

Prevalence, isolation and antimicrobial susceptibility of *Gallibacterium anatis* from Local Breed of Female Muscovy Ducks (*Cairina moschata*) in Maiduguri, Northeastern Nigeria

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ABSTRACT: The present study was carried out from the month of March to December, 2016 and aimed to isolate and investigate the prevalence of *Gallibacterium anatis* infection as well as to study the antimicrobial susceptibility pattern of the bacterium from extensively reared local breed of female Muscovy ducks in Maiduguri, Northeastern Nigeria. To accomplish this, a total of 250 samples (100 tracheal swabs, 100 cloacal swabs and 50 part of ovary) were collected from households where Muscovy ducks are reared and female Muscovy ducks from live birds market. Microbiological isolation and biochemical identification of the phenotypic characteristic consistent with *G. anatis* was used for the diagnosis of a positive sample and this revealed 75/250 (30.0%) positive isolates of the bacterium. The *G. anatis* was more frequently isolated from samples collected from house hold Muscovy ducks 51 (20.40%) than those from the live birds market 24 (9.60%) with a significant difference ($P < 0.0001$ at 95% CI; RR= 1.302). However, isolates were more frequently cultivated in samples of the tracheal swab 49 (19.60%) than those swabs collected from the cloaca 24 (9.60%) and ovary 2 (0.80%). *G. anatis* was discovered to be more frequent in the rainy season 51 (20.40%) when compared to the dry season 24 (9.60%) with a significant difference ($P = 0.0080$ at 95% CI; RR = 0.8466). Moreover, isolates revealed positive reactions to test with catalase, oxidase, phosphatase, sucrose and sorbitol, but show negative reactions to indole, urease, coagulase and maltose. The biochemical investigations differentiated the isolated strains into two biovars; haemolytic *Gallibacterium anatis* biovar 3 (4.0%) and a non-haemolytic *Gallibacterium anatis* biovar 72 (96.0%). Antimicrobial susceptibility test revealed multi-drug resistant of the *Gallibacterium anatis* isolated. The in-vitro antibiotic susceptibility testing revealed that isolates were highly susceptible to Cefotaxime, moderately susceptible to Ciprofloxacin, Doxycycline and Florfenicol. In conclusion, *G. anatis* is prevalent in extensively reared local breed of Muscovy ducks in the study area. Therefore, strict biosecurity measures should be practiced at all level of poultry production systems to curb the spread of the organism. Antimicrobial abuse should be avoided by poultry farmers and the guidance of a registered veterinarian should be sought whenever there is need for medications to avoid misuse and drug resistance.

Key words: Antimicrobial susceptibility pattern, *Gallibacterium anatis*, Maiduguri, Muscovy ducks, Northeastern Nigeria, prevalence.

INTRODUCTION

Poultry production forms an integral part of many rural families in developing countries of the world (Mwale and

Masika, 2009; Fentie et al., 2013; Angyiereyiri et al., 2015). Poultry production in Nigeria is largely dependent on the

exotic breeds of chickens, and the preponderance of scientific researches, vaccination awareness campaigns, improvement programmes and commercialization of poultry production are largely concentrated on exotic and village chickens while the other indigenous available poultry species such as guinea fowl, duck, turkey and pigeons are utterly neglected and rarely exploited for domestic and commercial purposes (Oguntunji, 2014). Generally, village poultry species reared in developing countries including Nigeria are given less attentions in terms of management system, feeding, housing and veterinary care, which can lead to low production and prevalence of diseases claiming substantial proportion of the flock among others (Oguntunji and Ayorinde, 2015). Dearth of researches on immediate factors that are responsible for declined relevance of duck, the management practices, mortality, constraints to accelerated duck production etc. are detrimental to the anticipated increased production of this waterfowl in Nigeria (Oguntunji and Ayorinde, 2015). Despite the fact that ducks are easily managed under village conditions, particularly if a waterway is nearby, and unlike the chickens, they are considered to be more resistant to some diseases that may cause huge loss and production decline in poultry production (Oluwayelu et al., 2007; Adegunloye and Adejumo, 2014). Nevertheless, ducks may suffer sub-clinical diseases, serve as reservoir of infectious diseases and also play a significant role in the maintenance and transmission of disease to other susceptible poultry species (Henning et al., 2010; Adegunloye and Adejumo, 2014; Cha et al., 2014). However, ducks do suffer from some diseases, mainly those traceable to mismanagement resulting from poor diet, stagnant unhygienic drinking water, mouldy feed, unhygienic bedding or overcrowded and filthy conditions (Kumar et al., 2004; Mbuthia et al., 2008). *Gallibacterium anatis* has been isolated from apparently healthy ducks in some parts of Africa (Sorour et al., 2015). *Gallibacterium anatis* infection is an emerging disease of poultry (Singh et al., 2016). The increasing global concern about *G. anatis* is the incomplete understanding of its growth kinetics, virulence markers, pathogenesis and vaccine(s) to control. *Gallibacterium anatis* (earlier known as *Pasteurella anatis*) is a commensal in upper respiratory tracts and the lower genital tracts of healthy chickens (Mushin et al., 1980). It has been reported to be associated with bacteremia, oophoritis, follicle degeneration, salpingitis, peritonitis, hepatitis, enteritis and respiratory tract diseases in chickens (Aarestrup et al., 2004; Jordan et al., 2005; Kristensen et al., 2011). *Gallibacterium anatis* mostly affects intensively farmed poultry birds causing loss in production with heavy mortality in broiler chicken and drop in egg production in layers with increased mortality (Bojesen et al., 2008). *Gallibacterium anatis* has also been reported to infect turkeys, geese, ducks, pheasants, partridges, budgerigars, peacock, cage birds, wild birds, cattle and pig (Christensen et al., 2003; Rzewuska et al.,

2007; Bisgaard et al., 2009; Gregersen et al., 2010). The bacterium has been reported to be associated with fatal bacteremia in immune-compromised human patient (Aubin et al., 2013). Poultry diseases caused by *Gallibacterium anatis* has been reported from all continents (Christensen et al., 2003; Bojesen et al., 2007). Its association with a variety of pathology makes it difficult to be diagnosed even after post-mortem in absence of pathognomonic lesion(s) and the disease is often confused with Fowl Coryza, New Castle disease and Bird Flu (Christensen et al., 2003).

Though the infection of *G. anatis* is treatable with antibiotics, the frequency of treatment failure is an emerging and recurrent problem. Multidrug resistant strains of *G. anatis* (Aarestrup et al., 2004; Bojesen et al., 2011) have shown resistance to sulpha drugs, novobiocin, tylosin, clindamycin, tetracycline and penicillin (Malik et al., 2005; Berge et al., 2006; Hendriksen et al., 2008; Guo et al., 2009; Johnson et al., 2011; Jones et al. 2013). Concerns have been shown for biosecurity measures towards control of disease, handling of pathogen and prevention of spread. *Gallibacterium anatis* has not been reported in local breed of the Muscovy ducks in Maiduguri, Northeastern Nigeria. Therefore, this present study was designed to isolate, determine the prevalence and antimicrobial susceptibility patterns of *Gallibacterium anatis* from local breeds of female Muscovy ducks in Maiduguri, Northeastern Nigeria.

MATERIALS AND METHODS

Study area

This study was conducted in Maiduguri, the capital and largest city of Borno State, Nigeria, located within the Sahel savannah zone of the Northeastern Nigeria. It lies approximately between Latitude 11° 5' and 11.83° N and Longitude 13° 09' and 13.50° E at about 350 m (1161 ft) above sea level with an ambient temperature range of 32 to 45°C (http://www.unimaid.edu.ng/About_Maid.aspx). The climate is hot and dry for a greater part of the year with a rainy season from June to September in the Northern part and May to October in the Southern part with a mean annual rainfall of about 650 mm. The mean relative humidity of Maiduguri ranges from 30 to 50% with the minimum been experienced in the months February and March when it drops to as low as 10% and reaches maximum in August when it rises to as high as 90% (http://www.unimaid.edu.ng/About_Maid.aspx).

Sample Population

Swab samples from the trachea and cloaca as well as ovary samples were collected from extensively reared local breeds of female Muscovy ducks within Maiduguri metropolis and those brought for sales/dressing at major

live birds market in the study area. Information on factors such as age and sex that seem to influence results in prevalence study were not included in this study; this is because of the challenges faced with consent for sampling from the duck owners in the study area. Information concerning type of management system employed in the rearing of ducks; availability of swimming ponds and the level of biosecurity around duck shelter 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 extensively reared local breeds of female Muscovy ducks in the study area was not known; so 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:

$$n = \frac{1.962 \times pq (1 - p_{exp})}{l^2}$$

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 study populations were selected for the sampling area.

Sample collection

During the periods of sample collections, village poultry farms in which ducks were also reared and live birds markets were visited on alternate days of the study period. Swabs samples were collected from the trachea and cloaca of live female 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. Samples were collected from five (5) different households rearing large numbers of female Muscovy ducks in a flock and two (2) live birds markets in the study area. Consent for sample collections was sought from the duck farmers/owner or sellers in each sampled farm/live birds market within Maiduguri metropolis for the detection of *Gallibacterium anatis* infection. Two hundred and fifty (250) samples were collected which comprised of One hundred (100) tracheal swabs, One hundred (100) cloaca swabs and Fifty (50) sample of ovary. At least Ten (10) female ducks were sampled from each ducks farm and Twenty five (25) female ducks from each selected live bird markets of the study areas during the study periods. All samples collected were labeled appropriately and transported to the Department of Veterinary Medicine Research Laboratory,

University of Maiduguri and the Microbiology laboratory, University of Maiduguri Teaching Hospital for processing and culturing.

Bacterial isolation and identification

Tracheal and cloacal swabs as well as sample of ovary were inoculated onto a plate of blood agar base (Oxoid), supplemented with 5% citrated bovine blood and incubated aerobically at 37°C for 24 to 48 hours. 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 hours of incubation at 37°C for both the haemolytic and non-haemolytic strains and only the haemolytic strains colonies were surrounded by a wide β-haemolytic zone (1 to 2 mm) after 24 hours 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).

Microscopic examination and Biochemical identification

Microscopic examination revealed Gram negative, rod-shaped or pleomorphic, non-motile characteristic of *G. anatis* as previously described by Christensen et al. (2003). Biochemical identification of *G. anatis* isolates showed catalase and oxidase positive, indole and urease negative.

Antimicrobial susceptibility testing

Antimicrobials susceptibility testing of *G. anatis* isolated was performed using disc diffusion test (Oxoid, UK). The antimicrobials used include Cefotaxime, Florfenicol, Norfloxacin, Ciprofloxacin, Gentamycin, Erythromycin, Ampicillin, Amoxicillin, Cephadrine, Doxycycline, Oxytetracycline, Sulphamethoxazole + Trimethoprim, Streptomycin, Lincomycin, and Spectinomycin. All isolates were cultured overnight on 5% citrated sheep blood agar at 37°C in micro-aerophilic condition. Then, the 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 hours and the interpretation was done according to the manufacturer.

Data Analysis

Data generated were entered into Microsoft Office Excel

Table 1. Overall prevalence of *Gallibacterium anatis* isolated from local breeds of female Muscovy ducks in Maiduguri, Northeastern Nigeria.

Number of positive samples (CI %)	Number of negative samples (CI %)	Total samples collected	Risk Ratio	Prevalence rate (%)
75/250 (30.0)	175/250 (70.0)	250	0.429	30.0

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.

Study location	Number of positive samples (CI%)	Number of negative samples (CI%)	Total samples collected	Risk Ratio	Prevalence rate (%)	95% CI L – U	P - value	Relative Risk
Live birds market	24 (16.0)	126 (84.0)	150	0.190	9.60	0.8021 – 0.9095	P < 0.0001	1.302
Households	51 (51.0)	49 (49.0)	100	1.041	20.40	0.5810 – 0.7374		
Total	75 (30.0)	175 (70.0)	250	0.429	30.0			

RR, Risk ratio (CI% infected ducks ÷ CI% uninfected ducks); L – U, Lower limit and Upper limit 95% Confidence interval; $p \leq 0.05$ was considered as significant.

spread sheet, Risk Ratios (RR) and 95% CI on the Relative Risk (RR) were calculated using the Fisher's exact test to determine strength and significance of associations between the seasons and infection as well as location of sample collection and infection from sampled ducks. The prevalence of *G. anatis* among the sampled population was calculated using frequencies and percentages in GraphPad prism® version 5.01 for windows (GraphPad Software, Inc., San Diego, California, USA) computer based program. The observed prevalence and 95% confidence intervals (CI) were evaluated and "P" values equals to or less than 0.05 were regarded significant.

RESULTS

The isolation of *Gallibacterium anatis* from the samples collected from extensively reared local breed of female Muscovy ducks in Maiduguri 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 75/250 (30.0%) 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 75/250 (30.0%) (Table 1).

Considering the isolation of *G. anatis* from apparently healthy local breed of Muscovy ducks based on the study locations where samples were collected, out of the 150 samples collected in Maiduguri live birds markets, 24 (16.0%) of the samples showed phenotypic characteristics consisted with *G. anatis*, with a prevalence rate of 9.60%. However, out of the 100 samples collected from

households/duck farms in Maiduguri, 51 (48.0%) exhibited phenotypic characteristics consisted with those of *G. anatis*, with a prevalence rate of 20.40%. There was statistical significant difference ($P < 0.0001$ at 95% confidence interval) between the prevalence rate of *G. anatis* isolated from samples collected from the live birds markets and those collected from households/duck farms in the study area. The risk of *G. anatis* infection among the infected ducks sampled from live birds markets and ducks farms was 0.19 and 1.04 times compared to the uninfected ducks respectively (Table 2).

The result of isolation of *G. anatis* from apparently healthy extensively reared local breed of female Muscovy ducks from the study areas based on the type of samples collected, revealed that the bacteria was more frequently isolated in samples of the tracheal swabs collected from the live birds markets 14 (5.60%) and household/duck farms 35 (14.0%) in Maiduguri when compared to isolation of *G. anatis* from the cloacal swabs collected from live birds markets 8 (3.20%) and duck farms 16 (6.4%). The isolation of *G. anatis* from the ovaries were the least frequent in the samples collected from the live birds markets in the study area 2 (0.80%) (Table 3).

The results of the distribution of *G. anatis* isolated from local breeds of female Muscovy ducks according to season of sample collection revealed that, the bacterium is more frequently isolated in samples collected during the rainy season 51 (20.40%) when compared to those collected during the dry season 24 (9.60%) in Maiduguri. There was significant statistical difference ($P = 0.0080$ at 95% confidence interval) between the samples collected during the two seasons. The risk of *G. anatis* infection among the infected ducks sampled was 0.69 times when compared to the uninfected ducks during the rainy season.

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

Study location	Type of samples	Number of samples collected (y)	Number of positive samples (x) (x/y %)	Prevalence rate (%)
Live birds market	Tracheal swabs	50	14 (28.0)	5.60
	Cloacal swabs	50	8 (16.0)	3.20
	Ovary samples	50	2 (4.0)	0.80
Households/farms	Tracheal swabs	50	35 (70.0)	14.0
	Cloacal swabs	50	16 (32.0)	6.40
Total		250	75 (30.0)	30.0

Table 4. Distribution of *Gallibacterium anatis* isolated from local breeds of female Muscovy ducks according to season.

Season of sample collection	Number of samples collected	Number of samples positive (CI%)	Number of samples negative (CI%)	Risk Ratio	Prevalence rate (%)	95% CI L – U	P-value	Relative Risk
Rainy	125	51 (40.80)	74 (59.20)	0.689	20.40	0.6368 – 0.7760	P = 0.0080	0.8466
Dry	125	24 (19.20)	101 (80.80)	0.238	9.60	0.7697 – 0.8939		
Total	250	75 (30.0)	175 (70.0)	0.429	30.0			

RR, Risk ratio (CI% infected ducks ÷ CI% uninfected ducks); L – U, Lower limit and Upper limit 95% Confidence interval; $p \leq 0.05$ was considered as significant.

However, the risk of *G. anatis* infection among the infected ducks sampled was 0.24 times when compared to the uninfected ducks during the dry season (Table 4).

The results of the biochemical identification test for *G. anatis* isolated from extensively reared local breeds of female Muscovy ducks in the present study revealed that the isolated organisms show positive reactions to test with catalase, oxidase, phosphatase, sucrose and sorbitol, however, demonstrate negative reactions to indole, urease, coagulase and maltose (Table 5).

The result of distribution of *G. anatis* isolated from extensively reared local breeds of female Muscovy ducks based on their haemolytic and non-haemolytic characteristics on blood agar revealed that the non-haemolytic strain 72 (96.0%) of the bacteria is more frequently isolated than the haemolytic strains 3 (4.0%) amongst the sample collected from the study area. The non-haemolytic strain of *G. anatis* was more frequently isolated from swabs samples collected from the trachea 46 (61.33%), followed by samples from the cloaca 24 (32.0%) and ovaries 2 (2.67%). While the haemolytic strain of the organism in the present study was only isolated from the tracheal swabs 3 (4.0%) (Table 6).

The *in-vitro* degree of antimicrobial susceptibility pattern of the isolated *G. anatis* to 15 different antimicrobials revealed that the isolated bacteria 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, Cephadrin, Oxytetracycline,

Sulpha. + Trimethoprim, Streptomycin, Amoxicillin, Ampicillin, Lincomycin and Spectinomycin (Table 7).

DISCUSSION

The research focused on diagnosis of *Gallibacterium anatis* infection on the phenotypic characteristics of the isolated organism on blood agar by samples collected from local breed of female Muscovy ducks in the study area. The bacterium has been considered an emerging pathogen of domesticated poultry species, semi-domesticated and wild domiciled birds in developing and developed countries, with no pathognomonic clinical signs (Singh et al., 2016). This is the first report of isolation of *G. anatis* in apparently healthy domesticated local breed of the female Muscovy ducks in the study area. This finding supported the fact that the organism exists among apparently healthy birds, although, the bacterium has previously been isolated from clinically sick ducks in Egypt by Sorour et al., (2015) and Abd El-Hamid et al., (2016). It has also been isolated from apparently healthy domesticated fowl (*Gallus domestica*) in Nigeria by Addo and Mohan (1985). The disease had been frequently isolated from diseased and apparently healthy layers and cockerels of exotic breeds of chickens as well as a wide range of semi-domestic birds including turkeys, geese, ducks, pheasants, partridges and cattle egrets (Bisgaard, 1993; Christensen et al., 2003; Rzewuska et al., 2007; Bisgaard et al., 2009; Gregersen et al., 2010; Paudel et al.,

Table 5. Biochemical identification for *Gallibacterium anatis* isolated from local breeds of female Muscovy ducks.

Biochemical Test	Number of samples tested (n=75)	Number of sample positive (%) (n=75)
Catalase	75	75
Indole	75	0
Urease	75	0
Oxidase	75	75
Coagulase	75	0
Phosphatase	75	75
Maltose	75	0
Sucrose	75	75
Sorbitol	75	75

Table 6. Haemolytic characteristics of isolated serovars of *G. anatis* from female Muscovy ducks on blood agar.

Type of Samples collected	Number of Positive samples tested (n = 75)	Type of <i>Gallibacterium</i> biovar isolated	
		Haemolytic (%)	Non-haemolytic (%)
Tracheal swabs	49	3 (4.0)	46 (61.33)
Cloacal swabs	24	0 (0.0)	24 (32.0)
Ovary samples	2	0 (0.0)	2 (2.67)
Total	75	3 (4.0)	72 (96.0)

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

Antimicrobials	Degree of Antimicrobial susceptibility of isolates			
	1	2	3	4
Gentamycin (CN- 10 µg)			+	
Erythromycin (E- 10 µg)				-ve
Amoxycillin (AML -30 µg)				-ve
Cefotaxin (CTX- 30µg)	+++			
Florfenicol (FFC- 30µg)		++		
Norfloxacin (NOR- 10 µg)			+	
Ciprofloxacin (CIP – 5 µg)		++		
Oxytetracycline (OT-30µg)				-ve
Doxycycline (DO- 30µg)		++		
Sulpha.+Trimethoprim (SXT - 25 µg)				-ve
Streptomycin (S- 30 µg)				-ve
Lincomycin (MY – 30 µg)				-ve
Spectinomycin (SH - 100)				-ve
Ampicillin (AMP - 10 µg)				-ve
Cephadrine (CE - 30 µg)				-ve

+++ , Highly susceptible; ++, Moderately susceptible; +, Fairly susceptible; -ve, Completely resistant.

2014; Sorour et al., 2015). The varying unhygienic environments (Plate 1 and 2) of rearing Muscovy ducks in the study area may possibly expose this species of birds to various infectious bacterial diseases including *G. anatis*.

This observation agrees with the finding of Bojesen et al. (2003) who reported up to 96.0% prevalence rate of *G. anatis* in free range scavenging domestic fowls and the high prevalent rate has been attributed to poor biosecurity.



Plate 1. Extensively reared local breeds of Muscovy ducks scavenging in unhygienic pool of water.



Plate 3. Local breeds of Muscovy ducks mixed with other village poultry species in a live bird market.



Plate 2. Extensively reared local breeds of male and female Muscovy ducks scavenging on rubbish dump.

The occurrence of the bacterium in free range local breed of female Muscovy ducks may pose health threat to chickens and other extensively reared range poultry species since the infection is horizontally transmitted. This agrees with the finding of Singh et al. (2016) who reported that the bacterium is naturally transmitted among poultry species and it is difficult to get rid of the infection on affected poultry farms. The isolation of *G. anatis* from apparently healthy local breed of female Muscovy ducks in

this study supported the findings of Sorour et al. (2015) who reported high prevalence of *G. anatis* in ducks and Bojesen et al. (2003) who in a similar study isolated the bacterium from apparently healthy chickens. Neubauer et al. (2009) and Sorour et al. (2015) have also reported isolation of *Gallibacterium* in pure cultures of sample collected from domesticated chickens and ducks with various pathological lesions.

G. anatis infection in this research was found to be more frequently isolated from swab samples collected from free range households ducks compared to swab samples collected from ducks in the live birds markets in the study area. This finding may probably be associated with the scavenging nature of the free range Muscovy ducks which might expose them to various organisms including *G. anatis* during scavenging on unhygienic contaminated environment or from horizontal transmission from other infected birds. This finding is in line with those of Bojesen et al. (2003) and Persson and Bojesen (2015) who have also frequently isolated the bacterium from domesticated birds reared under free range unhygienic environment compared to birds reared in an organized farm with modern facilities that maintained adequate biosecurity. From the results of this study, the isolation of *G. anatis* in samples collected from local breed of female Muscovy ducks in live birds markets may not be surprising, because it was observed that there are no discriminations of health status or screening for diseases among birds before mixing of different poultry species in live birds markets (Plate 3). This habit of live birds' sellers may facilitate horizontal disease transmissions from infected birds to susceptible uninfected ones. Although, there was a significant statistical difference ($P < 0.0001$ at 95% confidence interval) between the prevalence rates of *G.*

anatis isolated from samples collected from the live birds markets and those collected from household in which ducks are reared in the study area. This suggested that *G. anatis* infection may probably be more frequently isolated from extensively reared Muscovy ducks compared to ducks in the live birds market, even though ducks sold in the markets are usually sourced from households and other live birds markets.

The finding of our research revealed more frequent isolation of *G. anatis* from tracheal swabs collected from local breed of female Muscovy ducks sampled in live birds markets and free range ducks in households, compared to the frequency of isolation from cloacal swabs from both study locations while the bacterium was least isolated from the ovary in the present study. The tracheal region followed by the cloacal region might be the more preferable predilection site of the bacterium while the ovary might be considered the least preferred predilection site for the bacterium. This finding agrees with those of Paudel et al. (2013, 2014) and Sorour et al. (2015) who in a similar study have also reported frequent repeated isolation of *G. anatis* from the trachea and cloaca of apparently healthy ducks and chickens and have associated this to the commensal nature of the bacterium in the upper respiratory tract and lower genital tract of the birds. Bojesen et al. (2003) have also reported a significantly higher proportion of *G. anatis* positive samples collected from the tracheal region compared to the corresponding cloacal samples from the same bird in infected flocks.

The present study has revealed seasonal prevalence variation of the bacterium in the study area, *G. anatis* was more frequently isolated in samples collected during the raining season compared to samples collected during the dry season. This suggested that free range Muscovy ducks are more predisposed to *G. anatis* infection during the raining season compared to the dry season in the present study. There was significant statistical difference ($P = 0.0080$ at 95% confidence interval) between the prevalent rates of the bacterium in the samples collected during the two seasons. This finding may be associated with the abundance of unhygienic stagnant pool of water usually surrounding households in the rainy season, which may serve as bathing and dabbling pools for extensively reared ducks, such stagnant pool of water may be contaminated with various pathogens including *G. anatis*. This finding is consistent with those of Malik et al. (2005) who have 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, several researches have reported significantly higher isolation rate of bacterial diseases in poultry species during the rainy seasons 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 this research 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 supported those of Christensen et al. (2003) and Bojesen et al. (2007) who have 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. *Gallibacterium* genus can be differentiated from other genera of Pasteurellaceae with catalase, symbiotic growth, hemolysis, urease, indole, acid production from (+) D-xylose, (-) D-mannitol, (-) D-sorbitol, (+) D-mannose, maltose, raffinose and dextrin tests (Christensen et al., 2003; Bojesen et al., 2007).

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. In previous researches *Gallibacterium* isolates has been differentiated into *Gallibacterium anatis biovar haemolytica* and *Gallibacterium anatis biovar anatis* (Paudel et al., 2013; 2014). The two broad classification or biovars are described within *G. anatis*, as a haemolytic *biovar haemolytica* and a non-haemolytic *biovar anatis* (Kristensen et al., 2010). From the result of this present study the non-haemolytic strain of *Gallibacterium* was more frequently isolated from the infected samples compared to the haemolytic strain. Moreover, the non-haemolytic strain of *Gallibacterium* was more frequently isolated from swab samples collected from the trachea 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 have 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%).

The *in-vitro* degree of antimicrobial susceptibility pattern of the isolated *G. anatis* to 15 different antimicrobials in the present study revealed that the isolated bacterium 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, Cephradine, Oxytetracycline, Sulpha. + Trimethoprim, Streptomycin, Amoxicillin, Ampicillin, Lincomycin and Spectinomycin. This finding supported the antimicrobial susceptibility profile of *G. anatis* isolates from infected ducks and other poultry species which were reported from several investigations (Bojesen et al., 2011; Guo, 2011; Janda, 2011; Jones et al., 2013; El-bestawy, 2014; Sorour et al., 2015; Abd El-Hamid et al., 2016). Chuan-qing et al. (2008) have also reported that all *G. anatis* isolates were highly

sensitive to the third generation cephalosporin in antimicrobial resistance testing. Moreover, investigation has also revealed that *G. anatis* isolates were resistant to wide range of antibiotics and were susceptible to very few ones. However, the fact that the organisms remains susceptible to some antimicrobials such as Cefotaxime, Ciprofloxacin, Doxycycline and Florfenicol as demonstrated in the present research makes the organism treatable using chemotherapy, the most appropriate antibiotics with the guidance of a registered Veterinarian.

Conclusion

In conclusion, *Gallibacterium anatis* is exist among the free range local breed of female Muscovy ducks reared in the study area, with the non-haemolytic strain occurring more frequent in the trachea compared to isolation from the cloaca and ovary. The occurrence of this organism in swabs samples collected from adult female Muscovy ducks is attributed to natural horizontal mode of transmission of the organism since there was no previous report of the bacterium in the study area. However, there may be 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 swim and dab. Also, the unhygienic environment in which Muscovy ducks scavenge 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 non-haemolytic strain of the bacterium is more abundant in the sampled ducks and the isolated organism has demonstrated multidrug resistance, but susceptibility to a few ones, this is suggestive that the organism can be treatable with some antimicrobial chemotherapy.

Recommendations

The presence of the bacterium should be suspected in atypical 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.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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