BJVP
Brazilian Journal of Veterinary Pathology
ISSN 1983-0246

Original Full Paper

Relevance of Chinese Goose (*Anser cygnoides*) in Experimental Epidemiology of Newcastle Disease

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Submitted February 2nd 2012, Accepted May 25th 2012

Abstract

This study aimed to characterize the true epidemiological role played by the Chinese goose (Anser cygnoides) as a potential source of infection by the Newcastle disease virus (NDV). For this, Specific-Pathogen-Free chicks (SPF) were used and were housed with Chinese geese that had been inoculated with a pathogenic strain (velogenic viscerotropic, strain São João do Meriti) of NDV (DIE50=108.15/0.1 mL) pathogenic to chickens, by the ocular-nasal route. Each group was composed of 6 SPF Leghorn chicks and 3 geese. At 6 days (Group I) and 14 days (Group II) after inoculation of the Chinese geese with NDV, SPF chicks were put into direct contact with each goose group. Cloacal swabs were collected from both species (Chinese geese and SPF chicks) 6, 10 and 20 days after challenge to genome viral excretion by Reverse Transcription Polymerase Chain Reaction (RT-PCR). Chinese geese did not demonstrate any clinical signs of Newcastle disease (ND). They were refractory to the clinical disease with the NDV. However, NDV genome was detected 20 days after challenge. Therefore, NDV carrier status was demonstrated by Chinese geese. Moreover, 100% of SPF chicks housed with the infected Chinese geese had died by 6 (Group I) and 14 days (Group II) after challenge. Thus, the transmission of the pathogenic virus from the Chinese geese to cohabiting SPF chicks was evident within 20 days of the experimental infection. This reveals the epidemiological importance of Chinese geese as a potential transmitter of NDV infection to other commercial birds that could be raised in close proximity.

Key Words: Chinese geese, Anser cygnoides, Newcastle disease, epidemiology, NDV carrier, source of NDV infection.

Introduction

Newcastle Disease (ND) is caused by the virus Avian Paramyxovirus serotype 1 (APMV-1/NDV), which is a member of the genus Avulavirus, of the Paramyxoviridae family (5). This disease is an important viral infection of birds that can cause substantial losses to the poultry industry (1, 12). ND has been the main sanitation barrier for the free international trade of birds and poultry products between countries (11). The disease is distributed worldwide among 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 (6; 14). One of the affected species is the Chinese goose (Anser cygnoides Linnaeus, 1758, Anseriformes: Anatidae), which is produced commercially in several countries around the world for meat, fine feathers and down for use in the garment and household

linen industries (2). However, there is little information available about the true role played by Chinese geese as a potential source of NDV infection in other bird species.

Material and methods

Experimental birds and management

SPF chicks used were placed in direct contact with Chinese geese inoculated with a velogenic viscerotropic strain (São João do Meriti) of NDV (DIE50=108.15/0.1 mL). Each group was composed of three Chinese geese and six SPF Leghorn chickens. The birds were housed in isolators with filtered air and offered food formulated according to NRC recommendations (10) and water ad libitum.

Challenge

Chinese geese were challenged with a velogenic viscerotropic ND virus strain pathogenic to chickens. The virus had an intra-cerebral pathogenic index of 1.78 and embryonic death time of 48h, with a 50% embryo-infecting dose titer (EID50) of 8.15 log10/0.1mL. Distilled water was used as diluent for the inoculum that was instilled by ocular-nasal route, according to the Code of Federal Regulations (3). In order to evaluate the pathogenicity of the NDV challenge strain, a group of SPF chicks was used. At six days (Group I) and fourteen days (Group II) after challenge, six SPF chicks were housed together with the Chinese geese to ensure direct contact between the two species.

Viral and genome excretion

At 6, 10 and 20 days post-challenge, RNA extraction from cloacal swabs was performed from all birds (Chinese geese and SPF chicks). They were placed in phosphate buffer solution (pH 7.2). The QIAamp Viral RNA Mini Kit (QIAGEN Sciences®, Germantown, 20874, USA) was utilized 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, described by TOYODA (15). 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 SPF-chicks (100%) infected with the pathogenic strain of NDV died within 3 days after the challenge. They presented signs 48 hours after the challenge and lesions of ND, detected by isolation and identification of NDV. All Chinese geese infected with NDV showed no clinical signs or lesions of ND after challenge, being refractory to the clinical disease. Results of viral genome search for NDV in Chinese geese after challenge are shown in Table 1. The detection of the viral genome of NDV in the cloacal swabs taken from Chinese geese 20 days after challenge, emphasize the susceptibility of this species to NDV as well as would characterize the species as a NDV carrier. Similar studies performed by Lima et al. (7) demonstrated experimentally that Guinea Fowls (Numida meleagris galeata) did not show clinical signs when challenged with a sample of pathogenic NDV. However, the carrier status was demonstrated until 30 days after experimental infection with this pathogen. A similar situation has been demonstrated in other species. In studies with partridges (Rhynchotus rufescens), there was no clinical disease when inoculated with NDV.

Table 1. Post-challenge viral excretion results of Chinese geese (Anser cygnoides), from cloacal and tracheal swabs.

Birds	Viral genome excretion (RT-PCR)		
	6 dac	10 dac	20dac
Chinese geese (*Anser cygnoides*)	-	-	+

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

Therefore, there was viral isolation from 5 to 15 days after the challenge with the NDV, demonstrating, this way the state of carrier of the partridge NDV which happened until 15 days of the experimental infection with this pathogen (13). In quails (Coturnix coturnix japonica), all of them infected with NDV did not show clinical signs or lesions indicatives of NDV. The NDV was isolated from Japanese quail from five to 14 days after challenge (8, 9); and in budgerigars (Melipsittacus undulatus), infected with NDV didn't show clinical signs or lesions indicatives of NDV, being refractory to the clinical disease. 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, were detected antibody titles from HI test 13 and 19 days after challenge, confirming the state of carrier of the virus (NDV) in this specie (4). Under the epidemiological plan of ND, these results showed that the Chinese geese might be carriers of the virus, thus suggesting an important role of the specie Anser cygnoides in NDV epidemiology in regions of extensive poultry production. Table 2 shows that 100% of SPF chicks housed with the Chinese geese infected with a pathogenic NDV strain died between 5 and 6 days after the challenge. Moreover, all SPF chicks showed clinical signs from 2 to 3 days after the challenge and ND lesions, detected by RT-PCR. The main clinical signs were dyspnea, severe 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 cecal tonsils. Although Chinese geese did not show clinical signs of ND, they spread a sufficient

amount of the virus to induce an infection and the clinical disease in cohabiting SPF chicks. Transmission of the virus was demonstrated by the Chinese geese until 20 days after challenge with NDV virus to SPF chicks that were housed together. This calls attention to the epidemiological importance of the Chinese geese as a potential source of NDV infection transmission to commercial flocks that may be raised near this species.

Table 2. Results of clinical observation, macroscopic lesions and viral isolation of NDV from SPF chicks housed with Chinese geese (Anser cygnoides) 6 (Group I) and 14 (Group II) days after challenge.

Parameters	SPF chicks housed with infected Chinese geese	
	6 dac	14 dac
Clinical signs suggestive of NDV	+	+
First singns	48-72h	48-72h
Mortality (%) (patients and healthy)	100	100
Time to death	120-144h	120-144h
Lesions suggestive of NDV	+	+
Genome viral excretion (NDV)	+	+

dac= days after challenge + = positive results

Conclusion

Chinese geese (Anser cygnoides) were shown to be resistant to the development of clinical signs of ND when challenged with a velogenic viscerotropic strain of NDV. The virus carrier status of Chinese geese was demonstrated until 20 days after challenge with this pathogen, emphasizing the susceptibility of this species to NDV. All SPF chicks housed with the Chinese geese infected with a pathogenic strain of NDV died and presented signs and lesions suggestive of NDV. The Chinese geese spread a sufficient amount of the virus to induce an infection and the clinical disease in the SPF chicks with which they were housed. The relevance of Chinese geese in the epidemiology of ND was also demonstrated as a potential source of NDV infection in other domestic bird species, which should be considered in standardizing the biosecurity management measures to be performed by the poultry industry.

Acknowledgements

CEBEA – Commission on Ethics and Animal Well-Being. Protocol number 007605-08

To FAPESP for the assistantship and financial support (GRVM - FAPESP process numbers 2010/04543-0 and 2010/10907-5), CAPES (JMC) for the assistantship and financial support and CNPQ (ACP).

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