Research article

ANTIBODY PROFILES OF AVIAN LEUKOSIS VIRUS SUBGROUPS A/B AND J IN LAYER FLOCKS SUSPECTED TO HAVE MAREK'S DISEASE IN NIGERIA

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Previous reports indicate high seroprevalence of avian leukosis virus (ALV) p72 antigen in layer flocks suspected to have Marek's disease (MD) in Kaduna and Plateau States. However, the specific subgroups responsible for ALV infection in layers in the States are still unknown, hence the need for this study. Therefore, the objective of this study was to determine the antibody profiles of ALV subgroups A/B and J in layer flocks suspected to have MD in Kaduna and Plateau States. Sera from 7 and 16 layer flocks suspected to have MD in Kaduna and Plateau States respectively, were screened for the presence of antibodies to ALV subgroups A/B and J using IDEXX enzyme linked immunosorbent assay (ELISA) kits. Out of the seven layer flocks screened in Kaduna State, antibodies to ALV subgroup A/B was detected in six of the flocks (85.7%), while antibodies to ALV subgroup J was detected in only one flock (14.3%). Antibodies to both ALV subgroups A/B and J were detected in one flock (14.3%), which suggests co-infection of the two ALV subgroups. Out of the 16 flocks screened in Plateau State, antibodies to ALV subgroup A/B were detected in 15 flocks (93.8%), while antibodies to ALV subgroup J were detected in six flocks (37.5%). Antibodies to both ALV subgroups A/B and J were detected in five flocks (31.3%). The high detection of antibodies to ALV A/B suggests that ALV infection in layers is mostly due to ALV subgroup A or B in the study areas.

Keywords: antibodies, avian leukosis virus, Marek's disease

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INTRODUCTION

Avian leukosis virus (ALV) is a retrovirus and belongs to the Genus *Alpharetrovirus*, of the Subfamily *Orthoretrovirinae*, of the Family *Retroviridae*, and Order *Ortervirales* [1,2]. Avian leukosis virus in chickens is classified into 7 subgroups, designated A, B, C, D, E, J and K, based on envelope glycoprotein, viral interference patterns, and host range [3,4]. Subgroups A, B, C, D, J and K are exogenous ALVs and cause neoplasms in poultry, while subgroup E is an endogenous ALV and non-oncogenic [5]. The ALV subgroups A, B, C and D are known to cause lymphoid leukosis majorly, while subgroup J and K are responsible mainly for myeloid type tumours and haemangioma [6,7]. The ALV subgroups F, G, H and I are endogenous viruses found in ring-necked and golden pheasants, Hungarian partridge and Gambel's quail, respectively [8]. Transmission of ALVs is both horizontal and vertical [9,10].

Among ALVs, subgroups A, B and J are the most prevalent [11-14], causing neoplasms in chickens as single infections [13,15,16] or as multiple infections [12,15,17,18]. ELISA is a highly sensitive and specific diagnostic tool widely used to screen flocks of poultry for antibodies [19-21] or antigen [2,22] to virus-induced avian neoplastic disease viruses, especially ALV.

Previous serological surveys of ALV in Nigeria focused on the detection of p27 antigen [23-26] which is group specific to all ALVs, and cannot be used to differentiate between subgroups. Therefore, it became necessary to evaluate the involvement of specific subgroups of ALV in field cases of virus-induced avian neoplastic diseases in the study areas. The findings from this study may be helpful to stakeholders in the poultry industry in Nigeria in instituting control for virus-induced avian neoplastic diseases, thereby reducing economic losses incurred through virus-induced avian neoplastic disease associated mortalities and production problems in chickens. Results from this study will also add to the scarce literature on virus-induced avian neoplastic diseases, especially AL, in Kaduna and Plateau States, Nigeria.

MATERIALS AND METHODS

Ethical approval

This research related to animal use has been complied with all the relevant policies for the care and use of animals (University of Abuja Ethics Committee on Animal Use; UAECAU/2017/0020; Date: 04/05/2017).

Study area

The study was carried out in Kaduna and Plateau States, Nigeria. Kaduna State is in the North West and located between latitudes 9°03' and 11°32'N and longitudes 6°05' and 8°38'E of the Greenwich Meridian [27], while Plateau State is in North Central and located between latitude 80°24'N and longitudes 80°32' and 100°38'E [28].

Selection of the layer flocks for the study

The layer flocks studied in this research were selected using the purposive sampling technique as described by Crossman [29]. Seven and 16 commercial layer chicken flocks that were managed on deep litter in Kaduna and Plateau States, respectively, in which MD was tentatively diagnosed during postmortem examination were used for the study.

Collection of blood samples from layers for serology

Fifty-six and 125 blood samples were collected from layer flocks suspected to have MD in Kaduna and Plateau States, respectively. The number of layers sampled from each of the flocks was based on the number of sick layers available at the time of the farm visit and the willingness of the owners or managers of the farm to allow sampling of layers. One milliliter (ml) of blood was collected aseptically from each layer via the wing vein using sterile 23-gauge hypodermic needle and 2 ml syringe. The sampling of layers in Kaduna and Plateau States lasted 13 months, from February, 2017 to March, 2018. The blood collected from each layer was dispensed into plain blood sample bottles, allowed to clot and the serum dispensed into serum tubes. The sera were then stored frozen at -20°C [30] until used for ELISA. The sample bottles/ tubes were labeled appropriately with an indelible marker.

Enzyme linked-immunosorbent assay

Sera from the layer flocks were allowed to thaw to room temperature and tested for antibodies to ALV subgroups A/B and J using IDEXX ALV Ab and IDEXX ALV-J Ab ELISA kit, respectively, in accordance with the manufacturer's (IDEXX[®] Laboratories, USA) instructions. ELISA was carried out at the Viral Research Laboratory, National Veterinary Research Institute, Vom, Nigeria.

Each test sample (serum) was diluted 500 folds (1:500) with sample diluent prior to being assayed. The procedure for the detection of antibodies to ALV subgroup A/B using ALV Ab ELISA involved first labelling each well in the antigen-coated plates to correspond with that of the controls and test samples. 100 μ l each of undiluted negative control (NC) and positive control (PC) were dispensed in duplicate wells. Then 100 μ l of each of the diluted test samples were dispensed into appropriately labelled wells. The plates were then incubated for 30 minutes at 20°C. The content of the wells was discarded and each well was washed with 350 μ l of distilled water 5 times. Each plate was then tapped onto absorbent material (tissue paper) after the final wash to remove any residual wash fluid (distilled water) from the wells. 100 μ l of conjugate was dispensed into each well followed by another incubation (30 minutes at 20°C) and wash steps. 100 μ l of TMB substrate solution was then dispensed into each well followed by another incubation generation of the step solution was then added to each well. The absorbance values of the controls and

test samples were measured at 650 nm with an ELISA reader connected to a desktop computer.

The same procedure was followed to detect antibodies to ALV subgroup J using IDEXX ALV-J Ab ELISA. However, wash concentrate provided with the kit was used in the washing steps after it was diluted with distilled water at a ratio of 1:10.

Validity criteria

For each plate of the IDEXX ALV Ab, the mean absorbance value of the PC minus the mean absorbance value of the NC should be greater than 0.075, while the mean absorbance value of the NC should be less or equal to 0.150.

For the IDEXX ALV-J Ab, the mean absorbance value of the PC minus the mean absorbance value of the NC should be greater than 0.100, while the mean absorbance value of the NC should be less or equal to 0.150.

Calculation of sample to positive ratio

The sample to positive (S/P) ratio of each test sample was calculated using the formula below:

 $S/P = \frac{Sample - NCX}{PCX - NCX}$ Where $PCX = \frac{PC1 + PC2}{2}$ and $NCX = \frac{NC1 + NC2}{2}$

Interpretation of results

For IDEXX ALV Ab ELISA, test sample with S/P ratio greater than 0.40 was considered positive for antibodies to ALV subgroup A/B, while for the IDEXX ALV-J Ab ELISA, test sample with S/P ratio greater than 0.60 was considered positive for antibodies to ALV subgroup J.

Data analyses

The data were summarized using descriptive statistics [31] and presented in tables. The number of layer flocks as well as the number of layers per flock that were positive for ALV-A/B and ALV-J using ELISA, were summarized into percentages.

RESULTS

Antibody profiles of avian leukosis virus subgroups A/B and J in layer flocks suspected to have Marek's disease in Kaduna State

Out of the seven layer flocks screened, antibodies to ALV subgroup A/B was detected in sera from six of the flocks (85.7%), while antibodies to ALV subgroup J was detected in sera from only one flock (14.3%) (Table 1). Antibodies to ALV subgroups A/B and J were both detected in sera from one flock (14.3%), while antibodies to ALV subgroups A/B or J were not detected in sera from one of the flocks (14.3%). Of the seven layer flocks screened, five flocks (71.4%) had antibodies to ALV subgroup A/B only, while no flock had antibodies to ALV subgroup J only (Table 1).

Farm	Number of sera tested	ALV subgroup A/B positive (%)	ALV subgroup J positive (%)	ALV subgroups A/B and J positive (%)
Z1	9	2 (22.2%)	0 (0%)	0 (0%)
Z2	10	0 (0%)	0 (0%)	0 (0%)
Z3	6	2 (33.3%)	0 (0%)	0 (0%)
Z4	10	3 (30%)	0 (0%)	0 (0%)
Z5	9	5 (55.6%)	1 (11.1%)	0 (0%)
Z6	5	4 (80%)	0 (0%)	0 (0%)
Z7	7	7 (100%)	0 (0%)	0 (0%)
Total	56	23 (41.1%)	1 (1.8%)	0 (0%)

Table 1. ALV subgroups A/B and J antibodies in layers suspected to have Marek's disease in Kaduna State, Nigeria

A total of 56 sera from the seven layer flocks were screened for antibodies to ALV subgroups A/B and J, out of which 23 (41.1%) were positive for antibodies to ALV subgroup A/B, while one (1.8%) was positive for antibodies to ALV subgroup J. No serum tested positive for both antibodies (Table 1).

Antibody profiles of avian leukosis virus subgroups A/B and J in layer flocks suspected to have Marek's disease in Plateau State

Out of the 16 layer flocks screened, antibodies to ALV subgroup A/B were detected in sera from 15 of the flocks (93.8%), while antibodies to ALV subgroup J were detected in sera from six of the flocks (37.5%). Antibodies to ALV subgroups A/B and J were concurrently detected in the sera from five flocks (31.3%). Of the 16 layer flocks screened, 10 of the flocks (62.5%) had antibodies to ALV subgroup A/B only, while one of the flocks (6.3%) had antibodies to ALV subgroup J only (Table 2).

A total of 125 sera from the 16 layer flocks were screened for antibodies to ALV subgroups A/B and J, out of which 88 (70.4%) were positive for antibodies to ALV subgroup A/B, while 10 (8%) were positive for antibodies to ALV subgroup J. Seven (5.6%) sera tested positive to both antibodies (Table 2).

Farm	No. of samples tested	ALV subgroup A/B positive (%)	ALV subgroup J positive (%)	ALV subgroup A/B and J positive (%)
J1	15	9 (60%)	0 (0%)	0 (0%)
J2	10	4 (40%)	0 (0%)	0 (0%)
J3	10	7 (70%)	0 (0%)	0 (0%)
J4	10	5 (50%)	0 (0%)	0 (0%)
J5	6	2 (33.3%)	0 (0%)	0 (0%)
J6	10	9 (90%)	1 (10%)	1 (10%)
J7	7	5 (71.4%)	0 (0%)	0 (0%)
J8	10	10 (100%)	1 (10%)	1 (10%)
J9	6	4 (66.7%)	0 (0%)	0 (0%)
J10	4	4 (100%)	0 (0%)	0 (0%)
J11	4	0 (0%)	2 (50%)	0 (0%)
J12	2	2 (100%)	0 (0%)	0 (0%)
J13	5	3 (60%)	4 (80%)	3 (60%)
J14	7	5 (71.4%)	0 (0%)	0 (0%)
J15	10	10 (100%)	1 (10%)	1 (10%)
J16	9	9 (100%)	1 (11.1%)	1 (11.1%)
Total	125	88 (70.4%)	10 (8%)	7 (5.6%)

Table 2. ALV subgroups A/B and J antibodies in layers suspected to have Marek's disease in Plateau State, Nigeria

DISCUSSION

The antibody profiles of ALV subgroups A/B and J in layer flocks suspected to have MD in Kaduna and Plateau States were established. From our search, the only report on ALV subgroup A antibody in chicken in Nigeria was by Adene and Howes [32], who reported a 10.53% (4/38) antibody detection rate in layers in Ibadan. The higher detection rates of ALV subgroup A/B in layers in this study might be attributed to the lack of control measures for AL over the years leading to an increase in prevalence. Also, Adene and Howes [32] used the neutralizing antibody test as the diagnostic tool, while in this study ELISA technique was used, which may differ in sensitivity and specificity. In a study by Zhang *et al.* [33], in which the research was carried out in free range chickens in some cities in China, only 2.73% (20/732) of the sera screened for ALV subgroup A were positive.

Infection with ALV subgroup A/B in layer flocks seem to be widespread in the two States as 85.7% (6/7) and 93.8% (15/16) of the layer flocks tested in Kaduna and Plateau States respectively, had antibodies to ALV subgroup A/B. However, this pattern of widespread infection of ALV subgroup A/B in layer flocks was not the case with ALV subgroup J, as only 14.3% (1/7) and 37.5% (6/16) of the flocks screened

had antibodies to ALV subgroup J in Kaduna State and Plateau State, respectively. Previous reports on the serological status of ALV in commercial layer flocks in Nigeria indicated widespread infection with ALV [25,26]. However, the ELISA method used by the authors detected the group specific *p*27 antigen, which is shared by all ALVs including the non-oncogenic ALV subgroup E, and therefore could not be used to differentiate between infections by one ALV subgroup from another or multiple infections by ALV subgroups. Such widespread infection of layers with ALV has also been reported in indigenous chickens in Nigeria [23,24,26]. The widespread detection of antibodies to ALV subgroup A/B in the two States may result from vertical transmission of ALVs, which maintains the infection from one generation of chickens to another [9,20]. Also, reports suggest that ALVs could be transmitted through vaccination, as routinely used vaccines in poultry in Nigeria were found to be contaminated by avian neoplastic disease viruses [34].

In Kaduna State, 14.3% (1/7) of the layer flocks screened had antibodies to both ALV subgroups A/B and J, whereas in Plateau State, 31.3% (5/16) of the flocks screened had antibodies to ALV subgroups A/B and J, suggesting dual infections within flocks. While none of the serum samples of layers from Kaduna State had antibodies to both ALV subgroups A/B and J, 5.6% (7/125) of the serum samples screened from Plateau State had antibodies to both ALV subgroups A/B and J, 5.6% (7/125) of the serum samples screened from Plateau State had antibodies to both ALV subgroups A/B and J. Similar findings of mixed infection with ALV subgroups A/B and J in a flock have been reported [17,35]. Co-infection of avian neoplastic disease viruses may lead to increase severity of tumor development and mortality, or may provide an avenue for recombination between subgroups of ALV [6,36].

CONCLUSIONS AND RECOMMENDATION

To the best of our knowledge, this is the first report of the detection of ALV subgroups A/B and J antibodies in layers in Kaduna and Plateau States, Nigeria. We therefore recommend that an eradication program for AL through culling of ALV positive breeder flocks needs to be designed so as to reduce losses incurred by poultry owners due to virus-induced avian neoplastic disease associated mortalities and production problems. A detailed study to determine the involvement of avian neoplastic disease viruses in field cases of avian neoplastic diseases in Nigeria is also recommended.

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Authors' contributions

NAS, SBO, PAA and CN conceptualized the research. NAS, ICU, AS, SEA, MSM and ISI were involved in sample collection and processing, ELISA, and data analysis and presentation. All the authors made substantial inputs in drafting and editing the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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PROFILI ANTITELA SPECIFIČNIH ZA SUBGRUPE A/B I J VIRUSA AVIJARNE LEUKOZE U JATIMA NOSILJA SUMNJIVIH NA MAREKOVO BOLEST U NIGERIJI

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Prethodni izveštaji ukazuju na visoku prevalenciju prisustva p72 antigena virusa avijarne leukoze (ALV) u jatima nosilja za koje se sumnja da su obolela od Marekovu bolest (MD) u oblastima Kaduna i Plateau. Međutim, još uvek se ne poznaju subgrupe koje su odgovorne za ALV infekciju nosilja, što je i razlog realizacije ove studije. Otuda je cilj studije da se odrede profili antitela u odnosu na A/B i J subgrupe virusa avijarne influence, kod nosilja u jatima za koja se sumnja da su obolela od Marekove bolesti i u oblastima Kaduna i Plateau. Uzorci seruma iz jata nosilja za koja se sumnja da su obolela od Marekove bolesti, sakupljeni su iz sedam jata iz Kaduna kao i 16 jata iz Plateau. Svi su uzorci seruma ispitani na prisustvo antitela specifičnih za ALV subgrupe A/B i J i to upotrebom komercijalnog IDEXX imunoenzimskog test kit-a (ELISA). Od ukupno sedam jata iz Kaduna, u šest jata (85,7%) je dokazano prisustvo ALV subgrupe A/B, a samo kod jednog jata (14,3%) je dokazano prisustvo antitela na J subgrupu ALV. Antitela specifična za obe ALV subgrupe A/B i J, dokazana su kod jednog jata (14,3%), što ukazuje na mešanu infekciju sa obe subgrupe virusa. Od ukupno 16 jata koja su ispitana u Plateu, antitela specifična za A/B subgrupu ALV su ustanovljena kod 15 jata (93.8%) dok su antitela na J subgrupu ALV, dokazana u šest jata (37.5%). Antitela na obe subgrupe ALV, A/B i J, dokazana su u pet jata (31.3%). Visok stepen detekcije antitela A/B subgrupe ALV, ukazuje da je infekcija virusom avijarne leukoze kod nosilja većim delom posledica infekcije sa A ili B subgrupama ALV, u regionima u kojima su ispitivanja obavljena.