

MOLECULAR DETECTION AND RISK FACTOR ANALYSIS OF *CHICKEN INFECTIOUS ANAEMIA VIRUS* IN COMMERCIAL POULTRY FARMS IN MAIDUGURI, NORTH-EAST NIGERIA AND JOS SOUTH, NORTH-CENTRAL NIGERIA

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ABSTRACT

Chicken Infectious Anaemia Virus (CIAV) is an economically important emerging infection of poultry that causes immunosuppression and reduced egg production. This virus can lead to significant economic losses in the poultry industry due to increased mortality, reduced weight gain, and increased susceptibility to secondary infections, ultimately impacting productivity and profitability. This study investigated the prevalence of CIAV in commercial poultry farms in Maiduguri and Jos South local government area, Nigeria, by Polymerase chain reaction (PCR). The results revealed a total CIAV prevalence of 22.01% (17/77) in the study-area. Maiduguri recorded a higher prevalence of 35.48% (11/31) compared to Jos South, which had a prevalence of 13.04% (6/46). Age and location were identified as significant risk factors, with young chickens being 3.33 times more likely to test positive and chickens in Maiduguri being 3.67 times more likely to be infected. These findings highlight the need for targeted interventions including strict biosecurity protocols, and region-specific control measures to mitigate the spread and minimize economic losses in Nigerian poultry farms.

Keywords: chicken, infectious, anaemia, virus, Nigeria, Molecular

INTRODUCTION

The poultry industry is a rapidly growing sector in Nigeria, playing a significant role in poverty eradication and economic growth (Hamid *et al.*, 2017). However, the industry faces numerous challenges, including a lack of selection, low genetic potential, rising feed costs, and disease emergence (Sara *et al.*, 2024). One significant disease affecting poultry is Chicken Infectious Anaemia (CIA), which is caused by Chicken Anaemia Virus (CAV) and leads to severe anaemia and immunosuppression in chickens, resulting in substantial financial losses (Sara *et al.*, 2024; Syedah *et al.*, 2024).

The CAV has a worldwide distribution and infection of young chickens can lead to anaemia, decreased weight gain, transient immunosuppression and increased mortality rates

(Wani *et al.*, 2013; HyeSoon Song *et al.*, 2024). In contrast, infection in chickens older than 3 or 4 weeks often does not cause clinical signs but can still result in immunosuppression and secondary infections, leading to economic losses (Zeng *et al.*, 2023).

The CAV is a non-enveloped, icosahedral virus particle belonging to the Anelloviridae family, with a diameter ranging from 25 nm to 26.5 nm and a genome size of approximately 2.3 kb (Miller *et al.*, 2003; Noteborn *et al.*, 1991; Rosario *et al.*, 2017). First isolated in Japan in 1979 from a contaminated vaccine (Yuasa *et al.*, 1979), CAV has a broad host range. Although chickens are its natural host, the virus has also been detected in the feces of humans, mice, dogs, and other birds (Chu *et al.*, 2012; Fatoba & Adeleke, 2019; Fang *et al.*, 2017). Its detection in various hosts has

raised public awareness and driven the need for effective control methods (Spezia *et al.*, 2023; Rosario *et al.*, 2025).

Effective control of CAV relies on biosecurity measures, immunization, and genetic selection to increase resistance (Hoerr, 2010). While chickens are natural hosts of CAV, antibodies against the virus have been detected in other birds, including quail, ostriches, and turkeys (Emikpe *et al.*, 2005; MacLachlan & Dubovi, 2017; Kamdi *et al.*, 2020).

Despite the significant economic impact of CIA on poultry production, there is a notable scarcity of data on its prevalence in commercial poultry farms in Maiduguri North East Nigeria and Jos South LGA North Central Nigeria. This lack of data hinders the development of effective control measures and mitigation strategies.

MATERIALS AND METHODS

STUDY AREAS

This study was conducted in Maiduguri, Borno State, and Jos, Plateau State's capital.

Maiduguri, is situated between 10.20°N - 13.40°N latitude and 9.80°E - 14.40°E longitude, covers approximately 69,436 sq/km and shares international borders with Niger Republic, Chad, and Cameroon (Musa and Pindar, 2005)). Jos South Local Government Area, Plateau State, Nigeria, is situated between latitude 8°45'00" and 9°50'00" North of the Equator and longitude 8°41'00" and 8°58'00" East of the Greenwich Meridian. The area covers approximately 510 km², comprising three districts, Du, Kuru, and Vwang, with a population of approximately 306,716 persons, as recorded in the 2006 census (Ikegwonu, *et al.*, 2021).

SAMPLE COLLECTION

Between January 2024 and May 2025, a total of 77 pooled samples were collected from 77 commercial poultry farms in Maiduguri, Borno State (n = 31), and Jos South, Plateau State (n = 46). At each farm, 1--3 birds exhibiting poor performance and weakness were selected and humanely sacrificed and the spleen, liver & thymus tissues were harvested and pooled. The samples were stored at -20°C until further processing at the National Veterinary Research Institute, Vom Nigeria.

DNA EXTRACTION

Total viral DNA was extracted from processed tissue homogenates via a QIAamp Viral DNA Mini Kit (QIAGEN, Valencia, Calif., Germany). Briefly, the following steps were carried out according to the manufacturer's instructions: Twenty (20 µl) of proteinase K was pipetted into the bottom of a 1.5 ml micro centrifuge tube. Two hundred (200) µl samples (tissue homogenate) were then added to the micro centrifuge tube, and 200 µl of Buffer AL was added to the sample and mixed by pulse vortexing for 15 sec. The mixture

was then incubated at 56°C for 10 min. Briefly, the 1.5 ml micro centrifuge tubes were centrifuged to remove drops from the inside of the lid, and 200 µl of ethanol (96–100%) was then added to the sample and mixed again by pulse vortexing for 15 sec. After mixing, the 1.5 ml micro centrifuge tubes were then briefly centrifuged to remove drops from the inside of the lid. The mixtures were then carefully applied to a QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. The mixture was capped and centrifuged at 8000 rpm for 1 min. The QIAamp Mini spin column was placed in a clean 2 ml collection tube, and the tube containing the filtrate was discarded. The QIAamp Mini spin column was opened, and 500 µl of Buffer AW1 was added without wetting the rim. The cap/cover was closed, and the mixture was centrifuged at 8000 rpm for 1 min. The QIAamp Mini spin column was placed into another clean 2 ml collection tube (provided), and the collection tube containing the filtrate was discarded. Five hundred (500) microliters of Buffer AW2 was added, and the mixture was subsequently centrifuged at full speed at 14000 rpm for 3 minutes. The QIAamp Mini spin column was placed in a new 2 ml collection tube, and the old collection tube with the filtrate was discarded. This mixture was centrifuged at full speed for 1 min to eliminate the possibility of AW2 carryover of the Buffer. The QIAamp Mini spin column was placed in a new well labelled with a 1.5 ml microcentrifuge tube, and the collection tube containing the filtrate was discarded. Finally, the QIAamp Mini column was opened, 100 µl of Buffer AE was added, and the mixture was incubated at room temperature (15–25°C) for 1 min. The mixture was subsequently centrifuged at 8000 rpm for 1 min to elute the DNA. The eluted DNA was then stored in a freezer until analysis by PCR.

CONVENTIONAL POLYMERASE CHAIN REACTION

The extracted DNA was amplified via conventional PCR as previously described (Yao *et al.*, 2019), and the primers were amplified at 1,390 bp (VP1 gene). PCR was carried out in a 25 µl reaction mixture containing 12 µl of nuclease-free 5 µl of 5x Buffer, 2 µl of 10 µM forward or reverse, 0.5 µl of dNTPs, 1 µl of MgCl₂, 0.5 µl of Taq polymerase, 8.5 µl of purified DNA and 2 µl of purified DNA. The PCR amplification involved initial denaturation at 94°C for 2 minutes followed by 35 cycles of denaturation at 94°C for 0.5 minutes, annealing at 60°C for 1 minute, extension at 72°C for 1 minute and a final extension at 72°C for 7 minutes. PCR analysis was performed on a GeneAmp PCR system 9700 thermal cycler (Applied Biosystems, CA). After PCR, the amplicons were electrophoresed at 100 V for 40 minutes on a 1.5% agarose gel, stained with ethidium bromide and then visualized via a UV-transilluminator

employing a gel doc. A 5 µl 1.5kb DNA ladder was used as a reference for band size.

DATA ANALYSIS

Data generated from the study were analysed by Epi info version 7.2.5. The prevalence was reported as the proportion of positive samples for *Chicken Infectious Anaemia Virus* in poultry. Variables associated with CIAV infection were assessed via the chi-square test. The odds ratio was used to determine the degree of the association at the 95% confidence interval. Values of $P \leq 0.05$ were considered significant.

RESULTS

Conventional PCR successfully detected CIAV DNA in tissue samples from commercial chickens in Maiduguri and Jos South LGA, Nigeria. The viral VP1 gene, the primer set, amplified a 1,390 base pair DNA fragment, which was visible as a distinct band on an agarose gel. Six representative PCR amplicons of the expected size are shown in Figure I.

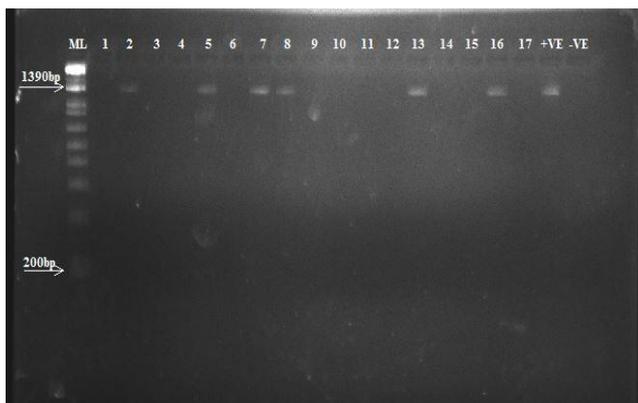


Figure I: Agarose gel electrophoresis of CIAV

The size of the target gene is 1390 bp. Lane: ML = 1500 bp molecular weight marker; -VE = negative control; +VE = positive control; L2 = 5, 7, 8, 13 and 16 = positive samples; L1, 3, 4, 6, 9, 10, 11, 12, 14, 15 and 17 = negative samples.

Out of 77 sampled analysed (31 from Maiduguri and 46 from Jos South: LG) 17 tested positive for CIAV (11 from Maiduguri and 6 from Jos South LGA: Table I).

Analysis of risk factors for the infection in the study-area is presented in Table II. Among the 28 young chickens sampled, 10 (35.7%) were positive, whereas 7 (14.3%) of the 49 adult chickens were positive ($p = 0.0446$; OR = 3.3333; 95% CI: 1.10--10.14). Among the breeds, 13 (23.6%) of 55 layers and 4 (18.18%) of 22 broilers were positive ($p = 0.07645$; OR = 1.3929; 95% CI: 0.40--4.86). By sex, 3 (15.79%) of 19 males and 15 (25.8%) of 58 females were positive ($p = 0.5352$; OR = 0.5375; 95% CI: 0.14--2.11). In terms of location, 11 (35.48%) of 31 chickens from

Maiduguri and 6 (13.04%) of 46 from Jos South: LGA were positive ($p = 0.0264$; OR = 3.6667; 95% CI: 1.18--11.35).

TABLE I: PREVALENCE OF CHICKEN INFECTIOUS ANAEMIA IN MAIDUGURI AND IN JOS SOUTH LOCAL GOVERNMENT AREA, NIGERIA

Location	No. Sample	No. (%) Positive	Confident Interval
Maiduguri	31	11 (35.5)	18.7-52.3
Jos South	46	6 (13.0)	3.3-22.7
Total	77	17 (22.1)	12.8-31.4

Table II: Risk Factors and Occurrence of Chicken Infectious Anaemia in Commercial Maiduguri and Jos South Local government area, Nigeria

Factor		No. Tested	No. (%) Positive	<i>p</i> value	Odd Ratio	95% CI
Age	Young	28	10 (35.7)	0.0446	3.3333	0.10-10.14
	Adult	49	7 (14.3)			
Breed	Layers	55	13 (23.6)	0.07645	1.3929	0.40-4.86
	Broilers	22	4 (18.18)			
Sex	Male	19	3 (15.79)	0.5352	0.5375	0.14-2.11
	Female	58	15 (25.8)			
Location	Maiduguri	31	11 (35.48)	0.0264	3.6667	1.18-11.35
	Jos South	46	6 (13.04)			

Note Age: Birds considered adult at 18 weeks old

DISCUSSION

The study revealed an overall prevalence of Chicken Infectious Anaemia Virus (CIAV) of 22.01% (17/77) in poultry populations in poultry farms with reduced productivity in Maiduguri and Jos South LGA. Prevalence of the infection varied between the two locations, with Maiduguri recording a higher prevalence of 35.48% (11/31) than Jos South LGA (13.04%: 6/46). The prevalence of CIAV has significant implications for poultry health, productivity, and economics, leading to immunosuppression, reduced growth rates, increased mortality, and decreased egg production, resulting in economic losses for farmers.

The findings in this study are lower than those of Jajere & Jajere (2022) and Shettima *et al.* (2024), who reported a detection rate of 42% in village chickens in Yobe South Senatorial District and Maiduguri, respectively. The higher prevalence reported in previous studies may be attributed to factors such as inadequate management practices, poor nutrition and healthcare, increased disease exposure due to

outdoor access, and inadequate health management, contributing to varying prevalence rates across studies.

There were significant associations between CIAV and age with young chickens being more likely to test positive for CIAV (35.7%) than adults 14.3% ($p = 0.0446$, $OR = 3.3333$). This finding agreed with the findings of Jajere & Jajere (2022), who also reported age as a risk factor for CIA in free-ranging village chickens in Yobe South Senatorial District, Nigeria, but disagreed with the findings of Shettima *et al.* (2024), who reported no significant association between CIAV infection and the age of village chickens in Maiduguri, Nigeria

Also, though female chickens had 25.8% CIA rate against males` 15.7% there was no significant association between CIAV infection and sex of the birds ($p = 0.5352$; $OR = 0.5375$; 95% CI: 0.14–2.11). Again, although layers had 23.6% against broilers` 18.18 % breed differences were not statistically significant ($p = 0.07645$; $OR = 1.3929$; 95% CI: 0.40–4.86).

The study revealed a significant location wise difference in CIA incidence, with Maiduguri recording a higher rate (35.48%) than Jos South did (13.04%) ($p = 0.0264$; $OR = 3.67$; 95% CI: 1.18–11.35). This significant difference highlights the importance of regional factors, such as management practices, climate, and vector distribution, in shaping CIA epidemiology. The odds ratio ($OR = 3.67$) indicates that the likelihood of CIAV occurrence is approximately 3.67 times greater in Maiduguri than in Jos South, underscoring the need for targeted interventions tailored to specific regions to effectively reduce CIAV's impact on poultry health. This finding is consistent with previous studies that have demonstrated similar geographic variations in CIAV prevalence dynamics (Kabir *et al.*, 2021; Jajere & Jajere, 2022; Di Francesco *et al.*, 2022), emphasizing the role of localized factors in disease transmission and the importance of region-specific control measures.

In conclusion, this study highlights the significant prevalence of CIAV in Maiduguri and Jos South, Nigeria, with an overall prevalence of 22.01%. These findings underscore the importance of age and location as risk factors, with younger chickens and Maiduguri chickens being more susceptible. To mitigate the spread of CIAV and minimize economic losses, poultry farmers should implement strict biosecurity protocols, vaccination programs, and good farming practices tailored to specific regions.

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