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PREVALENCE OF *MYCOPLAMA GALLISEPTICUM* AND *MYCOPLASMA SYNOVIAE* IN FREE RANGE CHICKENS AND WILD BIRDS IN OSUN, OYO AND KWARA STATES NIGERIA

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Abstract

Mycoplasma gallisepticum and *Mycoplasma synoviae are pathogens of the class mollicutes that pose economic and veterinary health concerns among poultry farmers and veterinary agencies. Listed by the World Organisation for Animal Health (OIE), these pathogens are responsible for various degrees of clinical manifestations in* poultry, wild birds and other free-living birds. This study was designed to examine the prevalence of *Mycoplasma gallisepticum* and *Mycoplasma synoviae infection among free-range chickens and wild birds in selected States in Nigeria. Using* rapid Mycoplasma field agglutination plate test as well as culture, we examined 541 blood samples collected from free-range (n = 501) and wild birds (n = 40), in three states in Nigeria; Osun, Oyo and Kwara States. Overall, the free-range chickens were positive for both *M. gallisepticum* and *M. synoviae* antibodies, while the wild birds were positive

for only *M. synoviae* antibodies. Results showed that 134 (24.7%) and 367 (73.3) free-range chickens were positive for *M. gallisepticum* and *M. synoviae* respectively, while 13 (32.5%%) wild birds were positive for *M. synoviae*. Kwara State had the highest number of *M. gallisepticum* (87; 23.6%) and *M. synoviae* (290; 78.6%) seropositive cases, while Osun (34; 28.8%) had the highest seroprevalence of *M. gallisepticum* infection. Results from culture showed that isolates of Mycoplasma were obtained in 46 of the 541 samples taken. Thus, a detection rate of 8.5% was obtained. Furthermore, these isolates were obtained from both the free-range chickens and the wild birds. Detection of antibodies to Mycoplasma shown in this study suggest infection of the free range local and wild birds by Mycoplasma; these birds could be possible sources of infection to commercial poultry birds.

Introduction

Mycoplasma gallisepticum (MG) and *Mycoplasma synoviae* (MS) are major pathogens responsible for avian mycoplasmosis among poultry birds, particularly in developing countries, and have been of great economic concern in the poultry industry (Raviv and Ley, 2013; Qadir *et al.*, 2020; Yadav *et al.*, 2021). MG and MS are particularly responsible for very high economic loss due to emaciation, low egg production, increased embryo mortality, reduced feed conversion efficiency, carcass condemnation, cost of prophylaxis and treatment, etc. Both are responsible for respiratory-related diseases, however, *Mycoplasma gallisepticum* (MG) causes respiratory disease in chickens and infectious sinusitis in turkeys while *Mycoplasma synoviae* (MS) elicit inapparent upper respiratory infection in chickens and turkeys, but may occasionally progress systemically and result in synovitis. For more than a decade now, MS has significantly increased in level of concern and veterinary importance due to the emergence of certain strains affecting eggshell quality, causing eggshell apex abnormalities and decreased egg production in poultry (Catania *et al.*, 2016; Matucci *et al.*, 2020; Yadav *et al.*, 2021).

Mycoplasma gallisepticum and *Mycoplasma synoviae* infection occur globally and with reported prevalence of 38.4% and 27.0% respectively (Chaidez-Ibarra *et al.*, 2021). South Asia and Sub-Sahara Africa have the highest prevalence while the least is observed in Europe and Central Asia regions (Chaidez-Ibarra *et al.*, 2021). Algeria, Saudi Arabia and Sudan are the least affected countries for *Mycoplasma gallisepticum*, whereas *Mycoplasma synoviae* is most prevalent in countries such as China, Egypt and Ethiopia (Chaidez-Ibarra *et al.*, 2021). *M. gallisepticum* and *M. synoviae* are the only two actiological agents of avian mycoplasma listed by the World Organization for Animal Health (OIE, 2018). The enormous economic and veterinary importance of these pathogens indicates the need for concern, intervention and control of the transmission, infection and endemicity of the avian mycoplasma within the study area.

Current measures to control *M. gallisepticum* infection include flock testing, eradication programmes, biosecurity programmes and vaccination. Free- range chickens (Gallus gallus) and wild birds are good indicators of prevalence of wild-type mycoplasmas in Africa since they are rarely vaccinated.

Mycoplasma infection is also prevalent in Nigeria, with reports of observed infections in poultry birds and herds in the northern and eastern parts of the country (Ahmed *et al.,* 2015; Kalu *et al*., 2015; Mera and Haruna, 2019). Unfortunately, there is paucity of data and research works on the prevalence, occurrence, pathogenic potential and distribution of mycoplasmas in free-range chickens in the mid-west region of Nigeria, a region where there are many commercial poultry farms. Thus, this research was designed to determine the seroprevalence of *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) among free-range chickens and wild birds in mid-west region of Nigeria.

Methods

Study Area

This study was carried out in 3 states (Kwara, Osun and Oyo) in Mid-West Nigeria. Kwara state is located in latitude 8.9669° N and longitude 4.3874° E, Osun state is located in latitude 7.5629° N and longitude 4.5200° E, while Oyo state is located in latitude 8.1574° N, and longitude 3.6147° E (Figure 1). These states, Kwara, Oyo and Osun are known for keeping and rearing of indigenous free-range chicken, sale and slaughter. Major farm animals commercially reared in these States include cattle, sheep, goats, poultry, etc., and they have various commercial poultry houses in different locations.

Sample Size Determination and Sampling

Sample size was ascertained using the formula described by Araoye, (2004) and Iloh *et al.* (2011), N=z2pq/d2 Where, N=sample size, z=1.96 confidence interval, p=prevalence. With the average prevalence unknown in the selected location p (prevalence) was set at 50%. Using this, the sample size was set at 541. A total of 541 blood samples were collected from free range chickens (501 samples) and wild birds (40 samples) across the three states. In Kwara state, 369 samples were collected, while 118 and 54 samples were collected from Osun and Oyo states respectively. The capture of the wild birds, caging using poultry feeds as baits was used, as the birds are normally seen pecking around the poultry sites. About 1-1.5 ml of blood was collected from wing vein of the birds using a sterile syringe for each bird. The collected blood samples were kept at room temperature for about 1-2 h and then centrifuged at 1500 rpm for 10 min and serum was collected and stored at "20°C prior to use as described by Uddin *et al.* (2016).

Detection of Mycoplasma antibodies

Sera samples obtained were screened for Mycoplasma antibodies using rapid Mycoplasma field agglutination plate test supplied by the Office International des Epizooties (OIE) reference laboratory, Pendik, in Istanbul, Turkey. Undiluted sera were heat activated at 56°C for 30 minutes on a water bath before testing the samples. Positive samples were re-tested at various dilutions and any sample positive at a 1:4 dilution or higher were regarded as positive.

Isolation and identification of Mycoplasma

For culture, swabs were collected from the 541 samples. Sterile cotton buds were used to swab the trachea of live birds. The swab sticks were cut, put in a screw capped bijoux bottles containing 2ml of mycoplasma broth medium and placed on dry ice and taken to the Mycoplasma Diagnostic and Research Laboratory, Department of Veterinary Microbiology, University of Ilorin where they were incubated at 37°C for 5 days in 5% CO_2 as described by Kalu *et al.* (2015). The bottles were examined every 24 hours for colour change of the media from alkaline (pinkish) to slightly acidic (orange-yellow). To check for colonies, 20 microlitre of each liquid culture was serially diluted to 1 x 10^{10} and the last portions were then inoculated on mycoplasma agar plates and incubated at 37°C in a glass anaerobic jar. The plates were examined every 24 hours for Mycoplasma like colonies using a stereoscopic microscope at a magnification of x180.

Growth inhibition test

For the identification of the mycoplasmas, growth inhibition test was performed following the standard procedures (Miles and Nicholas, 1998). A previously triple cloned mycoplasmas under test were carefully and aseptically selected and inoculated onto a freshly prepared PPLO broth Filtered through membrane filters aseptically. A pre-prepared 48-h broth culture (10^{-1} and 10^{-1}) was inoculated onto a pre-dried agar plates by allowing 50iL of each culture to run down a tilted

plate using the running drop technique and allow to dry. Two to three well-separated running drops was applied aseptically, and the agar cylinder was carefully removed using sterile surgical blade and forceps. The wells were carefully filled with about 60ìL of undiluted antiserum using a micropipette. Ensuring that the wells are not overfilled, set-up incubated at 37 °C and after 24 hours, the plates was examined for growth and looking out for a zone of inhibition, as measured from the lawn of mycoplasma growth to the disk, which ranges from >2mm to >5mm. The zone of inhibition on plates visible were recorded as positive and those examined daily for 7 days, with no zone of inhibition were recorded as negative.

Data analysis

All data were entered into Microsoft® Excel 2010 version after data cleaning was done. Descriptive statistics, analysis of variance and p value were determined using Statistical Package for Social Sciences (SPSS) version 22 (SPSS Inc., USA).

Results

Overall seroprevalence of *M. gallisepticum* among the total birds examined was 26.7%, while *M. synoviae* had a seroprevalence of 75.3% (Table 1).

Results showed that 134 (24.7%) and 367 (73.3) free-range chickens were positive for *M. gallisepticum* and *M. synoviae* respectively, while 13 (32.5%%) wild birds were positive for *M. synoviae* (Table 1). Furthermore, results showed that at odds ratio of 29.64 and 95% CI (1.81 - 485.80), there was a significant relationship between the local chickens and wild birds and seroprevalence of *M. gallisepticum* (Table 1). Similarly, at odds ratio of 5.683 and 95% CI (2.8514 - 11.3476), there was a significant relationship between the type of birds and seroprevalence of *M. synoviae* (Table 1). The seroprevalence of avian Mycoplasma in Osun state was 28.8%, while those for Kwara and Oyo states were 23.6% and 24.1% respectively (Table 2).

Also, results showed that there was no statistically significant relationship ($p < 0.05$)

Table 1: Seroprevalence of *M. gallisepticum* and *M. synovium* among the bird type

Bird Type	Total Sample Analysed	*Mycoplasma gallisepticum*				*Mycoplasma synoviae*
		Positive samples (%)	Negative samples (%)	OR (95% CI)	p-value	Positive samples (%)
				29.64 (1.81 - 485.80)	<0.0175	
Local chicken	501	134 (26.7)	367 (73.3)			367 (73.3
Wild birds	40	0 (0.0)	40 (100.0)			13 (32.5)

Key: OD- Odd ratio 95% CI- confidence interval

Table 2: Seroprevalence of *M. gallisepticum* and *M. synovium* in the study locations

State	Total Sample Analysed	*Mycoplasma gallisepticum*			*Mycoplasma synovium*		
		Positive samples (%)	Negative samples (%)	p-value	Positive samples (%)	Negative samples (%)	p-value
Osun	118	34 (28.8)	84 (71.2)		74 (62.7)	44 (37.3)	
Kwara	369	87 (23.6)	282 (76.4)		290 (78.6)	79 (21.4)	
Oyo	54	13 (24.1)	41 (75.9)		41 (75.9)	13 (24.1)	

between the states the samples were collected and avian Mycoplasma seropositivity. Results from culture showed that isolates of Mycoplasma were obtained in 46 of the 541 samples taken. Thus, a detection rate of 8.5% was obtained. Furthermore, these isolates were obtained from both the free-range chickens and the wild birds.

Discussion

This study observed an overall prevalence of 26.7% for *M. gallisepticum* and 75.3% for *M. synoviae* among free-range chickens. This confirms the endemicity of avian mycoplasma infections within the states surveyed in this study. Mycoplasmosis has been reported across different states in Nigeria Ahmed *et al.* (2008), Mera & Mudashir (2019) and Bakre *et al.* (2021) reported the prevalence of 91.83%, 88%, 743% respectively. These prevalence are significantly higher than the prevalence obtained in this study. Despite this, Hassan *et al.* (2014) reported a similar prevalence (24.8%) of infection for both infections in Pakistan, while Abbas *et al.* (2018) also observed a markedly high prevalence of 46.6% among poultry birds. However, their report on the prevalence of infection among wild birds (27%), is low, compared to the findings of this study. Further low prevalence of avian mycoplasma infection (1.7%), was reported by Michiels *et al.* (2016) among wild birds in Belgium. However, a study from Mozambique, reported prevalence of 48.8% and 84.5% for *M. gallisepticum* and *M. synoviae,* respectively. The prevalence of mycoplasmosis as seen in this study could be due to the following; limitations to routine vaccination and treatment of birds. Exposure of poultry birds to unvaccinated birds or insufficient spacing and/or isolation, is another factor that is very common in developing countries, which predisposes chickens and other birds to avian mycoplasma infection.

Results from this study showed the absence of *M. gallisepticum* among the wild birds examined. None detection of antibodies to *M. gallisepticum* among wild birds in this study contrasts the findings of Michiels *et al.* (2016), Abbas *et al.* (2018) that reported seropositivity among wild birds in Belgium and Pakistan (Michiels *et al.*, 2016; Abbas *et al,* 2018). Sawicka-Durkalec *et al.* (2020) however opined that the prevalence of infection among wild birds could be influenced by the type of assay used. Their study showed that the sensitivity of an assay, could significantly affect the results. Also, another factor that may be responsible for the absence of *M. gallisepticum* infection in wild birds as seen in this study could be lack of interaction or absence of cross-transmission between infected birds and the wild birds (Sawicka-Durkalec *et al.*, 2021). The disparity in ecological niche and adaptive environment may have created the gap of interaction between such birds in the study area, thus, minimizing the likelihood of infection.

Findings from culture further ascertained the presence of *Mycoplasma* sp. The presence of colonies with characteristics "fried egg" was indicative of mycoplasma. This was seen in 8.5% of the representative samples. The detection rate in this study is higher than the 3.8% reported by Kalu *et al.* (2015) in Nsukka, South East Nigeria. Unlike this study where isolates of mycoplasma were obtained from both the wild birds and free-range chicken, Kalu *et al.* (2015) reported that isolates were only obtained from layers while none was obtained from broilers. Different prevalence of Mycoplasma isolated from different bird types have been reported in the past. Gharaibeh and Al Roussan (2008) reported a higher *Mycoplasma gallisepticum* isolation rate in layers (38.1%) than in broilers (31.6%) in Jordan while, Heleili *et al.* (2011) recorded a higher rate in broilers (19.56%) than in layers (2.09%) in a study of chickens in Banta, Eastern Algeria.

There are significant implications of Mycoplasma in birds. Their presence in culture is an evidence of the organism's ability to propagate itself intracellularly (Ahmed *et al.*, 2015). The ability of Mycoplasma to persist as an intracellular parasite, is a major cause of avian mycoplasma. Their persistence in cells is a major protective mechanism used by Mycoplasma to evade the immune systems of animals, while having easy access to required nutrient (Vogl *et al.*, 2008).

In conclusion Serological testing showed that *M. gallisepticum* and *M. synoviae* are prevalent in free-range chickens with no history of vaccination in mid-west region of Nigeria. The study reveals that free- range chickens harbor these two mycoplasmas in the region while wild birds appear to harbour only *M. synoviae*. Detection of antibodies to Mycoplasma suggest infection of the free-range chicken and wild birds by Mycoplasma; these birds could be possible sources of infection to commercial poultry birds.

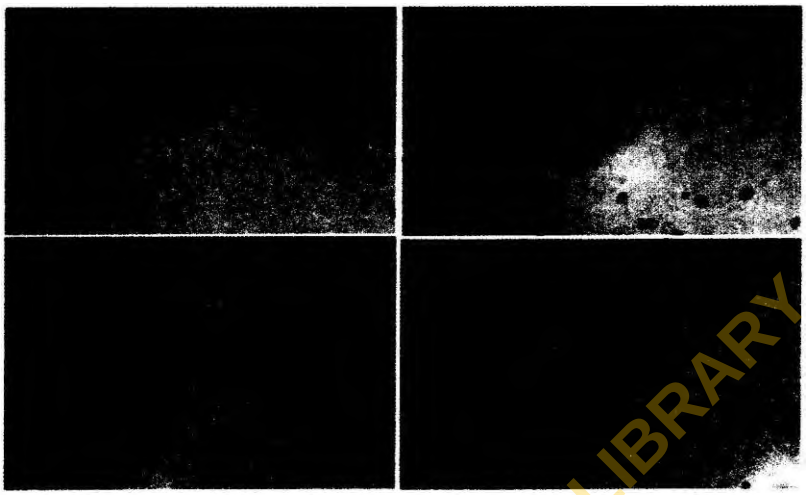

Figure 2: Presence of Mycoplasma colonies on PPLO agar plates.

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