Full Length Research Paper

Seroprevalence of avian leukosis virus antigen using ELISA technique in commercial exotic-layer chickens in Zaria and its environs

N. A. Sani[1*], S. B. Oladele[2], M. A. Raji[2] and N. D. G. Ibrahim[2]

[1]Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Abuja, Nigeria.
[2]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

This study examined the prevalence of avian leukosis virus (ALV) in commercial exotic-layer chickens in Zaria, Nigeria, and its environs. A total of 240 sera from eight commercial exotic-layer farms were tested for ALV p27 antigen using the antigen capture - enzyme linked immunosorbent assay (ac-ELISA), out of which 44 (18.33%) sera were positive. Of the eight commercial exotic-layer farms sampled, six were seropositive for ALV, while two were seronegative. The six farms seropositive for ALV had seroprevalence of 20.0, 11.4, 14.3, 8.6, 16.7 and 66.7%. All serum samples from exotic commercial layers that tested positive to ALV p27 antigen were lowly positive, except for farm VIII, where 11 of the 20 positive samples were lowly positive with EUs ranging from 11 to 22.6%, and 9 of the positive sera were moderately positive with EUs ranging from 28.7 to 62.8%. The average age of exotic layers from the ALV p27 antigen positive farms was 17.2 (SD=1.3) weeks. At the time of sampling of chickens from the six farms that tested positive to ALV p27 antigen, the farms had lost between 6.25 and 37.5%, and an average of 20.8% (SD=13.2) of the total chickens stocked. The average mortality rate per week for the ALV p27 antigen positive farms was approximately 2.6% (SD=1.7) with farm I having the highest mortality rate of 5%, and farm VIII lowest with a weekly mortality rate of 0.75%. These findings illustrate the potential severity of ALV infection and its economic consequences in this region.

Key words: Seroprevalence, avian leukosis virus, enzyme-linked immunosorbent assay (ELISA), chickens, Zaria.

INTRODUCTION

Avian leukosis (AL), Marek's disease (MD) and reticuloendotheliosis (RE), are avian oncogenic diseases of economic importance (Payne and Venugopal, 2000). Of these oncogenic diseases, avian leukosis is an insidious, but important disease of chickens (Fadly, 1990).

Avian leukosis is caused by members of the leukosis/sarcoma group of avian retroviruses, commonly referred to as avian leukosis viruses (ALVs) and was first discovered in Copenhegan nearly 100 years ago as

reviewed by Burmeister (2001). ALVs are classified into six subgroups; A, B, C, D and J are oncogenic and exogenous ALVs, while subgroup E is endogenous and regarded as having extremely low pathogenicity (Calnek et al., 1991). Avian leukosis virus subgroups A and B are most commonly associated with lymphoid leukosis and less commonly with erythroid leukosis in layers, while ALV subgroup J is mainly associated with myeloid leukosis in broilers (Payne et al., 1991). Avian leukosis virus, like other retroviruses, mutates at a high rate and can recombine between subgroups, resulting in new recombinant ALVs (Gingerich et al., 2002; Lupiani et al., 2003). The wide range of genetic and antigenic variations among isolates (Wu et al., 2010) coupled with an efficient mode of transmission have made it difficult to eradicate

*Corresponding author. E-mail:talk2nas2002@yahoo.com. Tel: +234-8032176723.

Table 1. Results of enzyme linked immuno-sorbent assay for avian leukosis virus antigen (p27) of chickens from eight commercial farms in Zaria and environs.

Farms	Location of farms	No. of sera tested	No. of sera positive	Percentage of sera positive
I	Funtua	35	7	20.0
II	Karau-Karau	35	4	11.4
III	Kaduna	35	5	14.3
IV	Zango	35	3	8.6
V	Shika	10	0	0.0
VI	Kaduna	30	5	16.7
VII	Kaduna	30	0	0.0
VIII	Kaduna	30	20	66.7

this group of retroviruses.

The enzyme-linked immunosorbent assay (ELISA) is useful for the detection of ALV group-specific antigen (p27), which is common to all of the subgroups. This assay is a sensitive, safe, rapid and easy to perform diagnostic tool (Smith et al., 1979). The ALV ELISA diagnostic method is reported to have 99.2% sensitivity and 100% specificity, and can be used clinically for screening purpose (Pham et al., 1999; Silva et al., 2007). Therefore, the level of avian leukosis viral proteins in serum and egg albumen, as well as the disease status of an entire flock can be monitored using ELISA (Crittenden et al., 1984).

Outbreaks of avian leukosis have been reported worldwide causing devastating loses to poultry farmers. However, there is a paucity of information as regards the status of this disease in Northern Nigeria. This survey for ALV using serological methods is expected to give an insight into the prevalence of the disease in Zaria and its environs.

MATERIALS AND METHODS

The study was carried out in Zaria and its environs. Zaria is located in Kaduna State within the Northern Guinea Savannah Zone of Nigeria between latitude 7° and 11°N, and longitude 7° and 44°E. The average rainfall ranges from 1,000 to 1,250 mm with temperatures ranging from 17 to 33°C (Saidu et al., 1994).

The prevalence of ALV in chickens from eight commercial exotic-layer farms in Zaria and its environs (Farms I, II, III, IV, V, VI, VII and VIII in Funtua, Karau-Karau, Kaduna, Zango, Shika, Kaduna, Kaduna and Kaduna respectively), that were diagnosed with avian tumuor at post-mortem, in the Poultry Unit of the Veterinary Teaching Hospital (VTH), Ahmadu Bello University (ABU), Zaria, was determined. The location of farms, age of chickens, vaccination history against Marek's disease, and mortality rate of the affected chickens were documented.

Two hundred and forty exotic layers were sampled by simple random sampling from the eight farms. Sampling of chickens from each farm was based on the cooperation of the farm owners. Two milliliters of blood was collected aseptically via the wing vein using sterile 23 gauge hypodermic needles and syringes. The sampling of exotic layers in Zaria and environs lasted four months, starting from May to August, 2010.

The blood collected from each bird was dispensed into a test

tube and transported on ice packs to the chemical pathology laboratory, Ahmadu Bello University Teaching Hospital (ABUTH), Shika, where the serological test (ELISA) was performed. The blood was allowed to clot, and in some cases centrifuged at 1,000 g for 10 min (WOAH, 2008) to clearly separate the serum from cellular components. The sera were dispensed into serum tubes and stored frozen at -20°C until used. Test tubes and serum tubes were labeled appropriately using indelible marking pen.

Sera were tested using colorimetric sandwich ELISA for the presence of ALV group specific antigen (p27), as described by the manufacturer, AffiniTech LTD (2009). The ELISA procedure was carried out at the chemical pathology laboratory, Ahmadu Bello University Teaching Hospital, Shika, Zaria.

The ELISA unit (EUs) for all samples tested on the Affini Tech ALV Antigen Detection Test Kit was calculated using the formula recommended by AffiniTech (2009).

$$\frac{Average\ Absorbance_{Test\ sample} - Average\ Absorbance_{Negative}}{Average\ Absorbance_{Positive} - Average\ Absorbance_{Negative}} \times 100 = ELISA\ Unit$$

The interpretation of the results was using the method described by AffiniTech (2009) and as modified by Emikpe et al. (2007). Enzyme-linked immunosorbent assay units (EUs) less than 10 were considered negative, while EUs greater than 10 were considered positive for ALV p27 antigen. Enzyme-linked immunosorbent assay units (EUs) 10-25 were considered weakly positive, EUs greater than 25-75 were considered moderately positive, while EUs greater than 75 were considered as strongly positive for ALV P27 antigen. Data obtained from this study were summarized using Statistical Package for Social Sciences (SPSS) version 16. The percentages, means and standard deviation of means were calculated.

RESULTS

In this study, a total of 240 sera from eight commercial exotic-layer farms in Zaria and environs, were tested for ALV p27 antigen by ELISA technique. Of the 240 exotic-layer sera from the eight farms sampled, 44 (18.33%) were found to contain ALV p27 antigen.

Thirty five serum samples from farm I were tested for ALV p27 antigen, out of which seven (20%) were positive with EUs ranging from 10.5 to 16.2%. Thirty five serum samples were also sampled from each of farms II, III and IV of which four (11.4%), five (14.3%), and three (8.0%) were positive for ALV p27 antigen, respectively (Table 1). The EUs ranged from 13 to 16.2% for farm II, 11.7 to

Table 2. Morbidity and Mortality rates of the eight commercial farms in Zaria and environs tested for avian leukosis virus antigen (p27).

Farms	Age of birds (weeks)	No. of birds per farm	No. of birds at sampling	Mortality	No. of birds sick at sampling	Mortality per week
I	17	900	600	300 (33.33%)	40	30 (5.00%)
II	16	4000	2500	1500 (37.5%)	150	80 (3.20%)
III	19	2000	1700	300 (15.0%)	20	25 (1.47%)
IV	16	400	370	30 (7.5%)	30	14 (3.78%)
V	18	600	400	200 (33.33%)	10	11 (2.75%)
VI	18	1600	1500	100 (6.25%)	50	20 (1.33%)
VII	14	2500	1800	700 (28.0%)	100	50 (2.78%)
VIII	32	1600	1200	400 (25%)	200	9 (0.75%)

Table 3. Results of Elisa units for chickens tested for avian leukosis virus antigen (p27) from eight commercial farms in Zaria and environs.

Farms	Number of sera tested for ALV p27 antigen	Number of lowly positive (EU= 10 – 25%)	Number of moderately positive (EU= >25 – 75%)	Number of strongly positive (EU= >75%)	Total number of ALV p27 antigen positive sera
I	35	7	0	0	7
II	35	4	0	0	4
III	35	5	0	0	5
IV	35	3	0	0	3
V	10	0	0	0	0
VI	30	5	0	0	5
VII	30	0	0	0	0
VIII	30	11	9	0	20

18.8% for farm III, and 12.2 to 19.5% for farm IV.

Farm VI had five (16.7%) out of 30 serum samples positive to ALV p27 antigen with EUs ranging from 11.6 to 18.9%, while 20 (66.7%) out of 30 sera samples from farm VIII were positive to ALV p27 antigen with EUs ranging from 11 to 62.8%.

All serum samples from exotic layers that tested positive to ALV p27 antigen were lowly positive except for farm VIII, where 11 of the 20 positive samples were lowly positive with EUs ranging from 11 to 22.6%, and nine of the positive sera were moderately positive with EUs ranging from 28.7 to 62.8% (Table 2).

The average age of exotic layers from the ALV p27 antigen positive farms was 17.2 (SD=1.3) weeks with the age of chickens (32 weeks) in farm VIII considered as outlier and therefore excluded from this calculation. Chickens of farms I, II, V, VII and VIII were not vaccinated against Marek's disease whereas farms III, IV and VI chickens were vaccinated against Marek's disease (Table 3).

At the time of sampling of the exotic chickens from the six farms that tested positive to ALV p27 antigen , the farms had lost an average of 20.8% (SD=13.2) of the total chickens stocked. The mortality at these ranged

from 6.25 to 37.5%, farm II having the highest mortality of 37.5%, and farm VI with the lowest mortality of 6.25%. The average mortality rate per week for the ALV p27 antigen positive farms was approximately 2.6% (SD=1.7) with farm I having the highest mortality rate of 5%, and farm VIII lowest with a weekly mortality rate of 0.75% (Table 2).

Avian leukosis virus p27 antigen was not detected in farm V and VI located in Shika and Kujama (Kaduna), respectively.

DISCUSSION

Avian leukosis is a major cause of serious economic losses to the poultry industry worldwide. Negative impact of the disease on chickens include reduced growth, unevenness of growth rates within flocks, and a greater susceptibility to developing serious disease when challenged by immunosuppressive viruses or secondary bacterial invaders (Bagust et al., 2004). Decreased egg production, decreased egg weight and shell thickness as well as fertility, hatchability, rate of growth and liveability problems have also been observed (Gavora et al., 1980,

1982). Since these production problems are common occurrences in the poultry industry in Nigeria, we undertook this study to determine the ALV status of commercial exotic-layer chickens in Zaria and environs.

A total of eight commercial layer chicken farms diagnosed at post mortem to have avian tumor, were screened for ALV p27 antigen using ELISA. Of these eight farms screened, six (farms I, II, III, IV, VI and VIII) were serologically diagnosed to have ALV with detection range of 8.6 to 66.7%, while two (farms V and VII) were sero-negative for ALV and an average ALV prevalence of 18.33% was recorded. These findings are similar to those previously recorded in Australia by Bagust et al. (2004) with a detection range of 0 to 75%. However, the overall prevalence recorded by these workers was 6.37%, which is lower than the overall prevalence of 18.33% recorded in this study. In another study by Olabode et al. (2009), a lower avian leukosis prevalence of 1.25% was reported for birds from Kwara State, Nigeria. However, diagnosis of AL in the study by Olabode et al. (2009) was by postmortem examination and histopathology. These diagnostic methods may not be relied upon in differentiating AL from other avian tumor causing diseases. The higher prevalence recorded in this study might be attributed to lack of control measures for this disease in Nigeria. As indicated by the nearly 75% of farms that were positive for ALV p27 antigen in this study, the lack of control measures may have led to widespread infection by ALV. In a recent study by Gao et al. (2010), a prevalence of 64.4% was reported which is higher than what was recorded in this study. This may be attributed to the more sensitive polymerase chain reaction (PCR) diagnostic method used in their study.

In this study, the highest ALV p27 detection (66.7%) was observed in farm VIII which also had birds that were the oldest (32 weeks old). The lowest levels of ALV p27 detection (8.6%) was for chickens sampled from farm IV, which was the youngest chicken age group bracket (16 weeks) of all the farms screened. This relationship is consistent with the findings of Wu et al. (2010) who reported high detection rates in older age groups of chickens. The higher number of ALV p27 antigen sero-positive chickens in the older age group (farm VIII) could be attributed to higher exposure time to the virus, and therefore higher transmission rates from infected chickens to uninfected chickens. The degree of infection was also higher in farm VIII, which was the only farm that had samples that were moderately positive for ALV p27 antigen. This might be attributed to prolonged infection of the chickens with the virus, thereby giving ample time for the virus to multiply in the chickens. The commercial layers in farm VIII were as old as the Nigerian local chickens in studies by Sani et al. (2011) and Emikpe et al. (2007), and hence the similarity in the seroprevalence results.

Avian leukosis is a disease of great economic importance. Mortality in chickens due to AL has been attributed to either the direct effect of the virus, in which case due to tumor induction by the virus in chickens, or due to the indirect effect of the virus through rendering the infected chickens immunosuppressed thereby making them more susceptible to opportunistic infections (Cox et al., 2004). A wide range of mortalities were recorded in this study with the lowest mortality as 7.5% (farm IV) and the highest mortality was recorded in farm II (37.5%). The high mortality recorded in this study is in agreement with the findings of Cox et al. (2004), and Latif and Khalafalla (2005), who reported 40% mortality in their study. Payne (1998) reported mortality rates of 6% to 8% per month.

The economic impact of this disease on the poultry sector is in no doubts enormous, and drastic steps must be taken to control this disease. A weekly loss rate of 1.5% due to ALV was reported by Bagust et al. (2004). Similarly, Latif and Khalafalla (2005) reported a weekly mortality of 2 to 3%. In this study, the average weekly loss rate was 2.59%. Weekly loss rate was observed to be lowest in farm VIII (0.75%) as compared to farm I (5%) and farm IV (3.78%). This is probably because most of the diseased chickens in farm VIII may have died earlier in the course of the disease with only a few remaining diseased chickens potentially responsible for the low weekly mortality rate. On the other hand, lower mortality amoung older birds could also be attributed to their higher resistance to developing ALV induced tumors (Mays et al., 2006).

In conclusion, this study has identified the widespread infection by ALV in the commercial exotic-layer farms in Zaria and its environs. Within the constraints of sampling and the detection method used here, the results of this study are generally consistent with previous reports from Nigeria and other countries. These rates of prevalence suggest a need for concerted efforts by the stakeholders in the poultry industry into combating the threat posed by ALV, a virus with high economic impact to the poultry industry.

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