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Evaluation of Experimental Vaccination Against Newcastle Disease in Lovebirds (*Agapornis roseicollis*): Investigation of the State of Virus Carrier

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Abstract: The aim of this study was to evaluate the importance of vaccination against Newcastle Disease (ND) in lovebirds (*Agapornis roseicollis*) and to investigate the state of carrier of the virus (NDV) in this species. There were used 48 lovebirds, distributed at random into 4 experimental groups: GI (Ulster 2C strain), GII (B1 strain), GIII (LaSota strain) and GIV (non-vaccinated group). At 12 months of age, all groups were challenged with a pathogenic virus (NDV) suspension ($EID_{50} = 10^{8.15}/0.1$ mL) and a group of Specific-Pathogen-Free (SPF) chicks were used as control of the virus. Cloacal swabs from each bird were collected after 9, 14 and 21 days post-challenge for detection of genome viral excretion by Reverse Transcription Polymerase Chain Reaction RT-PCR. Lovebirds of GI, GII and GIII did not demonstrate any signs of ND. They were refractory to the clinical disease. In lovebirds from the control group, NDV genome was detected 9 and 21 days after challenge. Therefore it was demonstrated the state of carrier of NDV by lovebirds. In birds from the vaccinated groups, genome viral excretion was not detected by RT-PCR. It was also demonstrated the importance of the vaccination in the suppression of the state of virus carrier of ND in lovebirds.

Key words: Newcastle disease, lovebirds, *Agapornis roseicollis*, NDV carrier, vaccination

INTRODUCTION

Lovebirds (*Agapornis roseicollis* Selby, 1836) are birds of the order Psittaciformes, common on captivity in Brazil (Lima, 2007; Silva *et al.*, 2009). Members of the genus lovebirds are small parrots native of African forests and savannas (Forshaw, 1989). Newcastle disease is an acute, highly contagious viral disease in domestic and wild birds; which can cause high level mortality in chickens. ND is one of the main sanitary barriers for the international trade of poultry and poultry products (OIE, 2008). The disease is caused by *Avian Paramyxovirus* serotype 1 (APMV-1/NDV), which is a member of the genus *Avulavirus*, of the *Paramyxoviridae* family (ICTV, 2010), has been demonstrated, by natural or experimental infection with ND virus, in at least 241 species from 27 of the 50 orders of birds (Kaleta and Baldauf, 1988). Historically, ND has been a devastating disease of poultry and the global economic impact is enormous (Alexander and Senne, 2008). However, there

is limited information about health programs in domestic birds in captivity. Considering that lovebirds can be a potential transmission source of NDV to other domestic bird species, this study aimed to evaluate the importance of vaccination against ND in lovebirds, as well as to investigate the state of NDV carrier in this species.

MATERIALS AND METHODS

Experimental birds and management: A total number of 48 lovebirds were distributed in a completely randomized experimental design with four treatments, with three replicates of 4 birds each. Birds were allocated in experimental cages, receiving water and food proper to specie *ad libitum*.

Vaccines: Birds were designated to treatments, according to vaccination strain as GI (Ulster 2C), GII (B1), GIII (LaSota) and GIV (control-non vaccinated).

Table 1: Results of challenge with viscerotropic velogenic Newcastle disease virus in lovebirds (*Agapornis roseicollis*) at 12 months of age and SPF chicks

Group	Vaccination (5 months of age)	Revaccination (6.5, 7.5 and 8.5 months of age)	Number of birds	Total protection
I	Ulster 2C	Ulster 2C	6	100.0
II	B1	B1	6	100.0
III	LaSota	LaSota	6	100.0
IV*	Control	- - -	6	100.0
SPF Chicks ("Specific-Pathogen-Free")			5	0.0

*Control group - not vaccinated

Table 2: Results of NDV genome excretion (by RT-PCR) in lovebirds (*Agapornis roseicollis*) after challenge

Group	--- Vaccination (5 months of age) ---		Revaccination (6.5, 7.5 and 8.5 months of age)		Viral genome excretion (RT-PCR)
			9 dac	14 dac	21 dac
I	Ulster 2c	Ulster 2c	-	-	-
II	B1	B1	-	-	-
III	LaSota	LaSota	-	-	-
IV	Control*	-----	+	-	+

*Control group - not vaccinated; - = negative genome viral excretion; dac = days after challenge

Commercial line recently manufactured live NDV vaccines (Ulster 2C, B1 and LaSota strains) were administered to each experimental group, as described by Paulillo *et al.* (1996). All birds, except those in the control group, were vaccinated at 5 months of age and revaccinated at 6.5, 7.5 and 8.5 months of age with the same vaccine strain that was applied in the first vaccination. Vaccine titers were obtained by determining 50% of the embryo-infecting dose in embryonated eggs of specific-pathogen-free breeders at 8 and 10 days of incubation. Titers of live vaccine strains Ulster 2C, B1 and LaSota were 7.15 log₁₀/0.1 mL, 7.2 log₁₀/0.1 mL and 7.35 log₁₀/0.1 mL, respectively. Birds were vaccinated and revaccinated by eye drop.

Challenge: At 12 months of age, two lovebirds from each repetition (six per treatment) were challenged with viscerotropic ND virus strain pathogenic to chickens. The virus had intra-cerebral pathogenic index of 1.78 and embryonic death time of 48h, with a 50% embryo infecting dose titer of (EID₅₀ = 8.15 log₁₀/0.1 mL). Distilled water was used as diluent for the inoculum that was instilled by oculo-nasal rout, according to the Code of Federal Regulations (1993). In order to measure the pathogenicity of the NDV challenge strain, a group of Specific-Pathogen-Free (SPF) chicks were used. The birds were housed in isolators with filtered air and offered food and water *ad libitum*.

Viral and genome excretion: At 9, 14 and 21 days post-challenge, RNA extraction from cloacal swabs was performed from all birds of each group to carry out virus isolation. There were placed in phosphate buffer solution (pH 7.2). The NucleoSpin[®] RNA Virus Kit was used, according to the manufacturer's protocol. RT-PCR was performed using primers targeting a conserved region of the NDV genome, described by Toyoda *et al.*

(1989). The primer sequence was as follows: P1F (sense) 5'-TTG ATG GCA GGC CTC TTG C-3' and P2R (anti-sense) 5'-GGA GGA TGT TGG CAG CAT Y-3'.

RESULTS AND DISCUSSION

Data about the challenge with viscerotropic velogenic NDV in lovebirds are shown in Table 1. The lovebirds of the control group (G IV) did not demonstrate signs and lesions of Newcastle disease, being refractory to the clinical disease with the NDV. In vaccinated lovebirds (Groups I-III), the percentage of the protection to the challenge was 100% (Table 1). On other hand, 100% of the SPF broilers died due to the NDV challenge, confirming the virus pathogenicity. The results of the genome excretion of NDV velogenic strain in lovebirds after challenge are shown in Table 2. In lovebirds from the control group (non vaccinated, GIV) the genome excretion of the NDV was detected 9 and 21 days after the challenge, by RT-PCR. It demonstrates the state of virus carrier by the lovebirds until 21 days after infection, which is important for the epidemiology of this disease. In contrast, genome excretion of the NDV was not detected by RT-PCR from vaccinated groups of lovebirds (GI to III). It suggests that vaccination can efficiently eradicate NDV in lovebirds and might be an important tool for the epidemiological control of ND dissemination to other birds. In addition, further studies should be developed to demonstrate the importance of the state of virus carrier of ND by lovebirds to the dissemination of this disease under field conditions.

Conclusion: Lovebirds (*Agapornis roseicollis*) showed to be refractory to the clinical signs when challenged with velogenic NDV. It was demonstrated the state of virus carrier of ND by lovebirds until 21 days after challenge. Vaccination against ND is essential to the suppression of the virus carrier state in these birds.

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REFERENCES

- Alexander, D.J. and D.A. Senne, 2008. Newcastle disease. In: Saif, Y.M. Diseases of Poultry. Iowa: Blackwell Publishing, pp: 75-100.
- Code of Federal Regulations, 1993. Animal and animal products. Washington: National Archives and Records Administration, pp: 818.
- Forshaw, J.M., 1989. Parrots of the world. Lansdowne: Willoughby, pp: 450.
- ICTV - The International Committee on Taxonomy of viruses. Available at: < <http://www.ncbi.nlm.nih.gov/ICTV/>>. Accessed in March 2010.
- Kaleta, E.F. and C. Baldauf, 1988. Newcastle disease in free-living and pets birds. In: Alexander, D.J. Newcastle disease. Boston: Kluwer Academi. pp: 197-246.
- Lima, R.G., 2007. Análise filogenética de psittaciformes (aves) com base em caracteres morfológicos siringeais e osteológicos. Tese (Doutorado em Ciências, na área de Zoologia) - Instituto de Biociências da Universidade de São Paulo.
- Office International Des Epizooties, 2008. Manual for animal disease reporting to the OIE. World Organization for Animal Health, Paris.
- Paulillo, A.C., G.S. Silva, L. Doretto Junior, M.V. Meireles, S.N. Kronka, J. Ariki, N.K. Sakomura and R.C. Ribeiro, 1996. Estudos zootécnico e imunológico de aves de corte submetidas a diferentes programas de vacinação contra a doença de Newcastle In: Reunião da Sociedade Brasileira de Zootecnia 33, Fortaleza, Brazil, Anais; pp: 388-390.
- Silva, A.S., D.L. Mahl, J.F. Soares, L. Faccio, S.L. Dau, R.A. Zanette and S.G. Monteiro, 2009. Parasitismo por *Isospora* sp. em *Agapornis fischeri* (Pássaro-do-Amor) criados em cativeiro no Brasil. Caderno de Pesquisa Ser. Bio., Santa Cruz do Sul, 21: 53-57.
- Toyoda, T., T. Sakaguchi, H. Hirota, B. Gotoh, K. Kuma, T. Miyata and Y. Nagai, 1989. Newcastle disease virus evolution. II. Lack of gene recombination in generating virulent and avirulent strains. Virology, 169: 273-282.