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Investigation of the State of Virus Carrier of Newcastle Disease in Budgerigars (*Melopsittacus undulatus*)

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Abstract: This study aimed to evaluate the importance of vaccination against Newcastle Disease (ND) in budgerigars (*Melopsittacus undulatus*) and to investigate the state of carrier of the virus (NDV) in this species. There were used 72 budgerigars distributed into four different experimental groups, vaccinated or not against ND: GI (Ulster 2C strain), GII (B1 strain), GIII (LaSota strain) and GIV (not vaccinated-control). At 11 months of age, all groups of birds were challenged with a pathogenic virus (NDV) suspension, $EID_{50} = 10^{8.15}$/0.1 mL. A group of Specific-Pathogen-Free (SPF) chickens were used as control of the virus. Cloacal swabs and blood samples were collected after 13 and 19 days post-challenge, respectively, for genome viral excretion by Reverse Transcription Polymerase Chain Reaction (RT-PCR) and antibody's level by the inhibition of hemagglutination test (HI). Budgerigars of all groups (including the control group-not vaccinated) did not demonstrate any signs of Newcastle Disease. They were refractory to the clinical disease with the NDV. Budgerigars from the control group (not vaccinated), showed antibody titers from HI test 13 and 19 days after challenge. Therefore, it was demonstrated the state of carrier of NDV in this species. The birds, from the vaccinated groups did not demonstrate genome viral excretion by RT-PCR and antibody titles were not detected by HI tests, respectively. It was also demonstrated the importance of the vaccination in the suppression of the state of carrier of NDV in budgerigars.

Key words: Budgerigars, *Melopsittacus undulatus*, vaccination, newcastle disease, NDV carrier

INTRODUCTION

Budgerigars also known as the common pet parakeet (*Melopsittacus undulatus* Shaw, 1805, Psittaciformes: *Psittacidae*) is widely acknowledged as the most common pet parrot in the world. They are intelligent and social birds and its natural habitat is in Australia (Lendon, 1973). Newcastle Disease (ND) is caused by *Avian Parainfluenzavirus* serotype 1 (APMV-1/NDV) viruses which is a member of the genus *Avulavirus*, of the *Paramyxoviridae* family (ICTV, 2010). The disease is world-wide distributed in a large range of hosts. Natural or experimental infection with ND virus has been demonstrated in at least 241 species from 27 of the 50 orders of birds (Kaleta and Baldauf, 1988; Spradbrow, 1988). Psittaciformes are highly susceptible to the Newcastle Disease Virus (NDV) (Erickson, 1977). However, there is little information available regarding health programs in this species. Because these birds are commonly kept as a pet in captivity, they might be important NDV carriers. Thus, this study aimed to evaluate the importance of vaccination against ND in this species and also to investigate the state of NDV carrier of budgerigars.

MATERIALS AND METHODS

Experimental birds and management: A total number of 72 (5 month-old) budgerigars were distributed in a completely randomized experimental design with four different treatments, with three replicates of six birds each. Birds were allocated in experimental cages, receiving water *ad libitum*. The diet comprised fresh fruits, seeds, vegetables and vitamin supplements.

Vaccines: Birds were designated to treatments, according to vaccination strain as GI (Ulster 2C), GII (B1), GIII (LaSota) and GIV (control-not vaccinated). Commercial 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, by eye drop, at 5 months of age and revaccinated at, 7, 8.5 and 10 months of age with the same vaccine strain that

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was applied in the first vaccination. Vaccine titers were obtained by determining 50% (EID_{50}) of the embryo-infecting dose in embryonated eggs SPF chickens was $10^{7.15}$/0.1mL = ED_{50} (Ulster 2C); $10^{7.20}$/0.1mL = ED_{50} (B1) and $10^{7.35}$/0.1mL = ED_{50} (LaSota).

Challenge: At 11 months of age, two budgerigars from each repetition (six per treatment) were challenge with viscerotropic DN virus strain pathogenic for chickens. The virus had intra-cerebral pathogenic index of 1.78 and embryonic death time of 48h, with title of EID_{50} = 8.15 log_{10}/0.1 mL. Two hundred microliters of the suspension of the virus were administered 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) chickens of 30 days old were used. The birds were housed in negative pressure isolators with filtered air and offered food and water *ad libitum*.

Serology: Blood samples of all budgerigars were collected 13 and 19 days post challenge with NDV. Sera samples were submitted to inhibition of hemagglutination (HI) test, according to Cunningham (1971).

Viral genome excretion: At 13 and 19 days post challenge, RNA extraction from cloacal swabs was performed from all birds of each group to carry out virus (NDV) isolation. They 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 (1989). P1F (foward) 5'-TTG ATG GCA GGC CTC TTG C-3' and P2R (reverse) 5'-GGA GGA TGT TGG CAG CAT Y-3'.

RESULTS AND DISCUSSION

Data about the challenge with viscetrotropic velogenic NDV pathogenic in budgerigars are shown in Table 1. Budgerigars of the control group (G IV) did not demonstrate signs of Newcastle Disease, being refractory to the clinical disease with the NDV, in contrast with the observation of Erickson (1977), were budgerigars exposed to NDV developed clinical signs such as apathy, inappetence and ruffled feathers after three days to two weeks of exposure. It is possible to suggest that this fact is linked with the recombination phenomenon present in populations and subpopulations of NDV viral particles, reflecting the resistance of budgerigars to ND. On the other hand, in vaccinated budgerigars (Groups I-III), the percentage of protection to the challenge was 100% (Table 1).

Furthermore, 100% of the SPF chickens died due to the NDV challenge, confirming the virus pathogenicity. The results of the genome excretion of NDV velogenic strain in budgerigars after challenge are own in Table 2. In budgerigars from the control group the genome excretion of the NDV was negative 13 and 19 days after the challenge, by RT-PCR. These results can be explained due to the intermittent elimination of the NDV. However, antibody titres were detected by the HI test 13 and 19 days after challenge, confirming the susceptibility of this species to the NDV. In contrast, genome excretion of the NDV was not detected by RT-PCR and HI test from vaccinated groups (G I to III) of budgerigars, after 13 and

Table 1: Challenge with viscerotropic velogenic Newcastle Disease virus in budgerigars

Group	Vaccination (5 months of age)	Revaccination (7, 8.5 and 10 months of age)	No. 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	Control	6	100.0
SPF chicken ("Specific-Pathogen-Free")		5	0.0	

*Control group: Non-vaccinated

Table 2: NDV genome excretion (by RT-PCR) and the immune response by HI test in budgerigars after challenge

			Viral genome excretion (RT-PCR) and HI			
			13 DAC		19 DAC	
Group	Vaccination (5 months of age)	Revaccination (7, 8.5 and 10 months of age)	RT-PCR	HI	RT-PCR	HI
I	Ulster 2C	Ulster 2C	Ø	0	Ø	0
II	B1	B1	Ø	0	Ø	0
III	LaSota	LaSota	Ø	0	Ø	0
IV*	Control	Control	Ø	5^1	Ø	5

*Control group-non-vaccinated
Ø : Negative genome viral excretion
1 : Titer (log_2)
DAC : Days after challenge

19 days post challenge. It suggests that vaccination can efficiently eradicate NDV in budgerigars and might be an important tool for the epidemiological control of ND dissemination to other birds. The serological results demonstrated the carrier status of NDV by this species until 19 days post challenge with this pathogen. Thus, data calls attention to the importance of the budgerigars from the epidemiological point of view as NDV carrier and the importance of vaccination on the suppression of this state.

Conclusion: Budgerigars (*Melopsittacus undulatus*) showed to be resistant to the development of clinical signs of ND when challenged with velogenic strain of NDV. It was demonstrated the state of virus carrier of budgerigars after 13 and 19 days post challenge. Furthermore, generally, the vaccination against ND is essential to the suppression the state of virus carrier of ND in budgerigars.

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