Isolation, antimicrobial susceptibility pattern and prevalence of *Gallibacterium anatis* in local breed of female Muscovy ducks (*Cairina moschata*) in Gombe, Northeastern Nigeria

Jallailudeen Rabana Lawal\(^1\), Juliana James Ndahi\(^2\), Yagana Bukar Majama\(^3\), Yagana Ahmed Gazali\(^2\), Jamila Dauda\(^4\) and Umar Isa Ibrahim\(^1\)

\(^1\)Department of Veterinary Medicine, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria.
\(^2\)Department of Veterinary Microbiology, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria.
\(^3\)Department of Veterinary Anatomy, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria.
\(^4\)Department of Veterinary Public Health and Preventive Medicine, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria.

*Corresponding author E-mail: rabanajallailudeen@yahoo.com*

Received 7 March 2017; Accepted 9 April, 2017

**ABSTRACT**

The study aimed to isolate *Gallibacterium anatis*, study its antimicrobial susceptibility pattern and investigate the prevalent rate of bacterium in local breed of female Muscovy ducks in Gombe, Northeastern Nigeria. A total of 250 samples were collected and analyzed for the study, out of which 83 were positive for *G. anatis* with an overall prevalent rate of 33.20%. The bacterium was found to be more prevalent amongst local breed of female Muscovy ducks sampled from households 48 (19.20%) compared to those from live birds market 35 (14.0%). *G. anatis* isolates were more frequently cultivated from samples of the tracheal swabs than from cloacal swabs and part of ovary samples. The prevalence of the bacterium was higher in the rainy season 68 (27.20%) than in the dry season 15 (6.0%). Isolates of *G. anatis* showed positive reactions to test with catalase, oxidase, phosphatase, sucrose and sorbitol but shows negative reactions to indole, urease, coagulase and maltose. The prevalence of biovars haemolytic *Gallibacterium anatis biovar* and non-haemolytic *Gallibacterium anatis biovar* were 8.43% and 91.57% respectively. The in-vitro antibiotic susceptibility pattern revealed that isolates were highly susceptible to Cefotaxime, moderately susceptible to Ciprofloxacin, Doxycycline and Florfenicol. In conclusion, *G. anatis* is prevalent in local breed of female Muscovy ducks in the study area. Therefore, adequate biosecurity measures should be put in place in all level of poultry production system to control the spread of the organism. The misuse of antibiotic by village poultry farmers should be discouraged to avoid drug resistance.

**Key words:** *Gallibacterium anatis*, antimicrobial susceptibility pattern, Local breed, Female Muscovy ducks, Gombe, Northeastern Nigeria

**INTRODUCTION**

Globally, the poultry industries have broadly being classified into the commercial poultry and the village poultry industries (Copland and Alders, 2005; Alders et al., 2009). Several studies have confirmed the benefits of...
village poultry production to the livelihood of mankind (Guèye, 2000; Nyoni and Masika, 2012; Mulugeta et al., 2013; Firaol et al., 2014; Opara et al., 2014; Yusuf et al., 2014). Among the significance derived from poultry production are provision of sustainable sources of high quality animal protein that ensures food security, where the poultry meat and eggs are used as food; income in form of cash to alleviate poverty from the sales of live birds and eggs and reduces rural-urban migration as it provides jobs opportunities for the youths (Dolberg, 2004; Ahuja and Sen, 2007; Sonaiya, 2009; Moges et al., 2010a, b; Alem et al., 2014; Islam et al., 2014; Yusuf et al., 2014). The major husbandry system for the village poultry provided by the farmers in rural and sub-urban areas in developing countries of Africa including Nigeria is mainly semi-intensive or extensive scavenging mixed poultry farming (Dinka et al., 2010; Augustine, 2010; Wilson, 2010; Fentie et al., 2013; Getu and Birhan, 2014). Unfortunately, this practice yields low and poor quality poultry products as well as predisposes the birds to various types of disease (Okene et al., 2011; Ochieng et al., 2012; Fentie et al., 2013; Alem et al., 2014). Otherwise, the organized large scale intensive poultry production system which provides appropriate housing, feeding, health and disease control program is considered highly expensive, capital intensive and barely unaffordable to the low income poultry farmers (Dinka et al., 2010; Mitleni et al., 2012; Talha, 2014; Tamir et al., 2015).

Meanwhile, commercial poultry production systems in Nigeria is largely dependent on chickens and the preponderance of most scientific researches, awareness campaigns, improvement programs and commercialization of poultry production has largely concentrated on the chickens while other domesticated poultry species such as ducks, pigeons, guinea fowls, turkeys and quails are utterly neglected and rarely exploited for domestic and commercial purposes (Fentie et al., 2013; Oguntunji, 2014).

The domestic ducks (Anas platyrhynchos) are one of the domesticated poultry species reared in most developed and developing countries including Nigeria (Adegunloye and Adejumo, 2014). The commercial exotic ducks rearing is not very popular in Nigeria when compared to the duck rearing systems in developed countries (Oluwayelu et al., 2007; Oguntunji, 2014; Oguntunji and Ayorinde, 2014). Various management activities which include malnutrition, inadequate housing facilities, poor veterinary health and inadequate management systems exert remarkable negative effects on the well-being and general performance of the domesticated duck flock in developing countries including Nigeria (Ugbomor, 2002; Alfred and Agbede, 2012; Sri Lestari and Siregar, 2015; Oguntunji and Ayorinde, 2014).

Although, ducks are considered to be more resistant to some diseases that may cause huge economic loses and production decline in chicken (Oluwayelu et al., 2007; Banga-Mboko et al., 2007; Adegunloye and Adejumo, 2014). They may still suffer from sub-clinical diseases and serve as reservoirs for other infectious poultry diseases (Kumar et al., 2004; Mbuthia et al., 2008; Henning et al., 2010; Adegunloye and Adejumo, 2014; Cha et al., 2014). Gallibacterium anatis which is an emerging pathogen of poultry and domiciled birds has been isolated from apparently healthy ducks in some parts of Africa (Sorour et al., 2015). The bacterium has recently been recognized as a major cause of pathological lesions in the respiratory organs and the reproductive tracts of domesticated poultry species (Christensen et al., 2003; Neubauer et al., 2009), causing diseases of the respiratory system involvement, drop in egg production in laying birds and increased mortality in infected birds (Bojesen et al., 2008). Multiple-drug resistance (Guo et al., 2009; Bojesen et al., 2011) and a substantial antigenic diversity (Vazquez et al., 2006) make it difficult to prevent the negative effects of Gallibacterium anatis using traditional antimicrobial agents and vaccine. Maintenance of strict biosecurity measures in poultry production systems remains the most reliable method to control the disease (Bojesen et al., 2003). In absence of pathognomonic lesion(s), the disease is often confused with Pasteurelosis, Salmonellosis, Newcastle disease and Bird flu (Christensen et al., 2003). The bacterium has not been reported in local breed of female Muscovy ducks in Gombe, Northeastern Nigeria. Therefore, the present study aimed to isolate, study the antimicrobial susceptibility patterns and evaluate the prevalence of Gallibacterium anatis from local breeds of female Muscovy ducks in Gombe, Northeastern Nigeria.

MATERIALS AND METHODS

Study area

This study was carried out in Gombe, the capital city of Gombe State, and Northeastern Nigeria. Gombe Township lies between Latitude 10° 08’ N and 11° 24’ E and longitude 11° 02’N and 11° 18’E of the Greenwich Meridian. The size of the town is 20,265 km², with an estimated population of 261,536 inhabitants. Gombe town is between 400-450 feet, above sea level. The climatic and edaphic factors favor crop and livestock agriculture. The occupation of most of the inhabitants is agriculture which includes food and cash crop production; village poultry, cattle, sheep and goat rearing under the extensive and semi-intensive animal husbandry management systems. The annual rainfall ranges between 850-1000 mm, with two distinct seasons. The rainy season which starts from May to October and dry season, from November-April. Average daily temperatures are 34°C in April and 27°C in August. The
relative humidity ranges from 70-80% in August and decreases to about 15-20% in December. The natural vegetation is typically that of the Sudano-Sahelian Savanna, which is composed of shrubs, herbs, grasses and sparsely distributed trees.

**Sample population**

Swab samples from the trachea and cloaca as well as part of the ovary were collected from local breeds of female Muscovy ducks reared within Gombe Township and those brought for sales/dressing at major live birds market within the study area. Information on risk factors such as age and sex of duck that may influence results in prevalence study were omitted in this study; for reasons that involved disapproval of sample collection from young and male ducks by the duck owners in the study area. Therefore, the study was modified and carried out by sampling only the adult female duck considering they are more abundant. However, information were source from the ducks farmers/owners and sellers concerning the type of management practice employed in the rearing of ducks; provision of dabbling pools and the level of biosecurity around ducks’ housing were observed and noted.

**Sample size determination**

The desired sample size for the study was calculated using the equation described by Thrusfield (2005), since the exact prevalence of *Gallibacterium anatis* in local breeds of female Muscovy ducks in the study area was not known; to maximize the sample size it was assumed that the expected prevalence was 50%, absolute precision was 5% and the confidence interval level was set to be 95% as shown below,

\[
n= \frac{1.96^2 \times pq (1 - p \ exp)}{l^2}
\]

Where, \(n\)= the required sample size, \(p\) = expected prevalence, \(q = 1 - p\); and \(l\) = absolute precision, that is the largest acceptable differences between the true and the estimated prevalence.

As a result, 250 samples were collected for the study.

**Sample collection**

During the periods of sample collections, households in which local breed of Muscovy ducks are reared and live birds markets were visited early in the morning on alternate days spread across the study period. Tracheal and cloacal swabs were collected from live female Muscovy ducks while sample of ovary were inclusively collected from slaughtered local breeds of female Muscovy ducks at the poultry dressing slabs of the selected live birds markets. Random samples were collected from five different duck flocks and two major live birds markets in the study area. Consent for sample collections was sought from the ducks farmers/owner or sellers in each sampled household and live birds market for the detection of *Gallibacterium anatis* infection. A total of 250 samples were collected which comprised of 100 tracheal swabs, 100 cloacal swabs and 50 parts of ovaries. At least 10 female Muscovy ducks were sampled from each ducks farm and 25 female ducks from each selected live bird markets of the study areas during the study periods. All samples were collected aseptically, labeled appropriately and transported in cold boxes with ice packs to the Department of Veterinary Medicine research laboratory, University of Maiduguri and the Microbiology laboratory, University of Maiduguri Teaching Hospital for microbiological analysis.

**Bacterial isolation and identification**

Swabs aseptically collected from the trachea and cloaca as well as sample of the ovary were inoculated onto a plate of blood agar base (Oxoid), supplemented with 5% citrated bovine blood and then incubated aerobically at 37°C for 24 to 48 h. The colonies of *G anatis* on blood agar appeared smooth and shiny, greyish, semi-transparent, circular slightly raised colonies with an entire margin and a butyrous consistency which is 1 to 2 mm in diameter after 24 h of incubation at 37 ºC for both the haemolytic and nonhaemolytic strains and only the haemolytic strains colonies were surrounded by a wide β-haemolytic zone (1 to 2 mm) after 24 h incubation adopting the standard protocol described by Christensen et al. (2003) and Bojesen et al. (2008). Such colonies were regarded as suspicious of *Gallibacterium*, therefore suspected colonies were further sub-cultured on blood agar to obtain pure cultures as described by Neubauer et al. (2009). Incubation was carried out at the Microbiology Laboratory, University of Maiduguri Teaching Hospital.

**Microscopic examination and Biochemical identification**

Microscopic examination and biochemical identification of *G anatis* were carried out using the standard procedures previously described by Christensen et al. (2003).

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility testing of *G anatis* isolated was performed using disc diffusion test (Oxoid, UK). The antimicrobials used were Cefotaxime, Florfenicol,
Norfloxacin, Ciprofloxacin, Gentamicin, Erythromycin, Ampicillin, Amoxicillin, Cephradine, Doxycycline, Oxytetracycline, Sulphamethoxazole + Trimethoprim, Streptomycin, Lincomycin, and Spectinomycin. All isolates were grown over night on 5% citrated sheep blood agar at 37°C in micro-aerophilic condition, then cultures were suspended in 0.85% NaCl to an optical density equivalent to that of McFarland 0.5 standards. Each isolate was then inoculated onto Mueller Hinton agar medium (Oxoid, UK), then 15 minutes later, the antimicrobial discs were applied. Plates were incubated anaerobically at 37°C for 24 h and the interpretation was performed according to the manufacturer.

**Statistical analysis**

Data generated from the study were recorded and coded using Microsoft Excel spreadsheet (Microsoft Corporation) and analyzed using GraphPad prism® version 5.01 for windows (GraphPad Software, Inc., San Diego, California, USA) computer based program. The prevalence of *G. anatis* among the sampled population was calculated as the number of positive samples divided by the total number of samples tested and was computed by percentages and Pearson’s Chi-square (χ²) test. The observed prevalence and 95% confidence intervals (CI) were evaluated, in all cases P<0.05 was considered as statistically significant.

**RESULTS**

In the present study, isolation and identification of *Gallibacterium anatis* in samples collected from local breed of female Muscovy ducks in Gombe were based on the phenotypic morphological characteristic exhibited by the colonies on blood agar plates and their biochemical reactions. Out of the total samples collected, *G. anatis* was isolated and identified from 83/250 (33.20%) samples which exhibited the entire phenotypic characteristic consistent with those of the bacterium. The cumulative incidence (CI%) of the bacterium in the infected samples was also 83/250 (33.20%), while the risk of *G. anatis* infection among the infected ducks sampled was 0.5 times compared to the uninfected ducks (Table 1). Table 2 shows the results of *G. anatis* isolation from local breed of female Muscovy ducks based on the study locations. Out of the 150 samples collected in live birds markets, 35 (23.33%) of the samples showed phenotypic characteristics consisted with *G. anatis*, with a prevalence rate of 14.0%. Out of the 100 samples collected from female Muscovy ducks in households, 48 (48.0%) were positive for *G. anatis*, with a prevalence rate of 19.20%. There was significant statistical difference (P= 0.0051 at 95% confidence interval) between the prevalence rate of *G. anatis* isolated from samples collected from the live birds markets and those collected from female Muscovy ducks in households in the study area. The risk of *G. anatis* infection among the infected female Muscovy ducks sampled from live birds markets and those samples from households was 0.30 and 0.92 times compared to the uninfected ducks respectively.

Table 3 shows the result of isolation of *G. anatis* from local breed of female Muscovy ducks based on the type of samples collected. The bacterium was more prevalent in the tracheal swabs collected from female Muscovy ducks sampled from households 31 (12.40%) and those from the live birds markets 18 (7.20%) compared to isolation from cloacal swabs from households 17 (6.8%) and live birds markets 10 (4.0%). The isolation of *G. anatis* from the ovaries 7 (2.80%) of local breed of female Muscovy ducks collected from live birds markets was the least prevalent. Table 4 shows the results of distribution of *Gallibacterium anatis* isolated from local breeds of female Muscovy ducks based on season of samples collection. The bacterium is more frequently isolated in samples collected during the rainy season 68 (27.20%) compared to those collected in the dry season 15 (6.0%). There was significant statistical difference (P < 0.0001 at 95% confidence interval) between the samples collected during the two seasons. The risk of *G. anatis* infection among the infected ducks sampled was 0.88 times compared to the uninfected ducks during the rainy season. The risk of the bacterium infection among the infected ducks sampled was 0.17 times compared to the uninfected ducks during the dry season.

Table 5 shows the result of haemolytic characteristics of isolated serovars of *Gallibacterium anatis* from local breed of female Muscovy ducks on blood agar, which revealed that the non-haemolytic strain of *G. anatis* 76 (91.57%) was more frequently isolated than the haemolytic strains 7 (8.43%). The non-haemolytic strain of *Gallibacterium anatis* was more frequently isolated from swabs samples collected from the trachea 44 (53.01%), followed by samples from the cloaca 26 (31.33%) and ovaries 6 (7.23%). The haemolytic strain of the organism in the present study was also isolated from the swab of the trachea 5 (6.02%) followed by swab samples from the cloaca 1 (1.20%) and ovaries 1 (1.20%).

Table 6 shows the results of the biochemical identification test for *G. anatis* isolated from local breeds of female Muscovy ducks in the present study, which revealed that the isolated organisms showed positive reactions to test with catalase, oxidase, phosphatase, sucrose and sorbitol, but demonstrated negative reactions to indole, urease, coagulase and maltose. Table 7 shows the result of *in-vitro* antimicrobial susceptibility pattern of the isolated *G. anatis* to 15 different Antimicrobials which revealed that the isolated bacteria were highly susceptible to Cefotaxime, moderately susceptible to Ciprofloxacin, Doxycycline and Florfenicol, as well as fairly susceptible to Gentamycin
Table 1. Overall prevalence of *Gallibacterium anatis* isolated from local breeds of female Muscovy ducks in Gombe, Northeastern Nigeria.

<table>
<thead>
<tr>
<th>Study Area</th>
<th>Number of positive samples (CI %)</th>
<th>Number of negative samples (CI %)</th>
<th>Total samples collected</th>
<th>Risk Ratio</th>
<th>Prevalence rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gombe</td>
<td>83/250 (33.20)</td>
<td>167/250 (66.80)</td>
<td>250</td>
<td>0.497</td>
<td>33.20</td>
</tr>
</tbody>
</table>

KEY: (CI %) = Cumulative Incidence of infected and uninfected duck sampled; RR = Risk ratio (CI% infected ducks CI% uninfected ducks).

Table 2. Isolation of *Gallibacterium anatis* from local breeds of female Muscovy ducks according to sampling location.

<table>
<thead>
<tr>
<th>Study location</th>
<th>Number of positive samples (CI %)</th>
<th>Number of negative samples (CI %)</th>
<th>Total samples collected</th>
<th>Risk Ratio</th>
<th>Prevalence rate (%)</th>
<th>95% CI</th>
<th>P-value</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live birds market</td>
<td>35 (23.33)</td>
<td>115 (76.67)</td>
<td>150</td>
<td>0.304</td>
<td>14.0</td>
<td>0.7471–0.8646</td>
<td>0.0051</td>
<td>1.2000</td>
</tr>
<tr>
<td>Households</td>
<td>48 (48.0)</td>
<td>52 (52.0)</td>
<td>100</td>
<td>0.923</td>
<td>19.20</td>
<td>0.5941–0.7500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>83 (33.20)</td>
<td>167 (66.80)</td>
<td>250</td>
<td>0.497</td>
<td>33.20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

KEY: RR = Risk ratio (CI% infected ducks CI% uninfected ducks); L – U = Lower limit and Upper limit 95% Confidence interval; p≤0.05 was considered as significant.

Table 3. Isolation of *Gallibacterium anatis* from local breeds of female Muscovy ducks according to type of samples collected.

<table>
<thead>
<tr>
<th>Study location</th>
<th>Type of samples</th>
<th>Number of samples collected (y)</th>
<th>Number of positive samples (x) (x/y)</th>
<th>Prevalence rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live birds market</td>
<td>Tracheal swabs</td>
<td>50</td>
<td>18 (36.0)</td>
<td>7.20</td>
</tr>
<tr>
<td></td>
<td>Cloacal swabs</td>
<td>50</td>
<td>10 (20.0)</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Part of Ovaries</td>
<td>50</td>
<td>7 (14.0)</td>
<td>2.80</td>
</tr>
<tr>
<td>Households/farms</td>
<td>Tracheal swabs</td>
<td>50</td>
<td>31 (62.0)</td>
<td>12.40</td>
</tr>
<tr>
<td></td>
<td>Cloacal swabs</td>
<td>50</td>
<td>17 (34.0)</td>
<td>6.80</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>250</td>
<td>83 (33.20)</td>
<td>33.20</td>
</tr>
</tbody>
</table>

and Norfloxacin, but were completely resistant to Erythromycin, Cephradin, Oxytetracycline, Sulpha + Trimethoprim, Streptomycin, Amoxicillin, Ampicillin, Lincomycin and Spectinomycin.

**DISCUSSION**

The present study focused on diagnosis of *Gallibacterium anatis* infection based on the phenotypic characteristics of the isolated organism on blood agar and a flock was considered infected when just one duck in the flock is tested positive for the organism. *Gallibacterium anatis* is an emerging globally reported bacterial pathogen mainly affecting the respiratory tract and reproductive organs of domesticated poultry species, semi-domesticated and wild domiciled birds that have attracted concerns of poultry veterinarian around the world (Singh et al., 2016), with main lesions reported in the ovary and oviduct leading to a drop in egg production (Bojesen et al., 2007). The disease have been frequently isolated from chickens as well as a wide range of other domestic birds including turkeys, geese, ducks, pheasants, partridges and cattle egrets (Christensen et al., 2003; Rzewuska et al., 2007; Bisgaard et al., 2009; Gregersen et al., 2010; Sorour et al., 2015). To the best of our knowledge this is the first isolation report of *Gallibacterium anatis* in apparently healthy local breed of female Muscovy ducks in the study area with an overall prevalence rate of 33.20%. This finding is higher than 26.0% and 23.43% reported by Sorour et al. (2015) and Abd El-Hamid et al. (2016) who also reported the prevalence of *G. anatis* in diseased ducks and chickens from Egypt respectively. However, our finding is absolutely lower than 96% reported from free range chickens from Denmark by Bojesen et al. (2003). The difference may be associated with variation in geographical regions and type of sample collected; prevalence proportions may be highly influenced by the birds production system, the biosecurity level observed in the flocks and whether the disease in the examined birds have been reportedly considered as pathogen or normal bacterial flora of the host. Bojesen et al. (2003) previously
Table 4. Distribution of *Gallibacterium anatis* isolated from local breeds of female Muscovy ducks according to season.

<table>
<thead>
<tr>
<th>Season of sample collection</th>
<th>Number of samples collected</th>
<th>Number of samples positive (CI%)</th>
<th>Number of samples negative (CI%)</th>
<th>Risk Ratio</th>
<th>Prevalence rate (%)</th>
<th>95% CI L – U</th>
<th>P-value</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainy</td>
<td>125</td>
<td>68 (54.40)</td>
<td>57 (61.60)</td>
<td>0.883</td>
<td>27.20</td>
<td>0.5763-0.7152</td>
<td>&lt;0.0001</td>
<td>0.7254</td>
</tr>
<tr>
<td>Dry</td>
<td>125</td>
<td>15 (12.0)</td>
<td>110 (72.0)</td>
<td>0.167</td>
<td>6.0</td>
<td>0.8295-0.9387</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td>83 (33.20)</td>
<td>167 (66.80)</td>
<td>0.497</td>
<td>33.20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

KEY: RR = Risk ratio (CI% infected ducks / CI% uninfected ducks); L – U = Lower limit and Upper limit, 95% Confidence interval; p ≤0.05 was considered as significant.

Table 5. Haemolytic characteristics of isolated serovars of *Gallibacterium anatis* from female Muscovy ducks on blood agar.

<table>
<thead>
<tr>
<th>Type of Samples collected</th>
<th>Number of Positive samples tested (n = 83)</th>
<th>Type of <em>Gallibacterium</em> biovar isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Haemolytic (%)</td>
</tr>
<tr>
<td>Tracheal swabs</td>
<td>49</td>
<td>5 (6.02)</td>
</tr>
<tr>
<td>Cloacal swabs</td>
<td>27</td>
<td>1 (1.20)</td>
</tr>
<tr>
<td>Part of Ovaries</td>
<td>7</td>
<td>1 (1.20)</td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>7 (8.43)</td>
</tr>
</tbody>
</table>

Table 6. Biochemical identification for *Gallibacterium anatis* isolated from local breeds of female Muscovy ducks.

<table>
<thead>
<tr>
<th>Biochemical Test</th>
<th>Number of samples tested (n=83)</th>
<th>Number of sample positive (%) (n=83)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>83</td>
<td>83</td>
</tr>
<tr>
<td>Indole</td>
<td>83</td>
<td>0</td>
</tr>
<tr>
<td>Urease</td>
<td>83</td>
<td>0</td>
</tr>
<tr>
<td>Oxidase</td>
<td>83</td>
<td>83</td>
</tr>
<tr>
<td>Coagulase</td>
<td>83</td>
<td>0</td>
</tr>
<tr>
<td>Phosphatase</td>
<td>83</td>
<td>83</td>
</tr>
<tr>
<td>Maltose</td>
<td>83</td>
<td>0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>83</td>
<td>83</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>83</td>
<td>83</td>
</tr>
</tbody>
</table>

Table 7. Antimicrobial susceptibility of *Gallibacterium anatis* isolated from local breeds of female Muscovy ducks.

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Degree of Antimicrobial susceptibility of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamycin (CN- 10 μg)</td>
<td>+</td>
</tr>
<tr>
<td>Erythromycin (E- 10 μg)</td>
<td>-ve</td>
</tr>
<tr>
<td>Amoxycillin (AML -30 μg)</td>
<td>-ve</td>
</tr>
<tr>
<td>Cefotaxin (CTX- 30μg)</td>
<td>+++</td>
</tr>
<tr>
<td>Florfenicol (FFC- 30μg)</td>
<td>++</td>
</tr>
<tr>
<td>Norfloxacin (NOR- 10 μg)</td>
<td>+</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP – 5 μg)</td>
<td>+</td>
</tr>
<tr>
<td>Oxytetracycline (OT-30μg)</td>
<td>-ve</td>
</tr>
<tr>
<td>Doxycycline (DO- 30μg)</td>
<td>++</td>
</tr>
<tr>
<td>Sulpha.+Trimethoprim (SXT - 25 μg)</td>
<td>-ve</td>
</tr>
<tr>
<td>Streptomycin (S- 30 μg)</td>
<td>-ve</td>
</tr>
<tr>
<td>Lincomycin (MY – 30 μg)</td>
<td>-ve</td>
</tr>
<tr>
<td>Spectinomycin (SH - 100)</td>
<td>-ve</td>
</tr>
<tr>
<td>Ampicillin (AMP - 10 μg )</td>
<td>-ve</td>
</tr>
<tr>
<td>Cephradine (CE - 30 μg)</td>
<td>-ve</td>
</tr>
</tbody>
</table>

KEY: +++ = Highly susceptible; ++ = Moderately susceptible; + = Fairly susceptible; -ve = Completely resistant.

mentioned that *G. anatis* is a common part of the normal flora of both the upper respiratory tract and lower genital tract of many avian species. But Neubauer *et al.* (2009) reported isolation of *Gallibacterium* in pure cultures of samples from birds with various pathological lesions. The isolation of *G. anatis* from apparently healthy local breed
Figure 1. Extensively reared local breeds of Muscovy ducks scavenging in unhygienic pool of water.

Figure 2. Constructed dabbling pool for local breed of Muscovy ducks (water may be contaminated by pathogens).
of female Muscovy ducks in this study is consistent with those of Addo and Mohan (1985) who reported the isolation of the bacterium from apparently healthy domesticated fowl (*Gallus domesticus*) in Nigeria. Moreover, Sorour *et al.* (2015) in a previous study reported predominantly high prevalence rate of *Gallibacterium anatis* in ducks than in different classes of chickens. The customary extensive management system practiced in rearing Muscovy ducks where ducks usually dabble in unhygienic pool of water (Figures 1, 2 and 3) in the study area may possibly expose this species of birds to several bacterial diseases including *G. anatis*. This observation agrees with the finding of Bojesen *et al.* (2003) who reported *G. anatis* in free range scavenging domestic fowls and the high prevalent rate has been attributed to exposure to contaminated environment with poor biosecurity.

From the result of the present study, *G. anatis* was more frequently isolated from samples collected from local breed of female Muscovy ducks in households 48 (19.20%) compared to in ducks sampled from the live birds markets 35 (14.0%) in the study area. And there was significant statistical difference ($P = 0.0051$ at 95% confidence interval) between the prevalence rate of *G. anatis* and the two sampling locations. This signifies that free range local breed of Muscovy ducks are more likely to get expose to the organism compared to those reared in a cages, even though most of the Muscovy ducks sold in live birds markets are usually source from households of nearby settlements. This finding is in agreement with a previous study of Bojesen *et al.* (2003) and Persson and Bojesen, (2015) who also recorded high prevalence of *G. anatis* in extensively reared scavenging birds compared to those reared in a cages and secured environment where adequate biosecurity is maintained. Moreover, the isolation of *G. anatis* in local breed of female Muscovy ducks from live birds markets may not be unexpected, because there are no discriminations of health status or screening for diseases before mixing of different poultry species in live birds markets (Figure 4).

The finding of our research revealed more frequent isolation of *G. anatis* from tracheal swabs collected from local breed of female Muscovy ducks sampled from households and live birds markets, compared to the
frequency of isolation of the bacterium from cloaca l swabs from both study locations while the bacterium was least isolated from the ovary in the present study. Since our samples were solely collected from apparently healthy local breed of female Muscovy ducks it is suggested that the tracheal followed by the cloacal region might be the more favorable predilection site of the bacterium while the ovary might be considered the least preferred predilection site for the bacterium. This finding is in agreement with a previous study of Paudel et al. (2013, 2014) and Sorour et al. (2015) who reported natural cases of the organism mainly targeting the upper respiratory and lower reproductive tract of birds. Our finding also buttress previous report by Bojesen et al. (2003) and Neubauer et al. (2009) who in a similar study reported a significantly higher number of tracheal swabs positive for G. anatis compared with the corresponding cloacal swabs.

The present study have revealed seasonal prevalence of the bacterium in the study area, G. anatis being more frequently isolated in samples collected during the rainy season compared to samples collected during the dry season. There was significant statistical difference ($P < 0.0001$ at 95% confidence interval) between the prevalent rate of the bacterium and the season. However, the risk of G. anatis infection among the infected ducks sampled was 0.88 and 0.17 times when compared to the uninfected ducks during the rainy and dry season respectively. This suggested that local breed of female Muscovy ducks will be more likely get exposed to G. anatis infection from contaminated environment during the raining season compared to the dry season. This finding may be associated with the abundance pool of stagnant water during the rainy season compared to its availability during the dry season, which may serve as dabbling pools for scavenging ducks, such pool of stagnant water may be contaminated with various pathogens including G. anatis. This finding is in agreement with those of Malik et al. (2005) who also reported variation in season to be one of the major factors that influence the increased in the susceptibility of domesticated poultry to infection by G. anatis. Moreover, there are several researches that have reported significantly higher prevalent rate of bacterial diseases in poultry species during the rainy season compared to the dry season (Mbuko et al., 2009; Yunus et al., 2009; Zdragas et al., 2012; Balami et al., 2014; Soo-Kyoung et al., 2016).

The finding of the present study also revealed that G. anatis isolated from local breeds of female Muscovy ducks in the study area shows positive reactions to test with catalase, oxidase, phosphatase, sucrose and
sorbitol, however, demonstrate negative reactions to indole, urease, coagulase and maltose. This finding agrees with those of Christensen et al. (2003) and Bojesen et al. (2007) who also reported similar reactions of G. anatis isolates which indicated that all typical G. anatis strains are catalase, oxidase, and phosphatase positive, and they can reduce nitrate. Christensen et al. (2003) and Bojesen et al. (2007) previously clarified that Gallibacterium genus can be differentiated from other genera of Pasteurellaceae with catalase, symbiotic growth, hemolysis, urease, indole, acid production from (+) D-xylose, (-) D-mannitol, (-) Dsorbitol, (+) D-mannose, maltose, raffinose and dextrin tests.

The identification of Gallibacterium organism and their classification into the two basic biovar in the present study relied on the type of phenotypic characteristics exhibited by the inoculated samples on bovine blood agar plates, which at the time of the study was the only detection method available. The finding of our research revealed that the non-haemolytic strain of Gallibacterium was predominantly isolated from pure culture of the positive samples compared to the haemolytic strain. It was noticed that the non-haemolytic strain of Gallibacterium was more frequently isolated from the tracheal swab followed by the cloaca and ovary in descending order of frequency.

This indicated that the non-haemolytic G. anatis is the most naturally abundant strain of the bacterium among free range Muscovy ducks in the study area. This finding is consistent with those of Sorour et al. (2015) who also reported significantly higher prevalence of non-haemolytic Gallibacterium anatis biovar anatis (69.2%) in duck compared to haemolytic Gallibacterium anatis biovar haemolytica (30.7%). However, our result is not consistent with those of Bojesen et al. (2003) who have reported the predominance of haemolytic Gallibacterium species in commercial chicken production systems.

The in-vitro susceptibility pattern of G. anatis isolates to 15 different antimicrobials in the present study revealed that these isolates of bacterium local breeds of female Muscovy ducks were highly susceptible to Cefotaxime, moderately susceptible to Ciprofloxacin, Doxycycline and Florfenicol, as well as fairly susceptible to Gentamycin and Norfloxacin, but were completely resistant to Erythromycin, Cephradin, Oxytetracycline, Sulpha + Trimethoprim, Streptomycin, Amoxicillin, Ampicillin, Lincomycin and Spectinomycin. The results of the present in-vitro antibiotic susceptibility pattern of G. anatis strains supported previous finding of Abd El-Hamid et al. (2016) who also reported high susceptibility of G. anatis isolates to Cefotaxime. On the other hand, the finding of the present study is also in agreement with Bojesen et al. (2011) and Abd El-Hamid et al. (2016) who also reported resistance of G. anatis isolates to Tetracycline and Sulfamethoxazole-Trimethoprim for the field strains. Bojesen et al. (2007) and Guo (2011) in a similar study previously also reported that all G. anatis isolates were susceptible to Amoxicillin-Clavulanic acid, Apramycin, Cefpodoxime, Cefitfur, Cephalotin, Chloramphenicol, Colistin, Florfenicol, Gentamycin, Spectinomycin, Streptomycin, Erythromycin, Tiamulin and Tilmicosin but their isolates were resistant to tetracycline, Sulfamethoxazole, Ciprofloxacin and Nalidixic acid. Chuan-qing et al. (2008) confirmed that all G. anatis isolates were highly sensitive to the third generation cephalosporin in antimicrobial resistance testing.

Elbestawy, (2014) reported a complete resistance of G. anatis isolates against 5 different antimicrobials; Oxytetracycline, Sulphamethoxazol + Trimethoprim, Streptomycin, Lincomycin and Spectinomycin and the isolates were sensitive to Chloramphenicol. Also, Janda, (2011) and Jones et al. (2013) agreed that G. anatis is a phylogenetically diverse organism from the family Pasteurellaceae and this can be supported by the fact that all isolates were resistant to Oxytetracyclin, Sulphamethoxazol-Trimethoprim and Streptomycin which is the commonly used antibiotics administered to domesticated poultry species for the treatment of Pasteurellaceae like infections.

Conclusion

Gallibacterium anatis is prevalent among the local breed of female Muscovy ducks reared in the study area, with the non-haemolytic strain being the predominant strain of the bacterium which was more frequently isolated from the trachea compared to the cloaca and ovary. The occurrence of the bacterium in swabs samples collected from apparently healthy adult female Muscovy ducks is attributed to natural infection probably from contaminated environment since there was no previous report of serious outbreak of disease caused by the bacterium in the study area. Although, ducks are considered hardy and they tend to tolerate most diseases that may cause serious lose in other poultry species. However, there may be the possibility of the organism causing mild form of disease in the infected birds without visible clinical signs. The bacterium may occur in both the rainy and dry season, but more frequently in the rainy season which was attributed to abundance of probably contaminated stagnant pool of water in the surroundings in which free range Muscovy ducks dab. Also, the unhygienic scavenging nature of Muscovy ducks might be considered as the most predisposing factor of the diseases transmission among free range Muscovy ducks. The indiscriminate mixing of several poultry species in live birds local markets might also contribute to the horizontal transmission of the organism. The in-vitro antimicrobial susceptibility of the isolated bacterium has demonstrated multidrug resistance, but susceptible to a few ones, this suggested that the bacterium can be treated with some antimicrobial chemotherapy.
The presence of the bacterium should be suspected in typical bacterial infections of poultry, especially where there is multidrug resistance to treatment with antibiotics. Isolation of the organism should be attempted in poultry diseases associated with uncertain clinical signs. To control disease transmission to susceptible birds, it is recommended that strict biosecurity measures should be maintained in all levels of poultry production systems. Molecular researches involving genotypic characterization of G. anatis in several poultry species and other geographical location should be conducted in Northern Nigeria.

ACKNOWLEDGMENTS

The authors wish to thank the Head, Department of Veterinary Medicine, University of Maiduguri and staff of the Postgraduate Research Laboratory of the Department of Veterinary Medicine and Veterinary Microbiology of University of Maiduguri for their support and technical guidance. We also wish to appreciate Dr. Abubakar Danjuma and entire staff of Microbiology Laboratory, University of Maiduguri Teaching Hospital for their technical assistance.

REFERENCES


Adegunloye DV, Adejumo FA (2014). Microbial Assessment of Turkey (Meleagris ocellata L) and Duck (Anas platyrhynchos L) Faeces (Droppings) in Akure Metropolis. Advances in Microbiology, 4, 774 – 779.


Guéye EF (2000). The role of family poultry in poverty alleviation, food


