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Full Length Research Paper

Aeromonas hydrophila infections in chickens affected by fowl cholera in Jos Metropolis, Nigeria

Dashe, Y. G.[1]*, Raji, M. A.[2], Abdu, P. A.[3] and Oladele, B. S.[4]

[1]National Veterinary Research Institute, Akure Zonal Office, State Veterinary Hospital, Akure, Ondo State, Nigeria.
[2]Department of Veterinary Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
[3]Department of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
[4]Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

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Many bacterial agents have been incriminated in cases of fowl cholera outbreaks in chickens. This study was conducted to determine the prevalence of *Aeromonas hydrophila* infection in chickens affected by fowl cholera between November, 2010 and October, 2011 in Jos, Nigeria. A total of 2000 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 fowl cholera and cultured for *Pasteurella multocida* and *Aeromonas hydrophila* organisms. Four hundred oropharyngeal swabs were also collected from 400 apparently healthy chickens for bacteriological analysis. Swab from each sample was cultured on 7% defibrinated sheep blood, MacConkey and casein sucrose yeast agar. Presumptive colonies of *P. multocida and A. hydrophila* were subjected to biochemical characterization and Microbact test. *P. multocida* 20 (1.0%) was isolated from all the tissue samples of clinically sick chickens, while *A. hydrophila* 11 (0.5%) was recovered from bone marrow, heart and liver of the sick chickens. *P. multocida* 5 (1.25%) was isolated from oropharynx of apparently healthy chickens, while *A. hydrophila* was not isolated at all from the apparently healthy chickens. It was concluded that *A. hydrophila* agent could have contributed to the aggravated clinical signs and mortality observed in clinically sick chickens within the study period.

Key words: *Aeromonas hydrophila*, *Pasteurella multocida,* chicken, fowl cholera, Jos.

INTRODUCTION

Fowl cholera is a contagious bacterial disease affecting both domesticated and wild avian species. The disease is caused by a gram negative coccobacillus bacterium called *Pasteurella multocida*. Fowl cholera occurs as a fulminating disease with massive bacteraemia and high morbidity and mortality (Glisson et al., 2003; Kwon and Kang, 2003). Chronic infections also occur with clinical signs and lesions related to localized infections. The pulmonary system and tissues associated with the musculoskeletal system are often the seats of chronic infection (OIE, 2008). In Nigeria, a great loss in poultry production as a result of this disease is still been recorded. Odugbo et al. (2004) recorded massive death in quails in Vom, Nigeria due to *P. multocida* serotype A: 4.

Aeromonas hydrophila is a gram negative rod, non lactose fermenting organism of the Family *Aeromonadaceae.* The bacterium is coccobacillus, indole and catalase positive. It also reduces nitrate to nitrite; this characteristic is similar to that of *P. multocida*. Disease caused by *Aeromonas species* is referred to as *aromoniasis* and is worldwide in distribution. All members of *A. hydrophila* complex such as *Aeromonas sobria*, *Aeromonas caviae* and *A. hydrophila* are isolated predominantly by fish, meat, foods and poultry samples and they contribute to the virulent taxons hybridization groups (Van-damme and Vandepitte, 1980). The disease

*Corresponding author. E-mail: yakubudashe@yahoo.co.uk. Tel: +234 8035778490.

is endemic in Nigeria and the most important disease of fish is zoonotic (Okpokwasili and Ogbulie, 2001). Mailafia et al. (2008) reported the isolation rate of *A. hydrophila* as 6.8% in humans in Zaria. *A. 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 (Zeinab, 2007; França et al., 2009). Aeromonas species have emerged as important human pathogens associated with food borne disease outbreaks and traveller's diarrhea with *Aeromonas hydrophila* mostly incriminated (Fukushema et al., 2007; Chang et al., 2008; Awaad et al., 2011). *A. hydrophila* has been reported to survive for a long time in the environment (Glunder, 2002).

Despite the devastating effect of *A. hydrophila* infections in poultry been reported in many parts of the world, there is scanty information regarding the bacterium in cases of fowl cholera in chicken in Jos, Nigeria. This study therefore seeks to highlight the possible complicating role of the organism in chickens affected by fowl cholera in Jos, Nigeria.

MATERIALS AND METHODS

Study area

Jos North and Jos South Local Government Areas of Plateau State, Nigeria were 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.

Plateau state is located between Latitudes 8.50°-100.46° N and Longitudes 8.20°-10.36°E in the North-Central zone of Nigeria.

Collection of samples

Systematic random sampling method (one in five; every 5th chicken was picked) was applied for the selection of 400 apparently healthy and 400 clinically sick chickens sample between November, 2010 and October, 2011. These samples were collected from Poultry clinics, poultry farms and live birds markets that were identified in Jos North and Jos South Local Government Areas for sample collection. Whole heart blood, femur, lung, spleen and liver (400 each) were collected from selected clinically sick chickens (suspected to be suffering from fowl cholera) and submitted for diagnosis at Central Diagnostic Department, NVRI, Vom, ECWA Veterinary Clinic, Bukuru, all in Jos South Local Government Area, and Plateau State Veterinary Hospital, Jos, in Jos North L. G. A.

Chickens with history of sudden death as well as those showing some of the following clinical signs: swollen combs and wattles, cyanotic combs, diarrhea, emaciation, torticolis, ocular and nasal discharges, were selected as presumptive cases of fowl cholera. Those chickens without the above clinical signs were not selected.

Chickens from 0-10 weeks were classified as chicks, 11-15 weeks were classified as growers and 16 weeks and above were classified as adults. On the other hand, 400 oro-pharyngeal swabs from apparently healthy chickens in Jos metropolis were collected from poultry farms and live birds markets. The samples collected were transported on ice to the Bacteriology Unit of the Central Diagnostic Laboratory, NVRI, Vom for culture and microbiological examination as described by CLSI (2009).

Culture and isolation of organism

The surface of each organ was seared with hot spatula and incised with a small sterile scapel blade. Swabs from these organs were inoculated directly onto selective medium such as Casein Sucrose Yeast (CSY) agar, blood agar and MacConkey agar. Cultures were incubated aerobically at 37°C for 24 h. Oro-pharyngeal swabs were cultured indirectly by inoculating them into 5 ml of brain heart infusion broth (BHI), incubated at 37°C for 24 h and then streak unto selective medium such as Casein Sucrose Yeast (CSY) agar and Blood agar. Presumptive *P. multocida* and *A. hydrophila* colonies were subjected to Gram and methylene blue staining for cellular morphology. Cultural and morphological examinations were conducted as described by Barrow and Felthan (2004). Organisms were further confirmed as *P. multocida* and *A. hydrophila* by biochemical tests according to CLSI (2009). Colonies representing each bacterium species were identified and characterized according to the methods described by Barrow and Felthan (2004).

The biochemical reagents used for the identification of the presumptive isolates of *P. multocida* and *A. hydrophila* were urease, simmons citrate, nitrate, indole, motility, methyl, Voges Proskauer and catalase.

Microbact

All *P. multocida* and *A. hydrophila* isolates recovered by biochemical test were further subjected to analytical profile test using commercially available kit (Macrobact GNB 24E kit) Oxoid according to the manufacturer's instruction.

Statistical analysis

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

Table 1. Distribution of *P. multocida* and *A. hydrophila* in tissues of chickens affected by fowl cholera and apparently healthy birds.

Sample	Infected chickens				Apparently healthy chickens			
	P. multocida	***A. hydro***	**NGS**	**Total**	***P. multocida***	***A. hydro***	**NGS**	**Total**
Bone m	4	1	395	400	-	-	-	-
Heart	8	4	388	400	-	-	-	-
Liver	4	6	390	400	-	-	-	-
Lung	3	-	397	400	-	-	-	-
Spleen	1	-	399	400	-	-	-	-
*Oropharynx	-	-	-	-	5	-	395	400
Total	20 (1.0%)	11 (0.5%)	1969 (98.5%)	2000 (100%)	5 (1.25%)	-	395 (98.75%)	400

* Oropharyngeal swabs were taken from apparently healthy birds only. Hydro - hydrophila; m - marrow; NGS - no bacterial growth.

Table 2. Identification of *A. hydrophila* isolated from clinically sick chickens by Microbact test in Jos, Nigeria.

Serial num.	Isolate id. Num	Octal num.	Percentage (%) identification	Probability index
1	JN3	545760761	99.37	<1/100,000,000
2	JN5	567640260	76.97	<1/100,000,000
3	JN43	557720661	99.95	<1/100,000,000
4	JN45	557663765	98.24	<1/100,000,000
5	JN48	547762770	98.42	<1/100,000,000
6	JN62	547610665	77.26	<1/100,000,000
7	JN64	557650661	98.72	<1/100,000,000
8	JN103	557600667	98.75	<1/100,000,000
9	JN152	567640261	76.97	<1/100,000,000
10	JN170	557663760	98.23	<1/100,000,000
11	JN180	547762770	98.42	<1/100,000,000

Key: J - Jos; N - Nigeria; Num - Number; Id - identification.

RESULTS

From the 2000 tissue samples consisting of bone marrow, heart, liver, lungs and spleen (400 each) examined, *P. multocida* 20(1.0%) was isolated from all the tissue samples of clinically sick chickens, while *A. hydrophila* 11 (0.5%) was recovered from bone marrow, heart and liver of the sick chickens (Table 1). The distribution of *P. multocida* in tissues was bone marrow 4 (10%), heart 8 (20%), liver 4 (10%), lungs 3 (0.75%) and spleen 1 (0.25%). *A. hydrophila* had bone marrow 1 (0.25%), heart 4 (10%) and liver 6 (1.5%). *P. multocida* 5 (1.25%) was isolated from oropharynx of apparently healthy chickens.

A. hydrophila was not isolated from apparently healthy chickens.

Twenty *P. multocida* isolates from clinically sick birds and five *P. multocida* isolates from apparently healthy birds were confirmed by Microbact GNB 24E kit. Eleven isolates of *A. hydrophila* were identified with probability index of 1/100,000,000 (Table 2).

DISCUSSION

Bacterial infections are of worldwide importance in commercially produced poultry and they have been reported to cause significant economic losses to the industry (Barnes et al., 2003). Many of such bacterial agents have been incriminated in cases of fowl cholera outbreaks in chickens. Complications of fowl cholera by several bacterial agents such as *Escherichia coli*, *Staphylococcus aureus, Proteus species, Klebsiella pneumoniae* among others in Jos and environs have been reported by Masdooq et al. (2008). In the present investigation, eleven isolates of *A. hydrophila* species were isolated from 3 different organs (bone marrow, heart and liver) of chickens with cases of fowl cholera. Although *A. hydrophila* has not been reported as an important poultry pathogen in Jos, Nigeria, the isolation of this agent in cases of fowl cholera in chickens in this study calls for re-examination of its roles in chickens. The low isolation of the bacterium in tissue samples of clinically sick birds and none at all from the oropharynx of

apparently healthy birds could probably be due to indiscriminate administration antibiotics by poultry farmers whenever they notice any sign of a disease. Although *P. multocida* is known to have tissue tropism (Glisson et al., 2003), the profound debilitation observed in chickens affected by fowl cholera might have been exacerbated by *A. hydrophila* and other bacterial agents not investigated in this study. The isolation of *A. hydrophila* from chickens affected by fowl cholera could be as a result of infection due to exposure to contaminated water, soil or dry fish which are not properly processed before they are incorporated into poultry feed. This finding has revealed that poultry products if not properly cooked could constitute a significant source of human infection. It is most likely that the biochemical similarities between *P. multocida and A. hydrophila* could have been responsible for mis-diagnoses of *A. hydrophila* by clinicians and laboratory workers.

This study has highlighted that *A. hydrophila* was isolated in visceral organs of chickens affected by fowl cholera outbreak between November, 2010 and October, 2011. The bacterium has significance in public health, as such, it is therefore necessary that all those involved in poultry industry should wear mask and hand gloves. Further study of the epidemiology of this pathogen in fish, animals, and humans in Nigeria is advocated in order to evolve effective ways of controlling aeromoniasis in Nigeria. More so, further study to elucidate the virulence factors and associated economic impact of *A. hydrophila* in future fowl cholera outbreaks in Jos, Nigeria is recommended.

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