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## ANIMAL HUSBANDRY & VETERINARY SCIENCE | RESEARCH ARTICLE

# Widespread exposure to infectious bronchitis virus and *Mycoplasma gallisepticum* in chickens in the Ga-East district of Accra, Ghana

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**Abstract:** Infectious bronchitis, a major challenge to the global poultry industry, is an acute and highly contagious disease of the respiratory and urogenital tract of chickens which causes significant economic losses to poultry producers. In addition, *Mycoplasma gallisepticum* (MG) is another respiratory pathogen that remains a concern to producers. This study investigated the sero-prevalence of IBV and MG in commercial chickens in Ga-East district of the Greater Accra Region, Ghana, using sera obtained from 440 broiler and layer chickens showing no signs of disease. IBV and MG specific antibodies were determined using commercial ELISA kits. Majority (85%) of the samples tested positive for at least one of the 2 pathogens investigated, with 30% testing positive for both. Overall sero-prevalence of IBV and MG were 85.5 and 29.5% respectively suggesting a higher IBV than MG field challenge in the study area. IBV prevalence was significantly higher in layers (100%) than broilers (42%). Age of bird had a significant influence on IBV prevalence among broilers. The MG prevalence in layers and broilers were 39.4 and 0% respectively. This data supports the need to institute control measures to mitigate IBV associated losses and improve poultry production in Ghana.

**Subjects:** Agriculture and Food; Plant & Animal Ecology; Laboratory Animal Science; Virology

**Keywords:** infectious bronchitis virus; *Mycoplasma gallisepticum*; sero-prevalence; chickens; Ghana

### ABOUT THE AUTHOR

Ayim-Akonor Matilda is an early career scientist in the Animal Health and Food Safety Division, CSIR-Animal Research Institute, Ghana with expertise in microbiological research of livestock and poultry diseases. Her research focuses on the epidemiology of infectious diseases of livestock and poultry and the development of appropriate control strategies to mitigate the effect of infectious pathogens on animal productivity. Ayim-Akonor also focuses on zoonotic diseases both of national and international interest.

### PUBLIC INTEREST STATEMENT

Infectious Bronchitis Virus (IBV) and *Mycoplasma gallisepticum* (MG) are two economically important infectious pathogens of poultry worldwide. Both pathogens cause huge economic losses to producers annually. Many poultry producing regions in the world use appropriate vaccines to reduce the effect of field challenges. In Ghana, there is no national plan to control IBV and MG infection mainly because of the limited information on the activity of these pathogens in the country. We observed a wide spread of IBV and MG in commercial poultry farms in Ghana and advocate for a need for stakeholders to institute control measures that will help to reduce IBV and MG associated losses and thereby improve poultry production in the country.

## 1. Introduction

Infectious bronchitis is an acute and highly contagious disease of the respiratory and urogenital tract of chickens. The disease is caused by a single-stranded enveloped RNA Coronavirus called infectious bronchitis virus (IBV) (Jackwood & de Wit, 2014). The respirotropic form of the disease is characterized by tracheal rales, cough and sneeze. Infections of the oviduct and kidneys by other IBV strains cause egg production losses, poor egg quality, urate deposits in kidneys and increased mortality among young birds (Cook, Jackwood, & Jones, 2012; Sellers, Telg, & Williams, 2008). *Mycoplasma gallisepticum* (MG) is the most economically important of the four avian pathogenic mycoplasmas (Ley, 2008). It is a pleomorphic bacteria with no cell wall (Ley, 2008). MG causes chronic respiratory diseases in chickens and infectious sinusitis in turkeys. In addition, infections with MG causes drop in egg production and poor hatchability of fertile eggs in layers and breeders respectively. Chickens of all ages are susceptible to MG infection but young birds are the most susceptible (Kleven, 2008; Ley, 2008).

Respiratory diseases remain a major challenge to the global poultry industry causing significant economic losses to poultry producers' worldwide annually (Yashpal, Devi, & Sagar, 2004). Reduced feed intake, poor growth and drop in egg production often characterise affected birds impairing productivity and reducing net income of producers. Respiratory infections most often spread quickly among flocks and can reach 100% morbidity in less than a week. Mortality also varies depending on the type and strain(s) of organism(s) involved, flock type and age of birds among others (Roussan, Khawaldeh, & Shaheen, 2009). Pathogens associated with respiratory diseases in poultry are bacterial and/or viral in nature, of which several species have been identified (Roussan, Khawaldeh, & Shaheen, 2009; Yashpal et al., 2004). IBV and *Mycoplasma gallisepticum* are two important respiratory pathogens of poultry that remain a concern to many poultry producers (Ley, 2008; Swayne & Halvorson, 2008).

IBV and MG infections are common in commercial poultry-producing regions of the world and both pathogens elicit similar respiratory signs that can complicate diagnostic efforts (Gelb & Jackwood, 2008; Ley, 2008). IBV and MG are economically important because, rather than increase mortality (which is often low in the absence of complications), these pathogens increase production inefficiencies and reduce net profit of producers. Among broilers, IBV and MG infections are associated with poor feed conversion efficiency, poor weight gain and an increase in carcass condemnation at slaughter (Gelb & Jackwood, 2008; Kleven, 2008; Ley, 2008). Suitable IBV and MG vaccines are used in some regions to protect birds against field challenges. MG is further controlled in an infected flock with appropriate antibiotics (Cook, 2008; Ferguson-Noel, 2014; Sandu, Jackwood, Sellers, Roney, & Williams, 2016).

The commercial poultry industry in Ghana contributes substantially to the animal protein needs of the society and it is the sole source of table eggs for the over 25 million inhabitants. Commercial birds are exclusively kept indoors, where higher inputs and labour are used. The industry also provides income and employment to many, particularly the youth and women (Food & Agriculture Organization [FAO], 2004, 2014). The industry is however faced with several production challenges that negatively impact productivity, reducing net profits and further threatening the survival of the industry. These challenges include outbreaks of respiratory diseases that most often remain unresponsive to antibiotic treatment, mortality, poor egg quality and reduced egg production (Adei & Asante, 2012; Anang, Yeboah, & Agbolosu, 2013; Kusi, Senyo, Isaac, & Kwamena, 2015), a situation that may be attributed to IBV and/or MG infection.

The presence of IBV in commercial poultry in Ghana was made evident when IBV was detected from trachea swabs of chickens in a respiratory disease outbreak (Ayim-Akonor, Arthur, Ohene, & Baryeh, 2013). MG antibodies have also been reported in layer birds in Ghana but an association between MG and respiratory disease outbreaks have not been found (Ayim et al., 2012; Ayim-Akonor, Baryeh, & Asante, 2013). These studies provided initial molecular and serological evidence of exposure to these two (2) pathogens in Ghana. In the present study, we investigated the extent of activity

of IBV and MG in chickens in the Ga-East district of the Greater Accra region during the rainy months of the year.

## 2. Materials and methods

### 2.1. Study design

Members of the Livestock and Poultry Farmers Association in the Ga-East District of the Greater Accra Region of Ghana were visited and given an explanation of the study. A written informed consent was either signed or thumb printed by farmers who agreed to participate in the study. A cross-sectional study design was used. 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.

#### 2.1.1. Flock selection and sampling

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.

On a farm with flock size between 50 and 500, 10 birds were randomly selected for sampling. Fifteen birds were selected from flock with 501–5,000 birds, and 20 birds were selected from birds with flock size >5,000. On multi-age farms, not more than two flocks were considered for sampling.

From each selected bird, 2 ml of blood was drawn with a sterile disposable syringe and needle from either the jugular or brachial vein. Blood was transferred into a 4 ml pre-labelled vacutainer tube contain 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. Tubes were transported to the laboratory on racks in an upright position.

Basic farm data about each flock collected from the farmer using structured questionnaire included flock type, age, flock size, health and vaccination history.

#### 2.1.2. Preliminary laboratory analysis

Upon arrival in the laboratory, tubes containing clotted blood were placed on the laboratory bench for at least 2 h for the sera to separate. Tubes were then centrifuged at 1,500 rpm for 3 min. Each serum was harvested into a pre-labelled 2 ml centrifuge tube with 1,000  $\mu$ l filtered tips. A new tip was used for each sample. Harvested sera were stored at –20°C until needed.

Samples were obtained over a three-month period (May to July). Ethical approval was obtained from the Ethics Committee of the CSIR-Animal Research Institute prior to sampling.

#### 2.1.3. Antibody detection

Each serum sample was individually for antibodies against IBV and MG using the Enzyme-linked immunosorbent assays (ELISA). Commercial IBV Ab and MG Ab Test kits from IDEXX Laboratories Inc. (USA) were used to detect antibodies in serum against these two pathogens. The IDEXX IBV Ab ELISA test kit detects total antibody response to IBV and not to a specific IBV serotype(s). For each test plate, the appropriate positive and negative controls (included in the test kit) were used. The manufacturer's instructions were followed with some modification. Briefly, test sera and ELISA test reagents were allowed to come to room temperature. Prior to been assayed, a 1:500 dilution of the sample was made with the appropriate sample diluent (IBV or MG) 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, 245  $\mu$ l of sample diluent was added to all wells except four wells marked for positive controls (2 wells) and negative controls (2 wells). A multi-channel pipette with barrier pipette tips was used. 5  $\mu$ l 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, 180 µl of sample diluent was pipetted into all wells except the control wells. 20 µl 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 with the test ID and date of analysis. 100 µl of undiluted positive and negative controls (provided by the manufacturer) were added to their appropriate wells in duplicates.

One hundred microliters (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 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 (OD)/absorbance value of each sample on the test plate was measured with a Biotek ELx808 Absorbance Reader at a wavelength of 630 nm. Nitrile gloves were worn throughout the assay. Laboratory bench surfaces and pipette aids were cleaned with 70% ethanol before the laboratory works were performed.

All samples were tested for IBV antibodies first, after which they were tested for MG antibodies. Each test was performed with the appropriate test kits and controls.

#### 2.1.4. Data analysis

The OD values were exported to excel. 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 ≤0.150 and the difference between PCX and NCX was greater than 0.075 for both IBV and MG tests. The Sample to Positive (S/P) ratio was calculated using the formula:

$$S/P \text{ Sample (OD)} - NCX(OD)/PCX(OD) - NCX(OD)$$

Endpoint titres were calculated using the formula:

$$\log_{10} \text{ Titre} = 1.09(\log_{10} S/P) + 3.36$$

IBV titers greater than 396 and MG titers greater than 1,076 were considered positive. Interpretation of all results was as directed by the manufacturer.

The prevalence of IBV and MG were individually calculated using the formula:

$$\text{Prevalence} = \frac{\text{No. of positive samples detected}}{\text{Total no. of samples analysed}} \times 100$$

$\chi^2$  test (SPSS 17.1) was also done to compare, separately, the prevalence of IBV or MG between broilers and layer flocks and also prevalence between flock ages. Probability < 0.05 was considered significant in all cases.

### 3. Results

A total of 21 farms, comprising of 29 flocks, were visited. Most of the farms (61.9%) had only one flock of either layers or broilers. Farms with two flocks constituted 38.1% of the total farms visited. None of the farms had more than 2 flocks.

Of the 29 flocks, 69% were layers and 31% broiler flocks (Table 1). The flock size varied considerably between and within the two-flock type. Flock sizes of layers ranged from a minimum of 250 to a maximum of 4,000 birds with an average of 1,230. Within the broilers, flock size ranged from 80 to 3,500 with an average of 751 birds (Table 1).

The layer birds' age ranged from 28 to 79 weeks, and broilers, 5 to 12 weeks (Table 1). All the 29 flocks were vaccinated for Newcastle Disease Virus and Infectious Bursal Disease Virus. Additionally, 21 flocks (made up of 86% layers and 14% broilers) had been vaccinated against Fowl pox. None of the flocks were vaccinated for IBV and/or MG. Thirty-one per cent (31%) of the flocks were reported to have had at least one episode of respiratory disease prior to sampling. Out of these, 67% were layer birds and 33% broilers.

A total of 440 sera samples were collected from the 21 farms and separately analysed for IBV and MG antibodies. Out of this number, 110 (25%) samples were from broiler birds and 330 (75%) were from layers. IBV antibodies were detected in a total of 376 samples. The overall IBV prevalence was 85.5%. Sero-prevalence of IBV varied between the two flock types. Within the layer flocks, positive levels of IBV antibodies were detected in all sera samples from the 20 flocks, resulting in a prevalence of 100%. However, a lower detection rate was observed in broiler flocks. Only 4 out of the 9 broiler flocks sampled tested positive for IBV antibodies. The prevalence within positive broiler flocks was 60% (1 flock) and 100% (3 flocks). IBV antibodies were detected in less than half (46) of the broilers sampled.

The overall prevalence of IBV in layers and broilers was 100 and 42%, respectively (Figure 1).  $\chi^2$  test indicated that the IBV sero-prevalence between layers and broilers were statistically significant ( $\chi^2 = 37.69, p < 0.001$ ). The prevalence of IBV among broilers varied within the different age groups. Five to eight-week-old broilers had IBV prevalence of 40% while nine to 12 weeks recorded a higher prevalence of 50%. The difference in IBV prevalence among these age groups was significant ( $\chi^2 = 44.55, p < 0.001$ ).

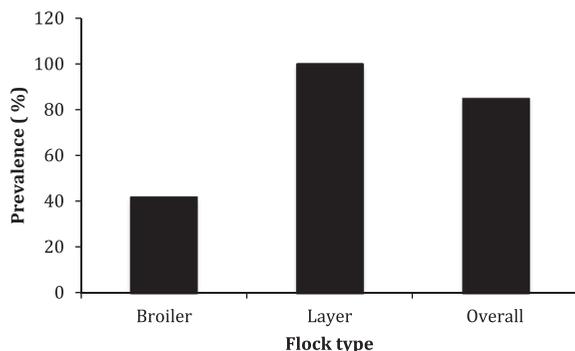
IBV antibody titre varied within the layer and broiler samples analysed. Among layers, none (0%) had IBV antibody titre levels that can be classified as low (titre level ranging from 397–1,000). Some (24%), however, had medium levels of antibody titre (titre levels ranging from 1,001–5,000) while the majority (76%) had high antibody titres (titres > 5,000) (Figure 3). On the other hand, titre levels in broilers included low, medium and high. Half (50%) of IBV positive broilers had medium level of antibodies, while low and high IBV antibody titres were detected in 26 and 24% of broiler samples respectively (Figure 2).

MG antibodies were less frequently detected in the samples than IBV. MG antibodies were detected in only one-third (130) of the 440 serum samples analysed. In all the broiler samples, levels of MG antibodies were below the positive cut-off levels and thus, were negative. All the MG positive samples were obtained from layers. The prevalence of MG in broilers and layers were determined to be 0 and 39% respectively (Table 2). The overall sero-prevalence of MG in the test samples was 30% (Table 2).

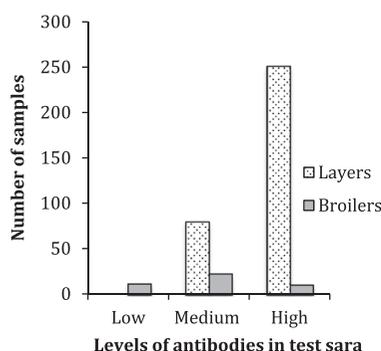
**Table 1. Basic flock description**

Flock type	Number of flock	Flock size range	Age range (weeks)
Layer	20	250–4,000	28–79
Broiler	9	80–3,500	5–12
Total	29		

**Figure 1. Sero-prevalence of IBV in layer and broiler flock.**



**Figure 2. IBV antibody titres levels in layers and broilers.**



**Table 2. Prevalence of MG in broiler and layer flock**

Flock type	Samples tested	MG positive	MG negative	MG prevalence (%)
Layer	330	130	200	39.4
Broiler	110	0	110	0
Total	440	130	310	29.5

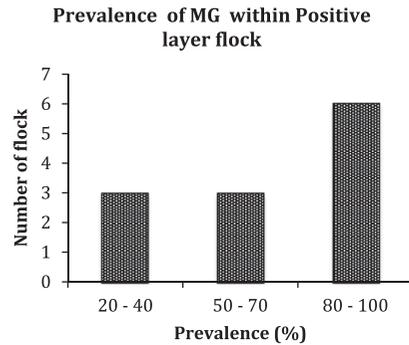
As indicated in Table 2, MG antibodies were detected in more than 50% (12/20) of the layer flocks. A great variation in MG prevalence was seen within the MG positive layer flock. A quarter (25%) of the flocks had MG prevalence between 20 and 40%. Another 25% had MG prevalence between 50 and 70% while the remaining 50% had MG prevalence between 80 and 100%, as shown in Figure 3.

Three hundred and seventy-six samples, representing 85% of the total samples, tested positive for at least one of the two pathogens. Thirty percent of the samples tested positive for both IBV and MG antibodies. Only layers were positive for both IBV and MG antibodies.

#### 4. Discussion

Poultry farms in Ghana are classified into three categories; small, medium and large-scale farms and based on a scale of 50–5,000 birds for small scale, 5,001–10,000 birds for medium scale and more than 10,000 birds for large scale (GAIN, 2013). In our study area, none of the farms visited was in the large-scale category. As single or multiple flock farms, only one farm had a total bird number that was classified in the medium scale category (6,984 birds) while the other farms were small-scale. Small-medium scale poultry producers continue to dominate the Ghanaian poultry industry. These farms are estimated to constitute more than 95% of the sector (GAIN, 2013). Small-medium scale farmers practice minimal biosecurity throughout the country and are prone to several production inefficiencies. It is worth mentioning, that most of the farms visited had land space and or existing infrastructure that could be used to expand production. The continuous production of birds below

**Figure 3. Variation in MG prevalence within layer flock.**



the maximum capacity of farms could be attributed to, among other factors, the high cost of feeding, unfavourable importation policies, diseases and limited technical know-how of farmers. Similar reports have been made in other parts of the country (Atuahene, Attah-Kotoku, & Mensah, 2010; Kusi et al., 2015).

Compared to broiler flocks, layer flocks were frequently encountered during the sampling period (Table 1). The Ghanaian market continues to be flooded with imported broiler meat from Europe and America, which is cheaper than what is produced locally (GAIN, 2013). In order to have a continual source of income, improve their livelihood and also contribute to the attainment of food security in the country, local poultry farmers have skewed the industry towards egg production, where there is very little to no competition with foreign entities. Broiler production is intensified during festive seasons such as Christmas when demand for live chicken generally increases in the country (FAO, 2014; GAIN, 2013). However, economic analysis of the sector has shown that despite the general and unique challenges facing both sectors, broiler and layer productions are profitable (Anang et al., 2013). Our sampling period (May–July) did not coincide with such a notable season, which could account for the fewer broiler flock encountered.

None of the birds in our study population were vaccinated for IBV and/or MG. This is because vaccination against these two pathogens is not permitted in the country. The recommended vaccination schedule by the Ghana Veterinary Services directorate for poultry production in the country are Gumboro (week 1 and 3), Newcastle disease (week 2, 10 and 16) and Fowl pox (week 7 and 12) (VSD, 2012). Other farmers in addition use coccidiostats to manage coccidial infections in their farms (Boamah, Agyare, Odoi, & Dalsgaard, 2016).

A substantial percentage of day old chicks (DOCs) used by local farmers are imported, either as DOCs or fertile eggs that are hatched in the country and supplied to farmers to augment that which is produced locally (FAO, 2014). Imported DOCs could have substantial amount of maternally derived antibodies of IBV and/or MG from the parent stock. All the birds sampled in our study were imported (data not shown). However, the least age of birds encountered was 5 weeks (Table 1) and maternal antibodies of IBV and/or MG if present would have depleted prior to our sampling (Gharaibeh & Mahmoud, 2013). The detection of IBV and/or MG antibodies in our samples therefore indicates a natural exposure of the flock to field IBV and/or MG.

ELISAs are suitable serological techniques used for the general monitoring of flock. The IDEXX IBV Abs ELISA test kit used in this study detects total antibody responses to IBV. The kit is not serotype specific. Circulation of IBV in poultry is of concern to farmers in Africa. IBV antibodies have been reported in both local and commercial chickens in African countries such as South Africa (Thekiso, Mbat, & Bisschop, 2003), Zimbabwe (Kelly et al., 1994) and Nigeria (Ducatez et al., 2004; Emekpe, Ohore, Olujonwo, & Akpavie, 2010; Komolafe, Ozeigbe, & Anene, 1990).

In our study, we obtained IBV prevalence of 86%, which denotes a widespread exposure of commercial chickens in the study area. The high IBV sero-prevalence detected supports earlier reports that IBV plays a significant role in respiratory disease outbreaks in poultry in Ghana (Ayim-Akonor, Arthur et al., 2013, Ayim-Akonor, Baryeh et al., 2013). Our results compare favourably to the 84% and 85% previously reported in commercial respiratory-disease free chickens in Nigeria by Ducatez et al. (2004) and Emekpe et al. (2010); but lower than that reported from Jordan (Roussan et al., 2009).

IBV prevalence in layer flocks was significantly higher than that in broiler flocks and further showed a larger proportion of samples with incredibly high levels of antibodies (Figures 1 and 2). This could be ascribed to layer birds spending more time on the farm than broilers and subsequently becoming re-infected in the absence of suitable control method.

Almost all the IBV positive broiler samples (92%) were obtained from farms that also had layer birds that tested 100% for IBV antibodies suggesting the endemic nature of the virus in layer flocks and further suggests active transmission of the virus between flocks on multi-age farms. IBV characteristically persist in the intestines of chickens and are constantly shed in faeces for several weeks, even after an infected flock has recovered from an infection (de Wit, Cook, & van der Heijden, 2011). A previously infected flock can serve as a local source of infection to IBV-free birds that are later placed on the farm. Between flocks on the same farm, airborne transmission via aerosol and mechanical transmission via farm workers, contaminated equipment, feeding troughs and other farm equipment could facilitate spread of the pathogen (Cavanagh & Gelb, 2008). Our IBV prevalence in layers (100%) is higher, compared to 92% reported from Nigeria (Emekpe et al., 2010).

The risk of acquiring an infection in an endemic area often increases with the time spent in that locality, and we detected age as risk factor. IBV positive antibodies were detected in our youngest flock (5 weeks) denoting a much earlier infection of broilers in an endemic environment. Birds of all ages are susceptible to IBV infection but young birds are severely affected with morbidity reaching about 100% (Gelb & Jackwood, 2008). Furthermore, we observed that as broiler birds stay longer in the field (9–12 weeks in our case), their risk of becoming infected increases and a significantly ( $p < 0.001$ ) higher IBV prevalence with an increasing high titres were observed (Figure 2) denoting a possible continuous exposure of multiple IBV infection on the farms.

The overall sero-prevalence of MG in the study area was found to be lower (30%) compared to IBV (86%) and differed significantly between the two flocks (Table 2 and Figure 1). MG prevalence in broilers was negligible (0%) but 39% prevalence was recorded in the layer flock. The present MG prevalence in layers is lower than the 59.1% reported earlier in the same study area by Ayim and co., (2012). This is likely attributed to the differences in serological methods used to detect the MG antibodies. The Serum Plate Agglutination test which measures IgM is quick, cheap and sensitive but also prone to false positives because of the cross-reacting antigens common to MG and *Mycoplasma synoviae* (MS). This lowers the specificity of the test (Avakian & Kleven, 1990; Kleven, 2008; Levisohn & Kleven, 2000). ELISA tests however detect IgG which are produced later after an infection and requires about 4 weeks to detect positives after a positive SPA result of 2 weeks. The specificity is high but sensitivity is low. Other tests including HI and culture are often recommended to complement these tests but none was performed in both cases.

MG antibodies were not detected even among broiler birds that were on the same farm with MG positive layers (data not shown). It is possible that such layers recovered from an MG infection before the broilers were placed on the farm and/or that the broilers were also infected but the test of choice here (ELISA) was unable to detect the infection within the relatively short broiler life/sampling time frame.

Respiratory signs among poultry are easily recognised by Ghanaian farmers who most often term them as CRD. Farmers rely on their own expertise to purchase antibiotics and apply on their farms, seeking veterinarian assistance often after treatment failures. The indiscriminate use of antibiotics

in flocks either as prophylaxis, treatment of infections or both in the industry (Annan-Prah, Agbemaflle, Asare, & Akorli, 2012; Boamah et al., 2016; Turkson, 2008) could have bactericidal and/or bacteriostatic effect on MG (if MG is the causative organism of the infection), and thus control the pathogen and further limit transmission between susceptible birds. This could account for the low MG prevalence observed overall and the low prevalence even among layers who spend longer time on the farm. Frequent usage of antibiotics by farmers could also account for the variation in MG antibodies detected within the same flock on a particular farm (Figure 3).

Overall, a much higher percentage (85%) of our samples tested positive to at least one of the pathogens of interest and 30% tested positive to both pathogens signifying the importance of IBV and MG in the study area. Almost all (99.9%) the farms visited were small scale. Small and medium scale farms practice very minimal biosecurity. Poor biosecurity on farms predisposes birds to various disease agents and promotes spread of infectious pathogens such as IBV and MG but the frequent use of antibiotics on farms could minimise the effect of MG in farms with no effect on IBV.

Although IBV and MG antibodies were detected in our study area, IBV appears to be more important than MG. This is because IBV was wide spread, detected in 18 of the 21 farms (86%) compared to MG, which was detected in 10 of the 21 farms (48%).

Our study was designed to account for past infections of IBV and MG in the flocks. Data is therefore skewed towards birds without active respiratory infections at the time of sampling.

## 5. Conclusion

IBV and MG circulate in chickens in the Ga-East district of the Greater Accra region. Compared to MG, IBV is widespread in the area and highly endemic in layer farms. Both layers and broilers of all ages are susceptible to IBV infection, but broilers can be infected at a much younger age when placed on farms that have IBV positive layer flock. Broilers kept longer than 8 weeks on the farm are at higher risk of IBV infections. The absence of IBV control measures contributes to spread of pathogens between susceptible species on the same farm. Layer birds rather than broiler birds are likely to be infected with MG. Frequent usage of antibiotics by farmers could mitigate MG effects and further reduce transmission between flocks.

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## Competing interests

The authors declare no competing interest.

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