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Activity of Mycoplasma synoviae in Commercial Chickens in Ghana: Serological Evidence

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Abstract
A total of 390 chicken sera were collected from respiratory disease free broilers, breeders and layers in the Ga-East district of Ghana and tested for *Mycoplasma synoviae* (MS) antibodies using commercial enzyme-linked immunosorbent assay (ELISA) kit from IDEXX Inc. USA. The overall sero-prevalence of MS was obtained to be 46.2%. The sero-prevalence among breeders and broilers were 0% each. MS sero-prevalence among layers ranged between 60% -85% and differed significantly among different ages ($p< 0.05$). The overall MS sero-prevalence among layers was 75%. It is concluded that *Mycoplasma synoviae* circulate in layer farms in the district. Layer birds at peak production age are at higher risk of infection.
Keywords: *Mycoplasma synoviae*, chicken, Sero-prevalence, Ghana

1. Introduction

Mycoplasma gallisepticum, *Mycoplasma synoviae*, *Mycoplasma meleagridis* and *Mycoplasma iowa*, are the four (4) recognized pathogenic mycoplasmas of poultry [1]. Of these, *Mycoplasma gallisepticum* (MG) is deemed the most economically important because of the huge economic losses associated with its infection. MG causes chronic respiratory diseases, increases carcass condemnation at slaughter due to airsacculitites, decreases egg production and egg hatchability [2]. The economic losses of MS have in the past been documented for strains associated with respiratory and arthropathic problems [3 – 8]. In recent times however, MS associated losses is gaining prominence in the global poultry industry due to the emergence of certain strains that cause abnormalities in the apex of egg shells and drops in egg production [9]. In countries with control and eradication programmes for MG, MS associated losses are hypothesised to be outrunning MG [10]. MS is widely distributed in commercial poultry farms around the world with infections reported in major poultry producing regions in Europe, Australia, America, Asia and some parts of Africa [1, 3, 5, 11-13]. MS strains exhibit varying host tropism and potentials of inducing diseases in susceptible hosts. Some strains affect the synovial membranes causing infectious synovitis. This primarily induces joint pathology, making it difficult for birds to access feed and water. Lameness and associated poor flock uniformity results in increased curling of birds and increased losses to producers [3, 5]. Other MS strains affect the oviduct of laying hens, resulting in egg production losses. The respiratory forms are often subclinical but certain strains can induce mild to severe respiratory problems and cause airsacculitis in chickens and turkey [4, 6]. Some strains could also interact with other respiratory pathogens such as Infectious bronchitis virus to elicit a more intense clinical outcome [14, 15]. MS can be transmitted vertically from an infected breeder hen to the progeny via the egg yolk and or horizontally via aerosol or contact with an infected bird and or contaminated farm personnel and equipment [1].

Poultry production is an economically important viable agricultural activity in Ghana. The sector consists of breeders, layers, cockerels and broilers. Together with the village chicken, the industry is the sole source of table eggs for the populace. Commercial production is intensified in three regions of the country [16]. The industry is generally faced with outbreaks of respiratory diseases, surges in egg production and poor egg quality. These among others reduce net profit of farmers and further hamper the growth of the industry. The presence of MS in the Ghanaian poultry industry is speculative and there exist no direct intervention to mitigate possible-MS-associated losses. We aimed to investigate MS circulation in the poultry population in Ghana by measuring the levels of MS antibodies in commercial chickens in the Ga-east district of the Greater Accra Region.

2. Materials and method

2.1 Study area

The study was conducted in the Ga-East district of the greater Accra region of Ghana over a period of 3 months. The study area has previously been described by Ayim and co [17]. Members of livestock and poultry farmers association were contacted. The study was explained to the farmers and their consent sort. Ethical approval was obtained from the Ethics Committee of the CSIR-Animal Research Institute prior to sampling.

2.2 Study design

A cross-sectional study design was used. Inclusion criteria for a farm were willingness of farmer to participate in the study, accessibility of farm and availability of birds. Additionally, the flock must be free of respiratory signs at the time of sampling. Sample size of 384 was computed using the formula $n= Z^2p(1-p)/d^2$, where n= sample size, Z= Z statistic at 95% level of confidence, p= estimated prevalence set at 50% and d= 5% precision of estimate [18].

2.3 Sampling

Ten (10) birds were randomly handpicked from each flock that met the previously outlined criteria. From each selected bird, 2mL blood was drawn from the jugular vein with a sterile disposable needle and syringe. Blood was transferred into a 4mL pre-labelled vacutainer tube containing no anticoagulant. The tubes were placed on racks and slanted at an angle of 45° to increase the surface area and facilitate clotting and sera separation. On multi-age flock farms, not more than 2 flocks were considered for sampling. Flock age, size, history, farm management practices including vaccination and biosecurity measures were collected for each flock sampled. Samples were transported to the laboratory in upright position and placed on the laboratory bench for at least 3h upon arrival. The tubes were then centrifuged at 1500rpm for 3 min. Each serum was harvested into a labelled 2mL centrifuge tube using barrier tips. A separate pipette tip and a new 2mL centrifuge tube were used for each serum. Harvested sera were stored at -20°C until needed.

2.4 Sera analysis

Sera were tested for MS antibodies using the IDEXX MS-ELISA antibody test kit. Sera and ELISA test reagents were allowed to come to room temperature before use. Prior to being assayed, a 1: 500 dilution of the sample was made with the sample diluent as recommended by the manufacturer. A 2 step dilution process was used to achieve this. A 96 well U bottom microtitre plate was labelled as D1. To this plate, 245µL of sample diluent was added to all wells except four (4) wells marked for positive controls (2 wells) and negative controls (2 wells). A multi-channel pipette with barrier pipette tips was used. 5µL of chicken serum was added to wells containing the sample diluent with single channel pipettes. Each sample was added to only one sample diluent in a particular well in D1. The samples were thoroughly mixed in the wells. Tips were changed in between samples. The resulting dilution of samples in D1 was 1:50. To a second plate labelled D2, 180µL of sample diluent was pipetted into all wells except the control wells. 20µL of samples in plate D1 were transferred to the corresponding wells in D2 after thorough mixing in plate D1. This gave a sample dilution of 1:10 and an overall dilution of 1:500. A multichannel pipette was used. The IDEXX antigen-coated plate was labelled. 100 µL of undiluted positive and negative controls (provided by the manufacturer) were added to their appropriate wells in duplicates. 100 µL of samples from plate D2 were transferred onto the appropriate well on the antigen-coated plate. The plate was incubated for 30 min at room temperature. Plate was manually washed five (5) times with deionised water and blotted dry on laboratory tissue paper after washing. 100 µL of conjugate was added to all wells and plate incubated on the laboratory bench (room temperature of 20°C) for 30 min (± 2 min). Washing and blotting were repeated as described above. 100 µl of TMB substrate was added to all wells and incubated at 20°C for 15 min (± 1 min). 100 µL of Stop Solution was added to all wells to stop the reaction. The bottom of each plate was cleaned severally with laboratory tissue to reduce and/ or prevent scattering of light from minute dust particles that could be present at the bottom of the plate. The optical density (absorbance value) was measured with a Biotek ELx808 Absorbance Reader at a wavelength of 630nm. Nitrile gloves were worn throughout the assay preparation. Laboratory bench surfaces and pipette aids were cleaned with 70% ethanol before the laboratory works were performed.

2.5. Data analysis

OD values were transferred onto an excel worksheet. The Positive Control Means (PCX) and Negative Control Means (NCX) for each test plate were calculated (Microsoft Excel, Microsoft Office 15). An assay was accepted to be valid when the NCX absorbance was less than or equal to 0.150 and the difference between PCX and NCX was > 0.075. The relative level of MS antibody of each sample was calculated with the sample- to-positive (S/P) ratio formula provided by the manufacturer as follows:

$S/P=Sample\ (OD) - NCX\ (OD)/PCX\ (OD) - NCX\ (OD)$, where S/P= sample –to-positive ratio, Sample (OD) = OD of test serum, NCX (OD) = mean OD of negative control, PCX (OD) = mean OD of positive control.

Serum samples with S/P ratios of less than or equal to 0.50 were considered negative. S/P ratios greater than 0.50 were considered positive and indicates exposure to MS. Interpretation of results was as provided by the manufacturer.

Data was exported and analyzed using SPSS 17.0.1 (SPSS Inc.). MS prevalence was calculated using the formula:

$$Prevalence = \frac{Number\ of\ positives\ detected}{Total\ number\ of\ samples\ analyzed}\ x\ 100\%$$

Chi-square test was performed to analyse prevalence between ages and flock types. $P < 0.05$ was considered significant in all cases.

3. Results and discussion

Samples were obtained from 39 flocks in 24 commercial poultry farms. With the exception of two (2) farms, none of the farms had a footbath. Three (3) types of flock, layers, broilers and breeders were encountered. Layer flocks were predominant and constituted more than half (61.5%) of the flock sampled. Broiler flock was almost half that of layer flock and made up 28.2% of the total flock. Breeder flock was the least found and constituted 10.3% of the sampled flock. Farms with more than one (1) flock were often encountered and constituted 63% of the total farms sampled. Majority (46.7%) of the multiple flock farms had only layer flocks. Broilers and breeders only on farms were 13.3% and 6.7% respectively. 33.3% of multiple-flock farms had both broilers and layers. Our data reemphasis the dominance of layer birds in the Ghanaian poultry industry, with occasional production of broilers during festive seasons [16]. Multi-age farms (63%) are common than single-aged farms as it offers farmers the possibility of continual production and a source of income throughout the year.

All the farms visited were small scale (flock size 50-5000) except one farm which had a total flock size of about 7000 birds, making it a medium scale farm. Small and medium scale farms form more than 90% of the farms in the industry. Characteristically, such farms apply very low to no biosecurity measures increasing the risk of infections of birds [16, 19]. The age of the birds varied within the different flock. The minimum age of birds sampled was 5 weeks and the maximum was 73 weeks. Among layers, flock age ranged between a low of 28 to a high of 73 weeks. Broiler age ranged from 5 - 12 weeks and breeders aged between 45 and 49 weeks (Table 1).

All birds were vaccinated against Newcastle and Gumboro. Additionally, all layer and breeder birds were vaccinated against Fowl pox. None of the birds had been vaccinated against *Mycoplasma synoviae* probably because MS vaccination is not a component of the scheduled vaccination of the Ghana Veterinary services [19]. All the birds were older than 4 weeks (5 weeks minimum age encountered) and therefore MS maternal antibodies if present would have depleted before we sampled [20]. Detection of MS antibodies in our samples will therefore be an indication of natural exposure of the birds to the pathogen on the field.

A total of 390 chicken sera were collected and analysed for MS antibodies. Positive titre levels were detected in almost half (180) of the analysed sera. The overall sero-prevalence of MS was determined to be 46.2% (Table 1). This report is the first evidence of activity of this pathogen in poultry in the study district. Our findings agree with reports in backyard chickens in Botswana (41%) [21] and Paraguay (53%) [22], but lower than that in commercial poultry from Algeria (66%) [23] and backyard chickens in Mozambique (85%) [12].

MS positive antibody levels were not detected in the broilers and breeders giving a sero-prevalence of 0% in each flock type (Table 1).

Table1. Prevalence of MS among different flock

Flock	Age (weeks)	Sera analysed n (%)	Number of positive sera	Number of negative sera	Sero-prevalence (%)
Layers	28 -73	240(61.5)	180	60	75.0
Broilers	5-12	110(28.2)	0	110	0
Breeders	45-49	40(10.3)	0	40	0
Total		390(100)	180	210	46.2

Layer birds were the most affected and more than two-thirds tested positive to MS. The sero-prevalence of MS among layers was 75% (Table 1). Despite the possible horizontal transmission of MS, all broilers on the same farm with MS positive layers were MS negative (data not shown). Most of the layers (82%) were reported to have had at least one episode of respiratory disease prior to sampling. Of these, more than half (62%) tested positive to MS. Respiratory infections are major challenges confronting poultry farmers in the country [24, 25]. These infections are caused by bacteria and or viruses, and when left untreated, could lead to high mortality and poor performance of birds, and consequently result in major financial loss to the farmer [26, 27]. To overcome these problems, poultry farmers in the country administer antibiotics as treatment options for respiratory infections and or as prophylaxis with little to no professional assistance [24, 25]. Farmers rely largely on their own experiences, that of their colleague farmers and also of drug sellers to purchase and administer these antibiotics to their animals [25, 28]. The scarcity of veterinary professionals together with the cost of investigative services (where available) is some of the key factors that compel farmers to execute these services on their own. This abuse of antibiotics may result in building-up resistance on farms, but can also have bactericidal and/or bacteriostatic effect on susceptible bacteria species. This could reduce and or prevent horizontal transmissions within and between susceptible flock on the same farm and could be the reason why

broilers were MS negative despite been in the same farm with MS positive layers. Again, MS infections in layers may have occurred and successfully treated with antibiotics before broilers were placed on the farm. The none detection of MS in some of the layers with history of respiratory disease could be that those infections were probably caused by other respiratory pathogens such as *Mycoplasma gallisepticum,* low pathogenic Avian Influenza virus and Infectious bronchitis virus among others [29]. In general however, we observed that layers on multiple-age farms were more susceptible to MS infections than layers on single-aged farms. This finding agree with reports of Fabienne and co [30] who observed that MS infection was more frequent in multi-age farms than single- aged farms.

The prevalence of MS among layers differed with the ages of the birds. MS antibodies were detected in the youngest layers (28 weeks) encountered. The prevalence of MS during the peak of egg production was also high (60%), which could impact negatively on profits of farmers if the strains involved affect egg production. The highest MS prevalence was obtained for layers between 40 and 59 weeks old. Layers older than 60 weeks had MS prevalence of 78%. This observed difference in prevalence within the various age groups (Table 2) was statistically significant ($p<0.001$)

Table 2. Sero-prevalence among different age groups of layer birds

	Age (weeks)	Sero-prevalence (%)
Layers	20-39	60.00
	40-59	80.00
	>60	77.80

$X^2 = 17.50$, $p<0.001$

The sero-prevalence of MS among layers in our study are generally high (60 -80%). This indicates the endemic nature of the pathogen on layer farms in the Ga-East district. Our prevalence in layers corroborates with that of Heleili et al [23] in Algeria and in Netherlands (73%) [31].

4. Conclusion

Our study shows that *Mycoplasma synoviae* circulates in poultry farms in the Ga-East district of the Greater Accra region of Ghana. Layer birds at peak of production are at high risk of MS infection.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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