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# Molecular detection of avian leukosis virus in commercial poultry farms with history of production losses and mortality in Jos South Local Government Area, Plateau State, Nigeria

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**Abstract**

Avian leukosis virus (ALV) is a significant poultry pathogen that causes substantial economic losses worldwide because of its impact on production, growth rate and immune function of birds. This study investigated the molecular prevalence of avian leukosis virus (ALV) in commercial poultry farms with a history of production losses and mortality in Jos South Local Government Area (LGA), Plateau State, Nigeria. Fifty-one pooled samples were collected from such farms and used for the study. Total viral DNA was extracted and screened for ALV by polymerase chain reaction (PCR). Results showed an overall prevalence of 80.4% (41/51), with significant variations in prevalence rates among different bird types (broiler chickens – 100%; layer chickens – 81.6%; turkeys – 33.3%). The occurrence of ALV infections was significantly ($p = 0.021$) associated with the egg laying status of the birds. Given the absence of effective vaccines against the disease, these findings underscore the need for stringent biosecurity measures and alternative control strategies to mitigate the impact of ALV on poultry production.

**Keywords**: Avian leukosis virus (ALV); Poultry; Molecular prevalence; Jos, Nigeria; PCR; Poultry production.

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## Introduction

Avian leukosis virus (ALV) poses a significant threat to the poultry industry worldwide, as it causes substantial economic losses due to its impact on production, growth rate and immune function. Infection with ALV leads to tumours, mortality, immunosuppression and reduced vaccine efficacy, with vertical transmission affecting offspring and compromising chicken quality (Wu *et al*., 2022). ALV can be transmitted both vertically and horizontally, inducing tumours, immunosuppression and reduced productivity in commercial poultry operations (Zhou, *et al*., 2019; Wu *et al*., 2022).

The avian leukosis virus belongs to the Alpharetrovirus genus of the Retroviridae family (Fadly, 1997). The virus is categorized into subgroups on the basis of viral envelope protein characteristics, with exogenous sub-groups including ALV-A, ALV-B, ALV-C, ALV-D, ALV-K, and ALV-J, the latter being the primary sub-groups responsible for avian neoplastic diseases worldwide (Zhang *et al*., 2020; Chen *et al*., 2022; Guo *et al*., 2023; Zhang *et al*., 2024). Notably, ALV subgroups A, B, J, and K have the widest epidemiological scope (Lv *et al*., 2019; Chen *et al*., 2022; Guo *et al*., 2023). Globally, ALV is prevalent in several breeding flocks, with sub-groups A, B and J being the most common in commercial poultry (Gao *et al*., 2014). Despite its significant impact, effective prevention and treatment methods remain elusive, as both attenuated and inactivated vaccines have proven ineffective in preventing ALV infection (Feng and Zhang, 2016).

Birds infected with avian leukosis virus experience stunted growth, uneven growth rates and heightened vulnerability to diseases caused by immunosuppressive viruses and secondary bacterial infections (Bagust *et al*., 2004). Other production challenges posed by the virus include reduced egg production, smaller eggs with thinner shells, lower fertility, poor hatchability and increased mortality rates (Gavora *et al*., 1980, 1982). Though there had been reports of production losses, poor weight gain, mortality and decreased egg production in farms in Jos South LGA of Plateau State Nigeria, there had been no reports in available literature on the occurrence/prevalence of ALV in the farms in the area. The present study evaluated the prevalence of ALV in poultry farms with reports of production losses, poor weight gain, mortality and decreased egg production in Jos South LGA of Plateau State, Nigeria, using molecular techniques.

## Materials and Methods

**Study Area:** The research was conducted in Jos South Local Government Area, Plateau State, Nigeria, which is situated between Latitudes 8°45'00" and 9°50'00" North of the Equator and Longitudes 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 (Ikegwuonu, *et al*., 2021). Owing to its favourable weather conditions, Plateau State is highly conducive for poultry production and various livestock farming activities. Additionally, the State serves as a significant centre for poultry farming in northern Nigeria, providing day-old chicks (DOCs) and other poultry products to numerous northern Nigerian States and Abuja, the Federal Capital of Nigeria. The poultry production system in this region primarily comprises smallholder backyard farms alongside some commercial farms, with chicken populations ranging from 50 – 50,000 per farm, which are focused mainly on egg production and seasonal broiler production.

**Sample collection:** From January 2024 to May 2025, samples were collected from 51 commercial poultry farms in Jos South LGA,

Plateau State, Nigeria. Birds displaying poor performance and weakness were purposively selected for sample collection. During necropsy, spleen, liver, and thymus samples were harvested from at least two birds in each farm sampled and pooled to create a group sample. A total of 51 of such pooled samples, representing 51 poultry farms, were tested for ALV.

**Tissue Processing:** Tissues, including the lungs, trachea, liver, and spleen, were harvested during necropsy. Approximately 1 – 2 g of each tissue sample was pooled and homogenized using a sterile glass mortar and pestle with an antibiotic mixture consisting of penicillin (2,000 units/mL), streptomycin (2 mg/mL), gentamicin (50 µg/mL), and amphotericin B (5 µg/mL) (PSGA). The homogenized tissues were then centrifuged at 3,000 rpm for 10 minutes. The supernatant was aliquoted into clean, sterile cryovials and stored in a freezer until DNA extraction.

**DNA Extraction:** Total viral DNA was extracted from processed tissue homogenates using 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 microcentrifuge tube. Two hundred (200) µl samples (tissue homogenate) were then added to the microcentrifuge tube, and 200 µl of Buffer AL was added to the sample and mixed by pulse vortexing for 15 seconds. The mixture was then incubated at 56°C for 10 minutes. The 1.5 ml microcentrifuge 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 seconds. After mixing, the 1.5 ml microcentrifuge 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 revolutions per minute (RPM) for 1 minute. 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 minute. 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) microlitres 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 minute 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 minute. The mixture was subsequently centrifuged at 8000 RPM for 1 minute 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 by Smith *et al*. (1998). A forward primer, Avian leukosis virus primer A-E (5'- GGATGAGGTGACTAAGAAAG-3'), and a reverse primer (5'-GGGAGGTGGCTGACTGTGT-3') were used for amplification, targeting a 295--326 bp region. The PCR mixture (25 µl) consisted of 12.5 µl of nuclease-free water, 5 µl of 5x Buffer, 2 µl of 10 µM forward and reverse primers, 0.5 µl of dNTPs, 1 µl of MgCl2, 0.5 µl of Taq polymerase, and 2 µl of purified DNA. The PCR amplification protocol involved initial

denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 48°C for 1 minute, and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes. The PCR analysis was performed on a GeneAmp PCR system 9700 thermal cycler (Applied Biosystems, CA). The amplicons were then electrophoresed on a 1.5% agarose gel at 100 V for 40 minutes, stained with ethidium bromide, and visualized via a UV transilluminator with a gel documentation system. A 5 µl 1 kb DNA ladder served as a reference for band size determination.

**Data analysis:** Data generated from the study were analysed via Epi info version 7.2.5. The prevalence was estimated as the proportion of positive samples for avian leukosis virus among the poultry farms sampled. Variables associated with avian leukosis infection were analysed using the chi-square test. Odds ratio was used to determine the degree of the association at 95% confidence interval. Values of $p < 0.05$ were considered significant.

## Results

The conventional PCR used for the study successfully detected ALV DNA in some of the tissue samples collected from the poultry farms surveyed. Specifically, the primer set used for the study amplified the expected 300-base pair (bp) DNA fragment, which was visualized as a distinct band on an agarose gel following electrophoresis (Figure 1).

The prevalence of avian leukosis virus (ALV) on the farms surveyed is presented in Table 1. Among the 51 samples tested, 41 (80.4%) were positive for ALV. The highest prevalence was observed in broiler chickens (100%) and cockerel chickens (100%), followed by layer chickens (81.6%), with geese (50%) and turkeys (33.3%) having lower prevalence.

Table 2 shows the age-based distribution of the prevalence of ALV among the farms sampled, which revealed that 76.2% (16/21) of young birds and 83.3% (25/30) of adult birds were positive, with no significant association ($p = 0.72$) between age and occurrence. Table 3 shows the prevalence of ALV based on egg laying status. The occurrence of ALV was significantly ($p = 0.02$) associated with the egg laying status of the birds, with the occurrence being significantly ($p = 0.02$) higher in laying birds (95.7%; 22/23) than in non-laying birds (64.3%; 9/14).

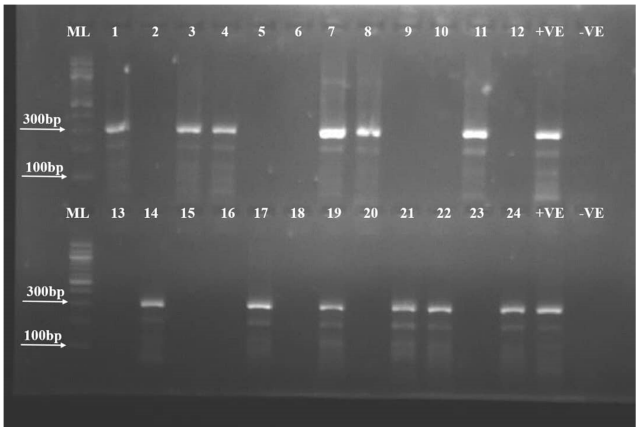


**Figure 1**. Agarose gel electrophoresis of ALV. The size of the target gene is 300 bp. Lane: ML= 100 bp molecular weight marker; -VE = negative control; +VE=positive control; L1, 3, 4,7,8,11,14,17,19.21,22 and 24= +ve samples; L2,5,6,9,10,12,13,15,16,18,20 and 23 = -ve samples.

**Table 1.** Prevalence of avian leukosis virus (ALV) in commercial poultry farms with history of production losses and mortality in Jos South Local Government Area, Plateau State, Nigeria.

| Category | Number of farms tested. | Number of farms positive for ALV | Percentage of farms positive for ALV |
|---|---|---|---|
| **Broiler chickens** | 7 | 7 | 100% |
| **Cockerel chickens** | 1 | 1 | 100% |
| **Layer chickens** | 38 | 31 | 81.6% |
| **Geese** | 2 | 1 | 50% |
| **Turkeys** | 3 | 1 | 33.3% |
| **Total** | **51** | **41** | **80.4%** |

**Table 2.** Age-based distribution of prevalence of avian leukosis virus (ALV) in commercial poultry farms with history of production losses and mortality in Jos South Local Government Area, Plateau State, Nigeria.

| Age | Number of farms tested. | Number of farms positive for ALV | Percentage of farms positive for ALV | P-value |
|---|---|---|---|---|
| **Adult** | 30 | 25 | 83.3% | 0.7218 |
| **Young** | 21 | 16 | 76.2% | |
| **Total** | **51** | **41** | **80.4%** | |

**Table 3. D**istribution of prevalence of avian leukosis virus (ALV) in commercial poultry farms with history of production losses and mortality in Jos South Local Government Area, Plateau State, Nigeria, based on egg laying status.

| Egg laying status | Number of farms tested. | Number of farms positive for ALV | Percentage of farms positive for ALV | P-value |
|---|---|---|---|---|
| **Laying birds** | 23 | 22 | 95.7% | 0.0211 |
| **Non-laying birds** | 14 | 9 | 64.3% | |
| **Total** | **35** | **31** | **88.6%** | |

## Discussion

Avian leukosis (AL) is a significant yet often overlooked disease affecting chickens worldwide (Fadly, 1997). Global outbreaks have been reported in susceptible avian species (Payne and Nair, 2012), resulting in substantial economic losses and food insecurity due to reduced production, uneven growth rates, immunosuppression, and increased susceptibility to secondary diseases (Kheimar *et al*., 2021).

This study revealed a high prevalence of avian leukosis virus (ALV) in commercial poultry farms with history of production losses and mortality in Jos South LGA, Plateau State, Nigeria, with an overall prevalence of 80.4%.

This result suggests that ALV currently persists and is highly prevalent in the chicken population in the study area. The high prevalence suggests that ALV might be a major contributor to the observed mortalities, decreased egg production and poor growth rates in affected farms. It should be noted that samples for the study were collected from birds/farms with history of production losses and mortality, which are signs of ALV infection; therefore, the true prevalence in the area studied might be lower than what was recorded in the present study.

Previous studies in Nigeria reported varying prevalence rates for ALV: Olabode *et al*. (2009) reported rates of 1.25% via histopathology, whereas Sani *et al*. (2012) and Bitrus *et al*. (2022) reported rates of 18.33% and 23.8%, respectively, via serological methods. Differences in sampling method, source and diagnostic methods may explain these disparities. Generally, PCR detection of proviral DNA is usually more sensitive than conventional methods (Garcia *et al*., 2003; Thapa *et al*., 2004), which may explain the higher prevalence observed in this study. In contrast, the use of histopathology by Olabode *et al*. (2009) might have resulted in a very low prevalence. Previous studies have reported that PCR is specific and more sensitive than most diagnostic techniques in distinguishing ALV from other infections that have similar lesions (Garcia *et al*., 2003; Thapa *et al*., 2004).

Interestingly, broiler chickens had the highest prevalence rate (100%), suggesting a higher susceptibility. Layer chickens had a relatively lower percentage (81.6%) than broilers, whereas geese and turkeys had significantly lower percentages (50% and 33.3%, respectively). These differences may be attributed to breed differences, management practices or exposure.

The age-specific prevalence was high in both young (76.2%) and adult birds (83.3%), with no statistically significant difference (p value = 0.7218). It is thought that the higher prevalence in adult birds might be due to more prolonged exposure. This finding aligns with those of previous studies (Wu *et al*., 2010; Bitrus *et al*., 2023), who also reported a higher prevalence in adult birds.

In the present study, a significant association was recorded between ALV infection and egg laying status, with laying birds being approximately 12 times more likely to be infected. The high prevalence in laying birds (95.7%) compared with non-laying birds (64.3%) underscores the need to investigate the underlying mechanisms involved. Our findings agree with those of Bitrus *et al*. (2023), who reported a prevalence of 86.5%, but they do not concur with those of Miheso *et al*. (2017) and Sani *et al*. (2012), who reported lower prevalences of 18.63% and 18.33%, respectively, in laying birds.

The high ALV prevalence in commercial poultry farms with history of production losses and mortality in Jos South LGA, Plateau State, Nigeria, has significant implications for poultry health, productivity and the economy. Improved biosecurity measures, vaccination programs, and control strategies are necessary to mitigate the spread of ALV since there are no effective vaccines against the virus. Further research is needed to investigate risk factors and develop effective control measures. Further molecular characterization is also essential for determining the specific ALV sub-group contributing to the high prevalence rate.

**Conflict of interest**

The authors declare that they have no competing interests.

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