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Isolation of *Aeromonas hydrophila* from Commercial Chickens in Jos Metropolis, Nigeria

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Abstract: *Aeromonas* species are increasingly incriminated in clinical cases in livestock and humans in Nigeria and the world at large. This investigative study was carried out between November, 2010 and October, 2011 in Jos, Nigeria to determine the isolation rate of *Aeromonas species* in clinically sick and apparently healthy commercial chickens. A total of 2000 postmortem samples consisting of bone marrow, heart, liver, lung and spleen (400 each) were aseptically collected from 400 clinically sick chickens suspected to be suffering from various clinical conditions and cultured for *Aeromonas* organisms. Four hundred oro-pharyngeal swabs were also collected from 400 apparently healthy chickens for bacteriological analysis. Swab from each sample was cultured on 7% defibrinated sheep blood and MacConkey. From the bacteriological cultures of the bone marrow, heart and liver of the sick chickens, a total 11 (0.5%) *Aeromonas hydrophila* isolates were identified by biochemical characterization and Macrobact™ test. *Aeromonas* organism was not isolated at all from the apparently healthy chickens. The co-occurrence of *Aeromonas hydrophila* with other pathogens in the sick chickens could have contributed to the observed exacerbation of clinical signs and mortalities in some of the investigated flocks during the study period.

Key words: *Aeromonas hydrophila*, chicken, Jos, Nigeria

INTRODUCTION

Bacteria of the genus *Aeromonas* and Family *Aeromonadaceae* are Gram negative rods, asporogenous and non-lactose fermenting organisms. Up to fourteen *Aeromonas* species have been discovered and *Aeromonas hydrophila* is perhaps the most commonly occurring member of the genus. The bacterium is coccobacillus, indole and catalase positive. It also reduces nitrate to nitrite (Martin-Carnahan and Joseph, 2005). All members of *Aeromonas hydrophila* complex such as *A. sobria*, *A. caviae* and *A. hydrophila* are isolated predominantly fish, meat, foods and poultry samples (Neyts *et al*., 2000) and they contribute to the virulent taxons hybridization groups (Van-damme and Vandepitte, 1980). Disease caused by *Aeromonas species* is refer to as *aeromoniasis* and is worldwide in distribution. The disease is endemic in Nigeria and the most important disease of fish that is zoonotic (Opkwasili and Ogbulie, 2001). Mailafia *et al*. (2008) reported the isolation rate of *A. hydrophila* as 6.8% in humans in Zaria. *Aeromonas hydrophila* is an opportunistic pathogen associated with hemorrhagic septicemia in cold blooded animals including amphibians, reptiles, fish and shellfish (Rippey and Cabelli, 1980). The organism has also been isolated in stagnant water and sewage (Hazen *et al*., 1978), rabbits (Okewole *et al*., 1989; Abdel-Gwad and Abdel-Rahman, 2004) as well as birds (França *et al*., 2009). *Aeromonas* species have emerged as important human pathogens associated with food borne disease outbreaks and traveler's diarrhea with *Aeromonas hydrophila* mostly incriminated (Awaad *et al*., 2011). Some authors have described the disease as diarrheal condition that affects infant and the elderly more severely (Nzeako and Okafor, 2002). Risk factors that predispose humans to the disease include: ingestion of contaminated food and drinking water (Handfield *et al*., 1996); pre-existing disease such diabetes (Kumar *et al*., 2012); immunosuppressive drugs and age (Okumura *et al*., 2011). From many parts of the world, there have been reports of the isolation of *Aeromonas* species from sick and dead birds, mostly captive wildlife species for example: a case of hemorrhagic septicemia of a captive ground-hornbill (*Bucorvus abyssinicus*) (Ocholi and Kalejaiye, 1990) and epidemic deaths of Mallard ducks (Zbikowski *et al*., 2006). There are other detailed reports of the isolation of Aeromonads from chickens meat (Dallal *et al*., 2012) and andor feces (Adeleke and

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Omafuvbe, 2011). In spite of the increasing cases of aeromoniasis in poultry been reported in many parts of the world, there is inadequate documented reports of the natural outbreaks the disease in commercial chickens; and in Nigeria little is known of the involvement of *Aeromonas* species in outbreak of chicken disease. This study was conducted in Jos, Plateau State of Nigeria with the aim of establishing the occurrence of *Aeromonas* species in sick and apparently healthy commercial chickens.

MATERIALS AND METHODS

Study area: Jos metropolis of Plateau State, Nigeria, is situated at 1, 238 m (4,062 feet) above sea level, in the North Central geo-political zone of the country and consists of two Local Government Areas (LGAs). Jos North LGA (9°55'N, 8°54'E) and Jos South LGA (9°46'N 8°48E). Jos North is the seat of Government and by extension the capital of Plateau State (80°24'N and longitude 80°32' and 100°38'E). The Jos terrain consists of a flat land resembling a table top, from which the name "Plateau" of the State is derived. According to the report of Ogbonna and Harris (2008), at Jos, the average monthly temperature ranges between 21°C and 25°C (69°F and 77°F), the average humidity is 60% and the average annual rainfall is 1400 mm (56"). Owing to it high altitude placement, Jos has a much cooler weather when compared with most other parts of Nigeria. The vegetation of the area is extensive highland savannah with short grasses interspersed with very few trees. There are two major season on the Jos Plateau: A long dry season that commences at about the middle of October. Jos receives an average of 1351 mm of precipitation annually and up to 96 percent of the rainfall occurs between April and September of each year (Iloeje, 2001). According to 2006 national Census of Nigeria Report (NPC, 2006), Jos North LGA has a land area of 291 km^2 and a population of 429,300, while Jos South LGA has a land area of 510 km^2 and a population of 306,716. The study area was purposively chosen for this study because of the availability of Veterinary hospitals and clinics, high concentration of poultry farms and willingness of poultry owners and clinicians to cooperate with the researchers.

Collection of samples: Purposive sampling technique according to Portney and Watkins (2008) was used in selecting three sampling points as follows: Central Diagnostic Laboratory of the National Veterinary Research Institute, located in Vom, Plateau State; Plateau State Veterinary Hospital, Jos and ECWA Veterinary Clinic, Bukuru. At these points, tissue samples were aseptically collected from sick chickens submitted for diagnosis. Breed of chickens sampled were Isa Brown, Hubbard, Marshall, Ross, Dominant black and others. One hundred and thirty three clinically sick chickens were collected at each sampling point to give a total of three hundred and ninety nine. One chicken was added at Central Diagnostic Laboratory of the National Veterinary Research Institute, Vom, to make up four hundred samples. The tissue samples collected during the postmortem examination of the carcasses were heart blood, femur, lungs, spleen and liver (400 each from clinically sick chickens, giving a total of 2000 tissue samples). From 400 apparently healthy chickens, samples of the oro-pharyngeal exudates (n = 400) were also collected with sterile swabs for bacteriological analysis.

Culture and isolation of organism: The surface of each organ was seared with hot spatula and incised with a sterile scalpel blade. Swabs prepared from these organs were inoculated directly onto Blood agar and MacConkey agar. The cultures were then incubated aerobically at 37°C for 24 h. Oro-pharyngeal swabs were cultured indirectly by first inoculating each sample into 5ml of brain heart infusion broth (BHI), followed by incubation of the broth mixture at 37°C for 24 h and then streaking loop samples of the broth culture unto media such as Blood and MacConkey agar. Cultural and morphological examinations were conducted as described by Barrow and Felthan (2004). Presumptive colonies of *Aeromonas* sp., (observed as cream colored, round, shiny, raised with beta hemolysis on Blood agar and yellowish orange, non-lactose fermenting colonies on MacConkey agar) were carefully selected and subjected to Gram staining prior to identification by cellular morphology. Organisms were further confirmed as *Aeromonas hydrophila* by biochemical tests according to CLSI (2009). Colonies representing each bacterial specie was identified and characterized according to the methods described by Barrow and Felthan (2004). The biochemical tests used for the identification of the presumptive isolates of *Aeromonas hydrophila* were urease, simmons citrate, nitrate, indole, motility, methyl, Voges Proskauer and catalase.

Microbact: All *Aeromonas hydrophila* isolates identified by the biochemical test reactions were further subjected to additional analytical profile test using Oxoid™ Microbact GNB 24E kit (a commercially available biochemical test kit in microplate format for identifying *Enterobacteriaceae* and miscellaneous Gram negative bacilli) and this was done according to the manufacturer's instruction.

Statistical analysis: The entry and sorting of primary data was performed with Microsoft excel, 2010. Descriptive statistical analysis was conducted using statistical package for social sciences SPSS (2004) (version 12.01). The results were summarized as percentages in tables and as Chi-Squared output of the dataset.

RESULTS

From the 2000 tissue samples consisting of bone marrow, heart, liver, lungs and spleen(400 each) examined, *Aeromonas hydrophila* 11(0.5%) was isolated from bone marrow, heart and liver of the sick chickens (Table 1). The distribution of *A. hydrophila* in tissues was as follows: bone marrow 1 (0.25%), heart 4 (10%) and liver 6 (1.5%). Only one species of *Aeromonas* (*Aeromonas hydrophila*) was recovered throughout the entire study. *Aeromonas hydrophila* was not isolated from apparently healthy chickens. The isolation rates of *Aeromonas hydrophila* in different breeds of chickens showed that 27% of the organisms were isolated from Isa brown, 18% were recovered from Hubbard and Marshall, while 9% were isolated from Rose (Table 2). There was no significant difference ($p>0.05$; $X^2 = 2.19$, df = 3) in the isolation rate of *Aeromonas hydrophila* and breed of chickens sampled. Eleven (11) *Aeromonas hydrophila* isolates from clinically sick chickens were confirmed by Microbact GNB 24E kit. Eleven isolates of *Aeromonas hydrophila* were identified with probability index of 1/100,000,000 (Table 3).

Table 1: Distribution of *Aeromonas hydrophila* in tissues of clinically sick chickens

	Clinically sick chickens		
Samples	*A.h* isolates	No. bacterial growth	Total
Bone marrow	1	399	400
Heart	4	386	400
Liver	6	394	400
Lung	-	400	400
Spleen	-	400	400
Total	11 (0.55%)	1989 (99.45%)	2000 (100%)

A.h: *Aeromonas hydrophila*

Table 2: Isolation rates of *Aeromonas hydrophila* in different breeds of clinically sick commercial chickens in Jos

Breed of chickens	No. *A. hydrophila* isolated	Percentage (%)
Isa brown	3	27
Hubbard	2	18
Marshall	2	18
Ross	1	9
Dominant black	3	27
Total	11	100

DISCUSSION

Significant economic losses are incurred in commercially produced poultry worldwide due to diseases caused by bacterial agents (Barnes *et al*., 2003). The isolation of eleven isolates of *A. hydrophila* species from 3 different organs (bone marrow, heart and liver) of clinically sick chickens in this study is an indication that avian species are susceptible to this organism. Despite the fact that *Aeromonas species* have not been reported as an important poultry pathogen in Jos, Nigeria; there are documented reports that the organism caused a fulminating disease which was characterized with high mortality in wildlife birds such as canary birds (Franca *et al*., 2009). Although there are inadequate documented reports on the prevalence, course sequelae of the natural disease in chickens in Jos, Nigeria, it is likely that the *A. hydrophila* infection of chicken reported in this study was horizontally transmitted via the oral route. Possible vehicles for the transmission include drinking water from contaminated sources and unhygienic feeds particularly those containing contaminated fish meals or similar substitutes. In a bid to lower the production cost of feed, poultry farmers in Nigeria occasionally resort to the use of locally dried fish offals and shellfishes as fish meal substitutes. Fishes and shellfishes may harbor pathogenic strains of *A. hydrophila*. Clark *et al*. (1973) and Hassan (2006) reported that formulation of poultry ration with poorly processed fish or shellfish meals could lead to the infection of birds, other animals and man, with fish-borne pathogens. Although in the present study, there was no further investigation to ascertain or attribute the primary cause of sickness to *A. hydrophila*, an experimental study by Awaad *et al*. (2011) of *A. hydrophila* infection in chickens using gentamicin resistant strains revealed that: up to 8.3% embryonic mortality was recorded after dipping of fertile eggs in infected broth culture, up to 13.3% mortalities of hatched chicks within the first week post-hatching; unthriftness of survivors characterized by lesions of omphalitis, enteritis, septicemia and depressed weight gain; but only an enteric infection in experimentally infected breed. Saif and Busch (1974) reported a synergistic relationship between *Salmonella infantis* and *A. hydrophila* in poults, with neither organism being able to induce disease in poults when injected singly, but causing death up to 30% of the subjects when injected together. The isolation of 11 strains of *A. hydrophila* species from 3 different organs sites (i.e., bone marrow, heart and liver) of necropsied chickens in Jos, Nigeria is an indication that some field strains of this bacterium present in the area, may solely or aided by other pathogens, provoke a fulminant, septicaemic infection of highly inbred chicken breeds such as the Isa Brown, Hubbard, Marshall, Ross and Dominant Black. Dashe *et al*. (2013) opined that *A. hydrophila* could have contributed to the aggravated clinical signs and mortalities in sick chickens with fowl cholera. The low frequency of isolation of *A. hydrophila* from tissue samples of clinically sick birds and none at all from the oropharynx swabs of apparently healthy birds could be due to indiscriminate administration antibiotics to birds by poultry farmers once any sign of disease is observed. It is common of poultry farmers in Nigeria to administer antibiotic cocktails comprised of several human and veterinary preparations to birds, particularly when conventional poultry drugs fail to mitigate the clinical

Table 3: Identification of *Aeromonas hydrophila* isolated from clinically sick commercial chickens by Microbact test in Jos, Nigeria

Isolate id. Number	Octal Number	Percentage (%) identification	Probability index
JN3	545760761	99.37	<1/100,000,000
JN5	567640260	76.97	<1/100,000,000
JN43	557720661	99.95	<1/100,000,000
JN45	557663765	98.24	<1/100,000,000
JN48	547762770	98.42	<1/100,000,000
JN62	547610665	77.26	<1/100,000,000
JN64	557650661	98.72	<1/100,000,000
JN103	557600667	98.75	<1/100,000,000
JN152	567640261	76.97	<1/100,000,000
JN170	557663760	98.23	<1/100,000,000
JN180	547762770	98.42	<1/100,000,000

Key J: Jos N: Nigeria Id: Identification

signs of disease (Olarinmoye *et al*., 2013). A disturbing but long-term consequence of this practice is the emergence and involvement of multidrug resistant phenotypes that are more difficult to treat in veterinary and human population with public health implications; this observation concurred with the reported by Dashe *et al*. (2013).

This study has highlighted that *A. hydrophila* was isolated from organs of clinically sick commercial chickens between November, 2010 and October, 2011, in Jos, Nigeria. The bacterium has public health significant; it is therefore necessary that all poultry and poultry products main for human consumption to be properly processed. Further study to elucidate the epizootiology and public impact of infections in poultry in Nigeria is recommended.

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