SHORT COMMUNICATION

Salmonella Gallinarum Infection in Poultry Affected by Highly Pathogenic Avian Influenza Virus H5n1 In Nigeria

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INTRODUCTION

Highly pathogenic avian influenza (HPAI) is a viral disease affecting almost all domestic and wild birds (Easterday et al., 1997; Alexander, 1999). The species of animals affected by avian influenza include 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. Avian influenza virus codes for 10 proteins including haemagglutinin (H), neuraminidase (N), protein matrix, RNP among others (Alexander, 1999; Swayne, 2003). There are 16 H and 9 N subtypes (Fouchier et al., 2005). Avian influenza depresses the host immune system thereby paving ways for opportunistic microbes to invade and exert an exacerbative effect resulting in high mortality in affected flocks (Aleksandr et al., 2004). Salmonella gallinarum is a Gram negative rod, non lactose fermenting organism of the Family Enterobacteriaceae. It is the etiologic agent of fowl typhoid which causes a serious threat to poultry industry particularly in tropical Latin America and many parts of Africa (Hall, 1977). The disease affects a variety of birds such as ducks, pheasants, quails, chickens, guinea fowls, turkeys and ostriches and it is a common problem in Nigeria (Oboegbulem et al., 1980). This study was aimed at isolating Salmonella gallinarum as well as highlighting the possible complicating role of the organism in natural outbreaks of HPAI (H5N1) that occurred in Nigeria.

KEY WORDS: Avian influenza, Salmonella gallinarum, poultry, Nigeria.

MATERIALS and METHODS

One hundred poultry were collected using simple random sampling from 114 commercial, backyard and free range flocks affected by HPAI in different parts of Nigeria. A total of 244,992 poultry were in the flocks.

Six samples consisting of the heart, intestine, liver, lungs, spleen and trachea were collected from each of the 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 (NVRI), Vom, Nigeria, using agar gel immuno-diffusion test, virus isolation in embryonated chick eggs, haemagglutination inhibition and reverse transcriptase polymerase chain reaction. All samples were kept in double transparent polythene bags, labeled and preserved at -70^{0}C at the Central Diagnostic Department, (NVRI), Vom. The samples were later transported in a leak proof insulated box packed with ice packs to the Department of Veterinary Pathology and Microbiology, Ahmadu Bello University, Zaria for bacterial isolation.

Bacterial Isolation

Swabs from the heart, intestine, lungs, liver, trachea and spleen were cultured on an enrichment medium (Selenite F broth) and was later incubated on 7% defibrinated sheep blood agar (BA). Colonies suspected to be *Salmonella gallinarum* were subcultured on MacConkey (MCA). All cultures were incubated aerobically at 37^{0}C for 24 h.

Identification of *Salmonella gallinarum*

Suspected *Salmonella gallinarum* isolates 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 by Edwards and Ewings, (1986). The biochemical reagents and tests used included: Triple sugar iron agar, urease, Simmons citrate and motility.

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 tissue samples obtained from highly pathogenic avian influenza H5N1 examined, 7(1.2%) of the samples yielded *Salmonella gallinarum*, isolated from liver samples of commercial layers 3 (0.5%), local chicken 2(0.3%) guinea fowls 1 (0.16%) and geese 1 (0.16%) between the ages of 20 and 44 weeks (Table I). *Salmonella gallinarum* was also recovered from the liver and spleen of apparently healthy commercial layer 1(0.2%) and guinea fowl 1(0.2%) (Table I). The organism was recovered from five different flocks with a total of 22,508 birds, mortality rate of 15.6% and proportional mortality rate of 5.6% (Table II). All the seven *Salmonella gallinarum* isolates were obtained from liver samples. No clinical sign or mortality was recorded in flocks with apparently healthy birds from which *Salmonella gallinarum* was isolated.

Table 1: Isolation and distribution of Salmonella gallinarum in tissues of birds affected by HPAI (H5N1) and apparently healthy birds.

	Infected birds		Apparently healthy birds	
Sample	S.gallinarum	NGS	S.gallinarum	NGS
Heart	-	100	-	6
Intestine	-	100	-	6
Liver	7	93	-	5
Lung	-	100	1	6
Spleen	-	100	-	5
Trachea	-	100	1	6
Total	7(1.2%)	593(98.8%)	2(3.3%)	58(96.7%)

Note: Salmonella gallinarum was isolated from 7 different birds affected by HPAI (one organ per bird).
NGS - No growth of Salmonella gallinarum

Table 2: Mortality and proportionate mortality rate associated with Salmonella gallinarum isolated from flocks affected by HPAI (H5N1)

Bacterium	No of flock affected	No of birds affected	No dead	Mortality rate(%)	Prop mort rate(%)
Salm. gallinarum	5	22,508	3,522	15.6	5.6
*NG S.gallinarum	109	222,484	59,559	10.1	94.4
Total	114	244,992	63,081	25.7	100

*N.G. - No growth of Salmonella gallinarum

DISCUSSION

Fowl typhoid is a common problem in many poultry farms in Nigeria due to poor management and unhygienic conditions (Chima and Ogbogu, 1998). Complication of avian influenza by bacteria has been reported by some workers (Lewis, 1997; Alexander, 2000). In the present study, *Salmonella gallinarum* was recovered from only 7(1.2%) liver samples of poultry affected by H5N1 during outbreaks in Nigeria. The result of this study as well as that of Kumbish *et al.* (2006), who isolated *Salmonella gallinarum* (1.1%) in a similar study on tissue samples of poultry affected by H5N1virus suggest that the organism was

one of the commonest bacteria that complicated avian influenza (H5N1) during outbreaks in Nigeria.

Although HPAI virus is known to have tissue tropism (Rott, 1993; Shinya *et al.,* 2004), the profound debilitation seen in poultry affected by HPAI might have been exacerbated by bacteria such as *Salmonella gallinarum,* among others, since the organism was found to have a proportionate mortality rate of 5.6% (mortality contributed by *Salmonella gallinarum* during HPAI outbreaks). Similarly, the 94.4% proportionate mortality rate in *Salmonella gallinarum* free flock could have been due to H5N1 virus and other unknown secondary agents which were not investigated for in this study. All the seven *Salmonella gallinarum* isolates were recovered from the liver, which supports the report by Robert (1975) that this organ serve as reservoir for *Salmonella gallinarum*. The recovery of *Salmonella gallinarum* from a number of poultry species between ages of 20 and 44weeks further agrees with the finding that fowl typhoid affects different types of poultry and is more prevalent in adult birds (Chima and Ogbogu, 1998). It is possible that the isolates of *Salmonella gallinarum* recovered from samples of apparently healthy commercial layer and guinea fowl could have been due to previous exposure or the birds might have been incubating S*almonella gallinarum* at the time of sampling. The finding of this study has shown that *Salmonella gallinarum* was one of the agents that occurred concurrently with natural outbreaks H5N1 in Nigeria and it has also contributed to mortality during the outbreaks. More study is required to further examine the role of *Salmonella gallinarum* in future HPAI H5N1 outbreaks in Nigeria

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