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Tracheal post-vaccinal reaction to different strains of Newcastle disease virus[1]

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ABSTRACT.- Rocha P.M.C, Barros M.E.G., Bandeira J.T., Braga J.F.V., Morais R.S.M.M., Souza F.A.L & Evêncio-Neto J. 2022. **Tracheal post-vaccinal reaction to different strains of Newcastle disease virus.** *Pesquisa Veterinária Brasileira 42:e06733, 2022.* Laboratório de Histologia, Departamento de Morfologia e Fisiologia Animal, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros s/n, Dois Irmãos, Recife, PE 52171-900, Brazil. E-mail: joaquim.evenciont@ufrpe.br

The aim of the present study was to evaluate the post-vaccinal reaction to two lentogenic vaccine strains of Newcatle disease virus (NDV) and a recombinant turkey herpesvirus (rHVT) vaccine expressing the fusion glycoprotein of NDV in broiler chickens through histomorphometric and histopathologic analyses of the trachea. The experiment involved 245 chicks housed in randomized blocks with three different enclosures under controlled conditions of temperature, light and ventilation. Each enclosure represented a vaccine strain and was divided into groups according to the administration route. Each block also had its own control group composed of unvaccinated birds. The vaccine strains PHY.LMV.42 (PL42) and La Sota (LS) were selected according to the Intracerebral Pathogenicity Index (ICPI) and the rHVT-NDV Serotype 3 strain (ST3) was selected for representing non-NDV infection. At two, four, seven, 14 and 21 days post vaccination, fragments from the middle third of the trachea were collected and submitted to routine histological processing. For the histomorphometric analysis, the slides were photographed, and the thickness of the tracheal mucosa was measured. Statistical analysis involved two-way ANOVA and Tukey's post-hoc test with a 5% significance level. For the histopathological evaluation, lesions were described as to the degree of intensity and distribution. At four and 14 days post vaccination with the LS strain administered by the ocular route, the means of thickening of the tracheal mucosa (20.85±7.31µm and 26.97±5.50µm, respectively) were significantly higher (p<0.05) than for all other strains, which was related to the severe histopathological lesions found in this group, characterized by hyperemia, hyperplasia of the mucous glands, moderate deciliation and multifocal lymphohistiocytic inflammatory infiltrate. At 21 days, broiler chickens vaccinated with the ST3 strain showed more discrete lesions and less thickening of the tracheal mucosa (23.23±7.62µm; p<0.05) in comparison with other studied strains. The lesions found in this group were only hemorrhage, deciliation and mild focal lymphocytic inflammatory infiltrate. The results of the histomorphometry and histopathology of the trachea indicated that vaccination with rHVT-NDV Serotype 3 strain induced lower degree post-vaccine tracheal lesions compared to other vaccine strains analyzed in this study.

INDEX TERMS: Newcastle disease, broiler, post-vaccinal reaction, trachea, lentogenic vaccine, enterotropic vaccine, vector vaccine.

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RESUMO.- [**Reação traqueal pós-vacinal à diferentes cepas do vírus da doença de Newcastle.**] Objetivou-se avaliar a reação pós-vacinal de duas estirpes lentogênicas do vírus da doença de Newcastle (VDN) e uma vacina recombinante de herpesvirus de perus (rHVT) que expressa a glicoproteína de fusão de VDN em frangos de corte por meio da histomorfometria e histopatologia da traqueia. Foram utilizados 245 pintos alojados em blocos ao acaso, sendo três galpões distintos em condições controladas de temperatura, luz e ventilação. Cada galpão representou uma cepa vacinal, onde foram divididos por grupos de acordo com a via de administração. Todos os blocos possuíam um grupo controle composto por aves não vacinadas. As cepas vacinais PHY.LMV.42 (PL42) e La Sota (LS) utilizadas foram selecionadas de acordo com o Índice de Patogenicidade Intracerebral (IPIC) e a cepa Sorotipo 3 (ST3), da vacina rHVT-VDN foi selecionada por não representar infecção do VDN. Aos dois, quarto, sete, 14 e 21 dias pós-vacinação, fragmentos do terço médio da traqueia foram coletados e posteriormente processados conforme rotina histológica. Para análise histomorfométrica da mucosa traqueal, as lâminas foram fotografadas e realizadas as mensurações da espessura da mucosa traqueal sendo aplicado teste de análise de variância a dois critérios (ANOVA) e utilizando o *post-hoc* de Tukey com nível de significância de 5%. Para a avaliação histopatológica foram observadas a presença de lesões microscópicas e estas foram descritas quanto ao grau de intensidade e distribuição. Aos quatro e quatorze dias pós-vacinação com a cepa LS administrada por via ocular, as médias do espessamento da mucosa traqueal (20,85±7,31µm e 26,97±5,50µm, respectivamente) foram significativamente maiores (p<0,05) quando comparada a todas as demais cepas utilizadas, isto se deve às severas lesões histopatológicas encontrados neste grupo, caracterizadas por hiperemia, hiperplasia das glândulas mucosas, deciliação moderada e infiltrado inflamatório linfohistiocitário multifocal moderado. Já aos 21 dias as aves vacinadas com a cepa ST3 apresentaram lesões mais discretas e menor espessamento da mucosa da traqueia (23,23±7,62µm; p<0,05) em comparação às demais cepas estudadas. As lesões encontradas neste grupo foram apenas hemorragia, deciliação e infiltrado inflamatório linfocitário focal discreto. Os resultados da histomorfometria e da histopatologia da traqueia indicou que a vacinação com a rHVT-NDV, cepa Sorotipo 3 induziu menor grau de lesões pós-vacinais na traqueia comparada a outras cepas vacinais analisadas nesse estudo.

TERMOS DE INDEXAÇÃO: Doença de Newcastle, frango, reação pós-vacinal, traqueia, vacina lentogênica, vacina enterotrópica, vacina vetorizada.

INTRODUCTION

Newcastle disease (ND) is a highly contagious viral infection that affects the nervous, respiratory and digestive systems of birds (Flores et al. 2006). The etiological agent of this disease is the ND virus, which belongs to the *Avian Orthoavulavirus 1,* genus *orthoavulavirus,* Avulavirinae, Paramyxoviridae (ICTV 2011).

ND is capable of infecting more than 240 bird species. It is disseminated throughout the world mainly through direct contact and has the potential to cause considerable economic losses in the poultry industry, resulting in the elimination of thousands of birds as well as restrictions regarding importations and exportations (Rauw et al. 2009, Dortmans et al. 2011). In Brazil, despite the gradual decline in the past years, the disease is exotic to the poultry industry, serving as a source of dissemination of the virus (Seal et al. 1998, Clavijo et al. 2000, Marks et al. 2014), mainly through the trafficking of wild birds (Orsi 2010).

ND can be prevented through vaccination. The commercial products currently available are attenuated live vaccines, vector vaccines and inactivated vaccines (Bermudez & Stewart-Brown 2003, Marangon & Busani 2006). The adverse effects of residual pathogenicity constitute the major disadvantage of attenuated live vaccines, especially in young birds. Vector vaccines are produced from immunogenic elements of the virus, especially surface proteins and vectors of the poxvirus (fox poxvirus) (Boursnell et al. 1990, Taylor et al. 1990, Iritani et al. 1991, Nagy et al. 1991, McMillen et al. 1994, Karaca et al. 1998) and herpes virus (turkey herpes virus) (Morgan et al. 1992, 1993, Heckert et al. 1996, Reddy et al. 1996). These vaccines eliminate post-vaccinal reactions and reduce the excretion of the virus in the field for a prolonged period (>70 weeks) without affecting the immune response to other diseases (Palya et al. 2014).

The expected post-vaccinal reaction is mild sneezing for three to five days in the majority of birds, followed by recovery (Bernardino 2004). An exacerbated post-vaccinal reaction implies a compromised performance and pathological conditions.

Knowledge on available vaccinal strains is important to the establishment of an effective vaccination program. The immune response tends to increase as the pathogenicity of the live vaccine increases. However, in the case of respiratory tropism vaccines, the post-vaccinal reactions are also increased at the same proportion (Tamas et al. 2004, Paniago 2007).

The trachea is one of the most affected organs during the ND vaccination process, as vaccines can cause damage to this organ. However, few studies have investigated the microscopic changes that occur. Therefore, the aim of the present study was to evaluate the effect of the post-vaccinal reaction to different lentogenic vaccine strains of Newcastle disease virus (NDV) and a recombinant turkey herpesvirus (rHVT) vaccine expressing the fusion glycoprotein of NDV in broilers through histomorphometric and histopathologic analyses of the trachea.

MATERIALS AND METHODS

Compliance with animal experimentation ethics. This study was conducted in accordance with the terms and conditions of the Ethics Committee on the Use of Animals of the "Universidade Federal Rural de Pernambuco" (UFRPE) and received approval from the committee (certificate number: 126/2014).

Experimental facilities. The experiment involved the use of 245 chicks with one day of life from *Mycoplasma gallisepticum* free breeder hens, lodged in random blocks in three separate enclosures with least 200 meters between each other, with controlled temperature and light in accordance with the standards required by the Cobb lineage, the natural ventilation being controlled through curtain management.

Each enclosure represented one block characterized by a vaccinal strain. The blocks were divided into three treatments according to the vaccine and administration route. Each block also consisted of a control group of unvaccinated birds. Two vaccines were selected

based on the Intracerebral Pathogenicity Index (ICPI): the enterotropic lentogenic PHY.LMV.42 (PL42) strain (Cevac Vitapest L®), ICPI≤0.16 ($10^{6.2}$ EID_{50}), administered via the ocular and spray routes; and the pneumotropic lentogenic La Sota (LS) strain (New-Vacin LaSota®), ICPI≤0.4 ($10^{6.2}$ EID_{50}), administered via the ocular and spray routes. Being one of the most recent vector vaccines against NDV, the rHVT-NDV Serotype 3 strain (ST3) vaccine expressing the F protein of NDV (Vectormune ND HVT®) was given subcutaneously. All vaccines were administrated at hatching day. In addition to these, the birds were also vaccinated against Marek's and Gumboro diseases.

All vaccines had a wide margin within the expiration date, were administered according to the manufacturer's recommendations and the evaluations of the unvaccinated control group served as a basis for estimating vaccine infection.

Sample collecting and processing. Five birds were euthanized per treatment at four, seven, 14 and 21 days post vaccination by cervical dislocation. Fragments were collected from the middle third of the trachea of each bird and fixed in 10% buffered formaldehyde 0.1M, pH 7.2. After 24 hours in the fixing solution, the fragments were placed in a 70% alcohol solution until the histological processing, following the routine protocol of the "Laboratório de Histologia" (Histology Laboratory) of the "Departamento de Morfologia e Fisiologia Animal" (Department of Animal Morphology and Physiology) of UFRPE for imbedding in paraffin. The blocks were cut into slices with thickness of 3µm on a rotary microtome (model RM2125RT, Leica®) and the slides were stained with hematoxylin and eosin (HE).

Histomorphometric and histopathologic analyses. The protocol described by Nunes et al. (2002) was used for the histomorphometric analysis of the tracheal mucosa. The slides were examined and photographed (magnification: 40X) with a DM500 Leica® trinocular optical microscope. The measurements of tracheal mucosa thickness were performed at ten points equidistant (100µm) from each other using the Image J® software. The protocol proposed by Sesti et al. (2013) was used with adaptations for the histopathologic analysis of the lesions. The tracheal sections were examined for the presence and intensity (mild, moderate and marked) of the following histopathological changes: flattening of mucosal epithelium, deciliation, hyperemia, hemorrhage, epithelial hyperplasia, mucous glands hyperplasia and tracheitis. The latter was characterized according to the predominant cell type(s) in the inflammatory infiltrate as mononuclear (lymphocytic, plasmacytic, histiocytic or its combinations) and/or heterophilic.

Data analysis. All statistical analyses were performed using the GraphPad Prism software (version 6.0). Comparisons of the thickness of the tracheal mucosa were performed using two-way analysis of variance (ANOVA), followed by Tukey's post hoc test at a 5% significance level. Descriptive semi-quantitative statistical analysis was also performed of the lesions found in the tracheal mucosa of the birds.

RESULTS

At four, seven and 21 days post vaccination, the histomorphometric analysis revealed significant differences in the thickness of the tracheal mucosa ($p<0.05$) within the groups in compared to the respective controls (Table 1). Significant differences were also found among groups vaccinated with the different strains (Table 1) and on different days post vaccination. This increase in tracheal thickness was mainly related to different degrees of hyperemia and tracheitis observed in the groups.

No significant difference ($p>0.05$) in thickness of the tracheal mucosa was found in the group vaccinated with the ST3 strain compared to its respective control group at any of the ages analyzed (Table 1).

The analysis by group did not reveal significant difference ($p>0.05$) regarding the thickness of the tracheal mucosa of birds vaccinated with the strain PL42 by ocular or spray route compared to its respective control group at two, four and seven days post vaccination. At 14 days post-vaccination, there was a significant increase in tracheal thickness ($p<0.05$) in both groups. In this case, in birds vaccinated via ocular

Table 1. Mean and standard deviation values of thickness of tracheal mucosa (µm) from broiler chickens vaccinated with different strains and administration routes and respective unvaccinated control groups at two, four, seven, 14 and 21 days post vaccination

Groups	Strains/Age (days)				
	rHVT-NDV Serotype 3				
	2	4	7	14	21
Control	11.45 ± 3.88^{a}	16.12 ± 5.48^{a}	16.25 ± 5.06^{a}	18.11 ± 5.07^{a}	26.14 ± 5.72^{a}
ST3	11.90 ± 3.15^{aA}	15.07 ± 5.30^{aB}	20.92 ± 7.38^{aB}	21.40 ± 5.53^{aB}	23.23 ± 7.62^{aB}
	PHY.LMV.42				
	2	4	7	14	21
Control	14.24 ± 4.14^{a}	14.06 ± 4.68^{a}	23.22 ± 6.32^{a}	18.29 ± 4.77^{b}	24.16 ± 5.13^{a}
PL42 - ocular	12.96 ± 3.19^{aA}	16.76 ± 6.91^{aB}	21.15 ± 7.34^{aB}	24.47 ± 7.27^{aB}	28.63 ± 7.19^{aA}
PL42 - spray	13.06 ± 3.16^{aA}	14.17 ± 3.98^{aB}	16.83 ± 3.09^{aB}	24.00 ± 5.61^{aB}	22.72 ± 5.94^{bB}
	La Sota				
	2	4	7	14	21
Control	11.07 ± 2.39^{a}	14.16 ± 2.79^{a}	24.87 ± 19.91^{a}	24.85 ± 7.66^{a}	25.72 ± 6.66^{b}
LS - ocular	13.75 ± 3.65^{aA}	20.85 ± 7.31^{bA}	22.35 ± 6.58^{aB}	26.97 ± 5.50^{aA}	21.21 ± 12.27^{bB}
LS - spray	13.73 ± 5.34^{aA}	14.37 ± 3.48^{aB}	25.86 ± 10.89^{aA}	23.88 ± 4.69^{aB}	33.04 ± 9.04^{aA}

$^{a, b}$ Means followed by different lowercase letters in columns differ significantly ($p<0.05$, Tukey's test) in the intragroup comparison of strains with their controls; $^{A, B}$ means followed by different uppercase letters in columns differ significantly ($p<0.05$, Tukey's test) in the intergroup comparisons; ST3 = rHVT-NDV Serotype 3 strain, PL42 = PHY.LMV.42 strain, LS = La Sota strain.

route, this increase was due to lymphohistioplasmocytic tracheitis (3/5), hyperemia (2/5) and hemorrhage (1/5). While birds vaccinated via spray with the same strain had lymphocytic tracheitis (4/5) and hyperemia (3/5). At 21 days, a significant reduction (p<0.05) in the thickness of the tracheal mucosa was found in the birds that received the vaccine via the ocular route compared to those that received the vaccine in spray form.

No significant difference (p>0.05) in thickness of the tracheal mucosa was found in the group vaccinated with the LS strain via the ocular or spray route compared to the respective control group at two, seven and 14 days post vaccination.

However, at four days post vaccination, the thickness of the tracheal mucosa was significantly higher (p<0.05) in the birds vaccinated using the ocular route. This thickening was related to epithelial hyperplasia (2/5), lymphohistiocytic tracheitis (2/5) and mucous gland hyperplasia (1/5). In this same group, 21 days after spray vaccination, the significant increase in the tracheal mucosa (p<0.05) was due to predominantly lymphohistiocytic tracheitis (3/5) and hyperemia (3/5).

In the intergroup comparisons, no significant differences (p>0.05) were found among the different vaccinal strains or different administration routes at two days post vaccination.

The analysis between groups revealed that birds vaccinated with the LS strain showed a significant increase (p<0.05) in post-vaccination tracheal thickness compared to the other groups. At 21 days post-vaccination, this increase was statistically equal to that of birds vaccinated with PL42 via the ocular route, both being higher than the others (p<0.05). In birds from the LS group, vaccinated via ocular route, the trachea was thicker (p<0.05) four days post-vaccination due to the epitelial hyperplasia (2/5), lymphohistiocytic tracheitis (2/5) and mucous gland hyperplasia (1/5). At seven days post-vaccination in LS group birds vaccinated by spray, the significant thickening (p<0.05) was related to hyperemia (3/5), hyperplasia of mucous glands (3/5), predominantly lymphohistiocytic tracheitis (2/5) and hemorrhage (1/5).

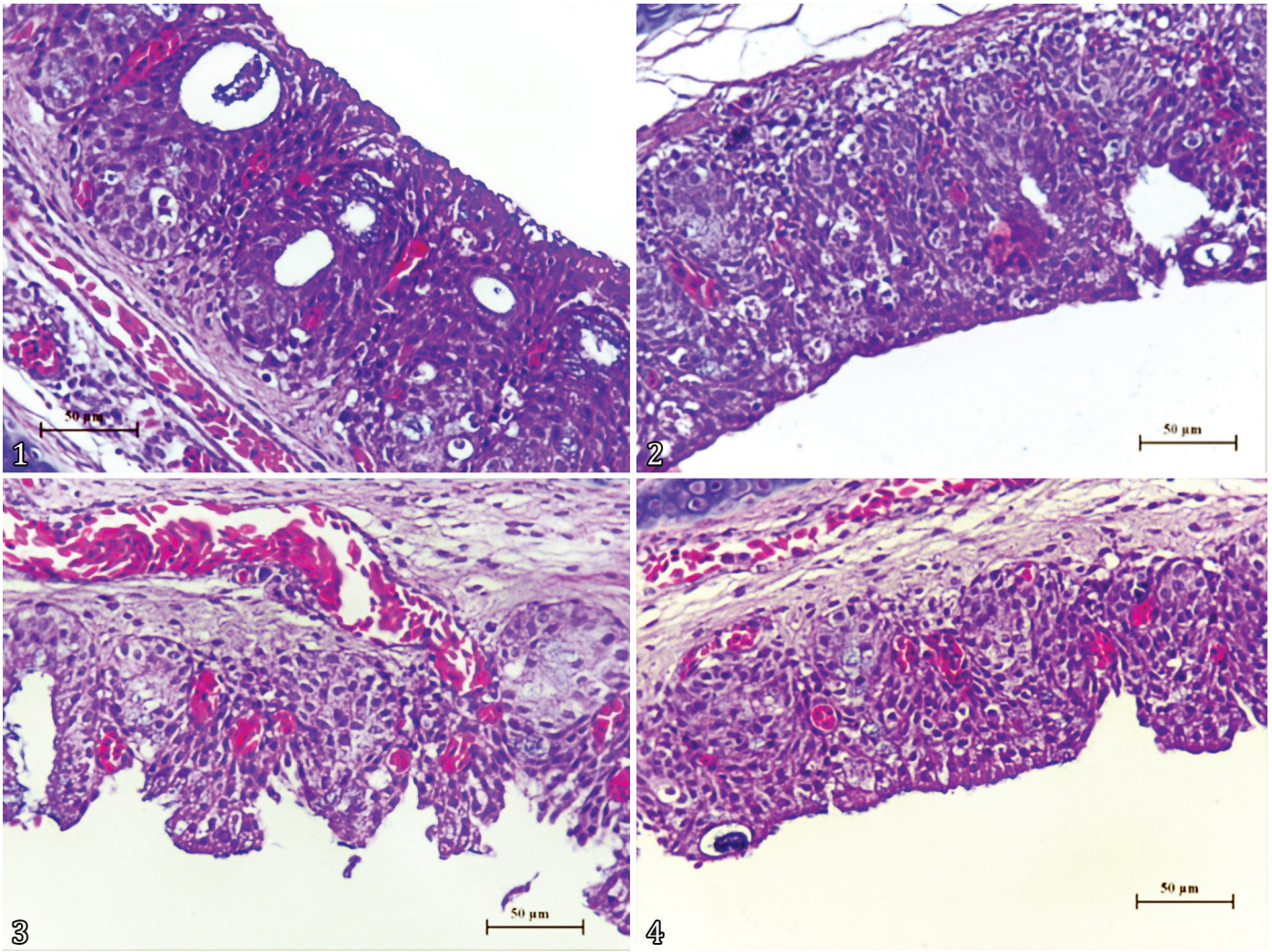

Fig.1-4. Histopathologic analysis of tracheal mucosa of birds at seven days post vaccination with LaSota strain via spray. (**1**) Hyperplasia of mucous glands and discrete hyperemia in lamina propria of trachea. HE, bar = 50µm. (**2**) Moderate multifocal lymphohistiocytic inflammatory infiltrate in lamina propria and respiratory epithelium and diffuse deciliation of epithelial cells. HE, bar = 50µm. (**3**) Moderate diffuse hyperemia in lamina propria of trachea and discrete epithelial hyperplasia. HE, bar = 50µm. (**4**) Epithelial hyperplasia and diffuse deciliation of tracheal mucosa. HE, bar =50µm.

Fourteen days post ocular vaccination, the brids from the LS group, had tracheal thickening due to hyperemia (4/5) and lymphocytic or heterophilic tracheitis (3/5). At 21 days post-spray vaccination, birds in the LS group had a significant increase (p<0.05) in tracheal thickness related to predominantly lymphohistiocytic tracheitis (3/5) and hyperemia (3/5). At this same age, the birds of group PL42 vaccinated via the ocular route had tracheal thickening associated with hyperplasia of mucous glands (1/5) and lymphohistiocytic tracheitis (3/5) (Table 1).

In addition to the relationship between tracheal thickness and lesions, the histopathology analysis allowed the determination of tracheal damage according to vaccine and administration route and the results are shown in Table 2. The birds vaccinated with the LS strain in spray form had more severe lesions among all strains and between administration routes at all ages tested. Epithelial hyperplasia, hyperemia, hyperplasia of the mucous glands, discrete to moderate deciliation and moderate multifocal lymphohistiocytic inflammatory infiltrate were found in this group, with some broilers exhibiting mild diffuse heterophilic inflammatory infiltrate. The birds vaccinated with the LS strain by the ocular route exhibited marked diffuse deciliation in comparison to the birds vaccinated using the spray route (Fig.1-4).

The birds vaccinated with the PL42 strain by the ocular route had more discrete lesions compared to the birds vaccinated with the LS strain (ocular and spray routes). Discrete focal hemorrhage, discrete diffuse hyperemia, discrete focally extensive hyperplasia of the mucous glands, discrete multifocal deciliation and discrete multifocal mononuclear (predominantly lymphohistiocytic) inflammatory infiltrate were found in this group (Fig.5-8).

The birds vaccinated with the ST3 strain had fewer and more discrete lesions in comparison with all the strains, with the occurrence of only hyperemia, hemorrhage, deciliation and discrete focal lymphocytic inflammatory infiltrate (Fig.9-12).

DISCUSSION

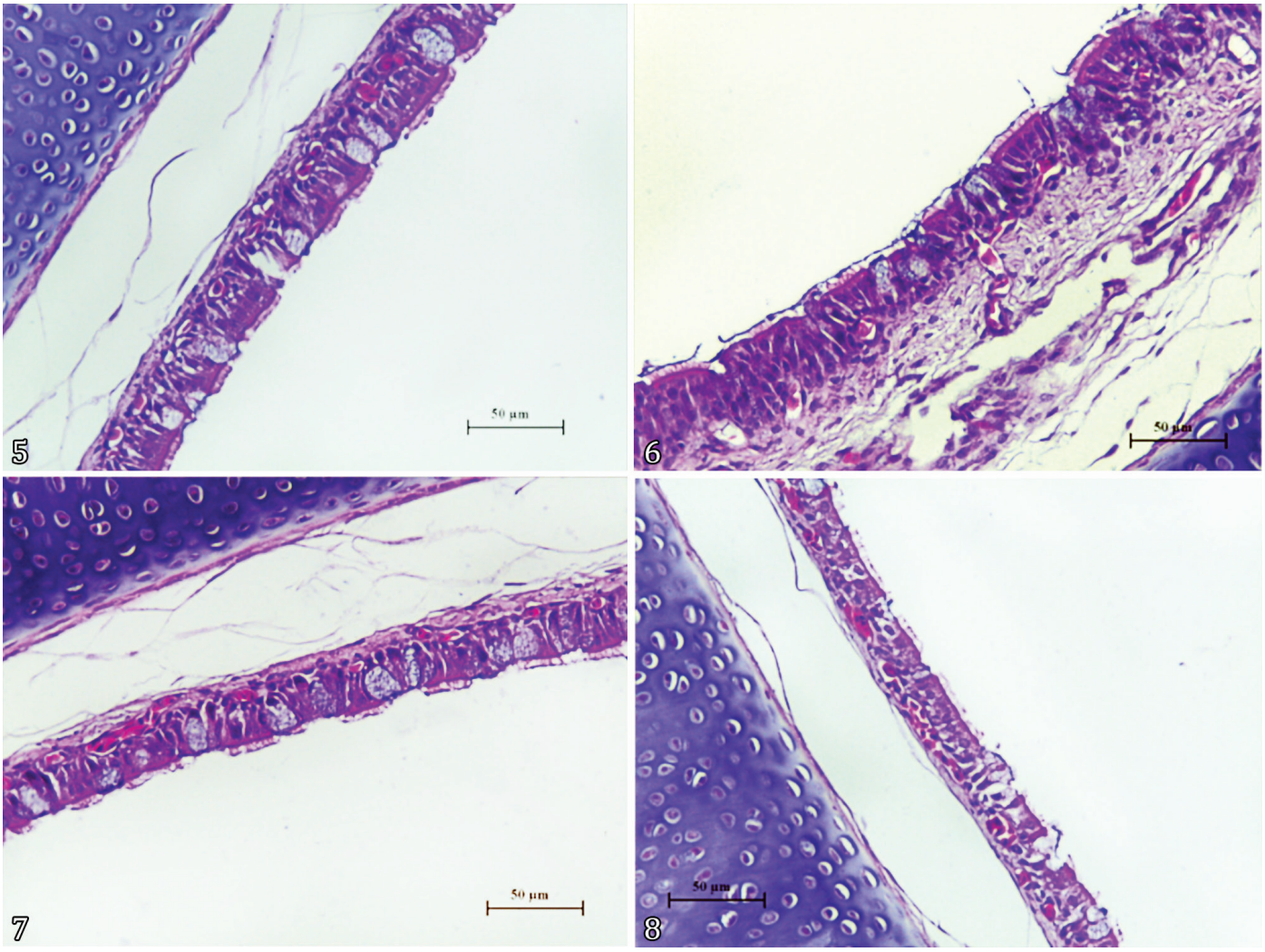

Fig.5-8. Histopathologic analysis of tracheal mucosa of birds at seven days post vaccination with PHY.LM.42 strain via ocular route. (**5**) Discrete diffuse hyperemia in lamina propria of trachea. HE, bar = 50µm. (**6**) Discrete focal lymphocytic inflammatory infiltrate in lamina propria and discrete presence of intraluminal mucus. HE, bar = 50µm. (**7, 8**) Hyperemia and discrete diffuse deciliation in lamina propria of trachea. HE, bar = 50µm.

Table 2. Tracheal histopathological lesions in broiler chickens vaccinated with different strains of Newcastle disease virus by different routes of application after two, four, seven, 14 and 21 days post-vaccination

Strain	DPV[a]	Group	Histopathological lesion																				
			Flattening of mucosal epithelium			Deciliation			Hyperemia			Hemorrhage			Epithelial hyperplasia			Mucous glands hyperplasia			Tracheitis		
			Mild	Moderate	Marked	Mild	Moderate	Marked	Mild	Moderate	Marked	Mild	Moderate	Marked	Mild	Moderate	Marked	Mild	Moderate	Marked	Mild	Moderate	Marked
ST3	2	Control	0/5	0/5	0/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		SC[b]	0/5	0/5	0/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
PL42	2	Control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5	0/5	0/5
		Ocular	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		Spray	0/5	0/5	0/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
LS	2	Control	0/5	0/5	0/5	2/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	3/5	0/5	0/5
		Ocular	0/5	0/5	0/5	3/5	1/5	0/5	0/5	3/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	1/5	0/5	0/5	3/5	0/5	0/5
		Spray	2/5	0/5	0/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
ST3	4	Control	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
		SC[b]	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
PL42	4	Control	0/5	0/5	0/5	4/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		Ocular	0/5	0/5	0/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	2/5	0/5	0/5	0/5	0/5	0/5
		Spray	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5	0/5	0/5
LS	4	Control	2/5	0/5	0/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
		Ocular	0/5	0/5	0/5	0/5	1/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5	1/5	0/5	2/5	0/5	0/5
		Spray	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
ST3	7	Control	0/5	0/5	0/5	1/5	0/5	0/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5
		SC[b]	0/5	0/5	0/5	2/5	0/5	0/5	1/5	1/5	0/5	0/5	2/5	0/5	1/5	1/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5
PL42	7	Control	0/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5
		Ocular	0/5	0/5	0/5	2/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	2/5	0/5	0/5
		Spray	0/5	0/5	0/5	2/5	0/5	0/5	3/5	1/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	1/5	0/5	0/5
LS	7	Control	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5	2/5	0/5	0/5
		Ocular	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
		Spray	0/5	0/5	1/5	0/5	1/5	0/5	2/5	1/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5	1/5	0/5	2/5	0/5

ST3	14	Control	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5
		SC[b]	1/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
PL42	14	Control	0/5	0/5	1/5	0/5	1/5	0/5	3/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5
		Ocular	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	3/5	0/5	0/5
		Spray	0/5	0/5	0/5	0/5	0/5	0/5	3/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	3/5	1/5	0/5
LS	14	Control	0/5	0/5	0/5	0/5	0/5	0/5	4/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	3/5	1/5	0/5
		Ocular	0/5	0/5	1/5	1/5	0/5	4/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	3/5	0/5	0/5
		Spray	0/5	0/5	2/5	0/5	0/5	0/5	4/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	3/5	0/5	0/5
ST3	21	Control	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5
		SC[b]	0/5	0/5	2/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
PL42	21	Control	0/5	1/5	0/5	1/5	0/5	3/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5
		Ocular	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	3/5	0/5	0/5
		Spray	0/5	0/5	1/5	0/5	0/5	3/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	3/5	1/5	0/5
LS	21	Control	0/5	0/5	0/5	0/5	0/5	4/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	3/5	1/5	0/5
		Ocular	0/5	0/5	1/5	1/5	0/5	4/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	3/5	0/5	0/5
		Spray	0/5	0/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	3/5	0/5	0/5

[a] Days post-vaccination, [b] subcutaneous; ST3 = rHVT-NDV Serotype 3 strain, PL42 = PHYLMV.42 strain, LS = La Sota strain.

Differences in the thickness of the tracheal mucosa occur due to the replication of pneumotropic lentogenic vaccinal strains with respiratory tropisms, replicating particularly in the mucosa of the trachea and causing inflammatory lesions that promote its thickening (Abdul-Aziz & Arp 1983). However, even with tropism by the intestinal mucosa, enterotropic lentogenic vaccines have low replication in the tracheal mucosa (Abdul-Aziz & Arp 1983, Alexander 1995, Borne & Comte 2003). Inspite the rHVT-NDV vaccine ST3 strain have predilection for lymphoid tissues (Heller & Schat 1987, Sanjay et al. 2017), the vaccinated broilers with this strand, were also submitted to histopathological and histomorphometric analysis, once there is only evidence of the lack of clinical signs and absence of the virus in the trachea (Esaki et al. 2013). The lack of difference between the birds vaccinated with the ST3 strain and respective control group offers further evidence that vector vaccines have low or no replication in the respiratory epithelium (Palya et al. 2012, Esaki et al. 2013).

The results for the birds vaccinated with the PL42 strain were similar to data described by Borne & Comte (2003), who found that the ocular route has higher than 98% effectiveness and leads to the ingestion of the vaccine through the palatal cleft, enabling a greater number of viral particles to arrive at the enteric epithelium (site of greater tropism of the PL42 strain). This virus subsequently colonizes the epithelium of the tracheal mucosa, causing it to thicken. The thickening of the tracheal mucosa found in the birds vaccinated with the LS strain is related to the capacity of this strain to penetrate more deeply into the airways and the fact that it is a pneumotropic strain, as reported in the study by Stewart-Brown (1995).

To assess the post-vaccination reaction in the trachea of birds subjected to ND vaccination, we compared the groups to each other. The results seen at four and seven days post vaccination were similar to results described by Nunes et al. (2002), in which the pneumotropic La Sota samples had greater thickness of the tracheal mucosa, coherent to it is ICPI. The superior quality of the ST3 was evident at 14 and 21 days, as demonstrated by the lesser thickness of the mucosa in comparison to the results of the other strains. On day 21 post vaccination, the response caused by the PL42 strain

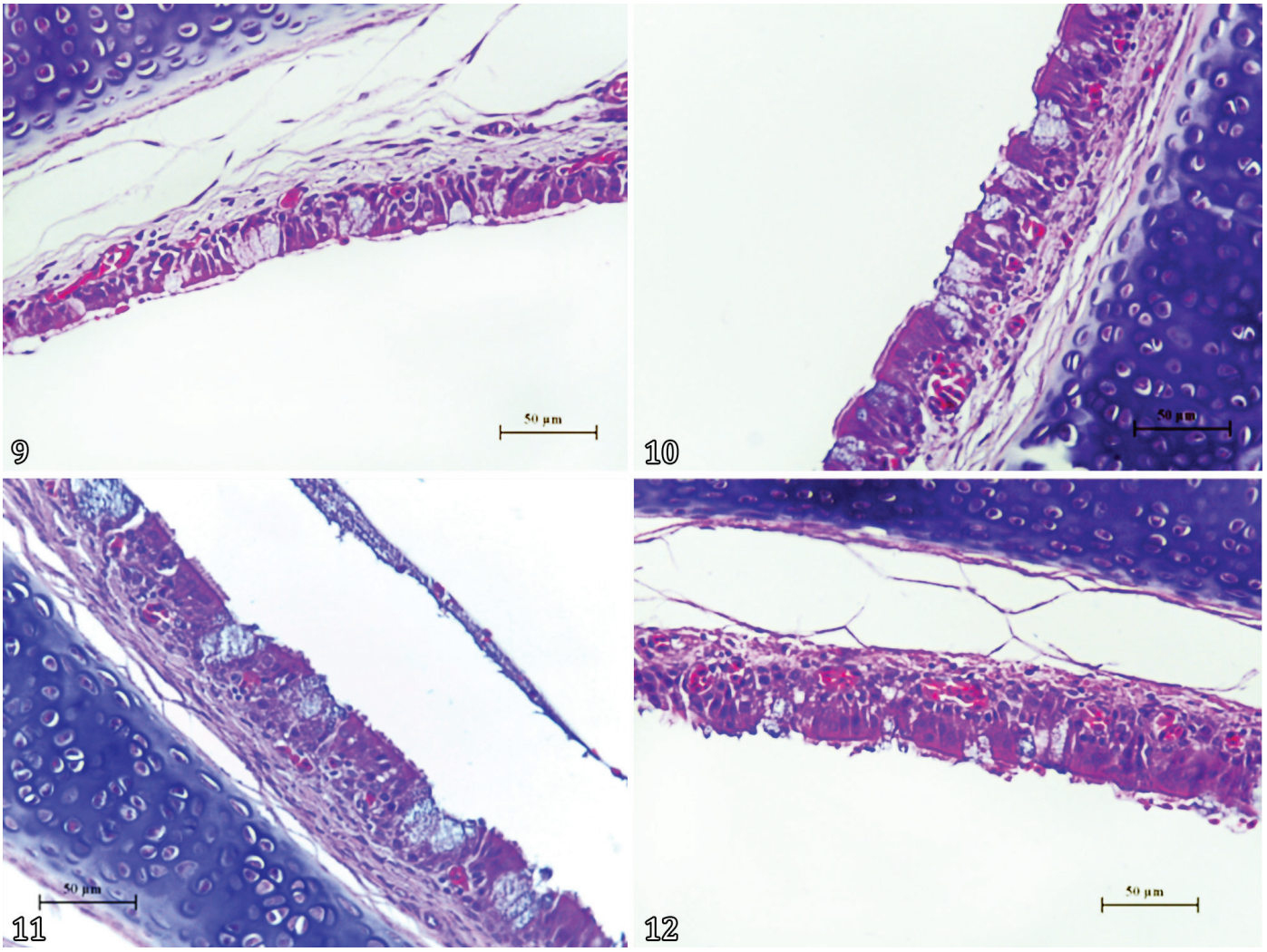

Fig.9-12. Histopathologic analysis of tracheal mucosa of birds at seven days post vaccination with Serotype 3 strain via subcutaneous route. (**9**) Discrete focal hyperemia in lamina propria of trachea. HE, bar = 50µm. (**10, 11**) Hemorrhage, deciliation and discrete focal lymphocytic inflammatory infiltrate in tracheal mucosa. HE, bar = 50µm. (**12**) Hyperemia and discrete deciliation in tracheal mucosa. HE, bar = 50µm.

shows that, although apathogenic enteric strains replicate mainly in the intestinal tract, replication in the respiratory tract occurs as well (Satra et al. 2011).

The LS strain administered in spray form was the most virulent of all strains studied and produced moderate vaccinal reactions. Thus, this strain is not recommended for preliminary vaccinations (Glisson & Kleven 1993, Alexander 1997).

The difference between the ocular and spray administration routes may be explained by the fact that ocular vaccination is one of the most efficient methods, as it ensures the administration of a complete dose to each bird. In contrast, the spray method has several critical points that can interfere with the efficiency of the vaccine (Bernardino 2004). The results found in the group vaccinated with the LS strain via spray are in agreement with data described by Gough & Allan (1973), who found that when the LS vaccine was inoculated via aerosol or spray, it induced varying degrees of post-vaccinal respiratory reactions.

Analyzing the tracheas in terms of histopathologic lesions, the present results are similar to those described by Barros et al. (2015), as the lesions from the LS strain were more severe than those from the PL42 strain, with the occurrence of hyperemia and diffuse inflammatory infiltrate, justifying the tracheal mucosa thickness measured in these groups. The mildest lesions were found in the birds vaccinated with the ST3 strain since this is a vector strain that offers protection from ND with the absence of a vaccinal reaction, because there is no colonization of the epithelium by the virus; moreover, the subcutaneous administration enables better control of the vaccinal process (Palya et al. 2014). Sesti et al. (2013) report similar lesions in chickens vaccinated with the vector Serotype 3 vaccine, with lower lesion scores (congestion, deciliation, epithelial hyperplasia and mononuclear inflammatory infiltrate) in comparison to conventional live strains.

Guy et al. (1990) e Miskinis et al. (2020) performed similar studies on vaccinated birds against infectious laryngotracheitis, in wich they found microscopic lesions similar to the found here, such as hyperemia, mucosa thickened due mild to moderate cell infiltration and/or normal epithelium except for foci of syncytia with intranuclear inclusion bodies, present in different intensities between the vaccinal strain. Proving that the histopathological lesions found are caused by tracheal defense mechanism of broiler chickens.

CONCLUSION

The results of the histomorphometry and histopathology of the trachea indicated that vaccination with rHVT-NDV strain Serotype 3 induced few microscopic lesions, which led to better histomorphometric measure post vaccination, consequently the tracheal mucosa remained preserved.

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Conflict of interest statement.- The authors declare that they have no conflicts of interest.

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