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Pathogenic Bacteria Associated with Respiratory Disease in Poultry with Reference to *Pasteurella multocida*

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Abstract: In this study, lung samples of chickens slaughtered at abattoir in Jos South, Nigeria were tested for the presence of *Pasteurella multocida* and other aerobic bacteria. The identity of *P. multocida* was proved by mouse pathogenicity test. A total of 3000 chickens were examined at post-mortem and 500 samples with pneumonia were collected. All lungs and heart blood from 15 different commercially reared chicken flocks showed respiratory disorders. Blood agar supplemented with 10% sheep blood was used for isolation of the agents. 32 (6.5%) *P. multocida* were isolated and identified. In addition, mouse pathogenicity test was carried out on *P. multocida* suspected isolates. Twelve (75%) of Isolates were all positive. This study showed that *P. multocida* is not the most prevalent bacterial infection among chicken population in Jos south.

Key words: *P. multocida*, mouse pathogenicity test, chicken, lung, heart blood

Introduction

Respiratory infection is the most serious disease affecting poultry and causes heavy economic losses in the poultry industry worldwide. In avian host, several micro organisms of the genus *Pasteurella* (*P. multocida*, *P. gallinarum*, *P. haemolytica* and *P. anatipestifer*), *Bordetella* (*B. avium*) and *Haemophilus* (*H. gallinarum*) are involved in respiratory disease complex (Hafez, 2002). *P. multocida* has been consistently found in the upper respiratory tract, spleen, lungs, blood and liver of infected birds (Hunter and Worbeser, 1980). *E. coli* associated with respiratory infection in chickens has also been reported (Elsukhon *et al.*, 2002). Tracheitis, exudative pneumonia, pleuritis, air sacculitis, pericarditis, sinusitis characterize the infection (Canal *et al.*, 2005).

Detection of *P. multocida* is important in the overall control and elimination of respiratory diseases from poultry flocks. Detection of *P. multocida* has mainly relied on mouse inoculation and in vitro culture in selective medium. Some media will not support the growth of all possible isolates, where as others are too insensitive (Kasten *et al.*, 1997). Mouse passage seems to represent a more efficient and widely accepted method of detecting *P. multocida*, although it may only select strains pathogenic for mice (Baldrias *et al.*, 1988). This study was aimed at isolation of *P. multocida* and other bacterial agents associated with Sinusitis, Pneumonia and air sacculitis in chickens slaughtered at a local abattoir and to confirm identification for *P. multocida* by mouse pathogenicity test.

Materials and Methods

A total of 3000 chickens were examined for presence of Pneumonia in lungs at a local abattoir in Jos south,

Nigeria and 500 samples were collected from suspected cases. The samples were obtained from fifteen different flocks. Samples were immediately transferred to the laboratory in sterile plastic bags where they were processed.

Culture: Swabs from the lungs samples were inoculated into blood agar supplemented with 10% sheep blood. The plates were incubated under aerobic condition for 24-48 h at 37°C. The plates were checked every day for suspected colonies. Identification and confirmation of bacterial species were assessed by observing the colonial morphology, Gram staining results and by the following biochemical tests; Catalase, Nitrate reduction, Urease activity, growth on MacConkey agar, H₂S production in triple sugar iron (TSI), Methyl red production, Coagulase test, Citrate and Carbohydrate fermentation from glucose, Trehalose, Xylose, Inositol, Salicin and Oxidase reaction (Carter, 1984). Isolates were stored at -80°C in nutrient broth with 10% glycerol for further analysis.

Mouse pathogenicity test: Swiss-Webster mice used in this study were obtained from the National Veterinary Research Institute, Vom, Nigeria. *P. multocida* colony was inoculated into nutrient broth and incubated at 37°C for 24 h. Mice (test mice) were inoculated parenterally with 1×10³ cfu mL⁻¹ of *P. multocida* strain. Control mice (inoculated with sterile normal saline). Both tests were kept for observation for 2 weeks.

Results

Culture results: After 48 h of incubation on blood agar supplemented with 10% sheep blood under aerobic conditions for 24-48 h at 37°C, grey to grey – white

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Table 1: Prevalence of single bacterial isolates from chicken lung and heart blood

<i>Escherichia coli</i>	94 (18.8%)
<i>Proteus</i> sp.	82 (16.4%)
<i>Staphylococcus</i> sp.	76 (15.2%)
<i>Streptococcus</i> sp.	34 (6.8%)
<i>Pastorella multocida</i>	32 (6.4%)
<i>Pseudomonas</i> sp.	18 (3.6%)
Yeast	12 (2.4%)
<i>Bordetella avium</i>	11 (2.2%)
Total	359 (71.8%)

Table 2: Prevalence of mixed bacterial isolates from chicken lungs and heart blood

<i>E. coli</i> + <i>Proteus</i>	7 (1.4%)
<i>E. coli</i> + <i>Staphylococcus</i> sp.	44 (8.2%)
<i>Proteus</i> sp. + <i>Staphylococcus</i> sp.	7 (1.4%)
<i>Staphylococcus</i> sp. + <i>Streptococcus</i> sp.	7 (1.4%)
<i>Pseudomonas</i> sp. + Yeast	6 (1.2%)
No growth	70 (14%)
Total	141 (28.2%)

*Number in parenthesis are percentages

colonies were subjected to biochemical reactions for isolation of typical *P. multocida* and other bacterial specie. Bacterial growth was observed in 430 out of 500 samples. The bacterial isolates were shown in Table 1 and 2.

Mouse pathogenicity results: *P. multocida* was isolated from 12 heart blood and lung samples of dead mice. However, *P. multocida* could not be isolated from 4 (25%) mice inoculated with strain that was culture positive. Heart blood and lung samples of dead mice were Gram stained and inoculated on to blood agar plates. The plates were incubated under aerobic condions for 24-48 h at 37°C and analyzed for the presence of *P. multocida*. Suspected *P. multocida* colonies were identified by biochemical methods.

Discussion

Respiratory infections are common among the chicken population. Several micro organisms are encountered in respiratory diseases of domestic poultry (Ozbey *et al.*, 2006). Isolation proportions obtained in this study were rather low (6.4%) compared to the total number of chickens examined. This may be due to the fact that most of our samples were taken from flocks in the acute phase of the infection and also due to poultry farmers in Nigeria misusing antibiotics flocks.

In addition to *P. multocida*, other pathogenic bacteria isolated and identified from lungs and heart blood samples of chickens include: *Staphylococcus* sp., *Streptococcus* sp., *E. coli*, *Bordetella avium*, *Proteus* sp., yeast and *Pseudomonas* sp.

This study showed that *P. multocida* is not the most prevalent bacterial infection among chicken population in Jos south. Intensive studies are needed to comprehend the epidemiology of disorders of the respiratory tract in the poultry within this region.

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