Prevalence of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* Antibodies in Free Range Chickens as Detected by ImmunoComb Assay

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Abstract

This study was conducted to detect the antibody titer *Mycoplasma gallisepticum* and *M. synoviae* on the free-range chicken project at the Tarlac Agricultural University and its beneficiaries in Santa Ignacia. A total of 110 blood samples from free-range chickens were collected, regardless of age, sex, and breed. These samples were tested for Mycoplasma infection using the ImmunoComb Assay.

Results showed that 56.4% were found positive for *Mycoplasma synoviae* and 46.3% positive for *Mycoplasma gallisepticum*. However, even though they were found positive, the antibody titer for *Mycoplasma synoviae* was only 1.82 ImmunoComb® unit in Tarlac Agricultural University – FRC Project, and 1N.34 ImmunoComb® unit in Santa Ignacia FRC beneficiaries. This finding indicate weak positive. Further, Mycoplasma gallisepticum with 1.40 ImmunoComb® unit in Tarlac Agricultural University – FRC Project, and 1.11 ImmunoComb® unit in Sta Ignacia FRC beneficiaries were found to be weakly positive. Based on the results of the study, the *Mycoplasma gallisepticum* and *Mycoplasma synoviae* tested were both prevalent but in low antibody titers on the Free-

range Chicken project at Tarlac Agricultural University and its beneficiaries in Santa Ignacia.

Keywords. *Antibody, Chicken*, *ImmunoComb®, Mycoplasma gallisepticum, Mycoplasma synoviae*

INTRODUCTION

Free-range chicken production nowadays is highly in demand as free-range chickens are believed to be good sources of nutritious meat and eggs for the consumers. The management system of production of free-range chickens is more like native chickens for they range in open fields for acquiring their food. Through this system of production, the chickens are highly susceptible to Mycoplasma infections.

One of the most serious diseases that affect the avian species is caused by Mycoplasma. Mycoplasma species that are most important in causing diseases in poultry farming are *M. gallisepticum, M. meleagridis,* and *M. synoviae*. The condition occurs worldwide and affects the production and performance of poultry farms. In some countries, this infection occurs rarely in commercial poultry.

In previous studies, Mycoplasma causes chronic respiratory disease in chickens and sinusitis in turkeys. Mycoplasma disease is characterized by respiratory rales, coughing, nasal discharge and conjunctivitis, and infraorbital sinusitis in turkeys. Increased carcass and downgrading condemnation caused by aereosacculitis, decreased growth and egg

production, and increased medication costs, make MG one of the costliest infection diseases (Raviv and Ley, 2013). Mycoplasma infection induces significant economic losses in poultry by reducing body weight gain, meat quality, and feed conversion rate in broilers, causing a significant decline in egg output in layers, and increasing embryo mortality in breeders. Blood and serum testing kits are used to determine the antibody titers of *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) in chicken flocks. ImmunoComb assay is a kit that detects the antibody of infection of Mycoplasma spp in chickens, this is used to diagnose the presence of the bacteria causing the low production of chickens.

Hence, this study was conducted to determine the presence of Mycoplasma in Free Range Chickens at Tarlac Agricultural University and its beneficiaries in Santa Ignacia.

METHODOLOGY

Management of Experimental Animals

A total of 110 free-range layer chickens aged five to eight months, both sexes, with an average of 120 kgs body weight, was used in the study**.** The experimental animals were housed at the Free-Range Chicken Project, Tarlac Agricultural University, and in the residents of the beneficiaries of the project in Sta. Ignacia, Tarlac. The chickens were semi-confined and were ranged in the morning to exercise and exhibit their natural behaviors. They were fed with commercial feeds added with forages in the morning and the afternoon. Water was also provided daily.

Vaccination and Health management

The chickens were vaccinated at the age of two weeks with the New Castles Disease vaccine B1B1. The vaccine was administered intraocular, one drop of NCD B1B1 in the eye per bird. Then they were administered with LaSota Newcastle disease vaccine at four weeks of age. In breeder and layer chicken flocks, the vaccine needs to be repeated at 3-month intervals to maintain a sufficient level of immunity. At the age of 18 weeks, the chickens were vaccinated with the NCD vaccine for further immunity, blood collection, and for the Immunocomb assay.

Biogal's ImmunoComb

Biogal" ImmonoComb Assay is a diagnostic test for specific antibodies in the animals' blood. The kit is similar to ELISA or Enzyme Link Immunosorbent Assay principle, which is used to test specifically Mycoplasma infection in chickens. This is produced by Biogal Galed Labs., Gale Kibbutz, Israel.

Development Process of Assay (Biogal's ImmunoComb)

Using paper disk

By using a paper disk, one of the chicken's veins was pierced. Then a specimen paper was taken and saturated a pre-punched disk with the blood. Using tweezers, the protective aluminum cover of wells in row A was slit open. Then a disk saturated with blood was punched out. This was followed by inserting the disks into the diluents which was succeeded by the extraction of antibodies. After that, next 2 consecutive wells for the control serum were opened. A 5 µl Positive Control Serum (C+) was taken and inserted

into well A next to the last sample. The serum was mixed into the well. Then the same steps were done with the Negative Control Serum (C-) in the following well. After that, one comb was removed from its protective wrapping and was inserted (printed side facing you) into Row A. Then, it was incubated for 10 minutes. To improve mixing, the researcher gently moved the Comb up and down at the start of each incubation (each row). This was repeated at least twice in all of the remaining rows. The cover of wells was pierced in Row B with the tweezers. The excess liquid was gently shaken off onto a tissue (follow the same procedure for the remaining rows at the end of each step). The comb was inserted into wells of row B and incubated for 2 minutes. The Comb was placed in Row D for 2 minutes, Row E for 2 minutes, and Row F for 10 minutes, allowing the color reaction process to develop. After the Comb had completed the cycle for Row F, it was transferred back to and incubated at Row E for two minutes for color fixation.

Reading Results with the Comb scale

When the comb was completely dry, it was aligned with the calibrated color CombScale provided in the kit. The tone of the comb with the purple-grey on the CombScale closely matches the Positive Control spot the most. Then, the yellow ruler was slid until the C+ mark appeared in the window above the color. The researcher held the slide in this position during the entire reading. In this step, it calibrated the C+ to S3, which was the “cut-off” point to which test spots were compared. The spots were read separately. After that, the researcher chose the most suitable color and read the titer in the yellow windows.

Reading and Interpreting the Results

The middle spot tested for MG and the lower spot tested for MS. The results were evaluated with each disease separately. MG and MS IgG levels were determined by comparing each specimen's color intensity to the Positive Control (C+). Specimens with identical or higher color intensity than the Positive Control were considered positive. The Negative Control consisted of non-immune sera and was read as zero (S=0). Non-specific reactions around S1 (i.e. false positive) occurred occasionally due to various reasons and may be associated with the use of certain commercial vaccines. To avoid misinterpretation of non-specific reactions and possible confusion with true low positive results, it is recommended to confirm results by retesting at a one-week interval. A test color darker than S6 indicates either an acute disease or a highly immune flock.

The Analysis of ImmunoComb® results using CombScore™ sheet

The number of samples was multiplied in a column by the corresponding S value. The answers for each column (S1, S2, etc.) were written in the open box under the column. The numbers (from the previous computation) were added to all the boxes and the sum was written in the total box. The total (from the previous computation) was divided by the sample size (number of birds tested) to arrive at the score. The score was the mean antibody titer of the test sampling.

Data Gathered

The following data were gathered in this study: the number of positive and negative results in detecting *Mycoplasma* infection using the ImmunoComb® Assay, the antibody titer of the test subjects that was found positive, and the mean antibody titer on the free-

range chicken project at the Tarlac Agricultural University and FRC beneficiaries in Sta Ignacia, Tarlac.

Results and Discussions

Detection of *Mycoplasma* infection using ImmunoComb® Assay

The result of the detection of *Mycoplasma* infection using ImmunoComb® Assay on Tarlac Agricultural University – Free-Range chicken (TAU-FRC) project and FRC beneficiaries in Sta Ignacia is shown in Tables 1 and 2. The results were based on the purple-gray color intensity seen on each comb card as shown in Figure 1.

Mycoplasma gallisepticum testing using ImmunoComb® Assay with their respective origin

Table 1 shows the summary results of *Mycoplasma gallisepticum* testing using ImmunoComb® Assay. A total of 46.3% or 51 samples were found positive and 53.7% or 59 samples were found negative with *Mycoplasma gallisepticum* from a total population of 110 free-range chickens from TAU and beneficiaries in Santa Ignacia.

Table 1. Summary of the results in Mycoplasma gallisepticum testing using ImmunoComb® Assay with their respective origin

Developing plate no.	MG n (+)	% in DP	Subtotal n (%+)	MG n (-)	% in DP	Subtotal n (%-)
1	3	30	2.7	7	70	6.4
2	0	0	0	10	100	9.1
3	10	100	9.1	0	0	0
4	10	100	9.1	0	0	0
5	2	20	1.8	8	80	7.3
6	0	0	0	10	100	9.1
7	4	40	3.6	6	60	5.4
8	7	70	6.4	3	30	2.7
9	3	30	2.7	7	70	6.4
10	2	20	1.8	8	80	7.3
11	10	100	9.1	0	0	0
TOTAL	**51**		**46.3**	**59**		**53.7**

Legend: MG n (+) – the number of positive samples of M. gallisepticum

Table 2 shows the summary of the results in *Mycoplasma synoviae* testing using ImmunoComb® Assay. A total of 56.6% or 62 samples out of 110 were found positive, while 43.6% or 48 samples were found negative.

The results showed that all experimental animals were healthy before blood collection, with no visible clinical signs of either *M. gallisepticum* or *M. synoviae*. This supported the claims of Seifi and Shirzad (2011). The absence of clinical disease in chickens in the early stage of *Mycoplasma* was also previously recorded by Levinsohn *et al.* (1989). The study by Talkington *et al.* (1985) stated that it was not uncommon for birds with mild or inapparent clinical signs to be infected with *Mycoplasma*. Ley (2003) also observed that the absence of visible clinical signs could happen even if serologic evidence were recorded. This was when the case had been encountered at a young age

and the chicken could have been partially recovered. The variation in seroprevalence of mycoplasmosis in poultry birds might be due to the replacement of breeding stock with the progeny of the same flock, seasonal influence, poor ventilation, contamination of litters, and no restriction on their movement of the caretaker, visitors, and such other persons as well as other biosecurity measures (Ombase *et al.*, 2018).

Table 2. Summary of the results in Mycoplasma synoviae testing using ImmunoComb® Assay with their respective origin

Developing plate no.	MG n (+)	% in DP	Subtotal n (%+)	MG n (-)	% in DP	Subtotal n (%-)
1	1	10	0.9	9	90	8.1
2	0	0	0	10	100	9.1
3	10	100	9.1	0	0	0
4	10	100	9.1	0	0	0
5	9	90	8.2	1	10	0.9
6	3	30	2.7	7	70	6.4
7	7	70	6.4	3	30	2.7
8	8	80	7.3	2	20	1.8
9	2	20	1.8	8	80	7.3
10	2	20	1.8	8	80	7.3
11	10	100	9.1	0	0	0
TOTAL	**62**		**56.4**	**48**		**43.6**

Legend: *MS n (+) – the number of positive samples of M. synoviae*
MS n (-) – the number of samples negative samples of M. synoviae

This study proves that *Mycoplasma gallisepticum* and *Mycoplasma synoviae* were present in the free-range chicken project at Tarlac Agricultural University and its beneficiaries in Sta Ignacia with seropositivity of 46.3% and 56.4%, respectively. In addition, this study also suggests that even though there were no clinical signs seen in those chickens tested, they could still have *Mycoplasma* infection.

Figure 1 shows that Sample 1.1 is positive for *M. gallisepticum* by the Comb Scale (Figure 2) that measured the antibody titer based on the intensity of the purple-gray spot present in the Combcard. The C+3 block, as seen in Figure 2, was aligned with the control serum (the squared portion in Figure 1). After which, the comb scale was placed at the top of the comb card number 1 for the interpretation of the purple-gray spot precipitated. Still, in Figure 1, the CombScale gave a reading of 4 ImmunoComb® units (S4) for sample 1.1 for *M. gallisepticum* and one (1) ImmunoComb® unit (S1) for *M. synoviae*. Aside from the aforementioned sample number, samples 1.7 and 1.8 yielded two (2) ImmunoComb® units and one (1) ImmunoComb® unit, respectively, both in *M. gallisepticum*.

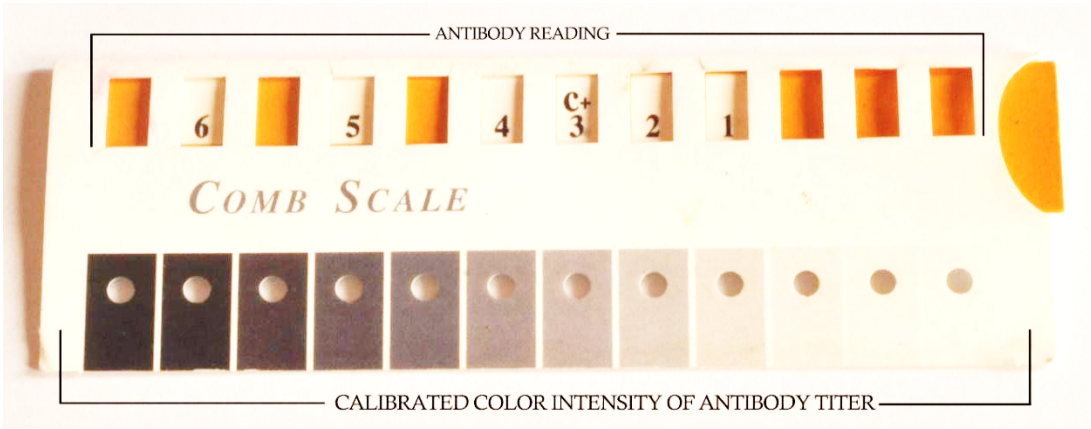

Figure 1. Comb card number 1, shows the purple-gray color results on each comb that corresponds to an antibody titer, the positive and negative control.

Detection of the antibody titer of *Mycoplasma gallisepticum* and *Mycoplasma synoviae*

The result of the detection of antibody titer of *Mycoplasma* infection using ImmunoComb® Assay in Tarlac Agricultural University – Free-Range Chicken (TAU-FRC) project and FRC beneficiaries in Sta. Ignacia is shown in Tables 3 to Table 6.

Antibody Titer for Mycoplasma gallisepticum in TAU- FRC Project

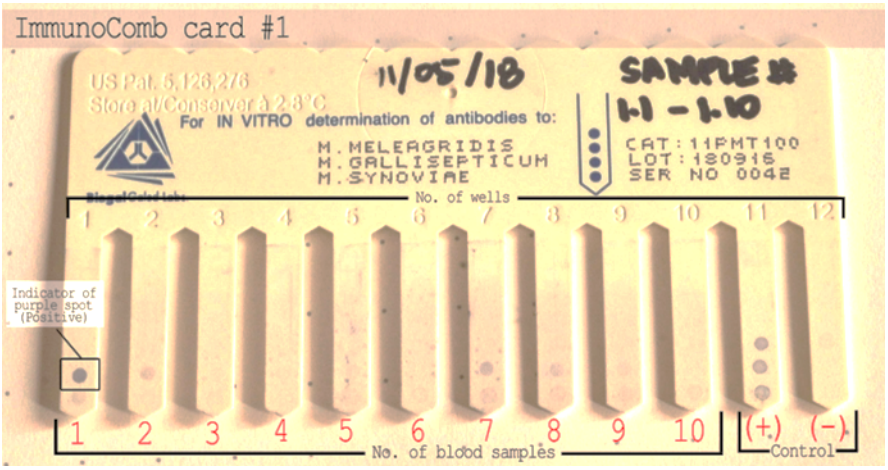

Figure 2. The Combscale shows the different color intensities that correspond to a particular antibody titer or level for each test.

Table 3 shows the summary of antibody titer for *M. gallisepticum* in 60 whole blood samples from the chickens of the TAU-FRC project. Developing plate number one scored 0.7 ImmunoComb® unit (S0.7) which was the fourth to the lowest titer recorded, i.e., Developing plates numbers 2 and 6, which yielded 0 ImmunoComb® units, respectively. Developing plate number 3 scored a 3.7 ImmunoComb® unit (S3.7) which was the highest of all the six (6) developing plates. Developing plates numbers 4 and 5 with 3.6 ImmunoComb® unit (S3.6), and 0.4 ImmunoComb® unit (S0.4), respectively. From the 60 whole blood tests for *Mycoplasma gallisepticum*, the mean antibody titer recorded was 1.40 ImmunoComb® unit (S1.40).

Table 3. Summary of antibody titer for *Mycoplasma gallisepticum* in TAU- FRC Project

Animal Samples	Developing Plates					
	1	**2**	**3**	**4**	**5**	**6**
1	4	0	4	4	0	0
2	0	0	4	4	2	0
3	0	0	4	4	2	0
4	0	0	4	4	0	0
5	0	0	4	3	0	0
6	0	0	4	4	0	0
7	2	0	3	3	0	0
8	1	0	4	3	0	0
9	0	0	3	4	0	0
10	0	0	3	3	0	0
MEAN	0.7	0	3.7	3.6	0.4	0
Mean Antibody Titer	1.40					

Antibody Titer for Mycoplasma gallisepticum in FRC Beneficiaries in Santa Ignacia, Tarlac

Table 4 shows the summary of antibody titer for *M. gallisepticum* with 50 whole blood samples of FRC beneficiaries in Sta. Ignacia, Tarlac. Developing plate number 2 scored the highest mean of 2.2 ImmunoComb® unit (S2.2); second highest was developing plate number 6 with a mean score of 1.8 ImmunoComb® unit (S1.8); followed by developing plate number 1 with a mean score of 1.2 ImmunoComb®unit (S1.2). Developing plates numbers 3 and 4 had the lowest mean score of 0.3 ImmunoComb® unit (S0.3) and 0.03 ImmunoComb® unit (S0.03), respectively. A total mean antibody titer of 1.11 ImmunoComb® unit (S1.11) was recorded for the 50 blood samples of chickens from Santa Ignacia.

Table 4. Summary of antibody titer for *Mycoplasma gallisepticum* in FRC Beneficiaries in Santa Ignacia, Tarlac

Animal Samples	Developing Plates				
	1	**2**	**3**	**4**	**5**
1	4	4	1	2	2
2	5	2	1	1	1
3	0	0	1	0	2
4	0	4	0	0	2
5	0	4	0	0	1
6	1	4	0	0	1
7	2	1	0	0	2
8	0	0	0	0	2
9	0	0	0	0	3
10	0	3	0	0	2
MEAN	1.2	2.2	0.3	0.03	1.8
Mean Antibody Titer			1.11		

Tables 3 and 4 show the summary of antibody titer for *Mycoplasma gallisepticum* with a total mean antibody score of 1.40 in the TAU-FRC project and 1.11 in Santa Ignacia. Though the antibody scores were low, presence of *the Mycoplasma gallisepticum* were detected with these healthy chickens. This finding was answered by Haghighi-Khoshkhoo et. al. (2011) who stated that the seroprevalence of *M. gallisepticum* in the Centernorth of Iran was low; only 4 of 40 (10%) flocks were positive. Malaysia and Burnham et.al (2003) observed that chickens produced good quality eggs and showed good performance although they harbored *M. gallisepticum* organism, despite having a high *M. gallisepticum* antibody. Other sources of infection of the *M. gallisepticum* may be from other birds that enter the farms. This was reported by Tan et. al (2016) in which free-flying birds in close contact with infected chickens may re-transmit the infection when in close contact with commercial chickens and also through fecal sheds.

Low infection of the *M. gallisepticum* in terms of age, in which experimental animals were from 5 to 8 months, was also observed in a similar report confirmed by Hossain et.al. (2007) and Talha (2003) who stated that the prevalence of *M. gallisepticum* infection significantly decreased with the increase of age. The highest infection in young chickens was due to the vertical transmission of the organisms.

In terms of breeds, all breeds of chickens are susceptible to *M. gallisepticum* infection. The prevalence varied widely among different breeds of chickens. These differences might have happened due to breed variation, the nature of poultry farming, operational practices, and other biosecurity measures of the farms (Ali et. al., 2015).

Another factor of infection is the size of the flock which can influence of infection of *M. gallisepticum* which corroborates the observation of Ali et.al. (2015) that the highest infection rate (69.63%) was tested in a large-scale flock (3,000 to 4,200 birds) compared to a small scale (1,300 – 1,600 birds). And this was also detected in the previous investigation of Heleili et. al. (2012) which documented 76.97% of MG infection in a herd containing 18000 birds from 20% in a herd with 500-1000 birds in Algeria. Hossain et.al (2007) stated that *the M. gallisepticum* infection rate was the highest (68.5%) in large flocks compared to small flocks (50.1%) in Rajshahi and surrounding districts of Bangladesh. Though the number of a flock in the TAU-FRC project and Sta Ignacia was on a small scale, *M. gallisepticum* infection might occur.

Summary of Antibody Titer for *Mycoplasma synoviae* in TAU- FRC Project

Table 5 shows the summary of antibody titer for *M. synoviae* in the same 60 whole blood samples from chickens of the TAU-FRC project. Developing plate number 1 scored 0.1 ImmunoComb® unit (S0.1) second to the lowest titer recorded. Developing number 2 yielded 0 ImmunoComb®unit. Developing plate number 3 scored a 3.9 ImmunoComb® unit (S3.9) which was the highest of all the six (6) developing plates tested for *Mycoplasma synoviae*. Developing plates numbers 4, 5, and 6 yielded 3.3 ImmunoComb® unit (S3.3), 3.3 ImmunoComb® unit (S3.3), and 0.3 ImmunoComb® unit (S0.3), respectively. From the 60 whole blood tested for *M. synoviae*, the mean antibody titer recorded was 1.82 ImmunoComb® unit (S1.82)

Summary of Antibody Titer for *Mycoplasma synoviae* from FRC Beneficiaries in Santa Ignacia, Tarlac

Table 6 shows the summary of antibody titer for *Mycoplasma synoviae* from FRC beneficiaries in Santa Ignacia, Tarlac. Developing plate number 2 scored the highest titer with 2.3 ImmunoComb® unit (S2.3), followed by developing plate number 5 with 2 ImmunoComb® unit (S2.0) and developing plate number 1 with 1.7 ImmunoComb® unit (S1.7). Developing plates numbers 4 and 3 had the lowest titer with 0.5 ImmunoComb® unit (S0.5) and 0.3 ImmunoComb® unit (S0.3), respectively. For the whole 50 blood samples, the mean antibody titer was 1.34 ImmunoComb® unit (S1.34) from Santa Ignacia FRC beneficiaries.

Table 5. Summary of antibody titer for *Mycoplasma synoviae* in TAU- FRC Project.

Animal Samples	Developing plates					
	1	**2**	**3**	**4**	**5**	**6**
1	1	0	3	2	2	0
2	0	0	4	3	3	1
3	0	0	4	3	3	1
4	0	0	4	3	3	1
5	0	0	4	3	3	0
6	0	0	4	3	3	0
7	0	0	4	4	4	0
8	0	0	4	4	4	0
9	0	0	4	4	4	0
10	0	0	4	4	4	0
MEAN	0.1	0	3.9	3.3	3.3	0.3
Mean Antibody Titer			1.82			

Table 6. Summary of antibody titer for *Mycoplasma synoviae* from FRC Beneficiaries in Santa Ignacia, Tarlac

	Developing Plates				
Animal Samples	**1**	**2**	**3**	**4**	**5**
1	4	5	1	3	2
2	5	2	0	2	1
3	0	1	0	0	2
4	0	4	0	0	2
5	1	4	0	0	2
6	2	3	0	0	2
7	2	3	0	0	2
8	0	0	0	0	1
9	3	1	0	0	3
10	1	0	1	0	3
MEAN	1.7	2.3	0.2	0.5	2
Mean Antibody Titer			1.34		

A low score of antibody titer results in Tables 5 and 6 of TAU-FRC Project and Santa Ignacia was related to the study of Feberwee (2008) in Dutch commercial farms

that *Mycoplasma synoviae* positive-farms seroprevalence was suggestively lower in layer type of chickens than in meat-type chicken because of the voluntary *M. synoviae* monitoring program aimed at detecting *M. synoviae* infection as early as possible. This was corroborated by Cortes et. al. (2021) who stated that the difference between the system of production of broiler and layer chickens' vaccination against Mycoplasma infection was more common in broiler chickens than in layer chickens. Therefore, reports of vaccination in layer chickens have not been studied. Kleven (1998) stated that the positivity of *M. synoviae* in chickens may often result in mild or even subclinical disease. Sui et al (2021) reported that some *M. synoviae*-infected chickens displayed no clinical signs, which led to the spread of *M. synoviae*, thereby increasing the probability of infection and coinfection with other pathogens.

In contrast with the results of this study, Cortes et. al. (2021) found that layer chickens had a high seroprevalence of *M. synoviae* with 95% and 74% in broiler chickens. Parallel with the results observed by Kapetanov et al. (2010), *M. synoviae* had high seroprevalence rates in adult flocks (90%) and flocks during the rearing period (40%) in 2009 in Serbia. Conversely, another previous investigation stated that lower seroprevalences in commercial layers of 69% (Buim et al., 2009) and 53% (Suzuki et al., 2009) were measured by ELISA.

Results seen in Tables 3 to 6 also showed that there was variability in the serological response of each chicken toward *Mycoplasma* infection. This supported the claim of Kleven (1998) who stated that *Mycoplasma gallisepticum* strains have recently been shown to have the ability to vary the expression of major surface antigens, thus expressing a continually changing "antigenic profile" to the immune system. In addition,

continual variability in the expression of such surface antigens also occurs *in vivo* and may be a major factor in the development of clinical disease and serological responses. Meaning, even with a strong immune response, it is most likely that *Mycoplasma* could still exist because of that variability. It may also help to explain “atypical” serological reactions found in infected flocks.

Razin et al. (1998) stated that Mycoplasma may not be recognized by the host immune system due to its intracellular location during its latency period. Mycoplasma will only induce disease after the host was affected by other disease-causing agents or an episode of host weakness.

Studies of the prevalence of mycoplasmosis in backyard chickens by Haesendonck et al. (2014) and Derksen et al. (2018) showed that the backyard poultry flocks would possibly act as reservoirs or amplifiers for poultry respiratory diseases serving as a continuous source of infection for industrial chickens. Viviana et. al. (2020) detected the presence of *M. gallisepticum* and *M. synoviae* in backyard poultry farms, confirming the potential role of this type of breeding to spread pathogens to commercial poultry production, especially in densely poultry-populated areas where backyard and commercial farms are close. And the result of their study in which all flocks tested were Mycoplasma positive as detected using PCR, suggested that backyard chickens should be tested periodically to determine the status of mycoplasma infection. Therefore, routine monitoring is essential to evaluate the immune status of a flock over time.

Vaccination Program against *Mycoplasma* infection

The decision to vaccinate or simply accept performance losses in commercial layers depended on several factors. The strain of *M. gallisepticum* in a farm must be taken into account as some strains of *M. gallisepticum* were mild while others were highly virulent. According to Butcher (2015), house construction was a major factor in knowing the severity of clinical disease; those layers that were kept in a closed-type house with poor ventilation would experience considerable performance losses. Also, flocks that were placed in open-sided houses and closed houses with excellent ventilation would not experience considerable performance losses. Thus, consideration of air quality where the layers will be housed before vaccination is a must.

Available live vaccines for *M. gallisepticum* were produced from the F strain, and more recently, strains ts-11 and 6/85, which were apathogenic with improved characteristics (OIE, 2012). Administration of the F strain by the intranasal or eyedrop route was preferred, but aerosol or drinking water administration may be used. The eyedrop method is recommended for ts-11, and a fine spray for 6/85. Pullets are generally vaccinated between 12 and 16 weeks of age. One dose is sufficient and vaccinated birds remain permanent carriers (Evans *et al.,* 2005).

Conclusions

Based on the results, *Mycoplasma gallisepticum* and *Mycoplasma synoviae* were both present on the Free-range chicken project at Tarlac Agricultural University and those in the residents of its beneficiaries in Sta Ignacia. The mean antibody titer of the whole test subjects results in *M. gallisepticum* and *M. synoviae* in the TAU-FRC project and its

beneficiaries in Santa Ignacia scored low, this means that under the interpretation given by the manufacturer of the test kit, the antibody titer or level was low or almost undetectable. This might be because the Mycoplasma infection present in the Tarlac Agricultural University – Free-range chicken project and Santa Ignacia, Tarlac was latent or the occurrence of the infection was still in its early course and have not yet severely progressed.

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