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Seroprevalence of *Mycoplasma synoviae* in chickens from some selected locations in Nigeria

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Abstract

Mycoplasma synoviae is the second most important species of *Mycoplasma* affecting chickens globally. It causes considerable economic losses to the poultry industry in Nigeria. Therefore, this study aims to assess the seroprevalence of *Mycoplasma synoviae* in chickens from the major poultry industry in seven selected cities in Nigeria. The birds were categorized based on the purpose of keeping them into commercial and breeder broilers and layers. A total of 14,326 sera were examined within four years (2018-2021) using an indirect enzyme-linked immunosorbent assay to detect the presence of antibodies to the *Mycoplasma synoviae*. The date of sample collection, age of bird, location, bird type, mean antibody titre and percentage positive for *Mycoplasma synoviae* antibodies were recorded. Kaduna has the highest seroprevalence rate with 54.4%, broiler breeder has 53.5%. The 30.1 to 40 weeks age group has the highest seropositive results with 13.5%, and highest seroprevalence was recorded in the year 2019 with 19.4%. This study demonstrated the presence of *Mycoplasma synoviae* in the study area in both commercial and breeder flocks of broilers and layer chickens, respectively, with broiler breeders across the states having the highest seroprevalence. This emphasises the importance of sero-monitoring in the poultry industry for early control of this endemic pathogen.

Keywords: Broiler chicken, Layer chicken, *Mycoplasma synoviae*, Nigeria, Seroprevalence

Introduction

Infectious diseases such as avian mycoplasmosis remain a major setback to the poultry industry worldwide. In Nigeria's poultry industry, mycoplasmosis is one of the most important disease problems that affect both commercial and indigenous breeds (Mera et al., 2020). *Mycoplasma* infection in chickens causes decreased feed efficiency, poor carcass quality, and sub-optimal egg production in

layer chickens, broilers, and other avian species (Hossain et al., 2007).

Several species of mycoplasma are reported to affect chickens. However, studies have shown that *Mycoplasma gallisepticum*, *M. synoviae*, *M. meleagridis*, and *M. iowae* are the four major pathogens that cause mycoplasmosis in birds (Bakre et al., 2021). Among these species, *M. synoviae* is a well-known cause of poor feed conversion, reduced

weight gain, and high mortality rates from air sacculitis or synovitis (Hossain *et al.*, 2007; Ley, 2008; Hong *et al.*, 2015). Hatcheries and commercial poultry farms are the major sources for the spread of avian mycoplasmosis, including the dreaded exudative tendinitis and synovitis caused by *M. synoviae*. This is based on the potential for vertical transmission from parents to their offspring (Hong *et al.*, 2015). Horizontal spread within flocks by direct contact also occurs (Torrubia-Diaz *et al.*, 2014). Diagnosis of avian mycoplasmosis requires comparative knowledge of the clinical signs, epidemiological data, post-mortem lesions, and isolation of the causal mycoplasma. Bacterial isolation and identification is the gold standard for mycoplasma diagnosis (Ferguson-Noel & Noormohammadi, 2020); the process is laborious, time-consuming and expensive. The rapid serum plate agglutination test (SPA) is widely used because of its simplicity and its low relative cost. Its lack of specificity is a major limiting factor (Kempf, 2015). Commercial enzyme-linked immunosorbent assay (ELISA) test kits are now commonly used for serodiagnosis and flock monitoring (Ferguson-Noel & Noormohammadi, 2020). In general, ELISA tests are slightly less sensitive but more specific than SPA tests; and less specific but more sensitive than Hemagglutination Inhibition (HI) tests (Kleven, 2008; Moreira *et al.*, 2017; Ferguson-Noel & Noormohammadi, 2020). Because of the diligent national poultry improvement programmes, the incidence of *M. synoviae* infection in commercial poultry sectors in developed nations such as the United States of America has substantially decreased (Michiels *et al.*, 2016). In Nigeria, however, effective implementation of these programmes is lacking,

resulting in a sustained risk of exposure to infectious diseases. Respiratory diseases, arthritis, and synovitis, among others, are common occurrences in poultry settings in Nigeria. However, attention has been focused on agents like Newcastle disease, Infectious bronchitis, *Mycoplasma gallisepticum*, and other bacteria that produce significant clinical diseases and obvious economic losses, while neglecting pathogens like *Mycoplasma synoviae* due to their putative low clinical relevance. This study was therefore undertaken to assess the seroprevalence of *Mycoplasma synoviae* infection in poultry farms in Nigeria to create baseline data that will equip poultry farmers, animal health professionals, and policy makers with information that will guide effective control and preventive measures.

Materials and Methods

Study area

An intensive serological monitoring of antibodies against *Mycoplasma synoviae* from major poultry farms in seven selected cities in Federal Capital Territory, Adamawa, Kaduna, Kano, Kwara, Niger, and Oyo over a four-year (2018-2021) period was conducted and analyses were done by the Kaduna Poultry Disease Laboratory (Olam Hatcheries Limited), Kaduna state, Nigeria (Figure 1).

Study design

The date of sample collection, age, location, bird type, vaccination history, mean antibody titres, antibody titre range, cumulative variance, and percentage positive for *Mycoplasma synoviae* (MS) antibodies were all recorded. The birds' age ranged from one day old (for newly hatched chicks) to 79 weeks for

commercial layers and breeders. Samples from different parts of the country were submitted for analysis, but most samples were from Kaduna, probably due to the location of the laboratory. The birds were categorized according to the purpose of keeping them in commercial and breeder flocks of broilers and layers. All blood samples were collected from apparently healthy flocks with no history of vaccination against MS.

Sample collection and storage

Blood samples were collected from 1-day-old chicks via cardiac venipuncture and from older birds via the wing vein (Poland & Raftery, 2019) to obtain sera for the determination of antibodies against MS. Sera were kept at a temperature of -18°C prior to use.

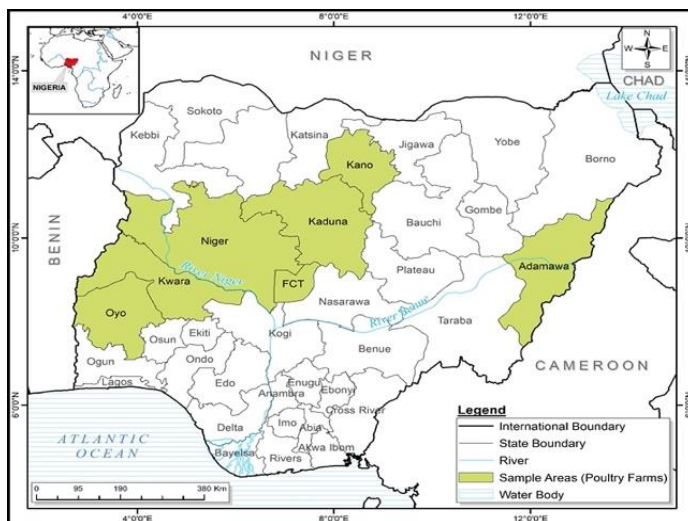


Figure 1: Map of Nigeria showing seven selected cities sampled (Poultry Farms)

Sample analysis

Antibodies against MS were detected in serum using two different sets of recombinant protein-based indirect enzyme-linked immunosorbent assay (ELISA) kits for the detection of anti-*Mycoplasma synoviae* antibodies. They are IDVet MS indirect antibody test kit (Innovative Diagnostic Veterinary Solution, Grabels, France) following the manufacturer's instructions with positive cut off titres of ≥ 737 and the BioChek MS antibody test kit (BioChek Smart Veterinary Diagnostics, Fokkerstraat, Netherland), also following the manufacturer's instructions with the positive cut off titres of ≥ 594 . The results were read using a BioTek ELX 800 absorbent microplate reader (BioTek Instruments Inc., Vermont, USA).

Data analysis

Tables and figures were used to analyse all the information obtained during this study. A Chi-square test was conducted with SPSS version 16.0 for

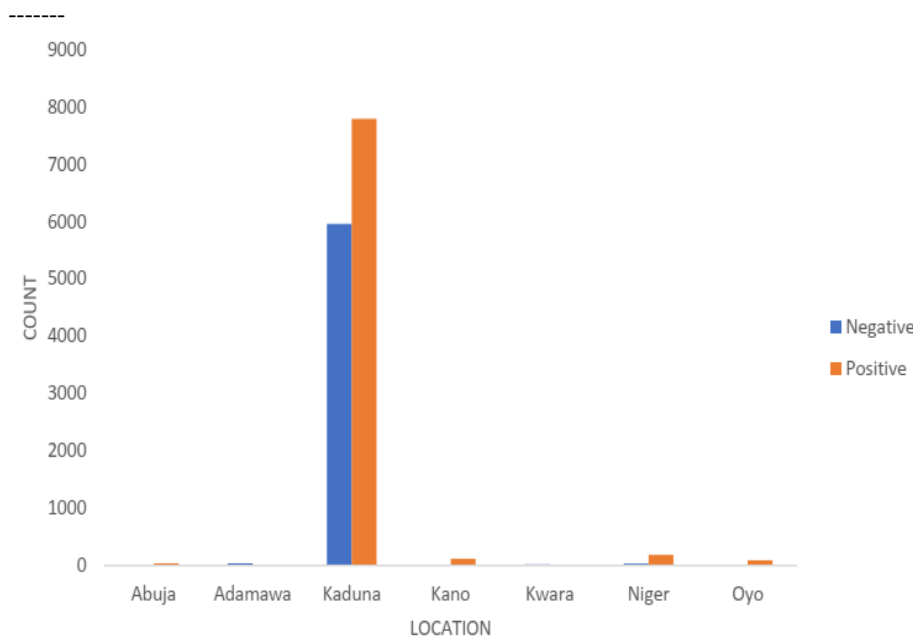


Figure 2: Seroprevalence of *Mycoplasma synoviae* infection based on location in commercial chickens in Nigeria

Table 1: Seroprevalence of *Mycoplasma synoviae* infection based on location in commercial chickens in Nigeria

Location	No. Negative (%)	No. Positive (%)	Total (%)	P-Value
Abuja	0 (0.0)	39 (0.3)	39 (0.3)	0.000
Adamawa	42 (0.3)	3 (0.0)	45 (0.3)	
Kaduna	5958 (41.6)	7795 (54.4)	13753 (96.0)	
Kano	13 (0.1)	120 (0.8)	133 (0.9)	
Kwara	18 (0.1)	16 (0.1)	34 (0.2)	
Niger	36 (0.3)	183 (1.3)	219 (1.6)	
Oyo	12 (0.1)	91 (0.6)	103 (0.7)	
	6079 (42.4)	8247 (57.6)	14326 (100.0)	

Windows® (2007), to determine the statistical difference in the disease prevalence rate. At the 95% confidence interval, $P < 0.05$ values were considered significant.

The prevalence rate was calculated using the formula:

$$\text{Prevalence} = \frac{\text{Positive samples}}{\text{Total samples analyzed}} \times 100$$

Results

The seroprevalence of *Mycoplasma synoviae* infection in some selected major poultry farms in Nigeria is presented in the tables and figures below. In Table 1 and Figure 2, out of the 14,326 sera collected from chickens of different breeds and age groups in the seven major cities within the study area, distribution of prevalence based on locations revealed that Kaduna alone accounts for 54.4% of the overall seroprevalence of 57.6% recorded. In contrast, other cities constitute the remaining 3.2%. Although the distribution of sera collected was not

proportionate, the difference was statistically significant ($p = 0.000$). The most frequent breed of chicken sampled was the broiler breeder with 53.5%, followed by the layer breeder with 25.9%, then commercial layer and commercial broiler with 12.9% and 7.7%, respectively. This was significantly ($p = 0.000$) true for the seroprevalence, with the broiler breeder having the highest seropositive of 29.0%, followed by the layer breeder with 15.5%, and commercial layer and commercial broiler

with 8.6% and 4.5% ($p = 0.000$), respectively (Table 2, Figure 3).

Although different ages from 1-day-old to 79 weeks of age were individually assessed and the differences were statistically significant ($p = 0.000$), the results presented here were compressed to age groups for ease of understanding. The highest seropositive rate was recorded in the 30.1 to 40 weeks of age group with 13.5%, followed by the age group 0 to 10 weeks with 12.8%, and the least encountered was in the older chickens of >70 weeks of age with 1.3% (Table 3, Figure 4).

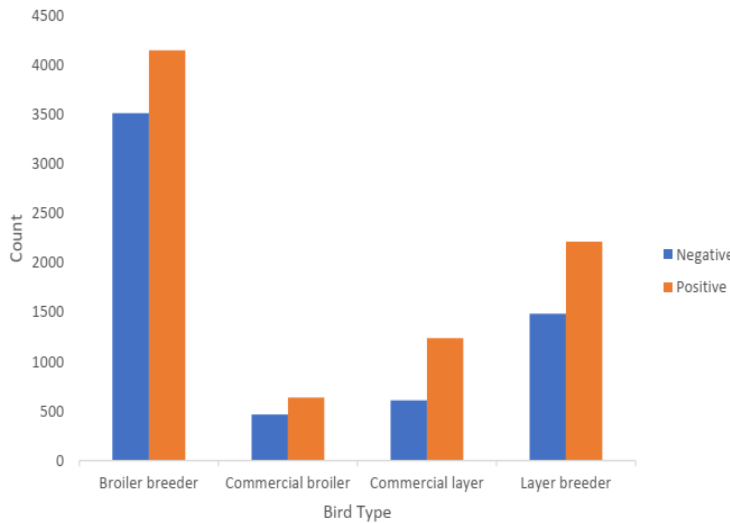


Figure 3: Seroprevalence and breed distribution of *Mycoplasma synoviae* in commercial chickens in Nigeria

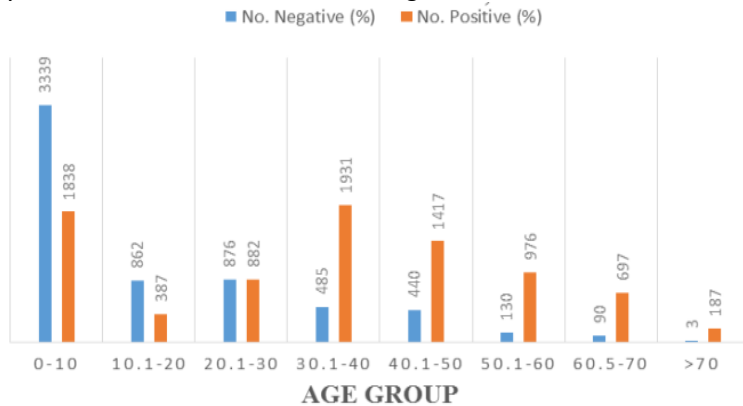


Figure 4: Seroprevalence of *Mycoplasma synoviae* based on the age of commercial chickens in Nigeria

In all, out of the 14,326 chicken sera examined for the detection of antibodies against different strains of *Mycoplasma synoviae* in the seven major cities across different regions in Nigerian, the overall seroprevalence of the *Mycoplasma synoviae* infection for the four years (2018 to 2021) period in chickens was observed to be 57.6% and the difference between the years understudied was statistically significant ($P = 0.00$). The positive sera were significantly higher in the year 2019 with 19.4%, this was followed by the year 2021 with 15.8% and the years 2018 and 2020 with 11.5% and 10.9%, respectively (Table 4, Figure 5).

Discussion

Mycoplasma synoviae infection in poultry often occurs as a subclinical upper respiratory infection of chicken and turkey (Ferguson-Noel & Noormohammadi, 2020). Although there are low clinical and economic impacts, arthritis, amyloid arthropathy, eggshell apex abnormalities, egg production losses often emerge (Feberwee *et al.*, 2008). With the associated clinical and economic impact as well as welfare concerns related to MS infection, there is increased awareness of the need to institute effective control measures.

Recently, MS infection has been reported to be endemic in the poultry industry of many countries worldwide (Moreira *et al.*, 2017). The high seroprevalence (above 50%) in this study and the detection of anti-MS antibodies each year during the period of the study suggested that *Mycoplasma synoviae* is prevalent and likely endemic in the poultry industry across different regions of Nigeria. This agrees with the findings of Sawale *et al.* (2023) and Gottstein *et al.* (2023), who reported a seroprevalence of 70.9% and 69.5% in India and Croatia, respectively, and

Table 2: Seroprevalence and breed distribution of *Mycoplasma synoviae* in commercial chickens in Nigeria

Bird Type	No. Negative (%)	No. Positive (%)	Total (%)	P-Value
Broiler breeder	3517(24.5)	4153(29.0)	7670(53.5)	0.000
Commercial broiler	467(3.3)	639(4.5)	1106(7.7)	
Commercial layer	609(4.3)	1238(8.6)	1847(12.9)	
Layer breeder	1486(10.4)	2217(15.5)	3703(25.9)	
Total	6079(42.4)	8247(57.6)	14326(100.0)	

further buttresses the fact that *Mycoplasma synoviae* infection is an important endemic disease in many countries. India (Sawale *et al.*, 2023) and Croatia (Gottstein *et al.*, 2023) showed higher seroprevalence rates compared to our study in Nigeria, indicating a potentially higher prevalence or circulation of *Mycoplasma synoviae* in poultry populations in those countries. However, the larger sample size (14,326) in our study provides a more robust estimate of seroprevalence compared to the smaller sample sizes in India (134) (Sawale *et al.*, 2023) and Croatia (1135) (Gottstein *et al.*, 2023).

Vaccination against MS is an uncommon practice in Nigeria (Ahmed *et al.*, 2015; Bakre *et al.*, 2021), it is reasonable to conclude that the anti-MS antibodies

detected were the result of challenges with wild strains of MS. The consistent detection of MS antibodies throughout the study period confirms the endemic status of MS in Nigeria. A previous study by Ahmed *et al.* (2015) also detected antibodies against MG and MS in local backyard chickens in north central Nigeria with a seroprevalence of 92%. Similarly, Fagbamila *et al.* (2022) recorded an MG/MS seroprevalence of 65% in commercial layers in the same region. This further suggests the presence of MS in different poultry settings in Nigeria.

Our findings showed that of all regions considered for the study, Kaduna has the highest seroprevalence of 54%. This could be because Kaduna has many chicken breeder operations, and most samples received came

from breeder farms. Vertical transmission of MS is believed to play the most important role in the spread of infection (Torrubia-Diaz *et al.*, 2014). This has made mycoplasmosis a disease of concern for most standard breeder farms and thus, the need for regular monitoring. The presence of laboratory facilities in Kaduna might have encourage consistency in sample analysis due to ease of accessibility. Breeders were also found to have higher seroprevalence. Concerns for vertical transmission to progeny

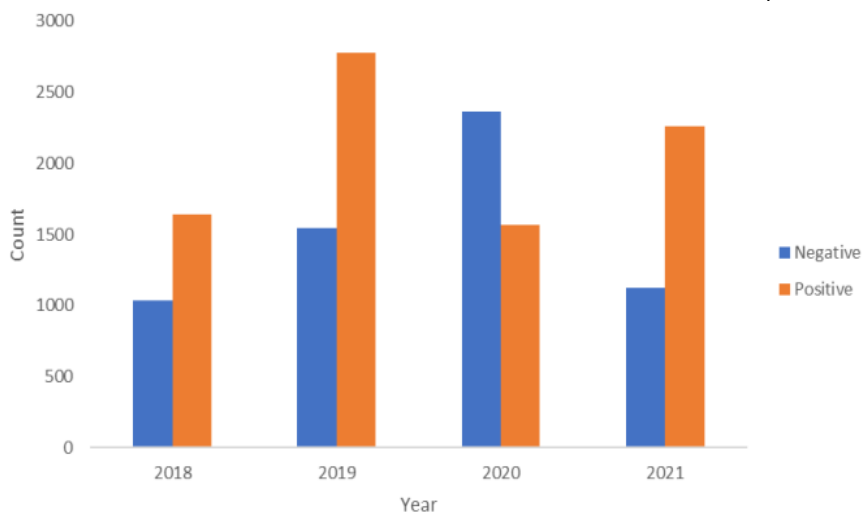


Figure 5: Seroprevalence of *Mycoplasma synoviae* based on the year of sera collection in commercial chickens in Nigeria

Table 3: Seroprevalence of *Mycoplasma synoviae* based on the age of commercial Chickens in Nigeria

Age group (weeks)	No. Negative (%)	No. Positive (%)	Total (%)	P-Value
0-10	3339 (23.3)	1838 (12.8)	5177 (36.1)	0.000
10.1-20	862 (6.0)	387 (2.7)	1249 (8.7)	
20.1-30	876 (6.1)	882 (6.2)	1758 (12.3)	
30.1-40	485 (3.4)	1931(13.5)	2416 (16.9)	
40.1-50	440 (3.1)	1417 (9.9)	1857 (13.0)	
50.1-60	130 (0.9)	976 (6.8)	1106 (7.7)	
60.5-70	90 (0.6)	697 (4.9)	787 (5.5)	
>70	3 (0.0)	187 (1.3)	190 (1.3)	
	6079 (42.4)	8247 (57.6)	14326 (100.0)	

Table 4: Seroprevalence of *Mycoplasma synoviae* based on year in commercial chickens in Nigeria

Year	No. Negative (%)	No. Positive (%)	Total (%)	P-Value
2018	1038(7.2)	1644(11.5)	2682(18.7)	0.000
2019	1547(10.8)	2779(19.4)	4326(30.2)	
2020	2366(16.5)	1565(10.9)	3931(27.4)	
2021	1128(7.9)	2259(15.8)	3387(23.6)	
Total	6079(42.4)	8247(57.6)	14326(100.0)	

which generated interest in monitoring might have contributed to higher prevalence in the breeder categories. The common practice for breeder farms in Nigeria is the multi-age poultry farming (Maduka *et al.*, 2016) which might significantly contribute to the higher prevalence of MS in breeders than in commercial layers and broilers. Empirical evidence has shown that this practice encourages the horizontal spread of mycoplasma (Ferguson-Noel & Noormohammadi, 2020). All – in, all – out farming is the common practice with small scale commercial broiler farming which dominate broiler production in Nigeria. This system is considered as an important component of the classic rules of biosecurity necessary for the successful control and eradication of mycoplasma (Kempf, 2015). This together with the short life span of this bird type (5 – 8 weeks) might have contributed to the lower seroprevalence of MS in this group of birds. This finding agrees with the findings of Matilda *et al.* (2018) who reported very low seroprevalence for MS in commercial broilers up to 12 weeks of age. In a separate study, Kleven *et al.* (2001) reported negative antibodies to MS in turkey up to 6 weeks post-challenge, though the bacteria were isolated by culture. El-Ashram *et al.* (2021) also reported negative antibodies against MS until at 16 weeks in commercial Ross, Lohman brown layers and local chickens. The use of antibiotics for therapeutic and preventive purposes is unfortunately a routine practice in most poultry farms (Endacott *et al.*, 2021). In breeders, this could limit the vertical transmission of MS to chicks with resultant lower prevalence in commercial broilers and layers at early stage of life. Another important finding in this study is the recording of the highest seroprevalence of MS in birds within 30 – 40 - week age group. This is the period of peak egg laying and is associated with stress and increased susceptibility to various infections including MS. Kleven *et al.* (2001) also reported high seroprevalence against MS beginning at 33 weeks of age. High seroprevalence was also recorded in the youngest age group (0 – 10 weeks). This most likely represent maternally derived antibodies against MS. This supposition is supported by the detection of antibodies in breeders. Detection of the lowest antibody titres in the oldest age group however cannot be clearly explained at the moment. Our hypothesis includes the possibility of acquiring sufficient mucosal immunity which has been shown to block the attachment of mycoplasma to epithelial surfaces (Ferguson-Noel & Noormohammadi, 2020). In a study by Cortés *et al.* (2021) seroprevalence increases with increase in the age of sampling. Vaccine strains of MS were detected with PCR in

these flocks which suggest the possible application of MS vaccine in the study area.

In conclusion, this study is the first to describe the comprehensive multi-state seroprevalence study of *M. synoviae* in Nigeria to the best of our knowledge. Our findings demonstrated the importance of serological surveillance which could ultimately support measures to prevent the dissemination of *M. synoviae*. It also demonstrated that the period of peak performance in laying birds could coincide with the period of maximum susceptibility to MS and thus high risk of vertical transmission of the pathogen to young flocks. This highlights the need for the implementation of effective control measures including the possibility of adopting effective vaccines against MS especially in breeding flocks.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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