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Evaluation of Experimental Vaccination in Chinese Goose (*Anser cygnoides*) Against Newcastle Disease: Investigation of the State of Virus Carrier

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Abstract: This study aimed the characterization of the importance of vaccination against Newcastle disease in Chinese geese (*Anser cygnoides)* and to investigate the state of carrier of the virus in this species. There were used 120 Chinese geese, distributed at random into 4 groups, vaccinated or not. At 60 days 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) chickens were used as control of the virus. Cloacal and tracheal swabs were collected after 6, 10 and 20 days post-challenge for genome viral excretion by RT-PCR (reverse transcription-polymerase chain reaction). Chinese goose of all groups did not demonstrate any signs of Newcastle disease. They were refractory to the clinical disease with the NDV. In Chinese geese from control group, NDV genome was detected 20 days after challenge. It was demonstrated therefore the state of carrier of NDV by Chinese goose. In geese, 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 carrier of NDV in Chinese geese.

Key words: Newcastle disease, chinese geese, *Anser cygnoides*, NDV carrier, vaccination

INTRODUCTION

Newcastle Disease (ND) remains as the most important poultry virosis with highly infectious ability, affecting domestic and wild birds. Routine vaccination combined with sacrifice of affected birds have helped to control the virulent disease caused by the ND Virus (NDV), classified as *Avian Parainfluenzavirus* type 1. Newcastle disease has been the main sanitary barrier for the free commerce of birds and its products between countries (Office International des Epizooties, 1996). The disease is world-wide distributed in a large range of hosts, with 27 of the 50 orders of birds reported to be possibly infected by this agent (Kaleta and Baldauf, 1988). One of the affected species is the Chinese goose also known as the swan goose (*Anser cygnoides* Linnaeus, 1758, Anseriformes*: Anatidae*), which commercial production is extensively distributed in several countries around the world for meat, fine feathers and down for use in garment and household linen industries (Buckland and Guy, 2002). However, there is little information available on sanitary control programs in this species. Because of the potential of these birds to produce high nutritive meat and the economic importance of feathers and down production, the massive raising of this species is increasing in many countries and this may cause high bird concentration in some areas that can lead to the dissemination of infectious diseases, such as ND. Thus, the aim of this study was to evaluate the importance of vaccination against ND in this species and also to investigate the state of NDV carrier of Chinese geese.

MATERIALS AND METHODS

Experimental birds and management: A total number of 120 days old Chinese geese were distributed in a completely experimental design with four different treatments, with three replicates of 10 birds each (Table 1). Birds were housed in boxes over litter, keeping distance between the other groups. Chinese geese nutrition was formulated according to NRC (1994) recommendations for each different growth phase.

Vaccines: 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). Birds were vaccinated 7 days of

Table 1: Distribution of Chinese geese in experimental groups

Group	Vaccination (7 days)	Administration route	Revaccination (28 days)
G1	Ulster 2C	Intra-conjuctiva	Ulster 2C
G2	B1	Intra-conjuctiva	B1
G3	LaSota	Intra-conjuctiva	LaSota
G4	Control*	-	-

*Non-vaccinated

Table 2: Results of challenge with viscerotropic velogenic Newcastle disease virus in Chinese geese at 60 days of age

Group	Vaccination (7 days)	Administration route	Revaccination (28 days)	Number of birds	Total protection (%)
G1	Ulster 2C	Intra-conjuctiva	Ulster 2C	6	100
G2	B1	Intra-conjuctiva	B1	6	100
G3	LaSota	Intra-conjuctiva	LaSota	6	100
G4	Control	-	-	6	100
G5	SPF chicken	-	-	12	0

age and revaccinated at 28 days 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, 7.2 and 7.35 log10/0.1 mL, respectively. Birds were vaccinated and revaccinated by eye drop.

Challenge: At 60 days of age, two Chinese geese from each treatment (six per repetition) were challenged with viscerotropic ND virus strain. The virus had intra-cerebral pathogenic index of 1.78 and embryonic death time of 48 h, with a 50% embryo infecting dose titer of 8.15 $\log_{10}$/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) chickens were used. The birds were housed in isolators with filtered air and offered food and water *ad libitum*.

Viral genome excretion: At six, 10 and 20 days post-challenge, RNA extraction from tracheal and cloacal swabs was performed from all birds of each group, using the Qlamp Viral RNA Mini Kit (Quiagen, USA), according to the manufacturer´s protocol. RT-PCR was performed using primers targeting a conserved region of the NDV genome, as described previously and used in other studies (Jestin and Jestin, 1991; Oberdörfer and Werner, 1998; Soares *et al*., 2005). 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 T-3'. cDNA synthesis and PCR were performed according to Jestin and Jestin (1991). Samples were analyzed by electrophoresis on 1.5% agarose (w/v) gels (Invitrogen®, USA) stained with ethidium bromide 0.5 µg/mL (Invitrogen®) and run at 100 V/50 min.

RESULTS AND DISCUSSION

Data about the challenge with viscerotropic velogenic NDV pathogenic for chickens in Chinese geese are shown in Table 2. None of the Chinese geese groups vaccinated or not, presented any sign of ND after challenge, which is consistent with the observations of Higgins (1971). On the other hand, 100% of the SPF broilers died due to the NDV challenge. Three days after challenge, the SPF chickens began to show clinical signs such as ruffled feathers, anorexia, depression, conjunctivitis, dyspnea, respiratory disorders, severe and green diarrhea and death. At necropsy, necrotic lesions were observed in the trachea accompanied by catarrhal exsudate in the lumen, petechial hemorrhages in the proventriculus and hemorrhages in the small intestine and cecal tonsils. NDV was isolated from these SPF chickens, indicating the ability of the NDV used in this trial to cause disease.

Peculiarly, Chinese goose of the control group did not demonstrate signs of Newcastle disease, being refractory to the clinical disease with the NDV. In vaccinated and non-vaccinated Chinese geese (Groups 1-4), the percentage of protection to the challenge was 100% (Table 2).

The results of the genome excretion of NDV velogenic strain in Chinese geese after challenge are in Table 3. In Chinese geese from the control group (non-vaccinated, Group 4) the genome excretion of the NDV was positive 20 days after the challenge, by RT-PCR, confirming the susceptibility of this species to NDV, according to Reis and Nobrega (1956). It demonstrated that Chinese geese are able to eliminate NDV 20 days after infection, which is important for the epidemiology of this disease.

Unfortunately, there is no information about the importance of Chinese geese as NDV carrier, or to the potential risk factor to disseminate ND to other poultry, such as turkeys, broilers, breeders, that may be raised close to Chinese geese. In contrast, genome excretion of NDV was not detected by RT-PCR from vaccinated

Table 3: Results of NDV genome excretion (by RT-PCR) in Chinese geese after challenge (60 days of age)

			Viral genome excretion					
			6 DAC		10 DAC		20 DAC	
Group	Vaccination (7 days)	Revaccination (28 days)	T	C	T	C	T	C
G1	Ulster 2C	Ulster 2C	-	-	-	-	-	-
G2	B1	B1	-	-	-	-	-	-
G3	LaSota	LaSota	-	-	-	-	-	-
G4	Control*	- - -	-	-	-	-	+	+

T = Trachea, C = Vent, DAC = Days after challenge, + positive genome viral excretion, - negative genome viral excretion, *Non-vaccinated

groups of Chinese geese. It suggests that vaccination can efficiently eradicate NDV in Chinese geese and can be an important tool for the epidemiological control of ND dissemination to other birds. In addition, further studies should be developed to establish the importance of carrier state of Chinese geese to the dissemination of this disease under field conditions.

Conclusion: Chinese geese showed to be resistant to the development of clinical signs of ND when challenged with velogenic NDV. It was demonstrated the relevance of Chinese geese in the epidemiology of NDV because these birds can shed the virus 20 days after challenge. Vaccination against ND is essential to control virus dissemination to other birds' species.

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