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Seroprevalence of *Mycoplasma gallisepticum* in apparently healthy layer chickens in commercial farms in Ibadan

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Abstract

Mycoplasma gallisepticum (MG) is a common poultry infection that causes chronic respiratory disease in chickens and turkeys, resulting in significant financial losses for poultry farmers and affecting the entire country. The primary goal of this study was to establish the seroprevalence of *Mycoplasma gallisepticum* in commercial layer birds in several local government in Ibadan namely; Afijio, Akinyele, Lagelu, Ido, Egbeda, Ona ara and Ibadan South west, between March and July, 2021. A total of 140 blood samples were obtained at random from commercial layer chickens (ISA brown) of various ages and flock sizes. The presence of antibodies against MG was detected using an indirect enzyme linked immunosorbent test (iELISA). Overall prevalence of 74.3% was recorded during this study with Deborah farm having the highest MG prevalence of 85%, Agboola, 80%, Brian, Betty and Cornerstone had 75%, Rehoboth had 70% and Abol farm had the lowest prevalence of 60%. The highest seroprevalence of MG antibody was 85% in 18-21 weeks, followed by 80% in 22-25 weeks, and lowest with 60% in 8-11 weeks. In the farm chosen, there was no significant change in relative flock size at $p < 0.05$. Moreover, because the disease is transmitted vertically, these findings suggest that commercial layers in Ibadan should be frequently examined for MG infection and carrier birds should be culled. Efforts should be focused on educating poultry producers on how to effectively control MG in layer farms in Nigeria through proper management and the use of appropriate preventive or therapeutic methods.

Keywords: Antibodies, Seroprevalence, *Mycoplasma gallisepticum*, Layer chicken, Poultry production

Introduction

Nigeria's animal business relies heavily on poultry farming (Buim *et al.*, 2009). It's a great way to get a lot of animal protein. Poultry meat is widely accepted around the world due to its lack of religious prejudice. Mycoplasmosis is still one of the most serious health issues impacting poultry in Nigeria (Ahmad *et al.*, 2015). The productivity of the commercial exotic and local breeds are hampered by the disease, which results in severe losses for the country (Silva *et al.*, 2008). *Mycoplasma synovae, Mycoplasma meleagridis, Mycoplasma gallisepticum*, and *Mycoplasma iowae* are four pathogenic organisms that cause mycoplasmosis (Hossain *et al.*, 2007). Gallinaceous and non-gallinaceous bird species are

infected with *Mycoplasma gallisepticum* which is the most virulent and commercially significant mycoplasma infection (Osman *et al.*, 2009). In meat-type birds, this bacteria manifests as chronic respiratory disease in chickens and infectious sinusitis in turkeys (Osman *et al.*, 2009). *Mycoplasma gallisepticum* is one of the aetiological agents in multifactorial illness complex, along with *Haemophillus paragallinarum* and other bacteria (Ley and Yonder, 1997). Reduced egg production in chickens, turkeys, and other avian species is one of the clinical indications of MG infection (Ahmad *et al.*, 2008). *Mycoplasma gallisepticum* infection also reduces feed efficiency, resulting in the production of poor carcass quality and unsatisfactory egg production in layers. The disease has spread all over the world. Clinical symptoms alone are insufficient to diagnose *Mycoplasma gallisepticum*. Serology and positive culture methods are frequently used. ELISA is the most effective serological test for detecting organisms in the environment quickly (Ahmed al., 2015). *Mycoplasma gallisepticum* is mostly controlled through vaccine, which is limited in other countries and unavailable in Nigeria despite the disease's severity. The main methods of eradication are tests and slaughter policies. Because of the growth of multiage complexes in the commercial layer industry, this policy appears to be untenable (Mera and Mudashir, 2019). The diagnosis and treatment of chronic respiratory disease in chicken have garnered attention due to its economic implications. The goal of this study is to assess the seroprevalence of *Mycoplasma gallisepticum* infection in chicken so that effective control measures may be implemented.

Materials and Methods
Study area
This study was conducted in seven selected local governments (Afijio, Akinyele, Lagelu, Ido, Egbeda, Ona ara, Ibadan South west) out of the total eleven local governments of Ibadan as shown in Figure 1. Local government areas are purposively selected for this study based on availability of farm and willingness of farm owner to allow samples to be collected from chicken. Ibadan is a capital city of Oyo state located on seven hills (elevation of 700 feet, about 100 miles from the Atlantic coast. It is the most populous city in Oyo state lies between latitude 7° 23' and 9° 25' N and longitude 3° 55' and 52° 50' E. Ibadan has a radius of 12 kilometers, with Mapo hall as its focal point. Ibadan has a tropical climate that is both wet and dry. The wet season in Ibadan lasts from March to

October. The city's dry season lasts from November to February. Ibadan's average total rainfall is 1420 mm, occurring over 109 days. The average maximum temperature is 26.5℃, the lowest temperature is 21.4℃, and the relative humidity is 74.5%. Economy activities in Ibadan include agriculture, commerce, handcrafts, manufacturing and service industries. There are a number of poultry farm settlement scattered across Ibadan especially at Akufo and Lalupon. Ibadan is rich with cheap sources of land and labour and are close to the commercial capital of the country, Lagos.

Sample collection
5 mls of blood samples were collected randomly from the jugular vein of commercial layer chicken (Isa brown) aged between 8 and 35 weeks with intensive and semi-intensive management from selected local government areas in Ibadan. Flock sizes ranged from 1500 to 3,000 birds, with most of the flocks containing 2000 to 2500 chickens. The chickens used for this study have no records of vaccination against *Mycoplasma gallisepticum*. The blood samples collected into sample bottles were allowed to stand in a slanting position for about 3 hours to allow for the separation of the serum from the cellular components of the blood. The serum was decanted in centrifuge tube and centrifuge at 2000rpm for 5 minutes to have a clear serum.

Figure 1: Map of Ibadan showing the study areas (Adefisan *et al.*, 2015)

The serum was then collected in another labelled sample bottles and then transported with ice packs to the CHI laboratory, Challenge, Ibadan for preservation at -20℃ until further processing for the serological study.

Indirect Enzyme Linked Immunosorbent Assay (ELISA) test was conducted to detect the antibodies against MG using (ProFLOK™ MG Ab, Manufactured by Zoetis inc, Kalamazoo, MI 49007, USA).

Based on the manual of the ELISA kit, the test is validated if: The normal control average optical density (OD) is less than 0.200 and the corrective positive control (CPC) is between 0.250 and 0.900. When S/P ratio value is ≥ 0.6 and antibody titre ≥ 744, the S/P ratio and antibody titer are calculated as follows:

S/P= (Sample OD) - (Average normal control OD)
　　　　　　Corrected positive control

MG ELISA titer
$Log_{10 (Titer)} = (1.464 \times LOG_{10} S/P) + 3.197$
Titer= Antilog of Log_{10} Titer

Data Analysis
Tables were used to present all of the information gathered during the research. To see if there was a statistical difference in the disease prevalence rate, Chi-square test was conducted with SPSS version 20. At the 95% confidence interval, $P < 0.05$ values were considered significant.

The prevalence rate was calculated using the formula:

Prevalence = Positive samples × 100
　　　　　　Total samples analyzed

Results
The seroprevalence of MG antibody in commercial layer farms at different ages in the local government areas of Ibadan, Oyo state was studied by Enzyme linked Immunosorbent Assay (ELISA). Out of the 140 sera samples collected and analyzed, only 104 sera samples were positive, this shows 74.3% with prevalence of 60%, 70%, 75%, 80%, 85%, 75%, and 75% for Abol farm, Rehoboth farm, Cornerstone farm, Agboola farm, Deborah farm, Brian farm and Betty farm respectively.

However, the overall results of prevalence of MG antibody shown in table 1, depicts details of 7 farms from where blood samples were collected.

Table 2 showed the prevalence of MG antibody was highest in 18-21 weeks with 85%, followed by 22-25 weeks with 80% and lowest in 8-11 weeks with 60%.

Table 3 shows no seroprevalence in relation to flock size with 500-1800 group having 75%, 1900-2200 group also having 75% and group 2300-2700 having 70%.

Discussion
In commercial layers in Ibadan, the overall seroprevalence of *Mycoplasma gallisepticum* (MG) infection was 74.3%. This indicates that *Mycoplasma gallisepticum* is endemic in commercial layer chicken in Ibadan. Similar findings were reported by Ahmed *et al.* (2008) and Mera & Mudashir (2019) who reported high seroprevalence of 91.83% and 88% in layer chicken in Niger and Sokoto state respectively. Other researchers (Ahmad, 2000; Wieliczko *et al.*, 2000) have also recorded high seroprevalence in Pakistan and Poland. However, in contrast to this, Feizi *et al.* (2013) reported a lower prevalence of 33.3% in northwest of Iran.

The highest prevalence of MG infection was found to be 85% in the 18-21 week age group, followed by 80% in the 22-25 week age group, and 60% in the 8-11 week age group. This study denoted that older birds were more affected than younger birds. These findings are totally different from the reports of Sarkar *et al.* (2005) and Hossain *et al.* (2010) who reported a maximum prevalence of 71.6% at 16-23 and 18-20 weeks of age, and the lowest seroprevalence of MG antibodies of 50.4% at 64 and 55 weeks of age. However, in contrast to this study, Ahmad, (2008) recorded maximum prevalence rate of MG infection with 74.60% in flocks aged 6 to 23 weeks, and the lowest prevalence rate was 33.17% in flocks aged 60-76 weeks.

The size of the flock has little effect on the serological findings. This study contradicts the work of Heleli *et al.* (2012), who reported 76.97% MG infection in a farm of 18000 birds in Algeria, compared to 20% in a flock of 500-1000 birds. Furthermore, Hossain *et al.* (2010) also reported that in Rajshahi and nearby regions of Bangladesh, the MG infection rate was higher (68.25%) in large flocks compared to small flocks (50.1%). Despite the fact that the majority of farms in the research area have small flocks and open shed poultry farms with poor management conditions, a smaller number of farms had satisfactory or good management. Other factors that may contribute to the high incidence in Ibadan include multiage production practice (where different age of flocks are kept together in the same farm), the building of chicken farms in the area and the ability of the microbes to persist longer in the area as well as a lack of basic bio-security measures.

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Table 1: Seroprevalence of *Mycoplasma gallisepticum* antibody by ELISA

No. of farm	Age (wks)	No. of sera tested	No. of positive samples (%)	Mean titer	CV (%)
Abol	8	20	12(60)	1126	69.56
Rehoboth	15	20	14(70)	1100	66.20
Cornerstone	12	20	15(75)	1115	60.56
Agboola	25	20	16(80)	900	60.32
Deborah	20	20	17(85)	1250	65.32
Brain	33	20	15(75)	1115	61.56
Betty	27	20	15(75)	800	58.66

Table 2: Seroprevalence of *Mycoplasma gallisepticum* (MG) antibody by age

Age of birds (weeks)	No. of sera tested	No. of positive samples	Prevalence (%)	*P*-value
8 – 11	20	12	60	0.062 *
12 – 15	40	29	72	
18 – 21	20	17	85	
22 – 25	20	16	80	
26 – 29	20	15	75	
30 – 33	20	15	75	

*Significant (p<0.05)

Table 3: Seroprevalence of MG antibody in relation to flock size

Flock size (No. of birds/flock)	No. of sera tested	No. of positive samples	Prevalence (%)	*P*-value
1500 – 1800	40	30	75	0.777[NS]
1900 – 22001	40	30	75	
2300 – 2600	20	14	70	
2700 – 3000	40	30	75	

NS-Not significant at p<0.05, MG =*Mycoplasma gallisepticum*

However, it has also been noted that the MG infection are more severe than previously thought.

According to the findings of this study, MG is endemic in layer chicken flocks in Ibadan, Nigeria. Efforts should be focused on educating poultry producers on how to effectively control MG in layer farms through proper management and the use of appropriate preventive or therapeutic methods. Furthermore, a more in-depth investigation of the prevalence and characteristics of MG across the country should be conducted to determine the disease's present state in Nigeria. In addition, the role of vaccination should be investigated in order to avoid unjustified antibiotic use in the treatment of infection.

Conflict of interest
The authors declare that there is no conflict of interest.

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