FACULTY OF VETERINARY MEDICINE
USMANU DANFODIYO UNIVERSITY
P.M.B. 2346, SOKOTO. NIGERIA

Sokoto Journal of Veterinary Sciences

ISSN 1595-093X

Dashe *et al.* /*Sokoto Journal of Veterinary Sciences* (2008). **7**(2): 55-57.

Klebsiella pneumoniae isolated from birds affected by natural outbreaks of highly pathogenic avian influenza (H5N1) in Nigeria

YG Dashe[1]*, HM Kazeem[2], PA Abdu[3], M Bello[4] and M Odugbo[5]

[1]*Central Diagnostic Department, National Veterinary Research Institute, Vom. Nigeria*
[2]*Department of Veterinary Pathology and Microbiology, A.B.U, Zaria, Kaduna State, Nigeria*
[3]*Department of Veterinary Surgery and Medicine, A.B.U, Zaria, Nigeria*
[4]*Dept. of Veterinary Public Health and Preventive Medicine, A.B.U, Zaria Nigeria*
[5]*Bacterial Research Department, National Veterinary Research Institute, Vom. Nigeria*

*Correspondence Author: Tel.: +234 8035778490; e-mail: yakubudashe@yahoo.co.uk

Abstract

A study was undertaken to examine the isolation rate of *Klebsiella pneumoniae* from birds affected by natural outbreaks of highly pathogenic avian influenza (H5N1) that occurred in Nigeria between December, 2006 and July, 2007. A total of 100 birds from 114 commercial, backyard and free range flocks infected with H5N1 virus within the study period were sampled. A total of 600 tissues (heart, lung, spleen, liver, trachea and intestine), 100 each from the 100 birds were collected for bacteriology. Data generated was entered into Microsoft excel, while descriptive statistical analysis was conducted using SPSS (Version 12.01). *Klebsiella pneumoniae* was isolated from 9 (1.5%) samples. The organism was isolated from the liver, lungs and trachea of commercial layers and turkeys. During the HPAI outbreaks, *Klebsiella pneumoniae* was isolated from 9 different flocks with a total of 21,805 birds, mortality rate of (7.3%) and proportionate mortality rate of (2.5%). The bacterium was not isolated from H5N1 free flocks which served as control. The result of this study indicated

that *Klebsiella pneumoniae* may have acted as a secondary pathogen to aggravate the clinical signs during H5N1 outbreaks that occurred in Nigeria.

Key words: Highly pathogenic, Avian influenza, H5N1, Klebsiella pneumonia.

Introduction

Avian influenza also called highly pathogenic avian influenza (H5N1) is a viral disease affecting almost all domestic and wild birds (Easterday *et al.*, 1997; Alexander, 1999). The species of animals affected by HPAI include: the birds, seal, whales, humans, horses and swine (Websters *et al.*, 1992). Avian influenza virus belongs to the Family Orthomyxoviridae which include the Genera influenza A, B and C. The 8 RNA segments of avian influenza A virus encodes for 11 proteins: haemagglutinin (HA), neuraminidase (NA), protein matrix (M1 and M2), non structural protein (NS1 and NS2), RNP, viral polymerase proteins (PB1, PB2, PA, PB1-F2) making the virus antigenic type specific (Swayne, 2003; Yuen, *et al.*, 2006). Presently there are 16 HA and 9 NA subtypes (Fouchier *et al.*, 2005). Avian influenza depresses the host immune system thereby paving way for opportunistic microbes to invade and exert an exacerbative effect resulting in high mortality in affected flocks (Alexander, 2000). About half of the death from avian influenza is believed not to be caused by the avian influenza virus alone, rather from secondary bacterial infections (Armin *et al.*, 2004; Anonymous, 2006). *Klebsiella pneumoniae* is a gram negative bacillus and a late lactose fermenting organism of the Family Enterobacteriacae. This bacterium is a common saprophyte in many parts of the environment and occasionally causes embryonic mortality and excess losses in young chickens and turkeys (Orajaka and Mohan, 1985). *Klebsiella pneumoniae* has been frequently recovered from birds in which it functioned as a primary pathogen and was associated with respiratory tract disease (Sandra and Duarte, 1998). The organism expresses both smooth lipopolysaccharide with O-antigen and capsular polysaccharide with K-antigen on its surface and both antigens contribute to the pathogenesis of this species. This study was aimed at isolating *Klebsiella pneumoniae* as well as highlighting the possible complicating role of the organism in natural outbreaks of HPAI (H5N1) that occurred in Nigeria.

Materials and Methods

One hundred (100) birds were collected using simple random sampling from 114 commercial, backyard and free range flocks affected by HPAI in the 6 geopolitical zones of Nigeria. A total of 244,992 poultry were sampled.

Six (6) samples consisting of heart, intestine, liver, lung, spleen and trachea were collected from each of 100 HPAI affected birds, giving a total of 600 specimens. Samples were collected over a period of eight months between December, 2006 and July, 2007. The presence of H5N1 subtype virus was confirmed by the Viral Research Department of the National Veterinary Research Institute, Vom, Nigeria, using agar gel immuno-diffusion test, viral isolation, haemagglutination inhibition and reverse transcriptase polymerase chain reaction. Similarly, 60 samples consisting heart, intestine, liver, lungs, spleen and trachea were collected from 10 HPAI virus free birds as control. All samples were kept in double transparent polythene bags, labeled and preserved at -700C at the Central Diagnostic Department, NVRI, and Vom. The samples were later transported in a leak proof insulated box packed with ice to the Department of Veterinary Pathology and Microbiology, A B U, Zaria for bacterial isolation and identification.

Bacterial Isolation

Swabs aseptically collected from the heart, lung, liver, trachea and spleen were cultured directly on 7% defibrinated sheep blood agar (BA) and MacConkey agar (MCA). All cultures were incubated aerobically at 370C for 24 h.

Identification of Organisms

Klebsiella pneumoniae isolate on BA and MCA were subjected to various techniques for identification according to the methods of Barrow and Felthan, (2004). Biochemical characterization was done according to standard method described Edwards and Ewings (1986).The biochemical reagents and tests used included: Triple sugar iron agar, urease, Simmons citrate, indole, motility, and Voges Proskauer..

Statistical Analysis

Data generated was entered into Microsoft excel, while descriptive statistical analysis was conducted using statistical package for social sciences SPSS (version 12.01).

Results

From the 600 samples, 9 (1.5%) tissues (obtained from 9 different birds) yielded *Klebsiella pneumoniae* (Table 1). *Klebsiella pneumoniae* was isolated from the liver, lung, and trachea as pure cultures. A total of 224,992 birds were affected by HPAI virus (H5N1) within the period of this study. Of all the bird types and species sampled, the organism was only isolated from 9 different flocks consisting (8 flocks of commercial layers and 1flock of turkeys) with a total flocks size 21,805 birds, mortality rate of (7.3%) and proportionate mortality rate (Table 2).

Klebsiella pneumoniae was not isolated from any sample of the HPAI free birds that served as control.

Table 1: Isolation and distribution of *Klebsiella pneumoniae* in tissues of birds affected by HPAI (H5N1)

Klebsiella pneumoniae*	**Tissue Samples**							
	Heart	**Intestine**	**Liver**	**Lungs**	**Spleen**	**Trachea**	**Total**	**(%)**
Isolated	-	-	1	6	-	2	9	1.5
Not Isolated	100	100	99	94	100	98	581	98.5
Total	100	100	100	100	100	100	600	100

**Klebsiella pneumoniae* was isolated from 9 different birds affected by HPAI (One organ per bird)

Table 2: Mortality and proportionate mortality rate associated with *Klebsiella* pneumonia isolated from flocks affected by HPAI (H5N1)

Klebsiella pneumoniae	Affected		Mortality		
	Flocks	Birds	Figures	Rate (%)	Proportional (%)
Isolated	9	21805	1582	7.3	2.5
Not Isolated	105	223187	61499	18.4	97.5
Total	114	244992	63081	25.7	100

Discussion

The role of infectious agents such as bacteria, fungi, mycoplasma and parasites in the complication of viral infections resulting in exacerbative condition and high mortality rate has been reported by Alexander, (2000). In the present study, the isolation of *Klebsiella pneumoniae* from 9 (1.5%) samples of birds affected by HPAI (H5N1) and none at all from the control birds' calls for a reassessment of the role of this organism in HPAI outbreaks in Nigeria. The findings in this study as well as that of Kumbish et al 2006, who reported 18% isolation rate of *Klebsiella pneumoniae* in a similar study conducted in Nigeria underscores the importance of this bacterium during HPAI outbreaks. However, the report of our finding is at variance with that of Lewis (1997), who isolated Escherichia coli in a study conducted on 8,000 nine-week-old Frazer Valley turkeys affected by H5N1 virus, as the only bacterium that complicates avian influenza (H5N1) during the outbreaks. It is most likely that the suppression of immune system in birds affected by AI (H5N1) could have favored the extra-intestinal infections of *Klebsiella pneumoniae* which accounted for the isolation of the bacterium from the liver, lungs and trachea of birds affected by HPAI virus (Yuen and Wong, 2005; Anonymous, 2006). *Klebsiella pneumoniae* has a capsule that may prevent the binding of antibodies or complement factors, thus enabling the organism to avoid detection by Neutrophils even in immunocompetent host (Timoney *et al.*, 1988). The capsule hinders phagocytosis, allowing the bacterium to multiply and spread. The high isolation rate of *Klebsiella pneumoniae* encountered in adult commercial layers and less from turkeys could be attributed to the large sample size obtained from this flock type, since commercial layers were mostly affected during the outbreaks in Nigeria (NADIS/PACE, 2006). The 2.5% proportionate mortality rate (mortality contributed by *Klebsiella pneumoniae* during HPAI outbreaks) is significant to the poultry industry in Nigeria. Similarly, the 97.5% proportionate mortality rate in *Klebsiella pneumoniae* free flocks could have been due to H5N1 virus and other unknown secondary agents which were not investigated for in this study. This study has shown that *Klebsiella pneumoniae* was isolated from flocks with H5N1 during outbreaks in Nigeria between December, 2006 and July, 2007. Most viral infections are exacerbated by secondary bacterial pathogens. It is possible that *Klebsiella pneumoniae* may have acted in concert with the primary viral infection to produced severe clinical signs during HPAI outbreaks in Nigeria. The role of this organism during natural outbreaks of HPAI (H5N1) needs to be investigated further.

References

Alexander D.J. (1999). Orthomyxoviridae Avian influenza. In: *F. T Jordan, editor, Poultry Diseases (4th edition)*. Baillierve Tindall: London, U.K: 156 – 165.

Alexander, D.J. (2000). A review of Avian Influenza in different bird species. *Veterinary Microbiology*, **74**:3-13. Anonymous, (2006): Food as possible source of infection with highly pathogenic avian influenza viruses for human and other mammals. *Efsa Journal*, **74**: 29.

Armin, R.W. E., Bert, K., Guus, K. (2004). Performance of gross lesion at post mortem for the detection of H5N1 outbreak during the avian influenza A virus (H7N7) epidemic in the Netherlands in 2003 *Avian Pathology*, **33**:418 – 422.

Barrow, G.I and Felthan, R.K.A, (2004): Cowan and Steels identification of medical bacteria 4th edition Cambridge University press Pp 50 – 145.

Easterday, B.C Hinshaw, V.S and Halvorson, D.A. (1997): Influenza. In: *Calnek, B.N Barnes, H.J Bear, C.W McDoughld, L.R and Saif, Y.M., editors, Diseases of poultry (10th edition)*. Iowa State University Press, Ames, Iowa. USA. pp.583 – 605.

Edwards and Ewings, (1986). *Identification of Enterobacteriaceace (4th edition)*. Elsevier Science Publication Campany Inc., NewYork, p.536.

Fouchier, R.A Muster. V. and Wallensten A. (2005): Characterization of novel influenza-A virus haemagglutinin Subtype (H 16) obtained from black headed gulls. *Journal of Virology*, **79**: 2814 – 22.

Kumbish, R.R., Bello, M.K., Jambalang, A.R. Hussaini, B.A Solomon, P., Ekong, P.S., Damina, M.S., Akanbi, B.O., Joanis, T.M.,Ularamu, H. G., Shitu, A. I. and Suleiman L. K, (2006): Bacterial and Parasitic agents associated with outbreaks of Highly pathogenic Avian influenza (H5N1) in Nigeria. *Vom Journal of Vet Sc.*, 0331-8753 Pp 23-31.

Lewis, R. J. (1997). Avian influenza in Frazer Valley turkeys. *Animals Health Center Newsletter, Diagnostic Dairy*, **7**: 8-9.

National Animal Disease Information and Surveillance/Pan African Control of Epizootics (NADIS/PACE), (2006). *Special "Bird flu" Edition*, Pp.1-7.

Orajaka, L. J.E. and Mohan, K. (1985). Aerobic bacterial flora from dead in shell chicken embryo from Nigeria. *Avian Disease*, **29**:583-589.

Sandra, O. J. and Duarte, C. J. H. (1998). Potential pathogens recovered from the upper respiratory tract of psittacin birds. *File// E: Klebsiella.htm. Accessed 20/3/2008*.

Statistical Package For Social Science (SPSS), Version 12.01, Incorporated Chicago United States of America.

Swayne, D.E. (2003): Viral diseases of Poultry. *Program and abstracts of the xiii congress of the World Vet Poultry Association, July 19 – 23 Denver U.S.A.* **56**: 51 – 54.

Timoney, J.F., Gillespie, J. H., Scott, F. W., Barlough, J. E (1988). In: Hagan and Bruner's microbiology and infectious diseases of domestic animals, 8th ed., pp. 10–12. Cornell University Press, Itaca, N.Y.

Websters, R.G., Bean, W.J., Gorman, O.T., Chamber I.M and Kawaok, Y. (1992): Evolution and ecology of

influenza A viruses. *Microbiology Review*, **56**:152 – 179.

Yuen, K. Y. and Wong, S. S. Y. (2005). Human infection by avian influenza A H5N1. *Hong Kong Medical Journal*, **11**(3):189-199.

Yuen, S., Yuen. K. and MRCpath, M. D. (2006). Avian influenza infection in humans. *Chest*, **129**:156-168.