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Infectious bronchitis virus circulation among poultry flocks inside Atlantic biome forest, Northwestern São Paulo, Brazil

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Abstract. Infectious bronchitis virus (IB) affects the respiratory system of poultry and wild birds, causing large economic losses to the poultry industry worldwide. This study aimed to evaluate the antibodies against IBV among unvaccinated flocks localized in northwestern São Paulo state. A total of 1000 serum samples from two regions (R1 considered industry-associated flocks; and R2 considered industry-independent flocks) were used. To investigate the presence of antibodies against IBV, the indirect ELISA® serological test was applied. Positivity for anti-IBV antibodies was observed in 34% of the samples, with the highest values being in R1 (41.51%) and R2 (52.63%). This study demonstrated the risk of no-IBV vaccination program since the IBV directly affects the production and susceptibility of flocks to IBV infection.

Keywords: Infectious bronchitis, poultry, prevention, serology, vaccination

Circulação do vírus bronquite infecciosa em lotes de frangos criados dentro do bioma da floresta Atlântica, na Região Noroeste de São Paulo, Brasil

Resumo. Bronquite infecciosa (BI) é causada por um vírus que afeta o sistema respiratório de aves comerciais e de vida livre, ocasionando grandes perdas econômicas na indústria avícola no mundo. O presente estudo objetivou avaliar a presença de anticorpos contra o vírus da bronquite infecciosa (VBI), em galpões de frangos de corte nunca vacinados. Um total de 1000 amostras de soro foram coletados de duas regiões (R1 considerada associada à indústria e R2 considerada independente). A quantificação dos anticorpos contra VBI foi realizada pelo kit comercial de ELISA indireto. Positividade de anticorpos para VBI foi observada em 34% das amostras, com valores altos na R1 (41.51%) e R2 (52,63%). Este estudo demonstrou o risco de não vacinar para o VBI, uma vez que o VBI afeta a produção e a susceptibilidade dos galpões à infecção pelo VBI.

Palavras-chave: Bronquite infecciosa, frangos, prevenção, sorologia, vacinação

Introduction

Brazilian meat exports, mainly poultry, have been expanding internationally (ANUALPEC, 2019; FAPRI, 2020). According to the Ministry of Agriculture, Livestock and Food Supply, by 2020, poultry is expected to make up 48.1% of world's meat exports, placing Brazil in the position of being the world's leading exporter of poultry (MAPA, 2017). On poultry farms, the high density of individuals may decrease environmental quality, decreasing poultry health (De Wit et al., 2011). Consequently, susceptibility to certain diseases, such as diseases affecting the respiratory system, may increase (De

Wit et al., 2011; Inoue & Castro, 2009). Listed on the International Organization of Epizootics (OIE), the infectious bronchitis virus (IBV) causes infectious bronchitis in chickens and has potential detrimental effects on poultry production (Balestrin et al., 2014).

Besides IBV, there are several other diseases that can also generate economic losses, such as Newcastle's disease, avian influenza, infectious laryngotracheitis, and swollen head syndrome (Bande et al., 2015). To avoid problems associated with morbidity, overweight, and decreases in egg production and mortality, IBV and other vaccines should be routinely administered, following properly prescribed protocols (Liu et al., 2009). IBV infection can attack an entire flock, and it is dependent on serotype virulence and poultry age, as well as either maternal or active immunity (Ladman et al., 2006; Yan et al., 2016). Immunizations for IBV is not well understood due to the variations in virulence and the number of serotypes of IBV (Cavanagh & Gelb, 2008). The aim of the present study was to measure the risk of unvaccinated flocks to have IBV antibodies in a producer area of São Paulo State, Brazil.

Material and methods

Study design and sites description

The study was carried out on broiler chicken slaughterhouse unit in the northwestern region of São Paulo, where there is no IBV vaccination protocol. A hundred broiler chickens that were at the final third of the production chain were selected, and 10 serum samples were collected from each aviary corresponding to 1000 samples. The birds were from the following commercial origin: Cobb 500, CobbSlow, Ross Ap 96 and Hubbard Flex. The birds were randomly housed that had three different pressure conditions: positive, negative and dark house. The farms were compared by their seroprevalence characteristics, and the effect of the distances between the flocks was analyzed. A 15 km distance was established as a boundary; all flocks that were within this radius were considered part of a single group, and a comparison was made among the groups with different radius distances. The sites were then divided into two groups based on the following characteristics: Region 1 (R1 industry-associated flocks): This group included 47 flocks that historically had fewer viral challenges. Most flocks were dark house or negative pressure aviaries, though a few had positive pressure, generally those that had been established for more than 25 years. This R1 was characterized by flocks with a higher degree of modernity. Region 2 (R2 no industry-independent flocks): This region included 53 flocks were considered less modern, and most had been in operation for more than 25 years.

Data collection and analysis

Blood samples were collected by venipuncture (1.0 mL / bird) of the brachial vein by using a 3-mL syringe. The samples were left at room temperature for approximately 24 hours for serum precipitation, and they were then separated and stored at -20 °C. The serological analysis was performed by the BIOVET laboratory using the ELISA® immunoenzymatic technique through the BIOCHEK commercial kit following the manufacturer's recommendations. The test measured the number of antibodies to IBV in the serum of the flocks. The microplate bottoms were sensitized with the inactivated viral antigen for IBV. The samples were diluted and added to the bottom of the microplates to assess the presence of the anti-IBV antibody. An Elx800 reader with a 405-nm filter (BIOTEK™) was used to read the titration microplates. Positive serum were those that had at least one sampled positive with an optical density (OD) ≥0.2.

Statistical analysis

Data were analyzed in the Statistical Analysis System (SAS, 2004) software by using a chi-square (X^2) with a statistical significance of 10% ($P \leq 0.1$).

Results

When comparing the positivity for IBV antibodies between R1 and R2, in the R1 region, 25.53% of the samples were positive, and in the R2 region, 41.51% of the samples were seropositive; thus, R2 had more seropositive samples than R1 ($p = 0.092$; Table 1; Figure 1). When we analyzed the relationship between bird housing and positivity, there was a statistically significant difference ($p = 0.05$) between the R1 and R2, with 29.63% and 52.63% of samples testing positive in R1 and R2, respectively (Table 2).

When we investigated the positive aviaries and their proximity to other farms, no significant difference was observed (p = 0.69).

Table 1. IBV positivity using ELISA regarding two different collection sites, Region 1industry-associated flocks and Region 2 industry-associated flocks

Results	Region 1*		Region 2*	
	Number	%	Number	%
Positive	12	25.53	22	41.51
Negative	35	74.47	31	58.49
Total	47	100	53	100

*P = 0.092.

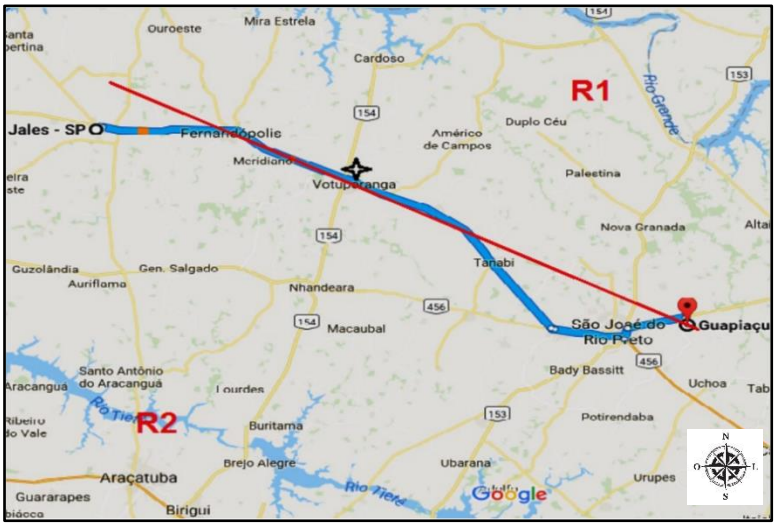

Figure 1. R1 and R2 classification to localize poultry flocks sampled through the highways that connect the cities of Jales to Guapiaçu/SP inside the Atlantic forest biome. Source: https://www.google.com.br/maps

Table 2. Positivity regarding the poultry housing (Industry associated or independent).

Results	Industry-associated*	Independent*
Positive	24	10
Negative	57	9
Total	81	19

*P = 0.05.

Discussion

The presence of a Brazilian genotype "BR-I genotype" has been reported throughout the territory of Brazil, as well as in other Latin American countries (Balestrin et al., 2014; Chacón et al., 2011). For this reason, this study investigated the presence of IBV in the northwestern region of São Paulo (P < 0.1). Extensive genetic diversity in IB viruses is found due to their fast replication and high mutation rates (Jackwood, 2012). Such considerations are extremely important for the prevention of the associated disease.

Both R1 and R2 exhibited antibodies to the virus, meaning that birds from both regions, regardless of their phenotypic differences, faced the virus at some point. (Pohjola et al., 2014) IBV study showed that both D274 and 4/91 genotypes of the virus were found in Finland, where no vaccination protocol has been implemented. They suggested that these genotypes may have arrived in the country from vaccinated animals elsewhere, considering bird migration as an important factor when studying IBV epidemiology (Pohjola et al., 2014). IBV in R2 could be associated with the different types of management protocols and vaccinations used in other industry-associated farms located in the surrounding areas. Positivity in R1, on the other hand, may have a relationship with either the presence of backyards or wild birds located in neighboring properties, since contact between wild birds and

poultry producing areas has been associated with the spread and maintenance of infectious diseases (Pohjola et al., 2014; Wang et al., 2013).

There was not an association between positivity and the distances between the regions. This result may be related to regulation 56, introduced by the MAPA, which states that poultry establishments may not impose adverse conditions that could interfere with the health and well-being of the poultry and that they maintain a minimum distance of 3 km apart (MAPA, 2017). Disease can be introduced through human transit, through animal/genetic material/biological product imports, or via transmission by migratory birds (MAPA, 2017). This information reinforces that care needs to be taken with sanitary control on farms, as it is an essential part of the poultry production chain (De Wit, 2000; Dixon, 2015). Studied Egypt's industry and showed that biosafety was crucial to the control of diseases. We believe that there must be a cause and effect relationship, indicating that the presence of sanitary measures may have decreased the exposure to viruses (Kouakou et al., 2015).

The low positivity for anti-IBV antibodies, representing 34% of the flocks in total, suggests the need for studies on the prevention of infectious diseases in poultry production. Both health and vaccination measures can influence the immunological protection of poultry lots. Vaccine serotypes that were recently developed may be favored by selection, mutation or recombination pressure, which needs to be considering when developing a vaccine control protocol for emergencies (De Wit, 2000; Bande et al., 2015).

Negative flocks are potential sources of concern, due to their probable susceptibility to the virus. Newcastle Disease virus (VCD) and VBI known as pathogenic agents were endemic to the country they studied, even though vaccination protocols were reported (Dixon, 2015). Although commercialization of live-attenuated and inactivated vaccines for IBV is available, vaccination protocols must be carried out with caution (De Wit, 2000; De Wit et al., 2011). At the same time, the absence or delay in developing specific vaccines for an emergency is important to avoiding errors in the protocol (Jackwood, 2012; Kouakou et al., 2015; Mendonça et al., 2009). Thus, vaccinations and sanitary management are necessary mechanisms to ensure the quality of animals and their products (De Wit, 2000; Dixon, 2015).

Conclusion

IBV antibodies were observed in the northwestern region of São Paulo, with higher positivity in the R2 region, which represented traditional poultry production flocks. Vaccination is an important mechanism in preventing IBV, along with adequate sanitary management, since the coronavirus is present in this region but there is no compulsory vaccination.

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