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Newcastle Disease Virus Infection in Sparrows (*Passer domesticus,* Linneaus, 1758) Captured in Poultry Farms of the Agreste Region of the State of Pernambuco

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■ **Keywords**

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ABSTRACT

Reservoir competence for the Newcastle Disease virus (NDV) was evaluated in sparrows (*Passer domesticus*, Linnaeus 1758) captured on a commercial poultry farm and a chicken hatchery in the State of Pernambuco, Northeastern Brazil. A total number of 103 birds collected from a poultry farm (24/103) and a chicken hatchery (79/103) were examined. Hemagglutination inhibition tests, isolation, and viral characterization were performed in all samples collected from each bird. Titers ranging from 1:2 to 1:64 were detectable in 10.68% of sparrows, but positive serology and viral isolation were obtained only from sparrows captured at the hatchery. Hemagglutination activity was inhibited by anti-avian paramyxovirus serotype 1 (APMV-1) serum, and this sample showed an intracerebral pathogenicity index (ICOI) of 0.21, which is similar to the B1 stock vaccine (0.20) used for vaccination in those farms. Therefore, it was concluded that the sparrows were infected by stock vaccine virus, and that these birds could be a reservoir for NDV. However, additional studies involving sequencing of the virus genome of stock vaccine must be carried out.

INTRODUCTION

The infection by Newcastle disease virus (NDV) has been recorded in several animal species, including reptiles and mammals, man inclusive (Paulillo & Doretto JR., 2000). Natural or experimental infection has already been established in 236 different bird species, representing 54% of the orders consisting the Bird class (Alexander, 1997).

In a study carried out with the birds of RioZOO Foundation, 194 birds from different families were studied as to antibody titers against NDV using hemagglutination inhibition (HI). Nine samples of birds belonging to the families Falconidae, Phasianidae, and Strigidae were found positive (Belluci *et al.*, 1999). Shortridge *et al.* (1978), working with two bird species belonging to the family Phasianidae, *Francolinus pintadeanus* and *Bambusicola thoracica*, obtained HI titers of 1:64 to 1:256, and intracerebral pathogenicity indexes (ICPI) between 1.75 and 1.88, demonstrating the presence of velogenic strains of NDV.

In Costa Rica, in a study with domestic and wild birds, 18.7% of 876 collected cloacal swabs presented hemoagglutinating agents. Strains similar to avian paramyxovirus (APMV) type 2 were recovered from two passeriformes (*Troglodytes musculus* and *Zonotrichia capensis*) and a backyard chicken, all derived from different regions (Goodman and Hanson, 1988).

In August, 1980, a group of cranes (*Anthropoides virgo*) was received by a zôo in Germany, directly imported from their natural habitat. Two days after they arrived, one crane died, with no clinical signs. During the next tem days, other five cranes, one flaming (*Phoenicopterus ruber*),

and a pied imperial dove (*Ducula bicolor*), also died, again with no clinical signs, NDV was isolated in chicken embryo fibroblast culture in all dead birds. Crossed serological reactions were obtained among isolates, and the NDV B1 strain was identified in HI tests and plate reduction test. Electron microscopy revealed particles bearing the morphology of the paramyxovirus, and the inoculation in naive birds resulted in severe symptoms and high mortality (Kaleta and Marschall, 1981).

Jorgensen *et al*. (2004) isolated, from domestic and wild birds, 21 strains of avian paramyxovirus type 1, which has low pathogenicity in chickens. In that study, virulence was determined through gene sequencing, and through intracerebral pathogenicity index in day-old chicks in some cases.

In a study conducted with 700 plasma samples of captive and free-living scavenger birds in Spain, Hofle *et al*. (2002) showed positive reactions in HI to avian paramyxovirus types 1, 2, and 3, with 120 belonging to type 1, 10 to type 2, and 4 to type 3. The prevalence of antibodies against avian paramyxovirus type 1 was significantly in captive birds as compared with free-living bids, with the predominance of species of the order Falconiformes.

During a Newcastle disease (NCD) outbreak in Southeast California, 17,593 free-living, wild, semi-domestic, and exotic bird specimens were sampled around the sites affected by the outbreak in order to determine their role in the epizootiology of this disease. NDV was isolated in 1.81% of the birds. Three sparrows and a crow directly associated to the involved flocks were the only free-living birds from which NDV was isolated (Pearson and McCann, 1975).

Contrary to the above studies, Chang *et al*. (1999) and Carrión *et al*. (2000) did not find antibodies against NDV in passeriformes and columbiformes captured in different areas of Peru. Garnett and Flanagan (1989), in Australia, did not obtain positive results in virus isolation tests, and NDV antibody research in 1235 birds belonging to 130 species. These studies merely show that researched birds did not have contact with the virus until the study was performed, which does not rule out their possible role in virus dissemination.

Gustafson and Moses (1953) demonstrated that sparrows (*Passer domesticus*) experimentally infected with NDV, and maintained in the same environment as chicken can infect chickens and themselves with NDV.

However, there are no studies on sparrows that live in or visit poultry houses in Brazil. Therefore, the aim of this study was to detect NDV infection in sparrows (*Passer domesticus*, Linneaus 1758) captured in broiler and broiler breeder farms located in the Agreste region of the state of Pernambuco, Brazil.

MATERIAL AND METHODS

A total number of 103 aves (sparrows) belonging to the order Passeriformes, family Passeridae, species *Passer domesticus*, were captured in a broiler farm and a broiler breeder farm located in the municipalities of Caruaru and Agrestina in the state of Pernambuco, respectively.

Serum samples were obtained from blood collected from the jugular vein, centrifuged for clarification, and stored at –20ºC until analyses were performed.

Hemagglutination (HA) and inhibition of hemagglutination teste were carried out according to the technique described in Regulation n. 182/94 of the Brazilian Ministry of Agriculture (Brasil, 1994), but using a erythrocyte solution at 1% prepared from sparrows' blood.

For viral isolation, a pool of 300 cloacal swabs was collected from sparrows captured in each farm. Swabs were placed in buffered saline solution (PBS), with pH adjusted to 7.0 – 7.4, and cold-stored until analyses were performed at the Laboratório Nacional Agropecuário – LANAGRO, Campinas, São Paulo. In the lab, virus isolation was carried out according to norm described in Regulation n. 182/94 of the Brazilian Ministry of Agriculture (Brasil, 1994).

Viral identification was determined by HI test using reference antisera (APMV-1 to APMV-9) produced by the laboratory of international reference on Newcastle disease – Veterinary Laboratory Agency – VLA, located in Weybridge, Surrey, UK. As APMV-5 does not produce hemagglutination, it was not used in the analyses.

Intracerebral pathogenicity index (ICPI) was obtained using *in vivo* test, as described in Regulation n. 182/94 (Brasil, 1994).

Flock age of broilers and broiler breeders when sparrows were captured were five days and 38 weeks, respectively. Broilers were not vaccinated for NDV, and broiler breeders received live vaccines for Newcastle (B1 strain) at two, four, eight, 10, 15, 20, and 38 weeks of age. The last Newcastle vaccination was administered in the drinking water during the weeks the sparrows were captured.

RESULTS AND DISCUSSION

None of the samples collected from sparrows captured in the broiler farm presented anti-VDN

antibodies. As to the broiler breeder farm, 03 (3.80%) samples showed positive titers against this virus, corresponding to 2.91% of the total samples analyzed (Table 1).

Table 1 – Absolute and relative frequencies of sparrows with positive reaction in the HI test according to the municipality, Recife, 2005

Municipality	N. captured birds	Negative		Positive	
		AV	RV (%)	AV	RV (%)
Caruaru	24	24	100,00	00	0,00
Agrestina	79	76	96,20	03	3,80
TOTAL	103	100	97,09	03	2,91

AV – Absolute value; RV – Relative value.

Titers lower than 1:16 are considered as unspecific; therefore, in the present study, only titer equal or higher than 1:16 were considered as positive. Titers of the three samples considered as positive were 1:16, 1:32, and 1:64.

In study carried out by Chang *et al*. (1999) in the province of Chancay, Peru, with 398 birds belonging to the order Columbiforme, the presence of antibodies against NDV was not detected, suggesting that the involved species had not previous contact with the virus. Later, in the same country, Carrión *et al*. (2000) carried out a serological survey in 462 birds, with 86 belonging to the species *Columba livia,* and 367 to the species *Eupelia cruziana*, and obtained the same results. The authors concluded that the investigated species did not act as reservoir of NDV to domestic species, and suggested further research with other bird species to try to identify possible reservoirs in the region.

On the other hand, research carried out with strain involved in 23 NDV outbreaks in broiler flocks in the UK indicated that sick pigeons belonging to the species *Columba livia* as responsible for NDV dissemination in 22 of these outbreaks. These birds contaminated the feed offered to the broilers with their feces (Alexander *et al*., 1985; Alexander, 1985; Biancifiori and Fioroni, 1983).

Waterfowl have also been identified as NDV carriers. In a serological survey conducted in Andalusia, southeast Spain, from 1990 to 1992, 579 sera from 24 bird families (18 aquatic and six terrestrial families) were collected. Antibodies against nine serogroups (APMV-1 ao APMV-9) were detected in aquatic species. In non-aquatic species, onlu antibodies belonging to the serogroups APMV-2 (with the highest prevalence, 60%), APMV-3, APMV-7, and APMV-8 were present. Among these species, there were 56 sparrows (*Passer domesticus*), out of which 38 (67.86%) reacted to APMV-2, 5 (8.93%) to APMV-3, 2 (3.57%) to APMV-7, and 11 (19.64%) to AMPV-8 (Maldonado *et al*., 1995).

Serological tests were also employed in cormorant species: *Phalacrocorax carbo* and *P. auritus* by Artois *et al*. (2002) and Farley *et al*. (2001), respectively. In the first study performed in France, at the end of the 1990s, 10 adult individuals of 53 sampled specimens showed antibodies against NDV. In the second study, carried out in the USA, two subspecies were sampled. A total number of 183 individuals of *P. a. auritus,* a migratory species, was tested, and 47% were positive. As to the second subspecies (*P. a. floridanus*), 45 birds were analyzed, and none were positive.

The interpretation of HI results in semi-domestic birds that share the environment of industrially reared birds presents some limitations. The presence of antibodies in the serum of these birds indicates that NDV infection may occur in nature with field species, or, in the case of sparrows, by inhalation or ingestion of vaccine virus particles. These findings were discussed in the article of Pearson & McCann (1975), when the results of HI tests in sparrow populations in the studied farms were presented.

Another study using parrot of the genus *Amazona* indicated that these birds may act as carriers and disseminators of the ND virus for at least one year, and that the epizootiology of this disease in free-living birds must be investigated using population studies (Cubas, 1993).

Pearson & McCann (1975) also carried out a complex study of the episootiology of the viscerotropic velogenic form of Newcastle disease in California, reporting the role of some semi-domestic, exotic, and wild bird species. Their resulted indicate a high frequency of semi-domestic waterfowl reacting in hemagglutination inhibition tests, with titers between 1:10 and 1:320. The percentage of birds reacting in the HI test was similar to that obtained by these authors in other semi-domestic birds.

In the present study, out of the samples submitted to isolation, only the pool collected from the broiler breeder farm showed positive results. ICPI was 0.21.

Takakuwa *et al*. (1998) carried out phylogenetic and virulence tests in 47 NDV strains isolated in the feces of waterfowl (ducks, geese, and teals) in Siberia, Canada and Alaska. The obtained results led to the conclusion that migratory populations of these species maintain lentogenic NDV strains, which are potentially virulent, circulating in wild environments, and these strains can be transmitted to commercial poultry flocks, acquiring pathogenicity after passage in these birds.

Vickers & Hanson (1982) isolated NDV from the cloaca of 5% of the waterfowl captured in Wisconsin from 1978 to 1980. Antibodies were detected in 8% of the tested birds, with no significant differences among ages and sex. Experimental infections performed by the same authors resulted in permanent shedding of the virus for months after exposure. The absence of detectable antibody levels in some experimental birds suggests that antibody prevalence may not be an indication of the true prevalence of infection in these species.

Migratory bird species are putative Newcastle disease virus hosts, and are involved in large outbreaks of this disease in some European countries, as reported by r Van Den (1996).

In order to confirm the circulation of non-vaccine strains in these species, it is necessary to isolate and to perform phylogenetic analyses of these strains in the region. This allows the establishment of disease control protocols. It is recognized that wild birds can act as virus reservoirs, and that the appearance of pathogenic variants and their dissemination in domestic and commercial birds is common, as discussed by Alexander (1997).

In Brazil, NDV was isolated in wild birds in the state of Rio de Janeiro, in addition of anti-NDV antibodies detection. This resulted in notifications to the Ministry of Agriculture, suggesting control measures for this disease in the studied area, as these birds were considered as reservoirs of the virus for commercial birds (Oliveira JR *et al.*, 2003).

In a study on the detection of Nile Fever virus carried out with migratory birds and birds residing in the municipality of Galinhos, state of Rio Grande do Norte, the presence of pathogenic and low-pathogenicity strains of the NDV in cloacal swabs of two migratory species (*Arenaria interpres* and *Calidris pusilla*). The report of this study suggests to the Ministry of Agriculture the serological follow-up of these birds every two years, as well as serological surveys in domestic bird farms (Araújo *et al.*, 2004).

In the state of Pernambuco, Bezerra (1996) researched hemagglutinins in the feces of birds raised in a zoo. Hemagglutination test results showed the presence of hemagglutinins in specimens collected from crested carcara (*Polyborus plancus*), macaws (*Ara ararauna*, *Ara chloroptera,* and *Anodorhynchus hyacinthinus*), ciconiformes (*Tigrisoma lineatum* and *Bubulcus ibis*), common moorhen (*Gallinula chloropus*), and scarlet ibis (*Eudocimus ruber*). These findings suggest the infection of NDV in the studied population. In the same state, Vieira & Saukas (1996) also worked with wild birds and sparrows in commercial poultry farms, and isolated NDV in lung and trachea samples from sparrows, and carcara feces.

Although sparrows have been imputed as NDV reservoirs in studies carried out in some countries, this is the first study carried out in Brazil using sparrows for anti-NDV antibodies, and virus isolation research. The obtain results cast a new light on the role of this species in the dissemination of pathogenic variants of the NDV to for domestic and commercial birds.

The isolation of NDV in cloacal swabs of sparrows from the broiler breeders farm, associated to the presence of anti-NDV antibodies in this species, emphasize the hypothesis that the sparrows were probably infected by ingesting virus particles derived from the water used for the vaccination of breeders. Although the genome of the isolated virus was not sequenced, it probably consists of the vaccine strain, as this farm uses B1 vaccine strain, which presents an ICPI of 0.2, being very close to the ICPI found for the isolated virus (0.21).

Gustafson & Moses (1953) demonstrated the experimental infection of sparrows, suggesting that these birds may transmit the virus to susceptible birds by co-habitation.

The results obtained in the present study show that, under natural conditions, sparrows are also infected by NDV, and thus can be considered as virus reservoirs for commercial poultry, as close contact between these two species was verified in the poultry houses used. Adequate management measures should be adopted in order to avoid the contact of this and other wild species with commercial poultry, reducing the risk of a NDV infection.

Funding to conduct a more comprehensive study are expected to perform serology and virus isolation in several wild and domestic species that live in broiler and broiler breeders farms in the state of Pernambuco.

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