OUTBREAK OF MYELOCYTOMATOSIS IN LAYER CHICKENS INVOLVING SOME COMMERCIAL FARMS IN NIGERIA: MORPHOHISTOLOGICAL LESIONS AND THE DETECTION OF ITS ANTIGEN USING ANTIGEN CAPTURE ELISA

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ABSTRACT

Aim: Avian leukosis is a worldwide viral disease complex of chickens with varying tumorous manifestations. Its outbreak in some commercial farms in Nigeria was studied.

Methods: Clinical signs of the leukotic diseases are mostly nonspecific. Investigations carried out include post mortem examination of carcasses, histopathology of tissues, and antigen detection using capture ELISA. 211 samples were collected and analysed. They include 18 cloacal swabs and 192 of the samples were sera and tissues collected from different flocks across the country with history of persistent mortality and in some cases evidence of neoplasm.

Results: 14 (77.7%) cloacal and 179 (92.7%) sera were positive. Overall 91% positivity was observed for p27 antigen that is common to all subgroups of ALV including endogenous and exogenous viruses.

Conclusion: Outbreak of myelocytomatosis was confirmed in some in layer chickens

Key words: Myelocytomatosis, Chicken, Antigen capture ELISA

INTRODUCTION

Avian leukosis is a worldwide poultry disease which may produce tumors or affect blood forming cells, lymphoid tissues, bone, kidneys and connective tissues caused by viruses belonging to the family Retroviridae (Chauhan and Sushovan, 2010 and Fadly 2003). They are further sub divided into Exogenous Avian leukosis virus (ALV) (A, B, C, D, E, and J) on the basis of differences in their viral envelope glycoproteins (Payne and Fadly, 1997 and Fadly, 2003) and endogenous ALVs (F, G, H and I) occurring in pheasants, partridges, and quails (Fadly, 2003) . Avian leukosis virus subgroup J (ALV-J) as reported by Shuhong and Zhizhong 2007, was first isolated in 1988 from white meat-type chickens in Great Britain and it could be differentiated from classical ALV subgroups A, B, C, D and E by neutralization and interference assays, as well as by envelope gene sequences. ALV-J induces mainly myelocytomatosis and nephromas in white meat-type chickens. So far, ALV-J has been recognized and reported in most parts of the world. The incubation period of about 14 weeks makes it more of a problem in breeders and commercial layers (Fadly, 2010).The avian leukosis complex (ALC) viruses are excreted through the secretions and excretions of the body such as feaces, saliva and eggs. Exogenous ALVs are transmitted through vertical and horizontal routes, a process that requires a fully infectious virus. In contrast, endogenous viral (ev) genes are inherited as host genes and may or may not be expressed (Fadly, 2000). Obvious clinical signs of the leukotic diseases are mostly nonspecific. However they may include inappetence, weakness, diarrhea, dehydration, and emaciation. In lymphoid leukemia (LL) especially, there may be abdominal enlargement. The comb may be pale, shriveled, or occasionally cyanotic. The burden of Avian...
Leukosis in Nigeria is under appreciated and currently there are no vaccination schedule or control policies to eradicate the virus. Deficiencies in prompt and rapid definitive laboratory diagnosis also contribute to ALV dissemination through hatcheries and breeder stock in Nigeria. In this study, we report the diagnostic investigation of farms that had recorded persistent mortalities following careful observations of gross lesions at post mortem examinations, histopathological examination of tissue sections and serology using antigen capture ELISA.

**MATERIAL AND METHODS**

The investigation was carried out on farms that reported outbreaks across Nigeria but more especially in Nasarawa State (Gora Village, Karu, Jikwoyi Phase IV,) and some farms in the Federal Capital Territory (FCT), Nigeria where higher and more frequent cases were found.

**Post Mortem Examination and Histopathology**

Carcasses and moribund birds were collected from the various farms listed above and examined for lesions. Tissue samples were collected and processed by the paraffin wax method and slides prepared for histopathological examination.

**Cloaca Swabs**

Intestinal content was swabbed into 1ml virus transport medium and frozen for 30 minutes. This was later brought to room temperature and the coarse materials allowed to settle. 100ul of resulting supernatant was pipetted and dispensed into cryovials for ELISA. Sera were separated from bloods and clarified by centrifugation before storing in cryovials for ELISA.

**ELISA**

Commercial ELISA reagents were allowed to attain room temperature and thoroughly mixed by inversion and shaking. Microtitre plate pre-coated with p27 antigen provided in the kit was labeled and demarcated appropriately with negative and positive controls. 100ul of sera and 100ul cloaca swab supernatant samples were dispensed onto separate wells and incubated for 60 minutes at room temperature. The samples were allowed to form a complex with homologous antisera to p27 antigen in a solid phase. Unbound materials are washed repeatedly with 350ul of distilled water, thereafter 100ul of anti-p27: horse radish peroxidase (HRPO) conjugate was added to bind to already attached p27 antigen in the well. This was thereafter incubated for 60 minutes at room temperature. Subsequently unbound conjugate was washed away with distilled water and enzyme substrate added to the well. 100ul of TMB substrate was added for color development for 15 minutes at room temperature and was stopped with 100ul of sulphuric acid. The intensity of developed color and absorbance in a spectrophotometer ELISA plate reader was measured at 650nm filter. The amount of p27 antigen present in the test sample is related to the intensity of color development.

**RESULTS**

**Clinical signs and gross lesions**

The moribund birds were humanely euthanized by cervical dislocation and examined. Lesions observed include old and fresh haemorrhages from the foot pad, shanks, skin of the breast and neck regions (Plate 1a and b). The hemorrhagic blisters measured 0.2 – 1.0mm in diameter. There was mild to moderate pallor of the carcasses of affected birds. Grayish white necrotic foci were observed in some of the livers of affected birds with slight splenic enlargement.

**Histopathological Examination**

Tissue samples of affected birds with or without obvious gross lesions were processed for histopathological examination. Microscopic examination of formalinized liver, spleen and kidney stained with Hematoxylin and Eosin (H & E) showed presence of well differentiated myelocytes with large, eccentrically placed basophilic vesicular nucleus. The cytoplasm of the cells were packed with acidophilic granules which were spherical in shape. In the liver, the myelocytes were found mostly around and within the blood vessels of the portal area and some in the hepatic parenchyma. In the spleen the myelocytes were widespread in the red pulp and were also found in the medullary regions of the kidneys.

**ELISA**

14 (77.7%) cloaca and 179 (92.7%) sera were positive. Overall 91% positivity was observed for p27 antigen that is common to all subgroups of ALV including endogenous and exogenous viruses.
**DISCUSSION**

Avian leukosis virus (ALV) infection of chickens is widespread especially in meat type chicken and known to be of significant economic importance. The occurrence of this disease in commercial egg type chickens is very rare however, the disease was reported in China in 12 different farms that sourced their chicks from the same breeder farm (Binrui et al., 2004). In this report however, we present a case of myelocytomatosis in commercial egg type chickens in Nigeria for the first time to the best of our knowledge and this is at variance with some authors Binrui et al., (2004) who reported in meat type chickens and claimed then that the condition was rare in commercial egg chickens. The affected layer breeder flocks were not reared with meat-type chickens, and the disease on the 12 farms occurred almost concurrently, suggesting vertical spread of ALV-J from the breeders was the main source of the infection. It is not known how the parent breeding stock became infected with ALV-J. The clinical signs, gross and histopathological findings in the case reported by some authors Binrui et al., (2004) are in agreement with our findings. Consequently, these similarities suggest that indeed what we are reporting in this outbreak is a myeloid leukosis (myelocytomatosis). To the best of our knowledge there is no previous report of such finding in meat type or commercial egg chickens in Nigeria. The occurrence of cutaneous capillary haemangiomas in birds in this case which occur as single/multiple nodules and several millimeters in diameter that bled easily and to an extent the birds died and very high mortalities were recorded. Those nodules were composed of thin-walled, irregularly-shaped blood vessels or less well-differentiated masses of endothelial-like cells and thin irregular clefts (Plate 1); this is in agreement with reports by another author and these tumors have also been described more commonly in chickens and budgerigars (Fadly, 2000). The occurrence of haemangiomas-like blisters in this case is also in agreement with similar (Fadly, 2000), however this is a case of natural or vertical infection which is also the first reported case in Nigeria. Out of 211 samples analysed, 18 were cloacal swabs and 192 of the samples were sera all collected from different flocks across the country with history of persistent mortality and in some cases evidence of neoplasm. Fourteen 14 (77.7%) cloacal and 179 (92.7%) sera were positive. Overall 91% positivity was observed for p27 antigen that is common to all subgroups of ALV including endogenous and exogenous viruses. This results showed a higher positive rate compared to the reports (Emikpe et al., 2007) which indicated 70.7% seroprevalence to ALV out of 128 sera collected from indigenous poultry in South West Nigeria. This present study was carried out among commercial birds, though there was no indication that they were vaccinated. Cloacal swabs analysed in this study detected p27 antigen in 77.7% of cases thus negating any possibility that the seropositivity may be due to vaccine antibody, but rather to the presence of ALV in the population as corroborated by some authors. In another study, ten years retrospective analysis of neoplastic cases diagnosed at the pathology laboratory in Kwara State of Nigeria, ALV had a higher prevalence of 58.23% over Marek disease (41.77%) (Olabode et al., 2009) further underpinning the endemicity of ALV in Nigeria.

**CONCLUSION**

Exogenous ALV can be transmitted vertically, congenital transmission through shedding in hatch able eggs is a major concern affecting the quality of chicks that are distributed around the country from major hatcheries. Hence hatchery operations in Nigeria require closer monitoring to prevent dissemination of ALV and associated losses to small holder farms. It is therefore imperative to design a comprehensive flock health management that will include vaccination
of laying birds and breeder flocks couple with ELISA test as suggested by some authors to identify and eliminate both endogenous and exogenous ALV from the poultry line that may congenitally transmit the virus to progeny chicks.

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REFERENCES


