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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Relevance of Lovebirds (*Agapornis roseicollis* Selby, 1836) in Experimental Epidemiology of Newcastle Disease

Gislaine Regina Vieira Martins[1], Antonio Carlos Paulillo[1], Elizabeth Moreira dos Santos Schmidt[2], Janine Denadai[1], Adriano Torres Carrasco[3] and Ivan Felismino Charas dos Santos[4]
[1]Department of Veterinary Pathology, FCAV - UNESP, Jaboticabal, São Paulo, Brazil
[2]Department of Veterinary Clinics, FMVZ - UNESP, Botucatu, São Paulo, Brazil
[3]UNICENTRO, Guarapuava, Paraná, Brazil
[4]Department of Surgery, Faculty of Veterinary, Eduardo Mondlane University, Maputo, Mozambique

Abstract: Studies were made to clarify the role that was played by the lovebirds (*Agapornis roseicollis*) in the epidemiological plan, under the perspective of its being a potential source of infection of Newcastle Disease Virus (NDV). The study used Specific-Pathogen-Free chicks (SPF) that were housed with lovebirds inoculated with a pathogenic strain (velogenic viscerotropic) of NDV pathogenic to chickens, by the ocular-nasal via. Each group was composed of six SPF chicks and four lovebirds. After five days of the inoculation of the lovebirds with NDV, SPF chicks were put together with each group of lovebirds. Cloacae swabs were collected after 9, 14 and 21 days post-challenge in both species (lovebirds and SPF chicks) for genome viral excretion by Reverse Transcription Polymerase Chain Reaction (RT-PCR). Lovebirds did not demonstrate any clinical signs of NDV. They were refractory to the clinical disease with the NDV. However, NDV genome was detected 9 and 21 days after challenge. This study shows that lovebirds can be carriers NDV. Moreover, 100% of SPF chicks allocated with the infected lovebirds demonstrated clinical signs and lesions suggestive of NDV. In these birds, NDV genome was detected 9, 14 and 21 days after challenge. Thus, the transmission of the pathogenic virus from the lovebirds to SPF chicks that were housed together was evident until 21 days of the experimental infection. This study reveals the importance of lovebirds from the epidemiological point of view as potential source of infection of the NDV to other avian species that could be raised near this species.

Key words: *Agapornis roseicollis*, Newcastle disease, Psittacidae, lovebirds, epidemiology

INTRODUCTION

A NDV causes a highly contagious disease in chickens that leads to substantial economic losses in the poultry industry worldwide. The NDV isolates vary widely in virulence and are divided into three broad pathotypes based on the severity of the disease produced in chickens: lentogenic (avirulent), mesogenic (moderately virulent) and velogenic (highly virulent) (Kumar *et al*., 2011). Lentogenic NDV strains are widely used as live vaccines around the world. However, they do not completely prevent infection or virus shedding, nor do they possess genetic markers to allow differentiation between infected and vaccinated birds (Kumar *et al*., 2011).

Lovebirds (*Agapornis roseicollis* Selby, 1836) belong to the order Psittaciformes and are common in captivity in Brazil (Lima, 2007; Silva *et al*., 2009). Members of the genus *Agapornis* are small parrots native of African forests and savannas (Forshaw, 1989). Infection with the NDV has been demonstrated by natural or experimental infection in at least 241 species from 27 of the 50 orders of birds (Kaleta and Baldauf, 1988). A high level of susceptibility to NDV has been reported in Psitttaciforms (Erickson, 1977). Senthuran *et al*. (2005), isolates four NDV obtained from a pigeon, lory, parrot and lovebirds were subjected to biological and molecular characterization. Although Paramyxovirus infections are uncommon in this order (Lamberski, 2004) an outbreak of NDV. In cockatiels was reported with 100% mortality (Bendheim *et al*., 2003).

There are protocols for Psittaciforms vaccination that many practitioners follow for lovebirds, but no information available on health programs concerning NDV vaccination for lovebirds. Because of the fact that this species may be kept as a pet in captivity, it represents a potential source of infection of NDV. Thus, this study is designed to evaluate the role that is played by lovebirds in the transmission of NDV to other domestic birds that might be housed with this species.

MATERIALS AND METHODS

Specific-Pathogen-Free (SPF) chicks were placed in contact with lovebirds inoculated with a viscerotropic strain of NDV. Each group was composed of four

Corresponding Author: Elizabeth Moreira dos Santos Schmidt, Department of Veterinary Clinics. FMVZ - UNESP, Botucatu, São Paulo, Brazil

lovebirds and six SPF chicks. The birds were housed in isolators with filtered air and offered food and water *ad libitum* proper to each species. The lovebirds were challenged with viscerotropic NDV 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 (EID_{50}) of 8.15 log_{10}/0.1 mL. Distilled water was used as diluent for the inoculum that was instilled by ocular-nasal rout, according to the Code of Federal Regulations (Code of Federal Regulations, 1993). In order to evaluate the pathogenicity of the NDV challenge strain, a group of SPF chicks was used. At five days after challenge with a viscerotropic strain of NDV, six SPF chicks were housed together with the lovebirds. The chickens are just exposed to lovebird droppings. At 9, 14 and 21 days post-challenge, RNA extraction from cloacal swabs was performed from all birds, lovebirds and SPF chicks. They were placed in phosphate buffer solution (pH 7.2). The NucleoSpin® RNA virus (Macherey-Nagel GmbH & Co, Düren, 52355, Germany) was used, according to the manufacturer's protocol. RT-PCR was performed using primers (Sigma-Aldrich Co®, St. Louis, 63103, USA) targeting a conserved region of the NDV genome, as described by Toyoda *et al*. (1989). The primer sequence was as follows: P1F (sense) 5'-TTG ATG GCA GGC CTC TTG C-3' aNDV P2R (anti-sense) 5'-GGA GGA TGT TGG CAG CAT Y-3'.

RESULTS and DISCUSSION

Lovebirds (100%) did not show any signs of NDV after challenge, being refractory to the clinical disease with the NDV. Results of viral genome search to NDV in lovebirds after challenge are shown in Table 1.

Viral RNA was detected in lovebirds at 9 and 21 days after challenge emphasizing the susceptibility of this species to NDV. On the other hand, the detection of viral genome of NDV in the cloacae swabs of lovebirds, characterize this species as NDV carrier until 21 days after infection with this pathogen ($EID_{50} = 10^{8.15}$/0.1 mL). These results, under the epidemiology of NDV, showed that the lovebirds might be carrier of the virus suggesting an important role of this species on the epidemiology of NDV. The genome excretion of the NDV in lovebirds was negative 14 days after the challenge (Table 1). This fact might be caused by the intermittent elimination of NDV.

Table 2 shows that 33.3% of SPF chicks allocated with the lovebirds infected with a pathogenic strain of NDV died from 48 to 72 hrs after the challenge and 100% presented signs and lesions of NDV, detected by RT-PCR 9, 14 and 21 days after challenge.

Main clinical signs were dyspnea, severe and green diarrhea, nasal-ocular discharge, depression and/or death. At necropsy, hemorrhagic lesions were observed in the proventriculus and necrotic lesions in the intestine and ceca tonsils. Although lovebirds did not show clinical signs of NDV, they spread a sufficient amount of virus to induce an infection and the clinical disease in SPF chicks allocated together.

Table 1: Results of viral excretion by RT-PCR of lovebirds (*Agapornis roseicollis*), after the challenge

	Viral (RNA) genome excretion (RT-PCR)		
Birds	9 DAC	14 DAC	21 DAC
Agapornis (%)	+(100%)	-(0%)	+(100%)

DAC: Days after challenge, +: Positive results, -: Negative results

Table 2: Results of clinical observation, macroscopic lesions and viral isolation of NDV of SPF chicks allocated with lovebirds (*Agapornis roseicollis*) 9, 14 and 21 days after challenge

	SPF chicks allocated with infected lovebirds		
Parameters	9 DAC	14 DAC	21 DAC
Clinical signs suggestive of NDV	+	+	+
Mortality (%)	2 (33.3)	2 (33.3)	2 (33.3)
Lesions suggestive of NDV	+	+	+
genome viral excretion (NDV)	+	+	+

DAC: Days after challenge, +: Positive results

NDV transmission from lovebirds to SPF chicks was demonstrated 9 to 21 days after challenging the lovebirds with NDV to SPF chicks that were housed together. This calls attention to the importance of the lovebirds from the epidemiological point of view as potential source of infection of the NDV to domestic birds that could be raised near this species, since the literature reports that NDV was introduced to poultry farms in several countries through imported psittacines (Bendheim *et al*., 2003).

Conclusion: Agapornis (*Agapornis roseicollis*, Selby, 1836) was resistant to the development of clinical signs of NDV when challenged with a velogenic strain of NDV. It was demonstrated the state of virus carrier of lovebirds until 21 days after challenge with this pathogen. It was also demonstrated the relevance of lovebirds in the epidemiology, as a potential source of infection of NDV to domestic birds, since they can shed the virus until 21 days after challenge.

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