











Circulation of Major Respiratory Pathogens in Backyard Poultry and their Association with Clinical Disease and Biosecurity

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SUMMARY

Raising backyard birds is a common practice in Brazil, mainly in the countryside or suburban areas. However, the level of respiratory pathogens in these animals is unknown. We sampled two hundred chickens from 19 backyard flocks near commercial poultry farms and performed ELISA to Infectious Bronchitis Virus, avian Metapneumovirus, *Mycoplasma synoviae* and *Mycoplasma gallisepticum*. We evaluated the association between the predictive ability of ELISA and Hemagglutination-inhibition (HI) by comparing results from eight flocks positive to *Mycoplasma gallisepticum* on ELISA. Besides, we assessed essential biosecurity measures in the properties (multiple species birds, rodent control, hygienic conditions, and water quality for the bird's consumption). We could access the vaccination program only on four properties; in three of them, the birds were supposedly vaccinated for IBV. Overall the properties had a poor score for the biosecurity measures, and the seroprevalence in backyard poultry flocks for IBV, a MPV, MS, and MG were respectively 87.5% (14/16), 89.5% (17/19), 100 (19/19) and MG 84.21% (16/19). We found low specificity and predictive value between ELISA and HI in MG analysis and a positive correlation between the presence of clinical symptoms and mean MG titers. Backyard chicken are pathogens' reservoirs and pose a risk for the commercial poultry farms in the region, and further efforts of the governmental entities and private sector of poultry production should consider these information to avoid future economic losses.

INTRODUCTION

Brazil plays a significant role in world poultry production, being the second biggest producer and the first exporter ABPA (2019). In the protein-producing business, the sanitary conditions of the herds are essential for long-term success. Respiratory diseases are an issue for poultry companies worldwide; yearly, companies expend millions of dollars in vaccines, diagnosis, and treatment. Economic losses are caused mainly due to a drop in egg production, increase of feed conversion, downgrading, and mortality, Buchala *et al.* (2006); Kleven (2008); de Wit *et al.* (2011).

Several people that live in the suburban area of Uberlândia, Brazil and its surroundings rear chickens in their backyard as a shared cultural trait; in some cases, chickens are slaughtered and sold to neighbors or acquaintances. Although several biosecurity measures are taken on commercial flocks, backyard or free-range production lack any veterinary protocol for disease prevention. In many cases backyard properties are located near commercial properties and may act as reservoirs for respiratory diseases to the commercial flocks, Derksen *et al.* (2018).



Among the avian mycoplasmas, *Mycoplasma gallisepticum* (MG) causes the most critical loss in poultry production. The outcomes of the infection are mainly respiratory diseases and drop in egg production in chickens, turkeys, and other avian species. Broilers and turkeys infected with MG show severe airsacculitis, coughing, rales, and impaired growth. Both MS and MG infections can cause massive condemnations caused by air airsacculitis at processing. Moreover, in chickens, MS infections cause synovitis Kleven (2008).

The Infectious bronchitis virus (IBV), a member of the family *Coronaviridae*, causes a highly contagious acute disease of the respiratory and urogenital tract of chickens leading to a decrease in egg production and quality. Besides, young chicks display acute respiratory disease, lesions in the trachea, and morbidity can easily reach 100% mortality, on the other hand, it is generally below 5%. Nevertheless, if concurrent infections and secondary pathogens are present, the mortality increases. Nephropathogenic strains cause enlarged kidneys with distended tubules and ureters that contain uric acid crystals, Buchala *et al.* (2006); de Wit *et al.* (2011).

Avian metapneumovirus (aMPV), previously referred to as avian pneumovirus (APV) and avian rhinotracheitis virus (ART) is an acute, highly contagious upper respiratory infection in turkeys and chickens. In chickens' laying flocks, particularly in broiler breeders, there is a marked drop in egg production, often preceded by respiratory signs. Severe respiratory distress is only observed in broilers when Metapneumovirus infection is associated with secondary pathogens such as IBV, mycoplasma, and *E. coli*. In such cases, birds may have swollen head syndrome, torticollis, incoordination, and depression (Gough & Pedersen, 2016).

Serology is broadly used to identify mycoplasma and virus infection in poultry flocks. The World Organization for Animal Health (OIE), recommends both HI and ELISA to evaluate serum titers for MG, and PCR to confirm the infection (OIE, 2019). Nonetheless, a previous study has found several false positives results caused by ELISA's low specificity to identifying antibodies against MS and MG Feberwee *et al.* (2005). Therefore, continuing to evaluate the feasibility of replacing HI tests by ELISA is essential.

This study aimed to evaluate the prevalence of serological titers of MG, MS, IBV, and aMPV in backyard chickens, to relate clinical symptoms to diseases, to verify simple biosecurity management, and to associate HI and ELISA results with flocks positive for MG.

MATERIAL AND METHODS

We randomly evaluated for biosecurity criteria and collected blood samples from birds in 19 rural poultry-producing properties in Uberlandia, Minas Gerais, Brazil, from March to October 2018 (Supplementary Table 1). This area is characterized by a semi-humid tropical climate with dry winter and rainy summer. The owners of the properties evaluated and registered in this study volunteered to be part of an extension and search project for sanitary assistance to small properties. We divulged the project on the radio, TV, and social media.

Supplemental table 1 – Number of total poultry and number of backyard chickens with blood sampled between the ages of 12 to 54 weeks.

Farm	Total of poultry	number of birds sampled
P1	45	5
P2	50	6
P3	38	6
P4	30	10
P5	200	12
P6	30	12
P7	40	12
P8	56	11
P9	120	12
P10	300	12
P11	80	12
P12	50	12
P13	50	12
P14	110	11
P15	200	10
P16	29	11
P17	200	12
P18	30	10
P19	110	12

We collected blood samples from 200 birds (*Gallus gallus*) aged between 12 and 54 weeks in clot activator (silica) vacuum collection tubes by puncturing the ulnar vein, using sterile and disposable needles and syringes. We stored the samples in isothermal boxes until the arrival at the Laboratory of Molecular Epidemiology of the Faculty of Veterinary Medicine from the Federal University of Uberlandia.

We collected blood serum by using automatic pipettes with unique tips and then sent them to the Animal Health Laboratory for serological analysis via indirect ELISA methodology IDEXX (2013). We performed ELISA according to the manufacturer's instructions, adding the samples in the plaque for sensitization, washing with a buffer and incubating at 18-26°C for 30 minutes. After that, we washed and added an enzyme-substrate, washed again, added the enzyme-substrate, and incubated at 18-26°C for 15



minutes. Then we added the Interruption Solution to stop the reaction IDEXX (2013).

We measured the serum samples' antibody absorbance values using an optical density spectrophotometer, and the software provided by IDEXX displayed the absorbance estimation for each sample. According to the manufacturer's guidelines, for IBV and aMPV analysis, antibody titers for these viruses higher than 397 are considered positive exposure, indicating either vaccination or natural exposure. Likewise, for MG and MS, antibody titers higher than 1077 are considered positive IDEXX (2013). We did not include vaccinated poultry in the disease's analysis.

We performed HI (Hemagglutination Inhibition test) for MG in bird's serum samples from eight properties (P) (11, 12, 13, 14, 15, 17, 18, and 19), as follows: using 96-well microplates, we diluted 25µl of the serum samples in PBS (pH 7.2) from 1: 2 to 1: 4096, then we added 25 µl of MG antigen 4 UHA (Hemagglutinating Units) in each well and incubated at room temperature (25°C) during 15 minutes. After that, we deposited 25 ul of 1% chick erythrocyte suspension (pH 7.2, optical density 0.33-0.35), incubated at room temperature for 30 minutes, and evaluated until which dilution the MG antibody in the sera was able to inhibit the hemagglutination caused by the antigen.

We assessed the farm properties regarding their biosecurity (supplementary Table 2) and the clinical aspects of respiratory diseases we considered that farms, where more than 25% of the birds showed clinical respiratory signs as positive. Besides, we georeferenced properties using google maps.

We have performed descriptive statistics using mean and standard deviation for the serological titers' analysis. To evaluate the correlation, we applied the Spearman test ($p < 0.05$). For the diagnostic test, we used the chi-square followed by the tests of sensitivity, specificity, positive and negative predictive value. We used the Graph Pad Prism 7.0 program and a significance level of 0.05.

RESULTS

Serological titers evaluated by ELISA for IBV, aMPV, MS, and MG

From the 19 farms studied, only in four properties the birds were vaccinated from 6 to 12 months before the sample's collections. The vaccines used were as following: P5 (Avian Pox, IBV, Newcastle and Infectious Bursal Disease), P9 (Avian Pox and Newcastle), P17 (Newcastle, Avian Pox, Marek, Coryza, IBV, and

Infectious Bursal Disease) and P19 (NewCastle, Avian Pox, Marek, Coryza, and IBV). In properties P5, P17, P19 the birds were supposedly vaccinated for IBV. In the properties where the owner declared that the poultry was vaccinated, there was no precise register when it occurred. Therefore, it is uncertain if the sampled birds were indeed vaccinated for IBV, thus these birds were left out of the disease's analysis.

We describe the mean antibody titers for the four diseases surveyed in Figure 1. According to the manufacturer, flocks that show ELISA's mean antibody titers above 397 are considered seropositive for IBV and aMPV and above 1077 seropositive for MG and MS. Therefore, excluding the three properties vaccinated for IBV, 82.3% of the birds and 87.5% of the flocks were seropositive for IBV (14/16), 77% of the birds and 89.5% of the flocks were seropositive for aMPV (17/19), P10 and P15 were seronegative for AMPV and P3 and P16 for IBV. We found the highest mean titers for aMPV in P3, P11, and P12, whereas P6, P11, P14, and P17 showed the highest mean antibody titers for IBV. All properties were seropositive for MS and 84.21% (16/19) to MG, respectively 87% and 73% of the birds were seropositive to MS and MG. Properties 2, 10, and 16 were seronegative. We observed the highest mean titers for MS in P3, P12, and P15 and MG on P17, P18, and P19.

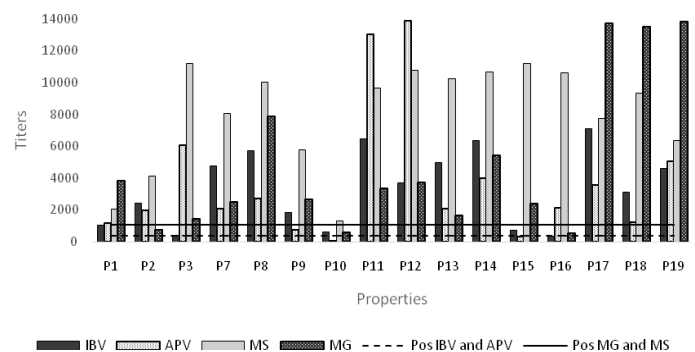


Figure 1 – ELISA antibody mean titers for IBV, aMPV, MS and MG. Serum samples collected from poultry between 12 and 54 weeks old.

Excluding the properties with vaccinated birds for IBV (P5, P17, and P19), 68.75% (11/16) of the properties were positive for all the diseases surveyed and 6.25% (1/16) for aMPV/MS/MG; (1/16) for IBV/MS/MG; (1/16) for IBV/aMPV/MS; (1/16) for aMPV/MS and (1/16) for IBV/MS.

Using scatterplot, we checked the distribution of the values for each analyzed property (supplementary Figure 1A-D). We found a high variation coefficient for the antibody titers for IBV (Supplementary Figure 1A), aMPV (Supplementary Figure 1B), and MG



Table supplementary 2A – Questionnaire about some aspects of biosecurity evaluated in the studied properties.

Question	P1	P2	P3	P4	P5	P6	P7	P8	P9
Breeding of different species? Which are?	yes (peacock, duck, broiler, hens, rooster, quail, turkey and goose)	yes (duck, broiler, rooster)	Yes (guinea fowl, broiler, hens, rooster)	Yes (guinea fowl, broiler, hens, rooster)	Yes (duck, broiler, rooster, call duck and goose)	no	no	yes (duck, broiler, hens, rooster)	no
Water source	artesian well (no treatment)	artesian well (no treatment)	urbanwater (treated)	artesian well (no treatment)	artesian well (no treatment)	artesian well (no treatment)	urban water (treated)	urbanwater (treated)	urban water (treated)
carcass disposal	near the dam	bury	common waste	common waste	burn	bury	common waste	in the woods without treatment	common waste or bury
veterinary monitoring if there are sick poultry	yes (owner is veterinary)	yes (project with university)	no	no	no	no	no	no	no
Source of technical information	owner is veterinary	project with university	don't look for information	don't look for information	internet and courses	talking with friends	don't look for information	don't look for information	internet
property cleaning	bad	reasonable	reasonable	bad	bad	bad	bad	bad	bad
perform vector control	no	no	controll of rats	no	controll of rats	not	controll of rats	no	controll of rats

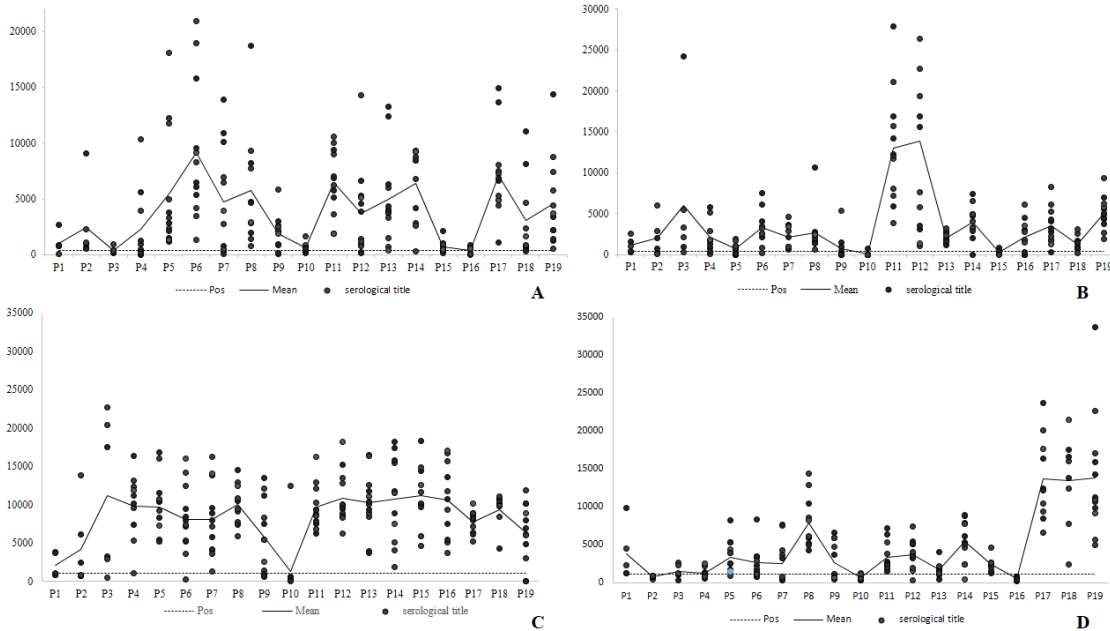
Table supplementary 2B– Questionnaire about some aspects of biosecurity evaluated in the studied properties.

Question	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19
Breeding of different species? Which are?	yes (duck, broiler, hens, rooster)	Yes (guinea fowl, broiler, hens, rooster and Wood-Rail)	Yes (guinea fowl, broiler, hens, rooster and quail)	Yes (guinea fowl, broiler, hens, rooster)	no	yes (duck, broiler, hens, rooster, canary and goose)	no	Yes (guinea fowl, broiler, hens, rooster and turkey)	no	no
Water source	urban water (treated)	urban water (treated)	head waters	headwaters	urban water (treated)	artesian well (no treatment)	urban water (treated)	artesian well (no treatment)	urban water (treated)	urban water (treated)
carcass disposal	common waste or bury	bury	burn	bury	bury	bury	bury	common waste	common waste	common waste
veterinary monitoring if there are sick poultry	no	yes (owner is veterinary)	no	yes veterinary hospital	no	no	no	no	no	no
Source of technical information	internet or veterinary store attendant	veterinary or veterinary store attendant	internet	veterinary hospital	veterinary store attendant	don't look for information	veterinary store attendant	internet and friends	internet	internet and courses
property cleaning	bad	reasonable	bad	bad	bad	reasonable	reasonable	reasonable	reasonable	reasonable
perform vector control	no	no	no	no	no	controll of rats and insects	controll of rats	controll of rats	no	controll of rats and insects



(Supplementary Figure 1D). The variation coefficient for IBV ranged from 46.29% to 147.89%; for AMPV, from 47.53% to 340%, and for MG, it ranged from

23.34% to 110.35%. Less variation was found for MS (Supplementary Figure 1C) from 19.51% to 26.66%.



Supplementary Figure 1 – Dispersion of IBV, aMPV, MS and MG antibody titers in the evaluated properties

To better understand the serological reaction for the surveyed diseases, we generated histograms, and the antibody titers were divided into groups as

recommended by the manufacturer. Histograms for IBV, AMPV, MS, and MG are in Figures 2 A, B, C, and D, respectively.

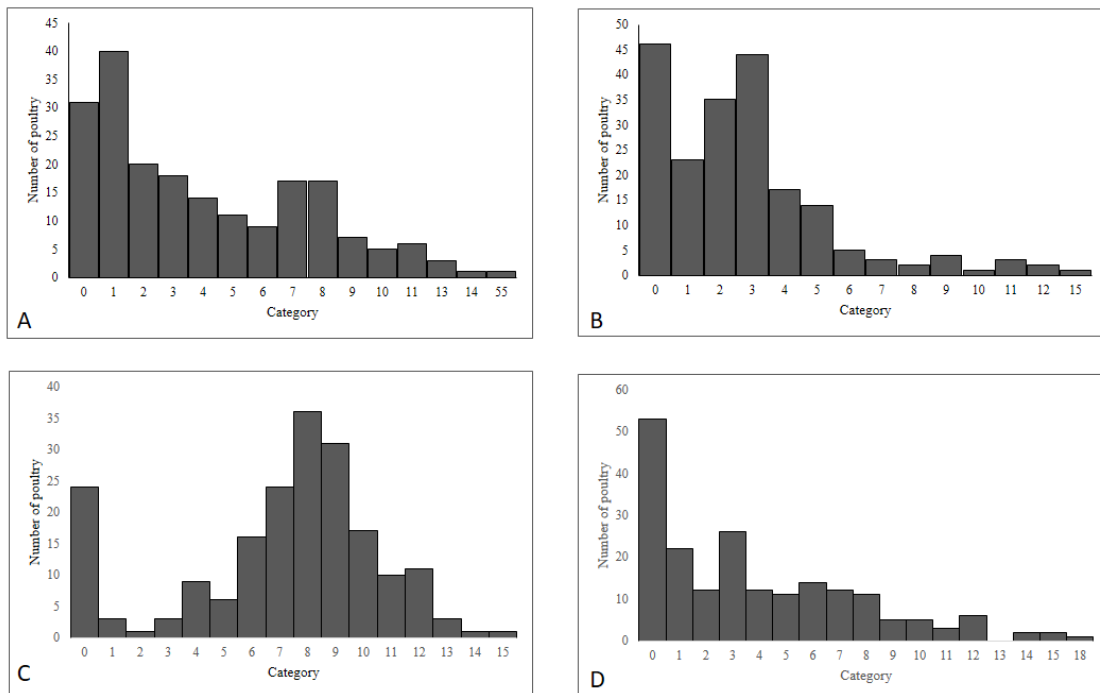


Figure 2 – Histogram of the distribution frequency of backyard chickens' antibody titers on ELISA for IBV (A), aMPV (B), MS (C), and MG (D).
 *IBV and AMPV titers value within each group: 0 (0 to 396); 1 (397-1000), 2: (1001 -2000), 3 (2001-3000), 4 (3001- 4000), 5 (4001-5000) and so on. MG and MS titers value for each group: 0 (0 - 1076), 1 (1077-1499), 2 (1500-1999), 3 (2000-2999), 4 (3000-3999), 5 (4000 -4999) and so on. Samples 'antibody titers within group 0 are seronegative, and others seropositive.



Correlation between serological titers evaluated by IBV, AMPV, MS and MG ELISA

We found a moderate correlation between seropositivity to aMPV and IBV, a weak correlation between aMPV and MG and MG and IBV; MS and IBV and MS and MG showed a very weak correlation (Table 1).

Table 1 – Correlation between the mean serological titers for IBV, aMPV, MS, and MG.

	AMPV		MS		MG	
	P	r	P	r	p	r
IBV	<0.0001	0.452	0.747	0.023	<0.0001	0.337
AMPV			0.013	0.175	<0.0001	0.2746
MS					0.336	0.06

Value classification of r: 0.00 < r > 0,19: very weak; 0.20 < r > 0.39: poor; 0.40 < r > 0.69: moderate; 0.70 < r > 0.89: strong.

HI results for MG

According to the Agriculture Ministry of Brazil, as well as for OIE, MG is a notifiable disease, and OIE (2000) considers HI the golden standard test to MG. We analyzed properties P11 to P19 for HI due to its proximity between commercial farms (Figure 3).

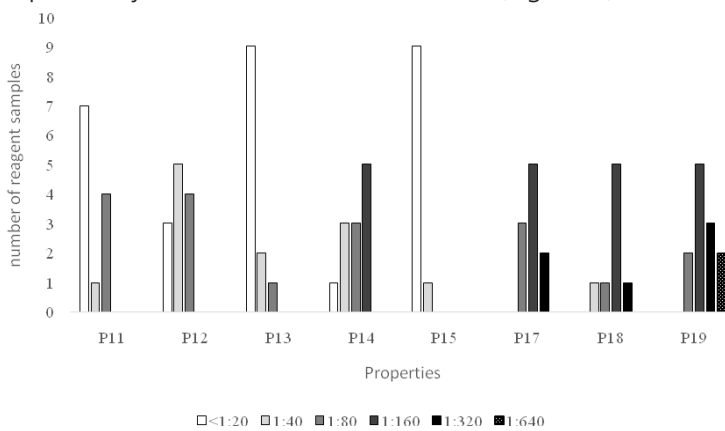


Figure 3 – Serological levels for the HI test for MG of the evaluated properties 11 to 19. Titers 1:20 are considered negative, 1:40 suspect and 1:80 or higher positive.

We found an association between HI and ELISA for MG disease in the diagnostic test (Table 2); the values for sensitivity and specificity were respectively 1, and 0.25, the positive and negative predictive values were 0.857 and 1, respectively.

Table 2 – Diagnostic test using HI for MG in the evaluated properties.

	P HI	N HI
P ELISA	72	12
N ELISA	0	4

P: positive; N: negative; p = 0.0008; sensitivity = 1; specificity = 0.25; positive predictive value = 0.857; negative predictive value: 1.

Correlation between the serological titers of the evaluated diseases and respiratory signs

We observed nasal secretion, sneezing and snoring symptoms in 13 from the 19 properties at the visit and sample collection (P1, P2, P4, P5, P8, P9, P12, P13, P14, P15, P17, P18, P19). We found a positive correlation between the presence of clinical symptoms and mean MG titers. For other diseases, there was no association (Table 3).

Table 3 – Correlation between respiratory symptoms and serological titers.

	IBV % (a/b)	AMPV	MS	MG
Respiratory symptoms and positive serum for the agent	71,4% (10/14)*	70,6% (12/17)	68,42% (13/19)	75% (12/16)
(p value)	0,230	0,923	0,724	0,039
Correlation (r)	0,288	-0,023	0,086	0,476

Respiratory symptoms: Snoring, sneezing and nasal secretion; a / b: seropositive birds with respiratory symptoms / total seropositive birds; * Flocks were not considered when birds were previously vaccinated for IBV (P5, P17 and P19).

Biosecurity

In all properties visited, there were multi-age poultry, chickens were the unique species on seven, 12 properties reared chickens and other birds such as ducks in 50% of the properties (6/12); guinea fowl in 50% (6/12); geese in 25% (3/12); turkeys in 16.66% (2/12); quails in 16.66% (2/12) and canary or call duck or peacock or wood-rail in 8.33%(1/12). The methods for disposal of the carcasses of dead birds were inadequate when compared with commercial chicken rearing; 36.84% of the properties (7/19) buried the dead birds, 31.57% (6/19) discarded on common waste, 10.52% (2/19) burned, 10.52 (2/19) buried or discarded on common waste, 5.26% (1/19) discarded on vacant areas, and 5.26% (1/19) discarded near a dam. Most of the properties (84.21%), were not accompanied by a veterinarian when sick birds were spotted. In two properties (P1 and P11), the owners were veterinarians; one (P13) would often take the sick birds to the veterinary hospital, however, without professional sanitary and nutritional management advice and one other (P2), participates in a project with the university. The properties' owners, when asked about the source of information to choose vaccination programs, diagnosis of diseases, and antimicrobial election, had often reported the use of websites, friends, family members, veterinary store attendants, and no information search. In table supplementary 2, it is possible to verify the biosecurity assessment. Besides, we analyzed essential BM, as shown in Table 4.



Table 4 – Biosecurity criteria and correlation between mean antibody titers and disease occurrence.

	Birds are perceived as sick often	Lack of rodent control	Facilities showed poor hygienic condition	Non-treated water	Multiple species of birds
% (a/b)	73.68% (14/19)	57.89% (11/19)	57.89% (11/19)	47.36% (9/19)	63.19 (12/19)
Correlation with serological titers ($p < 0,05$)	No	No	No	No	No
Correlation with frequent disease ($p < 0,05$)	No	No	No	No	No

Rating calculation was a/b: number of properties sorted according to variable/total number of properties. * No property was in a good state of cleanliness.

Location of evaluated properties

The localization of all studied properties was within a radius of 500 to 6 km from establishments of industrial poultry activities such as breeders, broilers, laying hen farms, and hatchery (Figure 4).

DISCUSSION

The serological analysis showed that 68.75% of the non-vaccinated flocks were positive for all the pathogens surveyed and 18.75% for three out of four. Therefore the results show a high seroprevalence of the

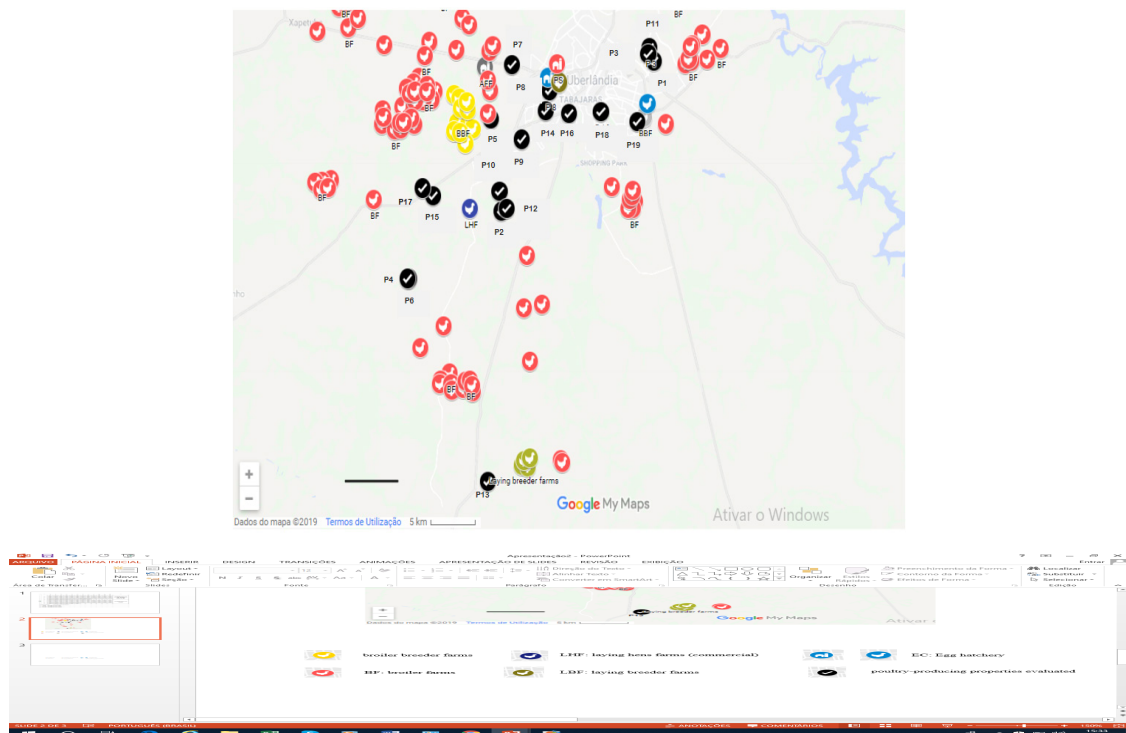


Figure 4 – Map of the evaluated properties and commercial farms. The graph shows the antibody titers per bird sampled on each property. A. IBV, B. AMPV, C. MS, D. MG. Pos: Titervalue considered positive. Mean: Mean of antibody titers found per property.

surveyed pathogens in the area studied. Nevertheless, we found a moderate correlation between aMPV and IBV and weak or very weak for other diseases, which may imply that the pathogens circulation is heterogenic between the properties.

The IBV seroprevalence (87.5%) could be either the result of the natural or live vaccine spread infection. The live vaccine virus could quickly spread into the air and reach the backyard birds since this type of vaccine is frequently applied in commercial poultry farms that are near the studied properties. Due to the lack of information and uncertainty about vaccination, it

is problematic to compare the vaccinated and non-vaccinated birds. The serum samples of supposedly vaccinated birds (P5, P17, and P19), showed lower seroconversion results when compared with others without vaccination history; however, the variability of titers showed that some birds in these flocks could be unprotected and having an active infection. A similar study performed in the Rio Grande do Sul State in southern Brazil has found 100% of properties with backyard chickens seropositive to IBV, reinforcing the spread of the virus in this type of property Santos *et al.* (2008). Reports have shown high seroprevalence



for IBV in backyard chickens in Belgium, the USA and Mexico, Gutierrez-Ruiz *et al.* (2000); Haesendonck *et al.* (2014); Derksen *et al.* (2018).

Veterinaries and poultry producers may underestimate the seropositivity to aMPV since it is not usually accessed in commercial chickens' flocks. It may be because the disease mostly leads to slight respiratory clinical signs that are exacerbated by bacterial infections such as *E. coli* or pathogens such as IBV and mycoplasmas, Gough & Pedersen (2016). The high seroprevalence found in this study, 77% of the birds and 89.5% of properties, shows the virus spread in the region and corroborates with other studies. In a study performed in a poultry hearing area in Bahia, Brazil, 77.1% of commercial flocks and 94.12% of backyard flocks surveyed were seropositive for aMPV, Sales *et al.* (2010). We found that the variation coefficient for aMPV within properties ranged from 47.53% to 340%; a high variation coefficient within the same farm could be explained by the diversity of the age of the poultry reared in the same place.

In this study, the totality of properties was seropositive for MS and 84% for MG; these data confirm the relevance of backyard chicken properties to the epidemiology of economically significant diseases such as MS and MG. A serological survey performed in Pernambuco, Brazil found that 53.33% of the studied backyard birds were seropositive for MG with 100% positive properties, Sá *et al.* (2015). Studies also reported high seroprevalence for MS and MG in backyard flocks in Belgium, the USA and Argentina, Xavier *et al.* (2011); Haesendonck *et al.* (2014); Derksen *et al.* (2018).

We considered that the correlation between the seroprevalence of MG and respiratory signs (nasal secretion, sneezing, and snoring) was moderate. When the *r* values are between 0.5 and 0.7, it represents moderate correlation, Mukaka (2012), we found $r=0.476$. We performed a transversal study between serological titers and birds clinical disease on the day of the visit. Admittedly, our analysis may contain bias as birds may have fallen ill at a very different time from the presence of serological titers, especially in the case of IBV and aMPV. Nevertheless, MG is a chronic and slow-spreading disease OIE (2018), hence we consider that in these free-range birds, there is a correlation between the clinical symptoms caused by MG and the increased serological titers. Moreover, we hypothesize that we could have found a correlation with the other studied diseases in a prior moment of the birds' lives.

It is crucial to consider that in MG infections, non-symptomatic birds are common and represent a threat

to poultry production since the infection remains silent and can spread without being noticed Kleven (2003). In our study, we had considered a respiratory disease only when the number of birds with such clinical signs exceeded 25% on the day of the visit. This criterion can also cause bias because only one sick bird can already be indicative of clinical disease when considering a slow spread disease as MG.

The ELISA results for MS showed the highest number of positive samples on higher groups and the lowest variation coefficient when compared to the other diseases surveyed. It means that active infection is occurring in the surveyed flocks. Lack of correlation between seroprevalence of MG and MS ($p=0.336$; $r=0.06$) shows a possible diverse source of contamination for these diseases.

The OIE terrestrial animal health code published in 2000, OIE (2000), stated that HI was the prescribed test to address MG in serum samples due to the high specificity of the test and thus a low number of false-positive results. However, in the current version of the document OIE (2019), HI is considered interchangeable with ELISA to diagnose MG. In this study, we suggest that ELISA should not replace HI tests. As shown in Table 2, ELISA showed low specificity and predictive value for MG analysis. Otherwise, HI is considered highly specific even for the differentiation between *Mycoplasma* spp. strains, Kleven *et al.* (1988). Feberwee (2005), and collaborators, also reported a high number of false-positive caused by the low specificity of ELISA tests for MS and MG diagnosis. Therefore, we suggest that ELISA for MG should be a screening test to diagnose and HI a confirmatory test along with the PCR analysis.

Biosecurity is the employment of procedures to reduce the risk of introduction and spread of pathogens FAO (2008). Hence, they act as bioexclusion and biocontainment measures to prevent infectious agents from entering and exiting the farm, Charisis (2008). In the properties visited, there was a high variability in the management and biosecurity measures. The multi-age and mixed-species farms are problematic because the all-in, all-out management cannot be performed. Thus, no effective sanitary cleaning and disinfection were made, probably causing the permanence of pathogens in the environment contaminating new birds that are eventually introduced in the property. The extensive production also allows contact with migratory birds that are often carriers of several pathogens such as avian influenza virus, Newcastle virus, *Campylobacter* spp., *Salmonella* spp., *Mycoplasma* spp., and coronaviruses, Muradrasoli *et al.* (2010); Lister (2008).



Studies have reported before the susceptibility of backyard chickens, due to its close contact with wild birds and their role as reservoirs of pathogens, Reed *et al.* (2003); Karabozhilova *et al.* (2012); Smith *et al.* (2012); Pohjola *et al.* (2015); Pohjola *et al.* (2016).

Rodents can serve as vectors and reservoirs of several poultry diseases; it is estimated that rodents can transmit about 35 different diseases affecting man and domestic animals. These diseases include mycoplasmosis, salmonellosis, colibacillosis, coryza, pasteurellosis, fowl cholera, erysipelas, leptospirosis, trichinosis, hantavirus pulmonary syndrome, among others. The rodents mainly contaminate the feed at poultry farms, Donald *et al.* (2015); Castillo *et al.* (2013). The lack of rodent control in the properties studied pose a risk either to the poultry flocks in the surroundings but also to people involved in the farm's activities.

Although, in our study, we did not detect the correlation between the biosecurity aspects evaluated and the increase in serological titers, it is well-known that biosecurity measures should be implemented in the properties to prevent diseases and thus guarantee better weight gain and feed conversion. Therefore, we believe that other types of evaluation in prospective longitudinal epidemiological studies, along with agent isolation evaluation, would be a more appropriate analysis. In the properties studied, birds were perceived by the owner as sick often in the majority of the farms surveyed (73.68%), this undoubtedly reflects the low hygienic conditions and the water quality, and these situations may lead to diseases caused by bacteria, coccidia or other parasites that were not evaluated in this study.

The impact of management and bird's origin can also be observed on P10, even though being reactive for IBV and also for MS, P10 had the lowest serological titers values. We justify that by the fact that the owner bought the birds from a specialized company in the production of selected backyard hens, they were at the same age (about six months old) and were also the first individuals to be allocated in the property.

The various species mixed in the same flock are certainly another vital risk to disease spread in the region, MS has been isolated from guinea-fowl, this species may also carry Coronavirus, Pascucci *et al.* (1976); Bouwman *et al.* (2019); Ducatez & Guerin (2015). In the past, MS and MG were isolated from geese, Benöina *et al.* (1988), and MG from quails, Murakami *et al.* (2002). Studies have already identified MS, MG, IBV and aMPV in ducks, Wu X *et al.* (2016);

Bencina *et al.* (1988); Sun *et al.* (2014). Therefore, these birds could act as a reservoir of such diseases and corroborate to the perpetuation of these pathogens in the backyard flocks, Henning *et al.* (2011); Gowthaman *et al.* (2012).

Regarding the mortality disposal, the majority of the properties buried the carcasses. Although the burial of livestock mortality may raise worry that infectious agents may enter both human food and animal feed chain and contaminate the environment, these concerns are more applicable when mass mortality happens. The burial of a typical farm carcass disposal may not raise such concern, especially when few birds are buried, Gwyther *et al.* (2011). To reduce the chance of soil and water contamination possibility, the use of hydrated lime (Ca (OH)₂) on the basis of the burial pits is advisable because it effectively reduces the survival of pathogens, Sanchez *et al.* (2008). On-farm burning of carcass disposals is frequent in many countries and there is no critical inconvenience to the environment, Gwyther *et al.* (2011). The disposal of bird's carcasses in open-air represents a significant risk to the pathogens spread in the property, and the region due to the ability of insects such as flies and dark beetles and rodents to carry and spread diseases within the farm and in commercial farms.

We emphasize that the high seroprevalence in the backyard flocks to MS and MG, the most economically significant diseases surveyed in this study, and the possibility to mycoplasma to be spread by air, Bradburry *et al.* (2008), cause concern due to the proximity to the commercial poultry farms that varied from 500m to 6Km. Therefore, we strongly suggest that commercial poultry farms employees must be oriented to avoid contact with backyard birds. Besides, other BM as to restrict as much as possible the visitors in the facilities, keep a register of visitors, and reinforce the rodent control in the area surrounding poultry houses should be adopted.

CONCLUSION

The high seroprevalence found for IBV, aMPV, MS, and MG in the backyard poultry surveyed in this study demonstrate the importance of such birds as pathogen's reservoirs. The high seroprevalence to MS and MG, along with the flaws in BM in the backyard properties, pose a risk of outbreaks in commercial poultry farms in the region, which could lead to significant economic losses. Although we found high serological titers for the diseases surveyed, only MG correlated with clinical



signs. ELISA and HI tests showed a low concordance index caused mainly due to false-positive results in ELISA; because of that, we recommend that ELISA and HI should be used respectively as screening and confirmatory tests rather than interchangeable tests.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors declare no competing interests.

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