

## Detection of Avian Leukosis Virus P 27 Antigen in Nigerian Indigenous Chicken

B.O. Emikpe O.A. Oladele D.O. Oluwayelu D.F. Adene O.G. Ohore and A.B. Bolarinwa  
 Faculty of Veterinary Medicine University of Ibadan, Ibadan

**Abstract:** Blood samples from 184 adult indigenous Nigerian chickens were collected at three locations in Ibadan, Nigeria. The serum from each sample was evaluated for Avian Leukosis Virus (ALV) P-27 antigen by the Antigen-ELISA method. An overall prevalence of 70.7% was obtained as it ranges between 63.3-90% in the three locations used. Fifty-six samples (21.9%) with ELISA units (EUs) less than 10 were regarded as negative. Out of the 128 positive sera, 24 were strongly positive, 70 were moderately positive while 34 were weakly positive. The overall mean EUs value was  $52.8 \pm 24.4$ . These chickens had no history of vaccination against any poultry virus including ALV and ALV has not been previously reported in indigenous chicken in Nigeria, making this the first evidence of the infection.

**Key words:** Avian leucosis virus, indigenous chicken, Nigeria

### INTRODUCTION

African livestock population statistics indicate poultry to be the most numerous species of farm animal and more than 80% of the population are kept in rural areas (Adene, 1997). The estimated chicken population in Nigeria is about 150 million, out of which the indigenous chickens constitute over 80% (Adene, 1997).

The Nigerian indigenous chicken is characterised by small body size, slow growth rate, late maturity and poor production ability (Ibe, 1990). The type of husbandry mostly practiced for village fowl production is free range or backyard systems (Gueye, 1998).

The movement of the birds is uncontrolled and they are rarely given any form of disease prophylaxis or health care. It is believed that these free wandering chicken acts as potential reservoirs and carriers of infection (Aden *et al.*, 1985; Javeeda *et al.*, 1990; Bounoubaa *et al.*, 1992).

Disease has been attributed as a major constraint to their production besides low genetic potential, poor housing and feeding (Saidu *et al.*, 1994; Ohore *et al.*, 2002, 2003). Of the various diseases reported on the Nigerian indigenous chicken, there had been dearth of information on neoplastic diseases Payne and Fadly (1997), especially Avian leucosis in this breed of chickens.

Avian leukosis is a neoplastic disease caused by Avian Leucosis Viruses (ALVs). The disease is characterised by transmissible benign and malignant tumours comprising of erythroid, lymphoid and myeloid leucosis (Aden, 1983). Avian leucosis have been divided into six subgroups, A,B,C,D,E, and J on the basis of their host range, viral envelope interference and cross neutralisation pattern (Aden, 1983).

Subgroups A,B,C,D are exogenous viruses capable of inducing tumours and subgroup E viruses are ubiquitous endogenous viruses of low pathogenicity. Subgroup J viruses are recombinant of exogenous and endogenous viruses (Aden, 1983).

The infection is of great economic importance as it results in poor weight gain, poor feed efficiency and it is both horizontally and vertically transmitted Aden D.F. 1983. The great concern about ALV infections in chicken flocks have encouraged the use of commercially available ELISA kits for detecting viral proteins (p 27) in serum and egg albumen as well as monitoring the disease status of different flocks (Payne *et al.*, 1991).

Despite the global spread, the ALV status of Nigerian indigenous chicken is yet to be determined. The aim of this study was therefore to investigate the presence and prevalence of ALV P 27 antigen in Nigerian indigenous chicken.

### MATERIALS AND METHODS

**Location/study area:** This study focussed on three communities-Ashi, Shasha, Molete representing 3 local government areas in Ibadan metropolis, a major urban centre in Oyo State, Southwest Nigeria. Southwest, Nigeria is the main port of entry of imported live chicken and the hub of major poultry breeders, which distribute poultry stocks throughout the country (Crittendam *et al.*, 1984).

Oyo State is located in Southwest of Nigeria, sharing borders with Ogun, Osun states of Nigeria and the Republic of Benin. It has a long wet season (March-October) and short dry season (November-February). There is no vaccination of chicken against ALV in Nigeria.

**Selection of bird and preparation of sera:** The approximate age of the indigenous chickens sampled ranged from 4-12 months. The birds were reared on free-range and they had a history of no vaccination against any poultry disease. The low sample size was due to the lack of interest and fear of mortality demonstrated by most chicken owners.

A total of 184 apparently healthy indigenous chickens from 35 flocks were sampled, one of five birds were randomly selected per household and bled via jugular venipuncture. Test sera were separated, kept in sterile plastic vials and stored at -20°C until used.

**Serology:** An ELISA commercial test kit donated by AFFINITECH, U.S.A. was used to detect ALV P-27 antigen following the instructions of the manufacturer. A serum dilution of 1:400 was used. Optical density values were read at 405nm with 630nm reference filter using Mortiscan® ELISA reader (Titertek).

**Calculation of result:** Average absorbance values of positive and negative controls were determined. Reactivity of each test sample was calculated using the following equation.

$$\text{Absorbance Test Sample} - \text{Average Absorbance (Negative)} = \text{Sample to positive ratio (SP ratio)} \times \text{Average absorbance positive} - \text{Average absorbance (Negative)}$$
$$\text{SP ratio} \times 100 = \text{ELISA Units (EU)}$$

The positive control value was set at 100 EU.

## RESULTS

**Interpretation of result:** EU values obtained were interpreted as recommended by the manufacturer, <10EUs were considered negative, 10-25 EUs were weakly positive; 25-75 EUs were moderately positive and >75Eus were strongly positive for ALV P 27 antigen.

At a titre of 10 EUs and above regarded as positive, a point prevalence of 70.7% ( $\frac{130}{184}$ ) was obtained in Nigerian indigenous chicken. In the three locations, the prevalence ranged between 63.3-90%.

The mean EUs values for positive reactors were 50.9±26.7 for Ashi, 42.1±20.4 for Shasha and 55.3±29.6 for Molete respectively while the overall mean EUs values was 52.8±24.4. The mean EUs distribution of positive samples for ALV antigen revealed 13.4% (24/184) as strongly positive.

## DISCUSSION

Serological tests for the detection of ALV have not been done in Nigeria, in spite of report of a clinical disease in commercial stock (Owoade *et al.*, 2003). This study

revealed a high prevalence (70.7%) for ALV P 27 antigen in indigenous chickens in Ibadan, Nigeria though no clinical disease has so far been reported in this breed of chicken. This report is of antigen detection unlike the antibodies in subgroups A, B reported in wild fowl and domestic chicken in Kenya and Malaysia (Aden, 1983). Despite the global spread of ALV (Aden, 1976) Tsukamoto this appears be the first report of antigen detection of ALV in Nigerian indigenous chicken.

A higher percentage (58.1%) of the chicken had more than 25 EUs, which is quite remarkable and is a strong indication of repeated exposure to the virus. The repeated exposure to the virus is possible through contact infection with congenitally infected hatch mates or contaminated formite or environment (Aden, 1983).

The viral antigen detected was due to natural infection, as the chickens were not previously vaccinated. The wide range of antigen titres suggests differences in the time of exposure and the dose of exposure to the virus. The kit used does not differentiates subgroups A, B, D, E and J, so the titres can not specifically state the subgroups that substantially contribute to the recorded EUs but all the subgroups A,B, D, E and J are capable of inducing tumours with varying pathogenicity. The reason for no reported clinical disease in Nigerian indigenous chicken is not known although reports showed that they are resistant to some poultry infection (Mdegela *et al.*, 2002). It is then pertinent to take cognisance of indigenous poultry as a possible carrier of avian leucosis viruses.

## ACKNOWLEDGEMENT

We are grateful to Affinitech Limited (USA) for donating the kit especially ms Leigh Nogy.

## REFERENCES

- Adene D.F., 1976. Studies on the type of neoplasm of poultry in Nigeria. *J. Nig Vet. Med. Ass.*, 4: 76-81.
- Adene, D.F., 1983. Serological survey of Marek's disease in exotic and local chicken in Nigeria. *Trop. Vet.* 1: 138-140.
- Adene D.F., 1997. Diseases of poultry in Nigeria: An overview of the problems and solutions. *Trop. Vet.*, 15: 103-110.
- Adene D.F., A. Oyejide and A.A. Owoade, 1985. Studies on the possible roles of naturally infected Nigerian local chickens and vaccines virus in the epidemiology of infectious bursal disease. *Rev. Elev. Med vet. Pays trop.*, 38: 122-126.
- Bouzoubaa, K., K. Lemaingner and J.G. Bel, 1992. Village chickens as a reservoir of *salmonella pullorum* and *salmonella gallinarum* in Morocco *Prev Vet. Med.*, 12: 95-100.

- Crittendam L.B., E.J. Smith and W. Okazaki, 1984. Identification of broiler breeders congenitally transmitting avian leucosis virus by enzyme immunosorbent assay. *Poult. Sci.*, 63: 492-496.
- Gueye E.F., 1998. Village egg and fowl meat production in Africa. *World Poult. Sci. J.*, 54: 73-87.
- Ibe, S.N., 1990. Increasing rural poultry production by improving the genetic endowment of rural population. Proceeding of an internetal workshop on rural poultry in Africa. Obafemi Awolowo University Ile-Ife, Nigeria.
- Javeed, T., A. Hameed and M. Siddique, 1990. Status of salmonella in indigenous chicken in Pakistan. *Veterinarski Arhiv*, 251-255.
- Mdegela, R.H., P.L.M. Msoffe, R.W., Waihenya, J.C. Kasanya, M.M.A. Mkanbo, U.M. Minga and J.E. Olsen 2002. Comparative Pathogenesis of Experimental Infection with Salmonella Gallinarum in Local and Commercial Chicken Trop. *Anim. Health Produc.*, 34: 195-204.
- Ohore, O.G., P.C. Ozegbe, B.O. Emikpe and D.O. Oluwayelu, 2002. The prevalence of antibodies to fowl typhoid in indigenous Nigerian chicken (*Gallus gallus domesticus*). *Bull. Anim. Hlth. Prod. Afri.*, 50: 63-65.
- Ohore, O.G., P.C. Ozegbe, B.O. Emikpe and V.E. Okojie, 2003. The survey of antibodies to Newcastle disease virus in apparently healthy adult Nigerian indigenous Nigerian chicken (*Gallus gallus domesticus*) in Ibadan using ELISA. *African J. Clin. Exp. Microbio.*, 3: 38-40
- Owoade, A.A., D.O. Oluwayelu, O.A. Fagbohun, N. Ammerlaan, M.N. Milders and C.P. Muller, 2003. Serologic evidence of chickens infectious anemia in commercial chicken flocks in Southwest Nigeria. *Avian Diseases*, 481: 202-205.
- Payne, L.N., S.R. Brown, N. Bumjstead, K. Howes, J.A. Frazier and M.E. Thouless, 1991. A novel subgroup of exogenous avian leucosis virus in chickens *J. Gen. Virol.*, 72: 801-807.
- Payne, L.N. and A.M. Fadly, 1997: *Disease of Poultry*. (10th Edn.), Iowa State University Press, Iowa.
- Saidu, L., P.A. Abdu J.U. Umoh and U.S. Abdullahi, 1994. Diseases of Nigerian indigenous chickens. *Bull. Anim. Hlth. Prod. Afr.*, 42: 19-23.
- Tsukamoto K.M., S. Hasebe H. Kakita, and Y. Hihara, Kono. Identification and characterisation of hens' transmission of avian leucosis virus ALV, by ELISA for detection infections ALV, ALV antigens and antibodies to ALV. *J. Vet. Med. Sc.*, 543: 859-864.