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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Relevance of Budgerigars (*Melopsittacus undulatus*) in Experimental Epidemiology of Newcastle Disease

Janine Denadai[1,4], Antonio Carlos Paulillo[3,6], Gislaine Regina Vieira Martins[1,4], Elizabeth Moreira dos Santos Schmidt[2] and Adriano Torres Carrasco[5]
[1]Aluna do Programa de Pos-graduacao em Medicina Veterinaria, Faculdade de Ciencias Agrarias e Veterinarias, UNESP, Jaboticabal, Brazil
[2]Profa. Assistente Doutora, Faculdade de Medicina Veterinaria e Zootecnia, Depto. Clinica Veterinaria, UNESP, Botucatu, Brazil
[3]Prof. Titular, Faculdade de Ciencias Agrarias e Veterinarias, Depto. de Patologia Veterinaria, UNESP, Jaboticabal, Brazil
[4]Bolsista FAPESP, Brazil
[5]Docente - Medicina Veterinaria, Universidade Estadual do Centro-Oeste - UNICENTRO, Parana, Brazil
[6]Research fellow PQ/CNPq, Brazil

Abstract: This study was carried out to clarify the real role that was played by the budgerigars (*Melopsittacus undulatus*) in the epidemiological plan, under the perspective of its being an infection source of the Newcastle Disease Virus (NDV). For this, the study used Specific-Pathogen-Free chicks (SPF) that were housed with budgerigars that were inoculated with a pathogenic strain (velogenic viscerotropic) of NDV (EID_{50} = $10^{8.15}$/0.1 mL) pathogenic to chickens, by the ocular-nasal via. Each group was composed by 10 SPF chicks and 5 budgerigars. After 5 days of the inoculation of the budgerigars with NDV, SPF chicks were put together with each group of budgerigars, so that there was a direct contact between both species. Cloacal swabs and blood samples were collected in both species (budgerigars and SPF chicks) after 13 and 19 days post-challenge, respectively, for genome viral excretion by Reverse Transcription Polymerase Chain Reaction (RT-PCR) and antibody's search by the inhibition of hemmaglutination test (HI). Budgerigars did not demonstrate any clinical signs of Newcastle Disease (ND). They were refractory to the clinical disease with the NDV. However, antibody titres from inhibition of Hemagglutination (HI) test were detected 9 and 21 days after challenge. Therefore, it was demonstrated the state of carrier of NDV in this species. In SPF chicks allocated with infected budgerigars, NDV genome was detected 13 and 19 days after challenge. Thus, the transmission of the pathogenic virus from the budgerigars to SPF chicks that were housed together was evident until 19 days of the experimental infection with this pathogen. This reveals the importance of the budgerigars from the epidemiological point of view as a potential source of infection of the NDV to commercial chickens that could be raised near this species.

Key words: Budgerigars, *Melopsittacus undulatus*, Newcastle disease, epidemiology, NDV carrier, source of infection of the NDV

INTRODUCTION

Budgerigars (*Melopsittacus undulatus* Shaw, 1805, Psittaciformes: *Psittacidae*) also known as the common parakeet 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). They report a high level of susceptibility in Psittaciformes to the NDV, including budgerigars (Erickson, 1977). However there is no information available on health programs for budgerigars. Because of the fact that this species may be kept as a pet in captivity, it represents a potential source of infection of NDV to other birds. Thus, this study was designed to evaluate the real role of the transmission of NDV to other domestic birds that may be housed together with this species.

Corresponding Author: Elizabeth Moreira dos Santos Schmidt, Profa. Assistente Doutora, Faculdade de Medicina Veterinária e Zootecnia, Depto. Clinica Veterinaria, UNESP, Botucatu, Brazil

Table 1: Results of viral excretion detected by RT-PCR and antibody (HI test) of budgerigars (*Melopsittacus undulatus*), after the challenge

	Viral genome excretion (RT-PCR) and antibody response (HI)			
	13 dac		19 dac	
Birds	RT-PCR	HI	RT-PCR	HI
Budgerigars (*Melopsittacus undulatus*)	-	+	-	+

dac = days after challenge; + = Positive results; - = Negative results

MATERIALS AND METHODS

Experimental birds and management: SPF chicks contacting with budgerigars inoculated with a viscerotropic strain of NDV were used. Each group was composed by 5 budgerigars and 10 SPF chicks. The birds were housed in isolators with filtered air and offered food and water *ad libitum* proper to each species.

Challenge: Budgerigars 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 48 h, with a 50% embryo infecting dose titer (EID_{50}) of 8.15 log_{10}/0.1mL. Distilled water was used as diluent for the inoculum that was instilled by the ocular-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 was used. At five days after challenge with a viscerotropic strain of NDV, ten SPF chicks were allocated together with the budgerigars for a direct contact with budgerigar's droppings.

Viral and genome excretion: At 13 and 19 days post-challenge, RNA extraction from cloacal swabs was performed from all birds (budgerigars and SPF chicks). 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 *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

All budgerigars infected with NDV didn't show clinical signs or lesions indicatives of NDV, being refractory to the clinical disease with this virus. In contrast, Erickson (1977) showed that 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. Results of viral genome research to NDV in budgerigars after challenge are shown in Table 1. The viral genome of NDV in budgerigars was not detected at 13 and 19 days after challenge. 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 state of carrier of the virus (NDV) in this species.

Table 2: Results of clinical observation, macroscopic lesions and viral isolation of NDV of SPF chicks allocated with budgerigars (*Melopsittacus undulatus*), 13 and 19 days after challenge

	SPF chicks allocated with infected budgerigars	
Parameters	13 dac	19 dac
Clinical signs suggestive of NDV	-	-
Mortality (%)	0	0
Lesions suggestive of NDV	-	-
Genome viral excretion (NDV)	+	+

dac = days after challenge
+ = positive results
- = negative results

These results, under the epidemiological plan of ND, showed that the budgerigars might be carrier of the virus suggesting an important role of this species on the epidemiology of NDV on regions of extensive poultry production. Table 2 shows that none of SPF chicks allocated with the budgerigars infected with a pathogenic strain of NDV died after the challenge and 100% did not show signs and lesions of ND after challenge. On the other hand, viral genome and antibody titres of NDV, by RT-PCR and HI test, respectively, were detected in SPF chicks at 13 and 19 days after challenge. Although budgerigars didn't show clinical signs of ND, they spread a sufficient amount of virus to induce an infection and the clinical disease in SPF chicks allocated together. It was demonstrated the transmission of the virus by budgerigars until 19 days after challenge with NDV to SPF chicks that were housed together. This calls attention to the importance of the budgerigars from the epidemiological point of view as a potential source of infection of the NDV to commercial chickens that could be raised near this species.

Conclusion: Budgerigars (*Melopsittacus undulates)* showed to be resistant to the development of clinical signs of ND when challenged with a velogenic strain of

NDV. It was demonstrated the state of virus carrier of budgerigars until 19 days after challenge with this pathogen. It was also demonstrated the relevance of this species in the epidemiology, as a potential source of infection of NDV to other domestic species, because these birds can shed the virus until 19 days after challenge and should be considered in the management of biosecurity measures for the poultry industry.

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