

## Serological evidence of mixed infections with avian influenza and Newcastle disease in village chickens in Jigawa State, Nigeria

Aliyu Mohammed Wakawa<sup>1\*</sup>, Paul Ayuba Abdu<sup>2</sup>, Jarlath Uduado Umoh<sup>3</sup>,  
Sa'idu Lawal<sup>2</sup>, and Rabi'u Babura Miko<sup>4</sup>

<sup>1</sup>Department of Veterinary Surgery and Medicine, Ahmadu Bello University, Zaria, Nigeria

<sup>2</sup>Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Nigeria

<sup>3</sup>Department of Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria

<sup>4</sup>Veterinary Division, Ministry of Agriculture, Jigawa State, Nigeria

---

**WAKAWA, A. M., P. A. ABDU, J. U. UMOH, S. LAWAL, R. B. MIKO: Serological evidence of mixed infections with avian influenza and Newcastle disease in village chickens in Jigawa State, Nigeria. Vet. arhiv 79, 151-155, 2009.**

### ABSTRACT

A serological survey for antibodies against avian influenza (AI) and Newcastle disease (ND) viruses in 250 village chickens confirmed to be suffering from highly pathogenic avian influenza (HPAI) was carried out in Jigawa State, Nigeria. The chickens had no history of vaccination against the two diseases. Sera were tested by the haemagglutination inhibition (HI) test for the presence of antibodies against the two virus antigens. Antibody prevalence against the viruses was noted as follows: AI (31.6%) and ND (38.8%), with mean antibody titres of  $0.32 \pm 0.1 \text{ Log}_2$  and  $0.39 \pm 0.2 \text{ Log}_2$ , respectively. The finding that 70.4% of the sera examined contained HI antibodies to both virus antigens indicates considerable combined activities of the two viruses among the village chickens during the HPAI outbreaks. It is suggested that this group of scavenging birds may play significant roles in the transmission of these two viruses to commercial poultry farms. The introduction and sustenance of routine vaccination of scavenging village poultry against ND is highly recommended. Nation-wide active surveillance of AI should also be conducted to define the true status of the disease in poultry in Nigeria.

**Key words:** antibodies, avian influenza, Newcastle disease, village chickens

### Introduction

Nigeria is the most populous country in Africa and is home to about 175 million domestic poultry. Poultry production in Nigeria can be classified into extensive and intensive systems. The extensive system accounts for the vast majority of poultry, more than 143 million, which are free ranging birds in the rural areas (SALMAN et al., 2007).

---

\*Corresponding author:

Dr. Aliyu Mohammed Wakawa, DVM, MSc., Department of Veterinary Surgery and Medicine, Ahmadu Bello University, Zaria, Nigeria, Phone: +234 803 704 4713; E-mail: drsaniiwakawa@yahoo.com

The estimated population of free range poultry in Jigawa State is about 4.2 million (ANONYMOUS, 2000). The village poultry provide an important source of income and high quality animal protein in the rural socioeconomy with little or no capital investment (BABA et al., 1998). Although, the first confirmed outbreak of H5N1 HPAI on the African continent occurred in January, 2006, at a commercial farm in Kaduna State, Nigeria (ADENE et al., 2006; WAKAWA et al., 2008), the source of the outbreak was unknown and inconclusive (SALMAN et al., 2007). As of May, 2007, 24 of 36 States in Nigeria had documented H5N1 HPAI outbreaks in poultry (MONNE et al., 2008). An assessment conducted by the United Nations Development Programme (UNDP) showed that the greatest adverse effect of HPAI was in the impoverished areas such as rural and semi-urban Nigeria. Eggs and chicken sales declined by more than 80% percent and more than 80% of workers on affected farms lost their jobs within 4 months of the announcement of the first outbreak. As of February, 2008, a total of 228 persons were reported to have died out of 362 persons that were infected with confirmed H5N1 HPAI worldwide (WAKAWA et al., 2008). One human death due to H5N1 HPAI was reported in a woman from the southern state of Lagos, Nigeria (MONNE et al., 2008).

Despite control measures that included quarantine, disinfection of infected premises, and movement restriction within the country, AI continued to spread within the country (ANONYMOUS, 2006). ND is endemic and was found to be the most important and destructive disease of village poultry in Nigeria, as it causes the death of millions of birds and economic losses to rural dwellers (ABDU et al., 2002; SA'IDU et al., 2004).

This study was designed to find serological evidence of concurrent AI and ND virus infections in village chickens with a view to determining their roles in the epidemiology of both diseases in both village and commercial poultry.

### **Materials and methods**

*Serum samples.* Blood samples were collected by simple random sampling from 250 village chickens confirmed to be suffering from H5N1 HPAI in different flocks, in the villages of Gumel, Maigatari, Auyo and Gagarawa of Jigawa State, Nigeria. The chickens bled had no history of vaccination against AI and ND. Sera were separated immediately by centrifugation at 447.2 g for 5 minutes (COLLEE et al., 1982) and stored at -4 °C until used. Confirmatory diagnosis of the outbreak was made by viral isolation from the livers, spleens, kidneys and intestines of clinically sick chickens submitted to the Virology Laboratory of the National Veterinary Research Institute (NVRI), Vom, Nigeria.

*Antigens.* AI type A H5 antigen, AI type A H5 positive serum, and ND "La Sota" vaccine, batch N° 85 (made by the NVRI, Vom, Nigeria) were used for detection of antibodies. The two antigens were obtained from the NVRI, Vom, Nigeria.

*Laboratory analyses.* A 1% suspension of chicken red blood cells (RBC) was prepared to be used as indicator in the haemagglutination (HA) and haemagglutination

inhibition (HI) tests (ANONYMOUS, 2004). The HA titres of the AI type A H5 and ND La Sota antigens, were determined as described by ANONYMOUS (2004) and diluted to contain 4 HA units. This concentration was used for the HI test. The HI antibody for each serum sample was determined and expressed in  $\log_2$ , and the mean antibody titre was calculated.

### Results

A total of 250 sera from the village chickens were tested for the presence of antibodies to AI and ND viruses; 176 (70.4%) were found to be positive for the presence of both of the virus antibodies (Table 1). Antibody prevalence against the individual virus antigen was noted as follows: AI (31.6%) and ND (38.8%). The mean antibody titres of the samples that tested positive for AI and ND antibodies were  $0.32 \pm 0.1 \log_2$  and  $0.39 \pm 0.2 \log_2$ , respectively. The minimum antibody titre for AI and ND were  $3 \log_2$  and  $2 \log_2$ , respectively. While the maximum antibody titres were  $8 \log_2$  and  $6 \log_2$ , respectively (Table 2).

Table 1. Prevalence and mean HI antibody titres for AI and ND in village chickens

N° tested	Total N° positive for both antibodies	N° positive for AI antibodies	N° positive for ND antibodies	Mean AI antibody titre ( $\log_2$ )	Mean ND antibody titre ( $\log_2$ )
250	176 (70.4%)	79 (31.6%)	97 (38.8%)	$0.32 \pm 0.1$	$0.39 \pm 0.2$

Table 2. Distribution of different HI antibody titres for AI and ND in village chickens.

Antibody titre ( $\log_2$ )	1	2	3	4	5	6	7	8	9	10	11	12	Mean $\pm$ SE
N° of reactions for AI	0	0	9	19	9	19	9	14	0	0	0	0	$0.32 \pm 0.1$
N° of reactions for ND	0	30	35	26	3	3	0	0	0	0	0	0	$0.39 \pm 0.2$

### Discussion

PAUL and SCHRIER (2001) reported that the apathogenic avian influenza virus could cause more severe disease in ND infected chickens. It is important to note that the results of this study indicate considerable activity of the AI and ND viruses in village chickens during the confirmed HPAI outbreaks. The chickens tested had no previous history of vaccination against AI and ND since this practice is not routine among this group of poultry in Nigeria (BABA et al., 1998). It is therefore inferred that antibodies detected in the village chickens investigated were as a result of natural infections of the birds by the

viruses. This group of scavenging birds may also play significant roles in the maintenance and spread of AI and ND viruses to the more susceptible commercial poultry (ABDU et al., 2002). It is also important to note that despite the high percentage of village chickens that were positive for the presence of both AI and ND antibodies, the mean HI titres of  $0.32 \pm 0.1 \log_2$  and  $0.39 \pm 0.2 \log_2$ , respectively were far too low to protect the birds against AI and ND when compared with the recommended minimum protective antibody titre against both AI and ND (i.e.  $4.0 \log_2$  (ANONYMOUS, 2004). The extremely low AI and ND antibody titres observed in the village chickens might be attributed to stress and the poor nutritional status of these birds. This is in view of the fact that this category of birds are predominantly on free range in Nigeria, and are not usually supplemented with feed high in proteins that could enhance their antibody responses (ABEGUNDE and MBIBU, 1987).

From the findings of this study, it can be concluded that concurrent infections of AI and ND may occur in free-ranging village chickens. Therefore, there is a need to introduce and sustain routine vaccination of village chickens against ND in Nigeria. It is also important that nationwide active surveillance of AI be conducted to define the true status of the disease in Nigeria.

---

#### Acknowledgements

The Authors sincerely acknowledge the contribution of Dr. T.M. Joannis of the NVRI, Vom, Nigeria, for the confirmatory diagnosis, and also for providing the antigens that were used for this research.

#### References

- ABDU, P. A., L. SA'IDU, B. D. J. GEORGE (2002): Diseases of local poultry in Nigeria. *Discov. Innov.* 14, 1-2.
- ABEGUNDE, A. A., H. N. MBIBU (1987): A survey for antibodies to avian influenza virus in Nigerian chickens. *Zariy. Vet.* 2 (1), 56-57.
- ADENE, D. F., A. M. WAKAWA, P. A. ABDU, L. H. LOMBIN, H. M. KAZEEM, L. SA'IDU, M. Y. FATIHU, T. M. JOANNIS, C. A. O. ADEYEFA, T. U. OBI (2006): Clinico-pathological and husbandry features associated with the maiden diagnosis of avian influenza in Nigeria. *Nig. Vet. J.* 1, 32-38.
- ANONYMOUS (2000): Salient agricultural information and investment opportunity in Jigawa State, Nigeria. Jigawa State Agricultural and Rural Development Authority (JARDA), November, 2000.
- ANONYMOUS (2004): Avian influenza. In: Office International des Epizootics (OIE) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. pp. 1-27.
- ANONYMOUS (2006): Special avian flu edition. National Animal Disease Information and Surveillance (NADIS INFO) Bulletin. Federal Department of Livestock and Pest Control Services, Abuja, Nigeria. 9, 1-3.

A. M. Wakawa et al.: Serological evidence of mixed infections with highly pathogenic avian influenza and Newcastle disease

- BABA, S. S., A. D. EL-YUGUDA, M. M. BABA (1998): Serological evidence of mixed infections with Newcastle disease and Egg drop syndrome 1976 viruses in village chickens in Borno State, Nigeria. *Trop. Vet.* 16, 137-141.
- COLLEE, J. G., J. P. DUGUID, A. G. FRASER, B. P. MARMION (1982): *Practical Medical Microbiology*, 13<sup>th</sup> ed., Mackie and McCartney. Churchill Livingstone. pp. 37-39.
- MONNE, I., T. M. JOANNIS, A. FUSARO, P. DE BENEDICTIS, L. H. LOMBIN, H. ULARAMU, A. EGBUJI, P. SOLOMON, T. U. OBI, G. CATTOLI, I. CAPUA (2008): Reassortant avian influenza virus (H5N1) in poultry, Nigeria, 2007. *Emerg. Infect. Dis.* 14, 637-640.
- PAUL, G., C. SCHRIER (2001): Apathogenic avian influenza viruses (subtype H9) are able to cause severe disease in NDV infected chickens. *Proceedings of the 7<sup>th</sup> International Congress of the World Veterinary Poultry Association*, 17-21 September. Cairo, Egypt. pp. 47-50.
- SA'IDU, L., L. B. TEKDEK, P. A. ABDU (2004): Prevalence of Newcastle disease antibodies in domestic and semi-domestic birds in Zaria, Nigeria. *Vet. Arhiv* 74, 309-317.
- SALMAN, M. D., S. GILLETTE, C. RIDKY (2007): Avian influenza in Nigeria. *Avian Influenza Epidemiology: A Training Workshop in Nigeria organized by United State Department of Agriculture (USDA), Colorado State University and Association for Veterinary Epidemiology and Preventive Medicine*. 10<sup>th</sup>-18<sup>th</sup> December, 2007, Jos, Nigeria. pp. 1-3.
- WAKAWA, A. M., L. SA'IDU, P. A. ABDU, T. M. JOANNIS (2008): Review of highly pathogenic avian influenza outbreaks in poultry in Zaria, Nigeria. *Sok. J. Vet. Sci.* 7, 1-4.

Received: 11 January 2008

Accepted: 21 December 2008

---

**WAKAWA, A. M., P. A. ABDU, J. U. UMOH, S. LAWAL, R. B. MIKO: Serološki dokaz mješovite infekcije virusom influence i virusom newcastleske bolesti u pilića seoskih gospodarstava u Jigawi, Nigerija. *Vet. arhiv* 79, 151-155, 2009.**

**SAŽETAK**

Istražena je prisutnost protutijela za virus influence ptica i virus newcastleske bolesti u 250 seoskih pilića u Jigawi u Nigeriji za koje je bilo potvrđeno da su bili zaraženi visokopatogenim ptičjim virusom influence. Pilići nisu bili cijepljeni protiv navedenih bolesti. Uzorci seruma bili su pretraženi testom inhibicije hemaglutinacije. Protutijela za virus influence ustanovljena su u 31,6%, a za virus newcastleske bolesti u 38,8% pilića. Srednja vrijednost titra protutijela za virus influence iznosila je  $0,32 \pm 0,1 \log_2$ , a za virus newcastleske bolesti  $0,39 \pm 0,2 \log_2$ . Dokaz protutijela inhibicije hemaglutinacije za oba virusa u 70,4% pretraženih uzoraka seruma upućuje na znatnu istodobnu aktivnost dvaju virusa u seoskih pilića za vrijeme pojave visokopatogenih ptičjih virusa influence. Smatra se da pretraživana skupina ptica ima značajnu ulogu u prijenosu spomenutih virusa na peradarske farme. Preporučuje se redovito cijepljenje seoske peradi protiv newcastleske bolesti. Radi određivanja stvarnog stanja influence u peradi potrebno je na nacionalnoj razini u Nigeriji donijeti upute za njezin nadzor.

**Cljučne riječi:** protutijela, ptičja influenza, newcastleska bolest, seoski pilići

---

