to the vaccinated birds. The unvaccinated day-old chicks in farms 8-10 showed a 100% antibody titre (Table 4).

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Table 4: Exposure Level Titre in Unvaccinated Poultry Birds Populations

Farm	Frequency	Exposure level (%)		GMT	Standard deviation
		@1:16	@1:128		
1	1	-	10	3.5	1.5
2	5	-	50	7.0	2.9
3	7	-	70	6.6	2.8
4	0	-	0	2.7	1.1
5	3	-	30	5.0	2.1
6	9	-	90	8.0	3.4
7	9	-	90	8.0	3.4
8	10	100	-	7.0	2.9
9	10	100	-	10.7	4.5
10	10	100	-	9.2	3.9

Figure 1 compares the antibody titres in unvaccinated day-old chicks, unvaccinated older birds and Vaccinated Birds. The vaccinated birds have a geometric mean titre of 9.9, followed by the day-old chicks, while the unvaccinated birds have the least 5.8 (Figure 1).

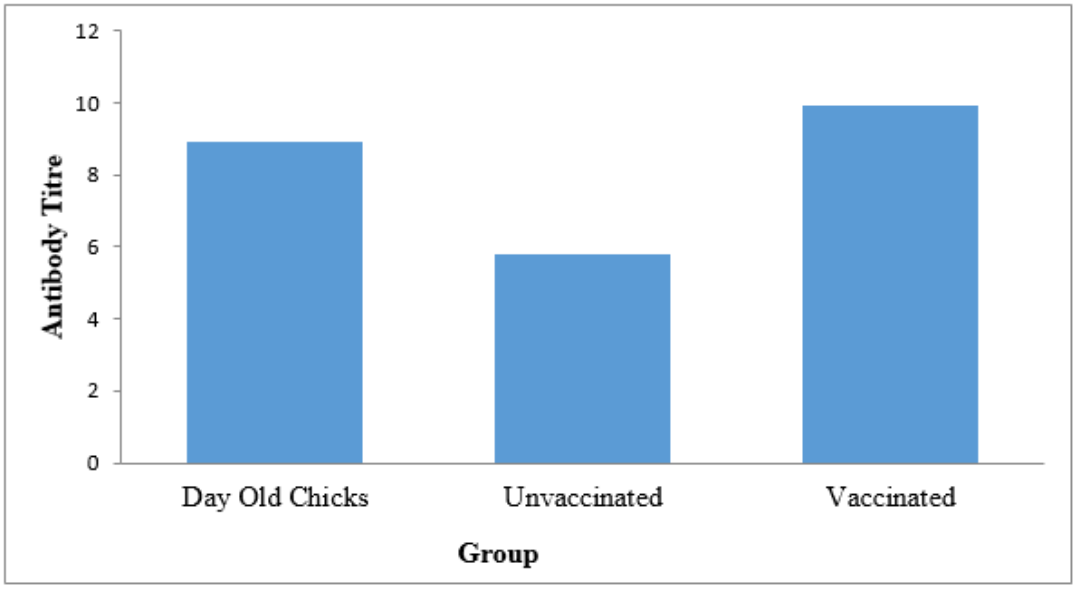

Figure 1: Mean Antibody Titre in Unvaccinated Day-Old Chicks, Unvaccinated Older Birds, and Vaccinated Birds

Table 5 shows the results of the bio-security/bio-safety information obtained from the structured questionnaire. The data revealed varied adherence to bio-security practices, with high compliance in areas such as controlled farm access (95%), proper feed storage (100%), and worker training on PPE usage (100%). However, significant gaps were noted in vehicle disinfection (10%) and disease outbreak reporting (70%).

Table 5: Bio-security/Bio-safety Practices of the Farms

Variables	Response Options	Number of Farms (n=20)	Percentage (%)
1. General Information			
Type of poultry birds	Broilers	10	50
	Layers	9	45
	Breeders	1	5
2. Bio-security Practices			

Controlled entrance?	Yes	19	95
	No	1	5
Visitors wear Protective Gear?	Yes	14	70
	No	6	30
Vehicles disinfect?	Yes	2	10
	No	18	90
Designated area for cleaning and disinfecting equipment?	Yes	19	95
	No	1	5
3. Bird Health and Hygiene			
Birds vaccinated often? (Frequency)	None	13	65
	Monthly	4	20
	Vaccination Programs	3	15
Bird enclosures cleaned and disinfected often? (Frequency)	Daily	9	45
	Weekly	10	50
	Monthly	1	5
Dead birds disposed properly?	Yes	20	100
	No	0	0
4. Feed and Water Management			
Feed stored at secure, rodent-proof area?	Yes	20	100
	No	0	0
Water Sources protected?	Yes	19	95
	No	1	5
Feed and Water tested regularly for contaminants?	Yes	16	80
	No	4	20
5. Manure and Waste Management			
Manure often removed from bird enclosures?	Daily	5	25
	2-3days	13	65
	Weekly	2	10
Manure stored in designated area?	Yes	16	80
	No	3	15
	Sometimes	1	5
Waste Disposal Facilities available?	Yes	14	70
	No	6	30
6.Bio-safety Practices			
Farm Workers wear PPE (gloves, masks, etc.)?	Yes	17	85
	No	3	15
PPE provided by farm?	Yes	17	85
	No	3	15

Workers trained on PPE usage?	Yes	20	100
	No	0	0
7. Disease Monitoring and Reporting			
Birds Health Records Maintained?	Yes	19	95
	No	1	5
Disease Outbreaks Reported to Veterinary Authorities?	Yes	14	70
	No	6	30
Post-Mortem Examinations conducted on deceased birds?	Yes	10	50
	No	10	50
Birds regularly tested for Diseases?	Yes	13	65
	No	7	35
Test recorded and reviewed?	Yes	16	80
	No	4	20

DISCUSSION

The vaccination history of birds and their antibody titre showed that farms with consistent vaccination practices displayed higher antibody titres ranging from 1,024 to 4,096. In contrast, farms with irregular or incomplete vaccination schedules showed reduced immunity, reflecting lower antibody titres ranging between 32 and 512. As observed from the study, the birds in farm 1 were vaccinated on 30/09/24, and the vaccination was not consistent between then and the time samples were collected (14/10/24). This resulted in low immunity, indicated by a low antibody titre value (32-256). Other farms showed higher antibody levels compared to Farm 1, where immunity dropped significantly after a single vaccination. This rapid decline in immunity underscores the importance of continuous vaccination to maintain adequate levels of immunity. The drop in antibody levels within such a short period suggests that a single vaccination, without a booster dose, may not be sufficient to ensure long-term protection against Newcastle Disease Virus (NDV). This finding emphasizes the need for regular revaccination, as immunity wanes over time, particularly when birds are exposed to potential infections. The birds in farm 6, among others, were consistently vaccinated, which likely contributed to a higher and more sustained level of immunity against NDV. These farms followed a regular vaccination schedule, which is critical for maintaining protective antibody titres. Hu *et al.* (2022) reported that it is vital to maintain current vaccination programmes, which are essential for monitoring virus dissemination in poultry and disease eradication in the long term. This result suggests that regular vaccinations are effective in maintaining immunity and protecting the birds from potential NDV infections. It also emphasizes the importance of adherence to vaccination schedules, particularly in controlling outbreaks and enhancing disease resistance in poultry populations, as agreed by Dimitrov *et al.*(2017), stating that incomplete or improper immunization often results in the disease and death of poultry after infection with virulent NDV.

The unvaccinated birds showed lower antibody titres when compared to the vaccinated birds. Some of the birds exhibited higher antibody titres, indicating that these birds were likely exposed to Newcastle Disease Virus (NDV) in their environment. Additionally, the presence of maternally derived antibodies in day-old chicks, observed in Farm 8-10, likely explains the higher titres found in younger birds, which gradually decrease as the chicks' age and lose the protection from maternal immunity. These findings are similar to those of Daodu *et al.* (2019), who reported that maternally-derived NDV antibody offered protection to the chicks for up to a month against NDV.

The protective level refers to the threshold of antibody titres necessary to confer immunity against the disease or provide immunity against NDV, while the geometric mean titre (GMT) provides a measure of the central tendency of antibody levels across the population. Protective levels typically range from a minimum value (e.g., 70% or 100%), depending on the vaccine used and the immune response required. According to Abraham-Oyiguh *et al.* (2014), Haemagglutination inhibition (HI) antibody titer between $0\log_2(1)$ and $3\log_2(8)$ is considered negative because they produce no antibody against the virus while HI antibody titer between $4\log_2(14)$ and $8\log_2(256)$ is considered positive for antibodies production against the virus and if the titres in the vaccinated birds exceed this protective threshold, it confirms that the birds are likely to be protected against NDV infection. In vaccinated birds, antibody titres consistently exceed the protective level (100%), indicating that the vaccination program is successful in inducing immunity. This is in contrast to the experiment carried out by Van *et al.* (2008), with the conclusion that a high fraction of birds (>85%) needs to have a high antibody titre 3log2 (8) after vaccination to ensure that no epidemic spread is possible in vaccinated populations. Higher GMT values in vaccinated birds (11.8) suggest that the vaccination is highly effective in boosting antibody levels, which would significantly reduce the risk of NDV outbreaks. However, Mayers *et al.* (2017) state that although vaccination programmes have reduced the impact of clinical disease, they have historically been ineffective in controlling the spread of virulent viruses and therefore do not always offer an adequate solution to the world's food security problems.

The exposure level reflects the titres resulting from natural infection or maternal antibodies passed from the mother hen to the chicks. For day-old chicks (Farm 8 - 10), the protective level is primarily determined by maternally derived antibodies, which offer initial

protection until their own immune system develops. The exposure level indicates the amount of environmental or viral exposure that the birds have had. For unvaccinated birds, the exposure level can vary widely, particularly if they are in environments where NDV is endemic (Dimitrov *et al*., 2017). Farms showing 70% and above antibody titres (Farm 3, 6 & 7) (Table 4) show the exposure level, which reflects the natural or environmental infection pressure the birds are subject to. For day-old chicks (Farm 8 -10), maternal antibodies from the hen provide early protection. These antibodies are usually strong immediately after hatching but gradually decrease as the chicks grow. The table shows that day-old chicks have a higher protective level because of these maternally derived antibodies, which offer them protection against NDV in the early stages of life (Liu *et al*., 2023). For day-old chicks, the protective level refers to the titre needed to offer sufficient immunity against NDV. In the case of unvaccinated chicks, these maternally derived antibodies play a crucial role in providing initial protection. The table shows that the titres in day-old chicks exceed the protective level as maternal antibodies transfer from the hen to the chick, ensuring protection during the first few weeks of life (Niewiesk, 2014). Over time, as the maternal antibodies decline, the protective level may drop. This is why the protection against NDV is considered temporary for unvaccinated chicks, highlighting the need for subsequent vaccination as maternal immunity wanes.

Figure 1 shows the mean antibody titres across unvaccinated day-old chicks, unvaccinated older birds, and vaccinated birds. The chart shows relatively high mean antibody titres in unvaccinated day-old chicks. In older birds without vaccination, the mean antibody titres are generally lower than in vaccinated birds or day-old chicks. This reflects the absence of active immunization and the diminishing maternal antibodies over time. Vaccinated birds show the highest mean antibody titres, demonstrating the efficacy of vaccination in inducing a robust immune response. In agreement with Oberländer *et al.* (2020), it is important to revaccinate chickens properly to boost the immune response until >85% of chickens of a flock have protective titers. The elevated titres ensure protective immunity, significantly reducing the likelihood of NDV infections in this group.

Most of the farms highly complied with feed storage (100%), water protection (95%), and proper disposal of dead birds (100%), as well as demonstrating good bio-safety practices, as 85% provided personal protective equipment (PPE) and trained workers on its use. This is similar to the works of Oberländer *et al.* (2020). Additionally, 95% of farms maintained health records, and 80% reviewed test results, indicating fair disease monitoring. However, notable weaknesses were identified. Vaccination uptake was low, with 65% of farms not vaccinating their birds. This could be as a result of the high cost of the vaccines, as reported by Dimitrov *et al*. (2017). Vehicle disinfection was poorly practiced, with only 10% compliance. Reporting of disease outbreaks was inconsistent, as only 70% of farms reported to veterinary authorities, and post-mortem examinations were conducted by just 50% of farms.
The risk factors associated with Newcastle Disease Virus (NDV) infection in the surveyed farms included low vaccination coverage, inadequate vehicle disinfection practices, inconsistent disease outbreak reporting, limited post-mortem examinations, and irregular testing for contaminants in feed.

Conclusion
This study successfully determined the immune status of poultry birds in Kaduna South L.G.A. by comparing vaccinated and unvaccinated populations. The findings showed that vaccinated birds generally exhibited higher antibody titres, indicating a robust immune response. Conversely, unvaccinated birds, including day-old chicks with maternally derived antibodies, demonstrated variable immune statuses, with some showing protective levels due to natural exposure.
The structured questionnaire showed valuable insights into bio-security and bio-safety practices across the surveyed farms. Most farms exhibited moderate to good bio-security measures, such as controlled access, proper feed storage, and PPE usage. However, gaps such as inadequate vehicle disinfection, low vaccination rates, and inconsistent disease outbreak reporting were identified.
Finally, the study identified several risk factors associated with NDV infection, particularly in unvaccinated commercial layers. These included exposure to contaminated environments, poor vaccination adherence, and insufficient disease monitoring practices. Addressing these risk factors through improved bio-security, regular vaccination programs, and better waste management practices is essential to prevent NDV outbreaks and enhance poultry health and productivity in the region.

Recommendation
It is recommended that antibody levels be monitored through adherence to regular vaccination programs and record-keeping. This can be achieved through awareness and education for farmers.
Bio-safety measures such as restricting movement of people, equipment, and birds between farms; Maintaining clean and disinfected environments; Quarantining new or sick birds before introducing them to the flock should be encouraged.
The government should support research and implementation of integrated Health Management in addition to emergency response plans for outbreaks.

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