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Immune response to Massachusetts and Brazilian variant strains of infectious bronchitis virus in broiler chickens

Resposta imune induzida pelo sorotipo Massachusetts e variante BR do vírus da bronquite infecciosa em frangos de corte

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Highlights

Both strains produced similar serological profiles.
Birds infected with BR Variant strain exhibited increased tracheal mucosa thickness.
Birds infected with Massachusetts H120 strain exhibited more lesions in the trachea.

Abstract

This study aimed to assess the virulence and immune response in broiler chickens vaccinated with the Massachusetts H120 strain and the BR Variant strain of bronchitis via serological, histomorphometric, and histopathologic analyses of the tracheal mucosa. We used 162 one-day-old chicks, housed in random blocks within two temperature and light-controlled enclosures. Both the Massachusetts H120 and BR Variant strains were administered via ocular and spray routes. Blood samples were collected at four, seven, 14, 21, and 28 days of age for humoral response analysis using ELISA, from which we

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derived an immune response profile for each vaccine group based on the geometric mean titer (GMT). Tracheal fragments were collected at four, seven, and 14 days of age and processed using standard histological techniques. Histomorphometric measurements were analyzed using ANOVA and Tukey's post hoc test ($p < 0.05$). The GMTs for both H120 and BR strains across the different administration routes were comparable in terms of humoral immune response. We observed significant histomorphometric differences ($p < 0.05$) among the strains and vaccine administration routes. The H120 group exhibited higher lesion scores across all ages. The results indicate that the BR Variant strain elicits a lower post-vaccinal reaction and provides superior homologous protection under the challenges of poultry farming in Brazil.
Key words: Histomorphometry. Histopathology. Humoral response. Post-vaccinal reaction.

Resumo

O objetivo deste estudo foi avaliar a virulência e a resposta imune em frangos de corte vacinados com o sorotipo Massachusetts H120 e variante BR através da análise sorológica, histomorfométrica e histopatológica da mucosa traqueal. O experimento utilizou 162 pintos de um dia, alojados em blocos aleatórios em dois recintos com temperatura e luz controladas. Os sorotipos Massachusetts e variante BR da bronquite, ambos foram administrados pela via ocular e spray. Para análise da resposta humoral foi utilizado o teste ELISA, as coletas de sangue foram realizadas aos quatro, sete, 14, 21 e 28 dias de idade e um perfil de resposta imune foi criado para cada grupo vacinal de acordo com a via de administração baseado na média geométrica de titulação (GMT). Fragmentos de traqueia foram coletados aos quatro, sete e 14 dias de idade e processados de acordo com o protocolo histológico de rotina. As mensurações histomorfométricas foram submetidas ao teste ANOVA com teste post hoc de Tukey ($p < 0.05$). Os GMTs obtidos com o sorotipo H120 e BR com diferentes vias de administração foram semelhantes em termos de resposta humoral. Foram encontradas diferenças histomorfométricas significantes ($p < 0.05$) entre os sorotipos e as vias de administração da vacina. O grupo H120 teve maiores escores de lesão comparados ao grupo BR em todas as idades analisadas. Os achados demonstram que a variante BR tem menor reação pós vacinal e melhor proteção homóloga com o desafio enfrentado nas atividades avícolas no Brasil.
Palavras-chave: Histomorfometria. Histopatologia. Resposta humoral. Reação pós vacinal.

Introduction

Infectious Bronchitis Virus (IBV) in chickens, classified within the genus Gammacoronavirus, family Coronaviridae, and order Nidovirales, causes avian infectious bronchitis (AIB). This condition leads to reduced weight gain and poor feed conversion in broiler chickens, increased carcass rejection at slaughter, and in breeding and egg-laying chickens, decreased egg production and poorer shell quality (M. F. S. Montassier et al., 2008; Mendonça et al., 2009; International Committee on Taxonomy of Viruses [ICTV], 2020). First diagnosed in Brazil in Minas Gerais in 1957, AIB has become the predominant poultry disease in the country, affecting broiler, breeder, and egg-laying chickens across all commercial

production regions (Mendonça et al., 2009; Assayag et al., 2012). IBV consists of a diverse group of genetic mutants—created through spot mutations, deletions, insertions, and recombination during viral RNA replication (Jackwood et al., 2003; Lai & Holmes, 2001). Epidemiological studies highlight the Massachusetts and BR strains, particularly the BR Variant strain, as the most prevalent in Brazilian poultry (Carranza et al., 2017; Balestrin et al., 2014).

Control of respiratory diseases like AIB primarily relies on biosafety measures, strict sanitary control of birds, and effective immunoprophylactic or vaccination programs using attenuated or inactivated vaccines. Evaluating humoral immune responses is crucial for these efforts (De Wit et al., 1992; Cavanagh, 2007). The enzyme-linked immunosorbent assay (ELISA) remains the most commonly used method to measure specific antibodies in serum and assess the level of post-vaccination protection (Wakenell et al., 2016).

Thus, this study aims to evaluate the virulence and humoral immune response in broiler chickens vaccinated with the Massachusetts H120 and BR variant strains of IBV through serological, histomorphometric, and histopathologic analyses of the tracheal mucosa.

Materials and Methods

Compliance with animal experimentation ethics

This study adhered to the ethical guidelines set by the Ethics Committee on Animal Use (CEUA) at the Universidade Federal Rural de Pernambuco (UFRPE), receiving approval under certificate number 126/2014.

Experimental facilities

We used 162 one-day-old chicks from a commercial hatchery, descendants of breeders vaccinated against bronchitis with the Massachusetts H120 and BR Variant strains. These chicks were randomly allocated to two aviaries, each holding 81 birds, and divided into three groups: a control group (non-vaccinated), a spray vaccinated group, and an ocular pathway vaccinated group. The aviaries, maintained according to Cobb lineage standards, were temperature and light-controlled.

Each aviary was designated a specific vaccine strain: Massachusetts H120 (Cevac Bron 120 L®) and the BR Variant strain (Cevac IBras L®), administered per the manufacturer's instructions.

Sample collecting and processing

We collected 2 mL of blood from five randomly selected birds at four, seven, 14, 21, and 28 days of age from the ulnar vein into non-anticoagulant tubes for serum extraction and subsequent humoral response analysis. The serum was stored at -20°C until analyzed.

At four, seven, and 14 days of age, five randomly selected birds per treatment were euthanized. Middle third tracheal fragments from each bird were fixed in 10% buffered formalin (0.1 M, pH 7.2). After 24 hours in the fixative, the fragments were transferred to 70% alcohol solution until histological

processing at the Histology Laboratory of the Department of Animal Morphology and Physiology at UFRPE. The tissue samples were dehydrated in increasing concentrations of ethyl alcohol, cleared in xylol, and embedded in paraffin. Sections 3 µm thick were cut on a Leica® rotary microtome (model RM2125RT) and stained with hematoxylin and eosin.

At 42 days of age, tracheal fragments from birds in the control and vaccinated groups were collected for RT-PCR analysis to check for cross-group contamination, following Chacón et al. (2011). The Massachusetts serotype was differentiated from Brazilian variants using a multiplex RT-nested PCR with primers XCE1 +, XCE2 -, XCE3 -, MCE1 + (specific for Massachusetts), BCE1 + (793B), and DCE1 + (D274). Samples not producing a 295 bp fragment corresponding to the Massachusetts serotype were considered variant strains.

Analysis of humoral immune response

Humoral immune response determination involved ELISA using the Idexx® IBV Ab Test according to the manufacturer's protocol, allowing for the calculation of the geometric mean titer (GMT) of IgGs, based on the method by Câmara et al. (2009) for evaluating vaccine response against Newcastle disease.

Histomorphometric and histopathologic analyses

Histomorphometric analysis of tracheal mucosa was conducted using protocols by Nunes et al. (2002). Tissue slides were examined under a DM500 Leica® trinocular optical microscope at 40X magnification, with mucosal thickness measurements taken at ten 100 µm equidistant points using Image J® software.

Histopathologic analysis of lesions followed the protocol by Sesti et al. (2003), with adaptations. Analyzed parameters included hyperemia, hemorrhage, epithelial hyperplasia, mucous and lymphoid follicle hyperplasia, mucous gland atrophy, deciliation, and mononuclear and heterophilic infiltrates, with intensity varying from absent to accentuated and distribution from absent to diffuse.

Slide analysis for both histomorphometric and histopathological assessments was conducted in a double-blind manner by two experienced pathologists, independently.

Data analysis

The titers against AIB from serum samples were transformed into geometric mean titers (GMT), with an immune response profile being determined for each vaccine group as a function of the administration route. The titers against AIB and tracheal mucosa thickness measurements were analyzed using two-way ANOVA with Tukey's post hoc test ($p < 0.05$) on GraphPad Prism software (version 6.0).

Results and Discussion

The Ministry of Agriculture and Livestock approved the use of Massachusetts serotype vaccines for Avian Infectious Bronchitis (AIB) in Brazil, which quickly became widespread. Initially effective, the

emergence of clinical signs in correctly vaccinated birds prompted investigations, confirming the presence of variant strains in Brazilian poultry stocks (Felippe et al., 2009; H. J. Montassier, 2020; Chacón et al., 2011). These variants, classified as the BR molecular group (Chacón et al., 2011), are prevalent across all regions of the country (Carranza et al., 2017). Therefore, our findings on the impact of the BR Variant strain's vaccine are critical for veterinarians to accurately interpret clinical signs and assess the immunological status of birds.

No statistical differences were observed in the serological analysis across groups. The geometric mean titers (GMTs) of birds vaccinated with the H120 strain via ocular and spray routes are shown in Figure 1. The GMTs between the inoculation routes and the control group were similar, except at seven days, where birds vaccinated via the ocular route exhibited higher GMTs. Figure 2 displays the GMTs for birds vaccinated with the BR variant strain. At four days, GMT values were higher in the ocular route group compared to the spray method and control group. However, from seven days onward, GMTs were comparable across all groups. Although initial responses to the BR variant were higher, GMT values converged with those of the H120 strain from the seventh day until the experiment's conclusion. Variability in serological profiles for both strains (H120 and BR variant) is detailed in Tables 1 and 2. RT-PCR results confirmed that vaccinated groups were only positive for their respective strains, and control groups tested negative for both.

Humoral immunity is assessed by detecting and measuring specific antibodies in serum (Tizard, 2014). This analysis allows us to identify current or past exposure to specific antigens. The presence of different immunoglobulin classes (IgA, IgM, IgY/IgG) in bodily fluids (Fairbrother et al., 2004) influences how these antibodies respond. This is why the initial results of the H120 strain administered through ocular and spray routes were similar to the control group. Unlike Newcastle Disease and Gumboro Disease in birds, maternal antibodies do not affect AIB immunity (Davelaar & Kouwenhoven, 1977).

Birds vaccinated through the eye (ocular route) at seven days old showed a higher initial immune response (GMTs) compared to the spray method. This difference might be due to the unique anatomy of birds. Unlike mammals, birds have a cleft palate and a nasolacrimal duct connecting their eyes, nostrils, and oral cavity (Mendes & Macari, 2005). This allows eye drops to potentially reach the digestive system and be absorbed by the gastrointestinal tract more efficiently than the spray. After the initial peak, antibody levels (GMTs) become similar between the ocular and spray groups (Mesquita et al., 2010). This suggests a natural decline in the initial antibody response over time. The same reasoning applies to the BR variant vaccine. Ocular administration again resulted in higher initial GMTs, likely due to better absorption. But once again, the levels become similar across groups after the initial peak.

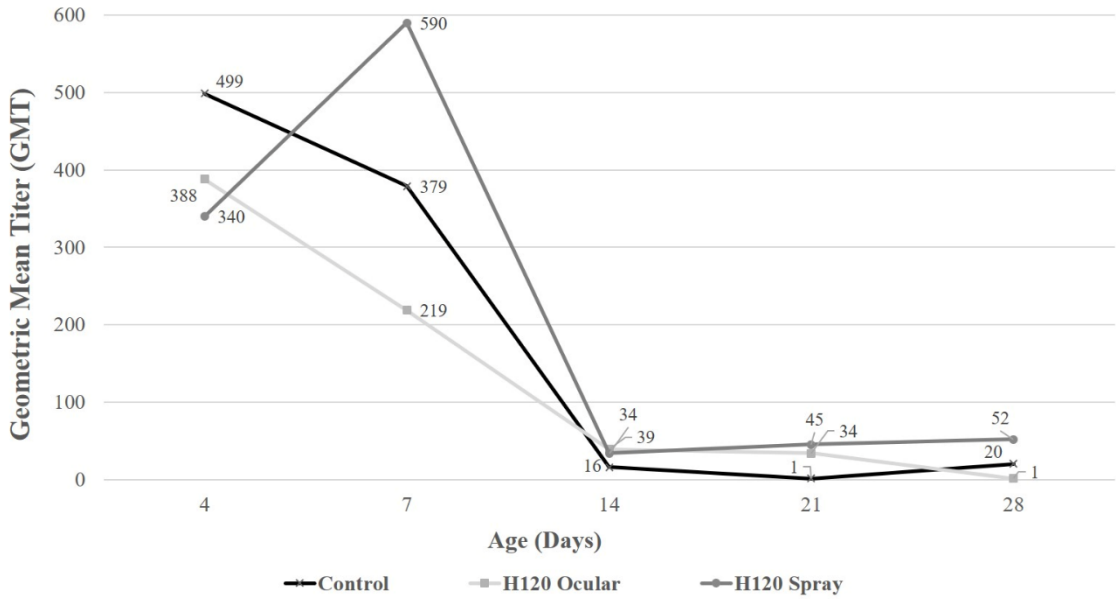

Figure 1. Profile of humoral immune response against avian infectious bronchitis virus (AIBV) in 162 broiler chickens vaccinated with the H120 strain via ocular and spray routes, compared to a control group. Chickens were vaccinated at different ages, and geometric mean titers (GMT) of IgGs were measured by an enzyme-linked immunosorbent assay (ELISA). ANOVA with Tukey's post hoc test ($p < 0.05$).

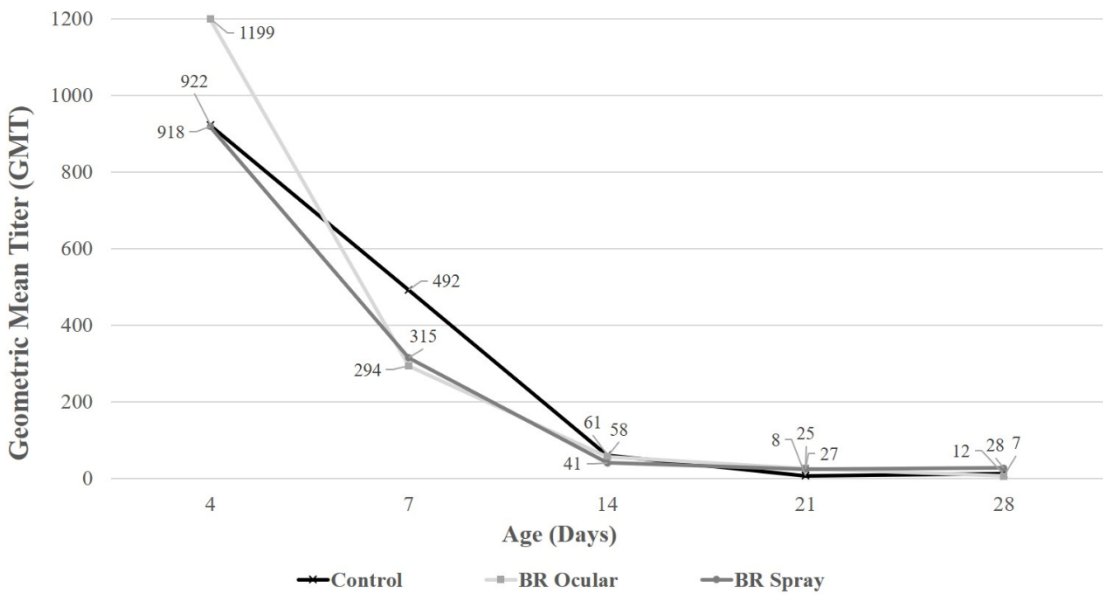

Figure 2. Profile of humoral immune response against avian infectious bronchitis virus (AIBV) in 162 broiler chickens vaccinated with the BR variant strain via ocular and spray routes, compared to a control group. Chickens were vaccinated at different ages, and geometric mean titers (GMT) of IgGs were measured by an enzyme-linked immunosorbent assay (ELISA). ANOVA with Tukey's post hoc test ($p < 0.05$).

Table 1
Coefficient of the serological profile in broiler chickens vaccinated with different Massachusetts H120 strains and a Control group, administrated via different routes at four, seven, 14, 21, and 28 days after vaccination

Day	Control	BR Ocular	BR spray
4	64.5	69.2	90.4
7	54.2	103.6	62
14		0	86.7
21	0	189.8	36.7
28	164.7	0	117.6

Table 2
Coefficient of the serological profile in broiler chickens vaccinated with different BR Variant strains and Control group, administered via different routes at four, seven, 14, 21, and 28 days after vaccination

Day	Control	BR Ocular	BR spray
4	89.5	59	39.3
7	35	46.1	65.8
14	85.4	32	87.1
21	86.4	125.7	79.8
28	117.4	107.4	110.1

Histomorphometric analysis of trachea mucosa in broiler chickens subjected to different vaccinal strains

At four days, birds vaccinated with the H120 strain via the spray method had a greater tracheal mucosa thickness (18.53 ± 3.09) compared to the ocular route (15.56 ± 3.07) and the control group (16.24 ± 4.06). By seven days, no significant differences were noted among the groups ($p > 0.05$). At 14 days, control group birds exhibited a greater mucosa thickness than those vaccinated with the H120 strain via either route (Table 3).

Table 3
Means and standard deviations for tracheal mucosa thickness (µm) in broiler chickens vaccinated with Massachusetts H120 and BR Variant strains via different administration routes and control group (unvaccinated) at four, seven, and 14 days after vaccination

Group	Strain/Age (days)		
	Massachusetts H120		
	4	7	14
Control	16.24 ± 4.06[b]	16.51 ± 4.82[a]	27.60 ± 8.81[a]
H120 - ocular	15.56 ± 3.07[b]	16.88 ± 3.68[a]	22.22 ± 8.79[b]
H120 - spray	18.53 ± 3.09[a]	21.53 ± 4.58[a]	25.64 ± 6.94[a]
	BR Variant		
	4	7	14
Control	15.61 ± 3.67[a]	15.43 ± 4.38[a]	26.61 ± 5.95[a]
BR variant - ocular	14.93 ± 2.47[a]	15.33 ± 3.29[a]	23.65 ± 6.42[a]
BR variant - spray	15.26 ± 4.06[a]	13.55 ± 3.14[a]	19.92 ± 4.43[b]

A: Means followed by different lowercase letters within columns differ significantly from each other ($p < 0.05$, Tukey's test).

For the BR variant, there were no significant differences in tracheal mucosa thickness at four and seven days compared to controls ($p > 0.05$). However, at 14 days, birds vaccinated via the ocular route displayed a greater thickness compared to the spray method (Table 3). No significant differences were found at seven days between groups vaccinated with the H120 and BR strains via both methods. At 14 days, however, the H120-vaccinated birds showed greater tracheal mucosa thickness compared to those vaccinated with the BR variant (Table 4).

Table 4
Means and standard deviations for tracheal mucosa thickness (µm) in broiler chickens vaccinated with different strains and administration routes at four, seven, and 14 days after vaccination

Group	Age (days)		
	4	7	14
H120 - ocular	15.56 ± 3.07[b]	16.88 ± 3.68[a]	22.22 ± 8.79[a]
H120 - spray	18.53 ± 3.09[a]	21.53 ± 4.58[a]	25.64 ± 6.94[a]
BR variant - ocular	14.93 ± 2.47[b]	15.33 ± 3.29[a]	23.65 ± 6.42[a]
BR variant - spray	15.26 ± 4.06[a]	13.55 ± 3.14[a]	19.92 ± 4.43[b]

A: Means followed by different lowercase letters within columns differ significantly from each other ($p < 0.05$, Tukey's test).

Although both strains exhibited similar serological profiles, the BR variant induced a stronger initial immune response, with GMT values at four days post-vaccination being approximately 145% higher than those observed in birds vaccinated with the H120 strain. Notably, the protective efficacy against homologous challenges differed significantly between the strains; the Massachusetts H120 strain provided only 40% protection against Brazilian variants, whereas the BR variant achieved complete protection (100%), as demonstrated by Chacón et al. (2016).

In birds vaccinated with the H120 strain, findings at four days included discrete diffuse hyperemia and accentuated multifocal to coalescent hyperplasia of the mucous glands (Figure 3a), along with multifocal moderate deciliation and a moderate focal to focally extensive mononuclear inflammatory infiltrate. At seven days, discrete focal hyperplasia of the mucous glands, multifocal moderate deciliation (Figure 3b), and multifocal moderate mononuclear inflammatory infiltrate were observed (Figure 3b). Lesions intensified by 14 days, featuring focally extensive discrete hyperplasia of the mucous glands and multifocal accentuated mononuclear inflammatory infiltrate (Figures 3c and 3d). Birds vaccinated with the BR variant exhibited lower histopathological lesion scores than those given the H120 strain. At four and seven days, diffuse discrete hyperemia, multifocal discrete deciliation, and multifocal discrete hyperplasia of the mucous glands were noted. By 14 days, there was diffuse moderate hyperplasia of the mucous glands and a multifocal discrete mononuclear inflammatory infiltrate (Figure 4).

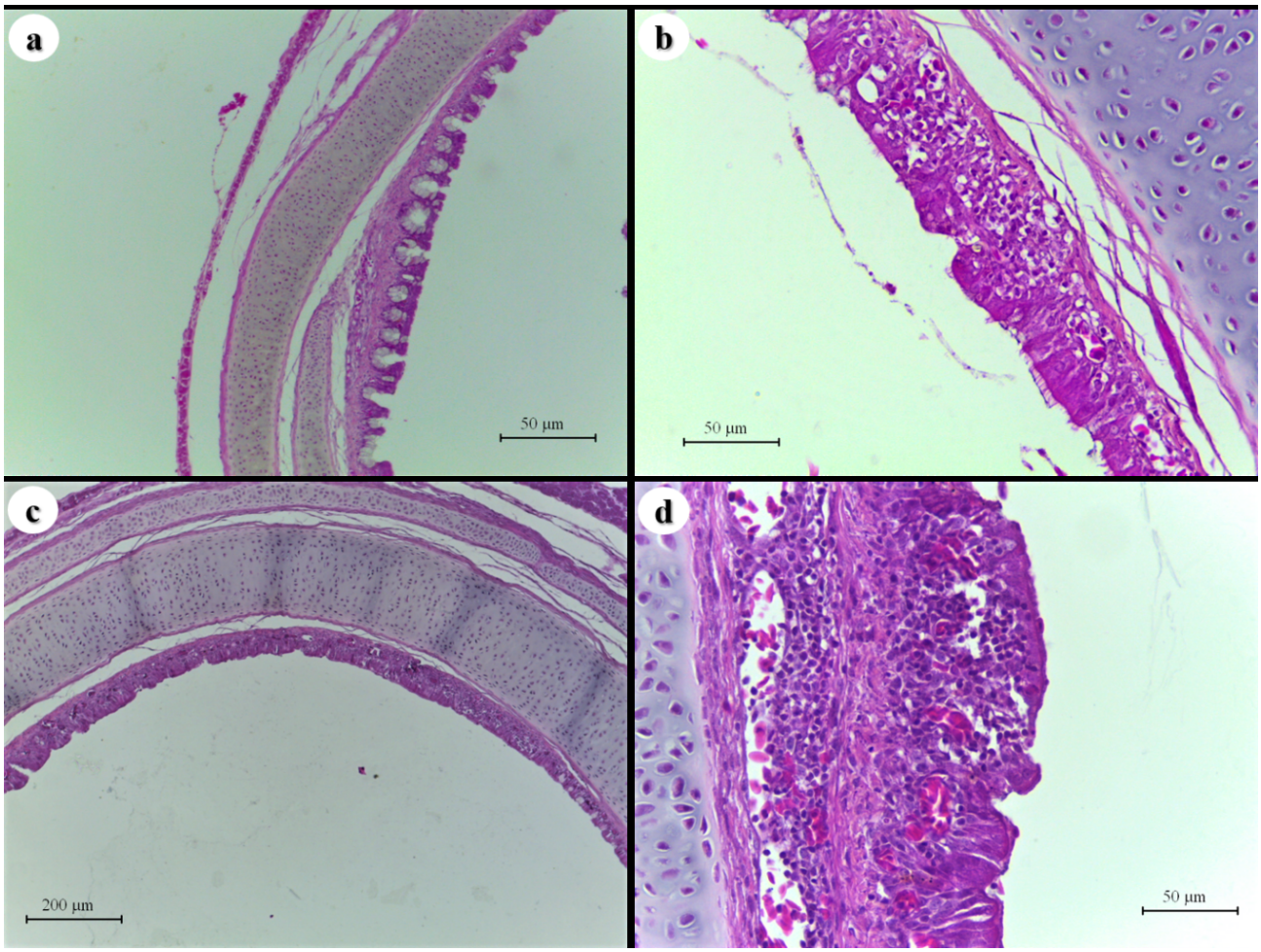

Figure 3. Histopathological analysis of tracheal mucosa in broilers vaccinated with the H120 strain at different ages.
(a) Accentuated multifocal to coalescent hyperplasia of the mucous glands in birds at four days after vaccination (H&E staining, 50 µm bar); (b) Moderate multifocal mononuclear inflammatory infiltrate in the lamina propria and respiratory epithelium, along with moderate deciliation of epithelial cells in birds at seven days after vaccination (H&E staining, 50 µm bar); (c) Accentuated multifocal mononuclear inflammatory infiltrate in the lamina propria of trachea in birds at 14 days after vaccination (H&E staining, 200 µm bar); (d) Accentuated mononuclear inflammatory infiltrate in the lamina propria of trachea in birds at 14 days after vaccination (H&E staining, 50 µm bar).

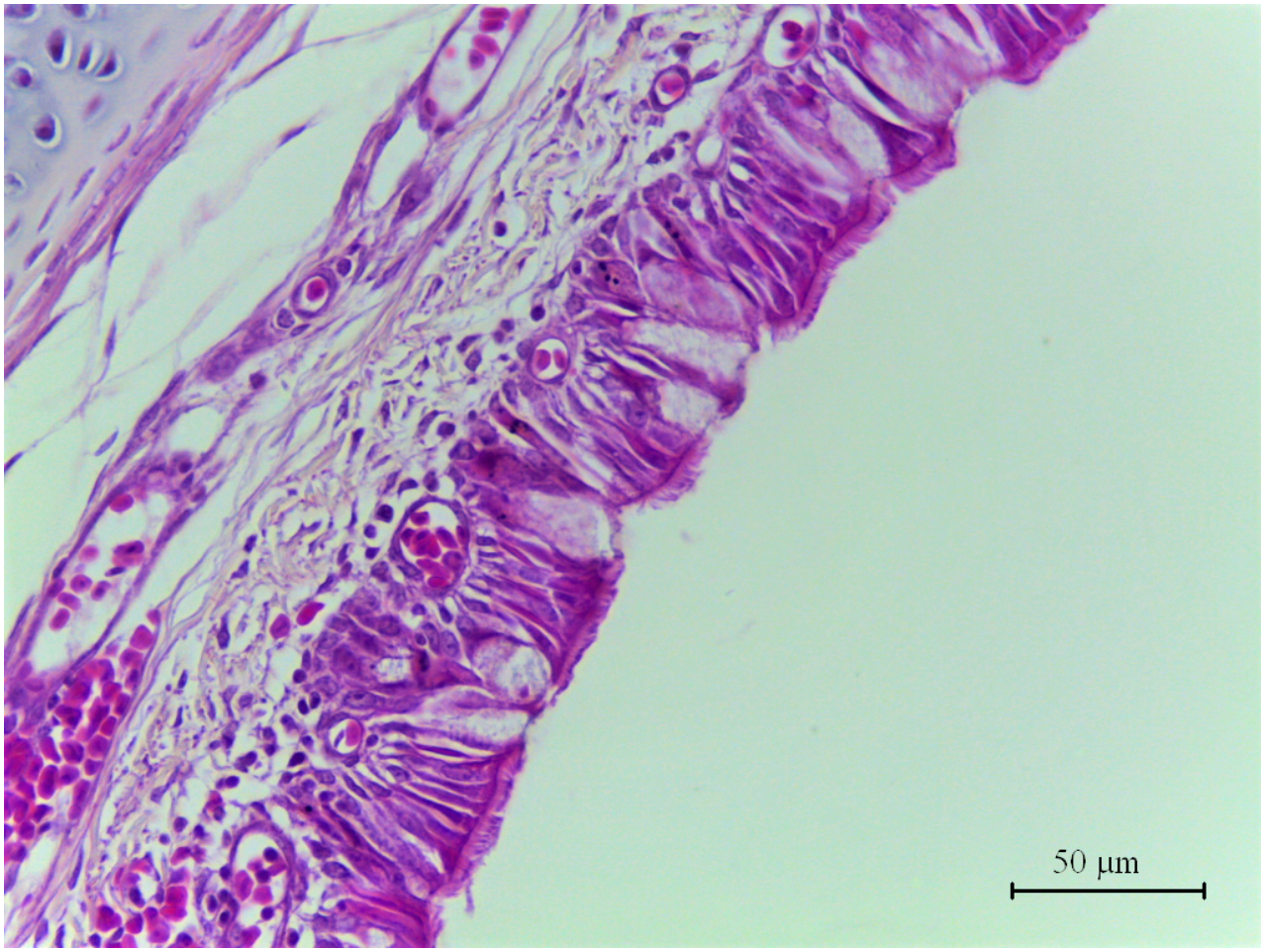

Figure 4. Histopathological analysis of tracheal mucosa in broilers vaccinated with the BR variant strain at 14 days of life. Diffuse moderate hyperplasia of the mucous glands and multifocal discrete mononuclear inflammatory infiltrate (H&E staining, 50 µm bar).

Histomorphometric analysis indicated that at 14 days of age, the tracheal mucosa thickness was greater in the control birds of the H120 group compared to those vaccinated via ocular and spray routes. This increased thickness can be attributed to environmental exposure to the vaccinal virus, as the control and vaccinated birds shared the same environment. Such exposure likely led to cross-contamination, facilitating transient protection in the initial days, a phenomenon previously documented by Macari et al. (2004).

For birds vaccinated with the BR variant via the ocular route, there was a noticeable increase in the thickness of the tracheal mucosa. This is likely attributable to the anatomical feature of the cleft palate, which allows the birds to ingest the vaccine, enhancing its effectiveness. This method, as noted by Borne and Comte (2003), promotes a robust vaccinal response without causing

significant epithelial damage, thereby maintaining the birds' resistance to other pathogens.

The similarity in tracheal mucosa thickness between the control birds and those vaccinated with the BR variant using the spray method suggests that the BR strain does not significantly damage the epithelium during viral replication. This observation aligns with findings by Chacón et al. (2016), who reported only mild tracheal damage in birds vaccinated with the BR variant, attributable to replication of the vaccinal virus in this organ. This is contrasted by the increased mucosa thickness observed at four and 14 days post-vaccination in birds inoculated with the H120 strain, which exhibited a more pronounced post-vaccinal reaction. Additionally, Chacón et al. (2016) highlighted similar trends in ciliostasis observed 12 days after vaccination, further confirming that the BR variant induces a milder post-vaccinal reaction compared to the Massachusetts strain.

The histopathologic analysis of the trachea revealed that the extent of damage varied with the vaccinal strain used. Birds vaccinated with the H120 strain exhibited higher lesion scores at all ages evaluated, characterized by moderate lymphohistiocytic tracheitis throughout the study period. In contrast, birds vaccinated with the BR variant showed only discrete lymphohistiocytic tracheitis, and this was observed solely at 14 days of age. This study is the first to conduct both histomorphometric and histopathologic analyses of the potential injuries caused by these vaccines to the tracheal mucosa, highlighting a significant differentiation in tissue response between the two strains.

The RT-PCR findings confirmed that there was no contamination between the groups, as the technique employed effectively differentiated the BR variant and H120 serotypes. Based on these results, the BR variant of avian infectious bronchitis elicited a humoral immune response comparable to that of the H120 strain. However, in terms of virulence and histopathology, the BR variant induced a milder post-vaccinal reaction and caused less damage to the trachea. Furthermore, the BR variant strain provided effective homologous protection against the challenges prevalent in Brazilian poultry stocks.

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Disclosure statement

The authors declare that they have no conflicts of interest.

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