SEROLOGICAL SURVEY OF MYCOPLASMA GALLISEPTICUM INFECTION IN LAYER CHICKENS IN THE GA-EAST DISTRICT OF THE GREATER ACCRA REGION

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ABSTRACT
Mycoplasma gallisepticum (MG) is among the most economically significant avian Mycoplasmas, causing chronic respiratory diseases in chickens. Limited knowledge exists about this pathogen in the poultry industry in Ghana. This study was carried out to investigate the prevalence of MG infection in commercial layer chickens in the Ga East district of the Greater Accra region of Ghana from March – October, 2010. A total of seven hundred and nineteen (719) apparently healthy individual chicken sera were collected from forty-nine (49) layer flocks. The Serum Plate Agglutination (SPA) test using Nobilis® MG antigen obtained from Intevert International, Boxmeer-Holland, was used to test sera for the presence of antibodies. The overall seroprevalence of MG was found to be 59.1%. Sero-prevalence of MG was high in all the different age groups (18-75 weeks). Infection was significantly higher ($X^2 = 202.8, p<0.00000$) in older birds (100.0%) than younger birds (15.6%). Sero-prevalence was again found to be significantly ($X^2 = 26.5, p<0.000001$) higher in larger flock sizes (81.3%) than smaller flock sizes (50.9%). These findings indicate a high MG seroprevalence in layer chickens in the Ga East district of the Greater Accra region. There is the need for the development of preventive and control strategies to help reduce MG infection.

INTRODUCTION
Poultry production is an ancient activity in Ghana. The industry has both indigenous and commercial chickens with the former constituting about 80% of the over 20 million poultry in the country (MoFA, 2002; VSD, 1998). The indigenous chickens are mainly found in the rural communities under the free range system receiving minimal food supplementation and negligible health care (Aboe et al., 2006; Awuni, 2002). These are sold to supplement the income of their owners and are also used for sacrifices. Commercial chickens are mainly in the urban communities and kept purposely for their meat and eggs in an intensive system. Majority of the farmers in this commercial activity are small scale, constituting about 60% of farmers in this industry (MoFA, 2002). Despite this, local productivity continues to be insufficient in meeting the needs of the people. This has resulted in an increase in the importation of meat and eggs in the past years (Asante, 2004). One of the major constraints in the industry in Ghana is the outbreak of diseases which re-
duces the gross profit of production and limits the supply of poultry products. Frequent outbreaks of diseases have significantly contributed to the folding up of many commercial poultry farms in the country. Diseases, respiratory in nature remain the topmost concern to farmers and is frequently encountered throughout the year. Although respiratory diseases are caused by a host of pathogens, Newcastle Disease Virus (NDV) has for a long time been regarded as the main pathogen accounting for about 80-90% deaths in poultry annually (Aboeet al., 2006; Awuni, 2002; VSD, 1998). Vaccination against NDV is done on regular basis in commercial farms yet; respiratory diseases of unknown etiology continue to affect chickens causing economic losses to poultry farmers.

Mycoplasma gallisepticum (MG) has been identified as the most pathogenic and economically significant of all avian Mycoplasmas. This pathogen continues to put a huge burden on the global poultry industry causing chronic respiratory diseases in birds (Ferguson et al., 2004; Kleven, 2003). Transmission is both vertical through infected eggs to chicks and horizontal through the inhalation of contaminated airborne droplets, feathers and dust. Contact between birds also aids in the rapid spread of the infection within flocks (Talha, 2003; Papazisi et al., 2002). Affected birds show respiratory signs of tracheal rales, nasal and ocular discharge, sneezing and coughing however, these symptoms are not exclusive to Mycoplasma gallisepticum and can thus be easily mistaken for other pathogens especially when laboratory tests confirmation is not done. Mortality is generally low with most infected birds suffering high morbidity, though naïve birds may suffer high mortality. In layers, there is a drop in egg production in infected birds. Work done in Brazil shows that compared to an MG-free chicken, an MG infected chicken lays 15.7% less eggs (Nascimento et al., 2005). Feed conversion ratio weight gain and egg hatchability are also reduced, with an increase in carcass condemnation at slaughter due to airsaculitis (Ferguson et al., 2004; Ley, 2003; Liu et al., 2001). All these make MG economically important (Ley, 2003). Affected birds may also be asymptomatic for life showing no obvious clinical signs but under conditions of management stress and/or the presence of other pathogens including attenuated vaccines, clinical signs may become evident. Infection with MG weakens the immune system and induces opportunistic infections (Hong et al., 2005). MG infection is very difficult to eliminate especially in farms where birds are of different ages. Strict adherence to biosecurity, surveillance and de-population of affected flocks could help in the prevention of the disease. Vaccines and antibiotics which reduce the effects of the infection can also be used (Ley, 2003).

Despite the worldwide distribution and the huge economic losses associated with this infection, Mycoplasma gallisepticum has not been well studied in the poultry industry in Ghana and thus no control measure(s) have been put in place to protect birds against this pathogen. This could be because most respiratory diseases in chickens in the country are attributed to Newcastle Disease Virus, and thus the possible association of other pathogens with respiratory diseases is masked. In this study the possible presence of MG antibodies in apparent healthy layer chickens not showing signs of respiratory diseases in the Ga East district of the greater Accra region is investigated.

**MATERIALS AND METHODS**

**Study Area**

The study was conducted in the Ga East district, an urban community in the Greater Accra region of Ghana where Small-scale commercial poultry production is intensively carried out. In addition livestock particularly cattle, sheep and goats are also kept by the farmers.

**Selection of farms**

An initial survey was conducted within the district to identify Commercial poultry farms where birds did not show signs of respiratory diseases. Farms where the farmer kept good farming records of his birds were then consid-
Serological survey of mycoplasma gallisepticum infection in layer chickens... Ayim et al.

Among other information, the age of the birds and the size of the flock were recorded. In farms where there were flocks of different ages, each flock was considered. Birds that were less than 5 weeks of age were not included.

**Sampling**

Fifteen (15) layer birds per flock that fit into the above criteria were randomly handpicked from the farms and blood samples collected from each.

**Blood sample collection**

Blood (1.0-1.5 ml) was aseptically drawn from the jugular vein of each bird with a disposable 2ml plastic syringe and needle. The blood was gently transferred into a labeled sterile 2ml Eppendorf tube and kept on racks in a cool box. The racks in the cool-box were gently tilted at an angle of about 45° to facilitate sera separation. The samples were then transported to the Molecular Biology laboratory of the CSIR-Animal Research Institute for immediate processing.

**Sera separation**

The racks bearing the blood samples in the tubes were put on the laboratory bench while still maintaining the 45° tilt. The samples were allowed to stand at this position for 1-2 hours after which the clean straw coloured serum found just on top of the clotted clumps was carefully collected with a 200µl sterile pipette tip into labeled sterile 1.5ml Eppendorf tube and immediately processed. A new pipette tip was used for each test sample.

**Serum plate agglutination (SPA) Test**

The SPA test was carried out at room temperature (25°C) with a crystal violet stained Nobilis® MG antigen (Intervet International B.V. Boxmeer-Holland) made of a suspension of killed and stained S-6 Adler Strain of Mycoplasma gallisepticum. The antigen was taken from the refrigerator (4°C) and allowed to stand at room temperature for 15 minutes prior to its usage. The procedure for the test was according to the manufacturer’s (Intervet International B.V. Boxmeer-Holland) instructions with some slight modifications. Briefly, a clean glass slide was obtained and both sides of the slide were cleaned with a paper towel. With the aid of a marker, a vertical line was drawn through one side of the slide to partition it into two. A sterile pipette with an appropriate tip was used to transfer 25µl of the fresh test serum onto one side of the line and another sample onto the other side. A new pipette tip was used for each sample. Twenty five microliter of MG antigen was then placed at the side of each test serum and a clean toothpick was then used to string the serum and the antigen together and to mix well. A new toothpick was used to do the same to the other test sample on the same slide. The slide was gently swirled for 5 seconds to mix well. The mixture was allowed to stand for 1 minute after which it was gently swirled again for another 5 seconds. The slide was allowed to stand for 55 seconds and the results immediately read. Samples that had definite clumps (agglutination) forming within 2 minutes were regarded as positive. Clumps formed slowly and usually from the periphery and could be seen even during swirling. The extent of clumping in the positive samples was not categorized. Samples that did not have clumps were considered negative.

**Statistical analysis**

Data was analyzed using the SPSS software (2007, version 16.0.1). The overall prevalence was calculated as:

\[
\frac{\text{the number of positivesera detected}}{\text{total number of sera tested}} \times 100
\]

The sero-prevalence of MG infection with respect to the age of birds and size of the flock were compared using the Chi-square test at a significance level of 0.05.

**RESULTS**

A total of forty nine (49) flocks were obtained from the twenty one (21) farms sampled. None of the farms at the time of sampling had more
than three flocks of different ages. Sixty seven percent of the farms had two (2) flocks of varying ages. Triple and single age flocks per farm formed 19% and 14% respectively of the total farms visited. Two types of production systems were encountered; the deep litter and the slatted floor. The deep litter system was the commonest production system found, accounting for approximately 86% (18 farms).

Blood samples and corresponding sera were obtained from a total of seven hundred and nineteen (719) birds. Out of this total sera analyzed, four hundred and twenty five (425) tested positive by the SPA test and two hundred and ninety four (294) were negative (Table 1). The overall prevalence was thus 59.1% (Table 1).

The age of the birds ranged from 18 -75 weeks old. There were more than two (2) flocks in each age group. The least number of flocks (3) were between 66-75 weeks old. Birds aged between 46-52 weeks old, had the largest number of flocks and formed 20% of the total flock (Table 1). There was a general increase in infection as the ages increased. The highest infection was found in the 66 weeks and above age group (100%) and the lowest infection (16%) was in the 18 – 24 weeks age group (Table 1).

The number of chickens per flock varied ranging from a least of 100 birds per flock to a maximum of one thousand three hundred (1300) birds. Most flocks (61.2%) had chickens between the ranges of 100-400 or 701-1000. 24.5% of the flocks had between 401 and 700 chickens. Only 7 flocks constituting 14.3% of the total flocks contained more than 1000 birds (Table 2). There was an increase in the seroprevalence of *Mycoplasma gallisepticum* infection as the number of chickens in the flock increased. The highest infection occurred in flocks which had more than 1000 birds in the flock and the least infection was in the 100-400 birds.

**DISCUSSION**

The results obtained showed that 59.1% of the sera tested from commercial layer chickens which showed no signs of respiratory diseases had antibodies against *Mycoplasma gallisepticum*. This is an indication that the birds have

| Table 1: Sero prevalence of *Mycoplasma gallisepticum* infection in chicken in relation to age |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Age (weeks)     | Number of      | Number of      | Sero-positive   | Sero-negative   |      |      |      |      |
|                 |   flocks       |   sera tested  |   n (%)         |   n (%)         |      |      |      |      |
| 18-24           | 6              | 90             | 14(15.6)        | 76(84.4)        |      |      |      |      |
| 25-31           | 6              | 83             | 26(31.3)        | 57(68.7)        |      |      |      |      |
| 32-38           | 5              | 70             | 34(48.6)        | 36(51.4)        |      |      |      |      |
| 39-45           | 8              | 120            | 62(51.7)        | 58(48.3)        |      |      |      |      |
| 46-52           | 10             | 150            | 106(70.7)       | 44(29.3)        |      |      |      | 202.8*|
| 53-59           | 5              | 75             | 53(70.7)        | 22(29.3)        |      |      |      |      |
| 60-66           | 6              | 86             | 85(98.8)        | 1(1.2)          |      |      |      |      |
| 66 and above    | 3              | 45             | 45(100)         | 0(0)            |      |      |      |      |
| Total           | 49             | 719            | 425(59.1)       | 294(40.9)       |      |      |      |      |

*Indicates significance at p value <0.00000
been exposed to the *Mycoplasma gallisepticum* pathogen. Birds in Ghana are not vaccinated against MG and therefore the presence of MG antibodies in the test sera could not be as a result of MG vaccination but rather exposure to field pathogen. The high sero-prevalence of 59.1% indicates a high activity of *Mycoplasma gallisepticum* in the layer chickens in the Ga East district. The high prevalence could be due to the limited knowledge of the pathogen in this country, hence the absence of any form of control/preventive measures to protect the birds against this pathogen. This result in this study is comparable to that reported by Hossain et al., (2007) who observed a 55.13% sero-prevalence of MG infection in layer chickens in the Greater Rajshahi district of Bangladesh and Sarkar et al., (2005) who reported 58.90% sero-prevalence of MG infection in layer chickens in some model breeder poultry farms in the Feni district of Bangladesh. This finding also corroborates reports of Godoy et al., (2001), Sikder et al., (2005) and Barua et al., (2006) who reported 59.1%, 56.86% and 60% sero-prevalence of MG infection in chickens respectively. The figure for the sero-prevalence of MG infection was, however, observed to be higher than that reported in Zaira, Nigeria (47.54%) by Abdu et al., (1983) and in selected farms in the south of Bangladesh (13-32%) by Biswas et al., (1992) and Amin et al., (1992). The systems of housing-deep litter and slatted floor, which accounted for more than 98% of the housing systems encountered in the present study (data not shown) could also account for the high prevalence. Such systems provide frequent and close contact between the birds. The infectious nature of the disease enhances the transmission of the pathogen directly from one bird to the other within the flock. The system also aids transmission of the pathogen from contaminated dust, airborne droplets and feathers through inhalation (Papazisi et al., 2002; Talha, 2003; Osman et al., 2009). The day old layer chicks used to raise these layers are imported and could be more susceptible to the strain of *Mycoplasma gallisepticum* in the country and hence the high prevalence. This study was however not designed to identify the strain (s) of MG present in the country.

Birds below 5 weeks of age were not included in the study. This was to avoid the possible detection of maternal antibodies (if any) which wanes away after 5 weeks (Tizard, 2000). The increase in age with a corresponding significant increase in infection prevalence shows the infectious nature of the disease and the possible horizontal spread of the pathogen in the layer chickens in the district. Most of the farms visited had at least two flocks of varying ages. Older chickens could serve as a potential source of infection to the younger ones. Farm hands could also aid in the transmission as they move from flock to flock on the same farm. More than 75% (data not shown) of the farms visited did not have foot bath and about 2/3rds of the few that had, did not have disinfectants in them. Ventilation was generally poor in most

### Table 2: Sero-prevalence of *Mycoplasma gallisepticum* infection in relation to flock size

<table>
<thead>
<tr>
<th>Flock size (number of Chickens/flock)</th>
<th>Number of flocks</th>
<th>Number of sera tested</th>
<th>Sero-positive n (%)</th>
<th>Sero-negative n (%)</th>
<th>$X^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>100-400</td>
<td>15</td>
<td>224</td>
<td>114(50.9)</td>
<td>110(49.1)</td>
<td></td>
</tr>
<tr>
<td>401-700</td>
<td>12</td>
<td>180</td>
<td>101(56.1)</td>
<td>79(43.9)</td>
<td></td>
</tr>
<tr>
<td>701-1000</td>
<td>15</td>
<td>219</td>
<td>132(60.3)</td>
<td>87(39.7)</td>
<td>26.5*</td>
</tr>
<tr>
<td>&gt;1000</td>
<td>7</td>
<td>96</td>
<td>78(81.3)</td>
<td>18(18.7)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>719</td>
<td>429(59.1)</td>
<td>294(40.9)</td>
<td></td>
</tr>
</tbody>
</table>

*indicates significance at p value <0.00001.
farms. The intensive nature of commercial poultry production coupled with the poor ventilation and the absence of bio-security in most of these farms could contribute to the significantly high infection of MG seen as the birds grew older (Dulali, 2003; Liu et al., 2001). A prevalence of 16% among the least age group (18-24 weeks) was high. It could be that the layer birds are exposed much earlier to the MG pathogen. The higher infection rates observed for older birds in this study agrees with studies carried out by Talha (2003) in Bangladesh, however, results contrary to these findings have been reported by Sarkar et al. (2005) and Hossain et al. (2007) in Feni and Rajshahi districts of Bangladesh respectively.

The prevalence of MG infection was generally high irrespective of the flock size (Table 2). There was, however, a significant increase in sero-prevalence of MG infection as the flock size increased (50.9% - 81.3%). Increasing the flock density also increases the frequency of contact between the birds of a particular flock. This high contact rate aids spread of infectious pathogens such as MG among the birds (Talha et al., 2003; Papazisi et al., 2002). The poor management practices in the farms and the general challenges involved in the management of large flocks could account for the high infection.

**CONCLUSION**

More than 50% of layer birds in the Ga East district of the Greater Accra region are infected with *Mycoplasma gallisepticum*. Older birds tend to be more infected than younger ones. The mode of transmission of the disease appears to be horizontal rather than vertical. Infection appears to be more prevalent in larger flock sizes than smaller flock sizes. The absence of bio-security and the generally poor management practices observed in the farms could contribute to the high infection. Further studies should be carried out to establish the association of MG with respiratory diseases in poultry in Ghana.

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Serological survey of mycoplasma gallisepticum infection in layer chickens...

Ayim et al.

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