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Experimental infection with Brazilian Newcastle disease virus strain in pigeons and chickens

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ABSTRACT

This study was designed with the goal of adding as much information as possible about the role of pigeons (*Columba livia*) and chickens (*Gallus gallus*) in Newcastle disease virus epidemiology. These species were submitted to direct experimental infection with Newcastle disease virus to evaluate interspecies transmission and virus-host relationships. The results obtained in four experimental models were analyzed by hemagglutination inhibition and reverse transcriptase polymerase chain reaction for detection of virus shedding. These techniques revealed that both avian species, when previously immunized with a low pathogenic Newcastle disease virus strain (LaSota), developed high antibody titers that significantly reduced virus shedding after infection with a highly pathogenic Newcastle disease virus strain (São Joao do Meriti) and that, in chickens, prevent clinical signs. Infected pigeons shed the pathogenic strain, which was not detected in sentinel chickens or control birds. When the presence of Newcastle disease virus was analyzed in tissue samples by RT-PCR, in both species, the virus was most frequently found in the spleen. The vaccination regimen can prevent clinical disease in chickens and reduce viral shedding by chickens or pigeons. Biosecurity measures associated with vaccination programs are crucial to maintain a virulent Newcastle disease virus-free status in industrial poultry in Brazil.

Introduction

Newcastle disease (ND) is an acute, highly contagious viral disease of poultry that can cause high mortality (up to 100%) in chicken, the most important natural host of the disease. The virus can also affect a wide variety of avian species causing severe disease. The disease is regarded as endemic in many countries, and is caused by an avian Paramyxovirus type 1 (APMV 1), a member of the genus *Avulavirus*, from the *Paramyxoviridae* family.[1] As demonstrated in intensive surveys, nearly 236 free-living species from 27 of the 50 orders of birds have been reported to be susceptible to either natural or experimental infection with ND.[2] On several occasions, Newcastle disease virus (NDV) was isolated from wildlife birds,[3] and most outbreaks of NDV arise in unvaccinated susceptible animals.[4]

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To keep ND under control, large-scale prophylactic vaccination is used in most member states of the European Union and elsewhere in the world.[4,5] Although vaccination in general provides good protection against clinical disease and mortality, it may not provide sufficient protection against virus transmission to prevent ND outbreaks.[6,7] The most common strains used worldwide in vaccination are LaSota and B1.[5] Conventional vaccine strains may protect against clinical disease caused by virulent NDV, but viral infection can still occur in vaccinated birds,[8,9] which may be the route of virus spread in poultry facilities farms.[10] However, vaccinated birds may show significantly reduction in virus shedding compared with unvaccinated ones.[9,10]

The susceptibility of pigeons (*Columba livia*) and other members of the *Columbidae* family to NDV has been reported by several authors.[11–15] It is now clear that the disease occurs in pigeons as a result of virus dissemination from affected chicken flocks, and it occurs in poultry flocks when the virus is disseminated from domesticated or feral pigeons.[16] The source of ND infection to chicken flocks may be food contaminated with feces of feral pigeons infected with NDV.[16,17]

Many aspects of ND infection in pigeons are unclear, and experimental infection could answer many questions about NDV epidemiology, such as virus pathogenicity, infectivity, and shedding. As previously described by experimental studies, adult pigeons infected via eye drops with a chicken pathogenic APMV-1 strain shed the virus both through the cloaca and the mouth for up to 21 days post-infection (dpi).[11] Pigeon Paramyxovirus type 1 (PPMV-1) isolates may be transmitted from infected pigeons to chickens that were in contact with them, with replication in the chickens (as demonstrated by the excretion of the virus by cloacal route), and antibody response against the virus.[16] In experimental infections conducted with a PPMV-1 strain, mortality rates greater than 70% were observed in pigeons, but no infected chicken died. In spite of these findings, there are few comparative studies on pigeons and chickens infected with the same PPMV-1 strain, making it difficult to determine the significance of these results.[15]

Thus, little is known about the course of the infection, the significance of humoral antibody response, viral shedding, clinical signs, and contact transmission of a Brazilian pathogenic NDV strain between pigeons and chickens (experimental infection). To some extent, viral replication complex may play a role in the pigeon-to-chicken transmission, although further studies are needed to investigate the factors that are determinant for interspecies transmission.[15]

Some studies were carried out to evaluate the prevalence of Newcastle disease in commercial birds in poultry-producing areas in Brazil,[18,19] and the results showed that industrial poultry produced in the nine Brazilian states analyzed was free of Newcastle disease.[18]

The nationwide efficiency of poultry production makes Brazil a competitive nation in international markets, even in the absence of economic subsidies. In order to guarantee better sanitary conditions for Brazilian avian products, the National Program for Poultry Health (PNSA) was implemented for the control of Newcastle disease in the country.[19]

Considering the potential risk of contamination of poultry species by pigeons carrying NDV, it is important to study the pathogenesis of the disease both in pigeons and in chickens. This study was designed to evaluate humoral immune response, viral shedding, and contact transmission after experimental infection of pigeons and chickens with a pathogenic NDV isolate of chicken origin under experimental and controlled conditions.

Materials and methods

Birds

In an attempt to reproduce natural conditions of NDV transmission, a total of fifty-two free-living adult domestic pigeons (*C. livia*) were used in this study. Animals were clinically healthy, had non-specific levels of hemagglutination inhibition (HI) antibodies (HI Titers ≤ 2), and were negative in reverse transcriptase polymerase chain reaction (RT-PCR) for the presence of NDV in cloacal swab samples. After capture, pigeons were housed in adequate facilities (away from chicken facilities) for 90 days to be adapted to captivity. Similarly, twenty-nine 90-day-old, SPF (specific pathogen free) chickens were used in the study. The two species were kept in separate facilities until the beginning of the experimental study.

On the day of experimental infection, pigeons and chickens divided in four experimental groups were moved to Negative Pressure Isolators (Alesco®, Brazil), under controlled laboratory and biosafety conditions.

All animal procedures were performed according to the Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation, and to the 2000 Report of the AVMA Panel on Euthanasia.[20]

Viruses

The experimental infection was performed using the Sao Joao do Meriti (SJM) strain (Gene Bank Number: EF534701), a highly pathogenic NDV strain (APMV-1) for chickens (mean death time in chicken embryos = 48 h; ICPI in day-old chicken = 1.78). The virus was propagated twice in the allantoic cavity of 9 to 10-day-old embryonated SPF eggs by inoculation of 0.1 mL of infectious allantoic fluid. A virus stock was produced by harvesting allantoic fluid from chicken embryos, freezing it at −70 °C, and storing it. This virus stock titer was $10^{9.0}$ median embryo lethal dose/mL (ELD_{50}), determined on day three before experimental infection. All virus dilutions were carried out with sterile phosphate buffered saline (PBS), pH 7.2.

Live vaccines containing LaSota (LS) NDV strain (New Vac-LS – Fort Dodge Saúde Animal Ltda®, Brazil) were used in the vaccination procedures. This strain is commercially used in the immunization of chickens in Brazil.

Experimental infection and sampling

Pigeons and chickens were randomly divided into four groups, as described below. After vaccination/experimental infection, birds were monitored daily for presence of any clinical signs, such as diarrhea, torticollis, incoordination, apathy, tremors, ocular and nasal discharge, abnormal posture, and flying