Ciência Rural
ISSN: 0103-8478
cienciarural@mail.ufsm.br
Universidade Federal de Santa Maria
Brasil

Santin, Elizabeth; Lima, Fabiana S.; Paulillo, Antônio C.; Nakaghi, Laura S. O.; Maiorka, Alex
The use of scaning electron microscopy in postvaccinal evaluation of tracheal epithelium of Coturnix coturnix japonica
Ciência Rural, vol. 33, núm. 1, janeiro-fevereiro, 2003, pp. 121-124
Universidade Federal de Santa Maria
Santa Maria, Brasil

ISSN 0103-8478

The use of scaning electron microscopy in postvaccinal evaluation of tracheal epithelium of *Coturnix coturnix japonica*

Emprego da microscopia eletrônica de varredura na avaliação pós-vacinal em epitélio traqueal de *Coturnix coturnix japonica*

Elizabeth Santin [1,2] Fabiana S. Lima [1,2] Antônio C. Paulillo [1,3] Laura S. O. Nakaghi [1] Alex Maiorka [1,2]

ABSTRACT

*This study aimed at evaluating the use of scanning electron microscopy in the study of the post-vaccinal respiratory reaction of the tracheal epithelium of quails (**Coturnix coturnix japonica**) immunized against Newcastle disease. A number of 36 quails were distributed into four groups: T1 – control birds (non-vaccinated); T2 – birds vaccinated with Ulster 2C strain; T3 – birds vaccinated with B1 strain; T4 – birds vaccinated with LaSota strain. Regardless the experimental group, birds did not show detectable clinical signs of post-vaccinal respiratory reaction. However, the analysis of tracheal fragments by scanning electron microscopy showed that birds vaccinated with B1 and LaSota strains developed epithelial sloughing of the trachea, whereas those vaccinated Ulster 2C strain did not develop this change, demonstrating intact tracheal epithelium, similar to the control group.*

Key words: ***Coturnix coturnix japonica****, Newcastle disease, scanning electron microscopy, respiratory post-vaccinal reaction.*

ABSTRACT

*Este experimento foi realizado para avaliar o emprego da microscopia eletrônica de varredura no estudo da reação respiratória pós-vacinal em epitélio traqueal de codornas (**Coturnix coturnix japonica**) imunizadas contra a doença de Newcastle. Foram utilizadas 36 codornas que foram distribuídas em quatro grupos, sendo: T1 – grupo de aves controle (não vacinado), T2 – grupo de aves vacinadas com a estirpe Ulster 2C, T3 – grupo vacinado com a estirpe B1, T4 – grupo de aves vacinadas com a estirpe LaSota. Independente do grupo experimental, as aves não apresentaram sinais clínicos detectáveis de reação respiratória pós-vacinal. Entretanto, na análise de fragmentos traqueais, ao microscópio eletrônico de varredura, observou-se que as codornas vacinadas com as estirpes B1 e LaSota desenvolveram descamação epitelial da traquéia, enquanto as aves vacinadas com a estirpe Ulster 2C não desenvolveram tal alteração, mostrando um epitélio traqueal íntegro, semelhante ao grupo controle.*

Palavras-chave: ***Coturnix coturnix japonica****, doença de Newcastle, microscopia eletrônica de varredura, reação respiratória pós-vacinal.*

INTRODUCTION

The species ***Coturnix coturnix japonica***, popularly known as Japanese quail, are chicken-like ground birds, and are susceptible to the experimental infection by the Newcastle disease virus – NDV (REIS & NOBREGA,

[1] Faculdade de Ciências Agrárias e Veterinárias, Departamento de Patologia Veterinária, Universidade Estadual Paulista, Via de Acesso Prof. Paulo D. Castellane, km 05, 14884-900 ,Jaboticabal, SP. Autor para correspondência: E-mail: paulillo@fcav.unesp.br
[2] Bolsista da FAPESP.
[3] Bolsista do CNPq.

Recebido para publicação 04.12.01 Aprovado em 27.02.02

1956; LANCASTER, 1964) . Some studies carried out with this animal species emphasize the isolation and the identification of NDV in outbreaks induced by pathogenic samples (HIGGINS & WONG, 1968; HASHIMOTO et al., 1969; LUY et al., 1987). Despite the great affinity of NDV to the upper respiratory system of birds, particularly to the trachea (ABDUL-AZIA & ARP, 1983), the action of this virus has not been yet described on the tracheal epithelium of quails, as it has in broilers (DORETO et al., 1999).

The present study aimed at comparatively studying the post-vaccinal respiratory reaction in the tracheal epithelium of quails (***Coturnix coturnix japonica***) by the use of the lentogenic NDV strains Ulster 2C, B_1 and LaSota.

MATERIALS AND METHODS

Experimental birds and food

A number of 36 sexed males, six-week-old quails (***Coturnix coturnix japonica***) were used. Birds were housed in layer cages made of galvanized wire, and equipped with nipple drinkers and trough feeders. Nine birds were housed per cage unit. Birds received water and feed *ad libitum*. The diets were based on corn and soybean meal, according to the nutritional recommendation of the NATIONAL RESEARCH COUNCIL (1994). Overall, the birds were submitted to similar conditions and the usual management procedures used in quail production systems.

Experimental design

The 36 birds were randomly distributed into four groups of nine birds each according to the following treatments: T1 – group of control birds (non-vaccinated); T2 – group of birds vaccinated with Ulster 2C strain; T3 – group of birds vaccinated with B_1 strain; T4 – group of birds vaccinated with LaSota strain. During the experimental period, the four group of birds were kept as isolated as possible from each other in order to avoid the influence of treatments among groups.

Vaccine

Lyophilized vaccines were used, with the lentogenic NDV strains Ulster 2C, B_1 and LaSota, respectively. The determinations of the 50% infecting dose (EID_{50}) of the vaccinal strains studied (REED & MUENCH, 1938) were: EID_{50} (Ulster 2C) = $10^{7,15}$/0.1mL, EID_{50} (B_1) = $10^{7.35}$/0.1mL, EID_{50} (LaSota) = $10^{7.20}$/0.1 mL. Vaccines were administered once by eye drop when the birds were six weeks of age by reconstitution of the lyophilized vaccines diluted in distilled water in a proportion of 30ml/1000 vaccine doses/1000 birds, which corresponded to an eye drop vaccine dose of 0.03mL (PAULILLO et al., 1987).

Clinical, necropsies and scanning microscopical analysis

All groups were observed twice a day and clinical signs were recorded. Three, five and eight days after vaccination, three birds from each group were submitted to necropsy to collect fragments of the medial portion of the trachea in order to evaluate destruction level of tracheal epithelium resulting from vaccinal virus replication.

Tracheal fragments, measuring 5 x 8mm, were fixed in a solution of glyceraldehyde at 3% in 0.1M, pH 7.6 phosphate buffer for 2 hours at a temperature of 4°C. The fragments were then post-fixed in a osmium tetra-oxide solution at 1% for 30 minute at a temperature of 4°C. After that, using the same buffer, the material was washed consecutively for six times, dehydrated by immersion in increasing concentrations in ethyl alcohol and put through the drying chamber of a critical point drier, using carbon dioxide. The material was then set on the appropriate metallic stub, covered with a layer of 30nm of gold and scanned in a scanning electron microscope (Model Jeol JSM 25SII), operating at 15KV.

RESULTS

Regardless the experimental group, none of the birds presented detectable clinical signs of post-vaccinal respiratory reaction. However, the observation of the scanning electron micrographs (Figure 1) of the tracheal fragments revealed a clean tracheal epithelium, consisting of ciliary cells, in the control group. As to the material from birds vaccinated with Ulster 2C strain (T2), in all sample

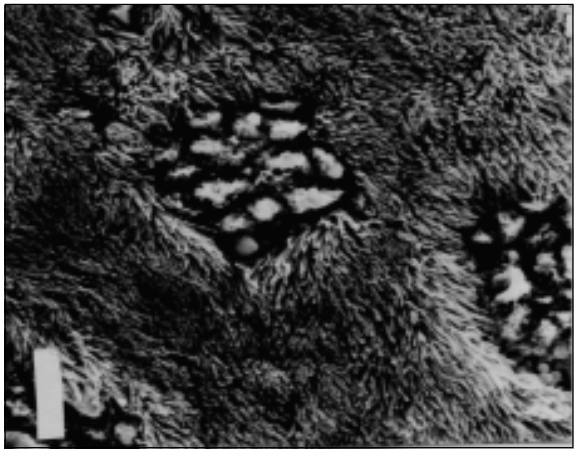

Figure 1 - Scanning electron microscopy of tracheal epithelium of ***Coturnix coturnix japonica*** at 5 days post vaccination, group control (T1). Clean tracheal epithelium. Bar = 10μm.

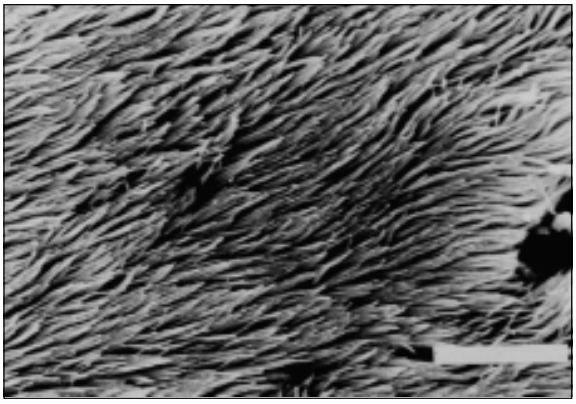

Figure 2 - Scanning electron microscopy of tracheal epithelium of ***Coturnix coturnix japonica*** at 5 days post vaccination, group vaccinated with NDV strain ulster 2C (T2). Clean tracheal. Bar = 10µm

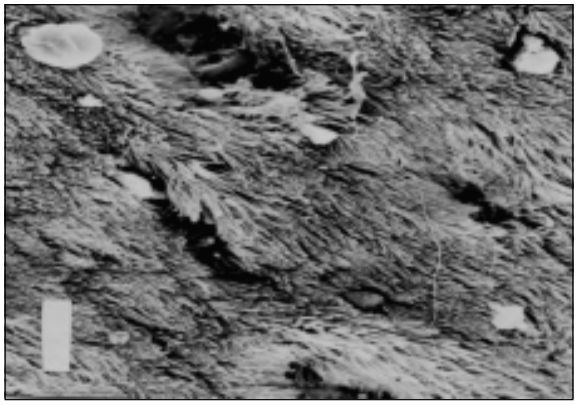

Figure 4 - Scanning electron microscopy of tracheal epithelium of ***Coturnix coturnix japonica*** at 5 days post-vaccination, proup vaccinated with NDV strain LaSota (T4). Epithelium sloughing of the trachea with extensive regions of ciliary epithelium removal. Bar = 10µm

collections (three, five and eight days after vaccination), the scanning electron micrographs (Figure 2) showed intact epithelium with intact ciliary cells, very similar to the epithelium of the control birds. In contrast, the material collected from birds vaccinated with B_1 and LaSota strains (Figures 3 and 4) showed marked epithelial sloughing, with extensive regions of ciliary epithelium removal. These changes were more evident five days after vaccination when the several sloughing and extensive areas without ciliary cells was observed (Figures 3 and 4). Eight days after vaccination, bird vaccinated with B_1 and LaSota strains (Figures 5 and 6) started showed a reduced area of ciliary epithelium removal.

DISCUSSION

Groups of quails vaccinated by eye drop with B_1 (T3) and LaSota (T4) strains did not show clinical signs of post-vaccinal reaction, contrasting with reports in broilers (DORETTO et al., 1999), in which these types of vaccines have the disadvantage of inducing undesirable respiratory reactions (ALLAN, 1971). In broilers, the severity and the extension of these clinical signs depend on a series of factors, which include the vaccinal virus strain, the presence of ***Mycoplasma gallisepticum***, and the route of vaccine administration (BEARD ***et al***., 1993). Despite the

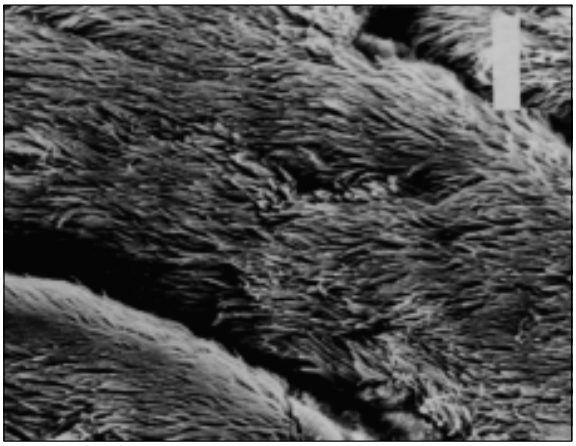

Figure 3 - Scanning electron of tracheal epithelium of ***Coturnix coturnix japonica*** at 5 days post-vaccination, group vaccinated with NDV srain B_1 (T3). Epithelium showing areas of ciliary epithelium removal. Bar = 10µm

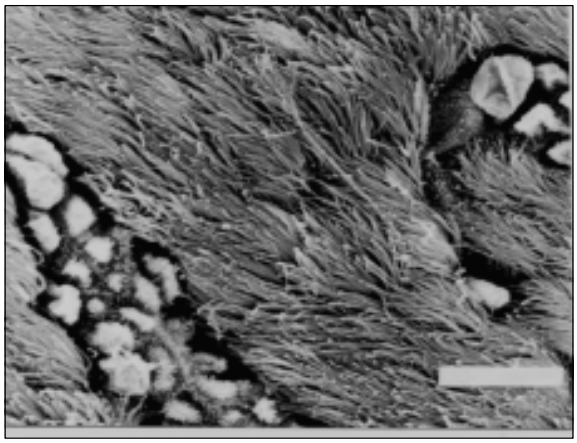

Figure 5 – Scanning electron microscopy of tracheal epithelium of ***Coturnix coturnix japonica*** at 8 days post vaccination, group vaccinated with NDV strain LaSota (T4). Epithelium sloughing of the trachea with area of ciliary epithelium removal than the same group at 5 days post-vaccination. Bar = 10µm.

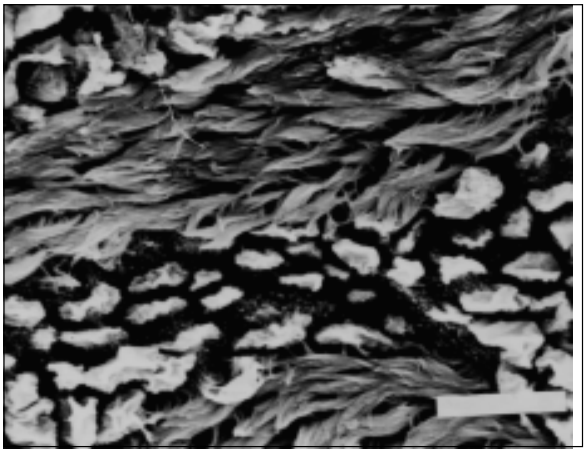

Figure 6 - Scanning electron microscopy of tracheal epithelium of ***Coturnix coturnix japonica*** at 8 days post-vaccination, group vaccinated with NDV strain B_1 (T3). Epithelium sloughing of the trachea with reduced area of ciliary epithelium removal than the same group at 5 days post-vaccination. Bar = 10μm.

Despite the absence of clinical signs of respiratory reaction, the analysis of the tracheal fragments of birds vaccinated with B_1 and LaSota strains showed epithelial sloughing of the trachea three days after vaccination. Therefore, we may speculate that the absence of clinical signs may result from the absence of concurrent infections by other agents, such as ***Mycoplasma gallisepticum***, which can intensify such reactions. It is possible that the controlled environmental conditions of the present experiment prevented these concurrent infections. In addition, it must be noted that quails, particularly male, perform several noises when housed in confined environments, which may also have rendered the characterization of few and discrete respiratory symptoms difficult.

The group of quails vaccinated by eye drop with Ulster 2C strain (T2) did not show clinical signs of respiratory reaction and no epithelial sloughing at tracheal evaluation, despite the passage of this strain through the upper respiratory tract. This can be explained by the fact that Ulster 2C strain is the only vaccinal NDV strain with intra-cerebral pathogenicity index (ICPI) is zero (ICPI 0.0) and therefore, it is less pathogenic than vaccinal strains B_1 (ICPI 0.2) and LaSota (ICPI 0.4) currently used (ALEXANDER, 1997).

CONCLUSIONS

The species ***Coturnix coturnix japonica*** did not show evident clinical signs of post-vaccinal respiratory reactions to NDV, regardless the vaccinal strain used by eye drop. Quails vaccinated by eye drop with B_1 and LaSota present this change. Scanning electron microscopy proved strains developed epithelial sloughing of the trachea, whereas those vaccinated with Ulster 2C strain did not present this change. Scanning electron microscopy proved to be a safe and efficient method in the evaluation of post-vaccinal respiratory reaction in the tracheal epithelium of quails (***Coturnix coturnix japonica***).

ACKNOWLEDGEMENTS

The authors thank Fundação de Amparo à Pesquisa do Estado de São Paulo for their financial support. Proc. 98/15865-5 e 98/16199-9-FAPESP.

REFERENCES

ABDUL-AZIA, T.A.; ARP, L.H. Pathology of the trachea in turkeys exposed by aerosol to lentogenic strains of Newcastle disease virus. **Avian Diseases**, v.27, p.1002-1011, 1983

ALEXANDER, D.J. Newcastle disease and other avian paramyxoviridae infections. In: CALNEK, B.W. et al. **Diseases of Poultry**. Ames : Iowa State University, 1997. p.496-519.

ALLAN, W.H. The problem of Newcastle disease. **Nature**, v.34, p.124-131, 1971.

BEARD, C.W.; VILLEGAS, P.; GLISSON, J.R. Comparative efficacy of the B-1 and VG-GA vaccine against velogenic viscerotropic Newcastle disease virus in chickens. **Avian Diseases**, v. 37, p.222-225, 1993.

DORETTO, L. et al. Utilização da microscopia eletrônica de varredura na avaliação da reação respiratória pós-vacinal em epitélio traqueal de pintos. **Revista Brasileira de Ciência Avícola,** v.1, p.149-152, 1999.

HASHIMOTO, K.; KANETO, S.; ONOSHI, H. An outbreak of Newcastle disease in Japanese quails **(*Coturnix coturnix japonica*)**. **Bulletin National Institute Health**, v.58, p.7-14, 1969.

HIGGINS, D.A.; WONG, F.S.F. Newcastle disease in a flock of Japanese quails. **Veterinary Record**, v.83, p.437-440, 1968.

LANCASTER, H.J.E. Newcastle disease control by vaccination. **Veterinary Bulletin**, v. 34, p. 57-76, 1964.

LUY, S. et al. Occurrence of Newcastle disease in Taiwan from 1970-1985. **Poultry Abstracts**, v.13, p.21-41, 1987.

NATIONAL RESEARCH COUNCIL (NRC). **Nutrient requirements domestic animals**. Nutrient requeriment of poultry. Washington : National Academic, 1994. 55p.

PAULILLO, A.C. et al. Doença de Newcastle. IV. Ensaio experimental de diferentes vias de vacinação com a estirpe lentogênica LaSota em frangos de corte. **Ars Veterinária**, v.3, p.73-79, 1987.

REED, L.J.; MUENCH, H.A. A simple method of estimating percent and points. **American Journal of Hygiene**, v.27, p.493-497, 1938.

REIS, J.; NÓBREGA, P. **Tratado de doenças das aves**. 2. ed. São Paulo : Edições Melhoramento, 1956. 123p.