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## Aquatic Birds Surveillance for Newcastle Disease Virus (NDV) in Khozestan Province

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### ABSTRACT

**Background:** Wild aquatic birds are considered as reservoir hosts for Newcastle disease viruses (NDVs) and may act as vectors for transferring these viruses to poultry, causing outbreaks of disease. Recent studies suggested that the velogenic viruses arise from avirulent strains originating from wild birds.

**Objectives:** The primary objective of this study was to determine whether the aquatic birds of Khozestan province were infected with NDV.

**Materials and Methods:** In winter, 24 Muscovy ducks from Shoshtar, 20 geese from Dezful and 51 geese from Khorramshahr (Aquatic birds of Khozestan province) were bled, and birds' sera were collected and used in HI assay and antibody titer to NDV were determined. Beta procedure of hemagglutination inhibition (HI) assay was performed in U-bottomed 96-well microtiter plates with 0.5% chicken erythrocytes.

**Results:** All serum samples tested for antibodies against hemagglutinin by HI assay had positive results, and all of these birds had high levels of antibody titer to the NDV.

**Conclusion:** The results in the present study clearly showed that the aquatic birds of Khozestan province population in Iran carries NDVs, and may act as one of the important reservoirs for this virus. So it was concluded that detection and elimination of these birds can be a valuable tool for the control of Newcastle Disease outbreaks.

**Keywords:** Disease Vectors; Newcastle Disease Virus; Hemagglutination Inhibition Tests

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Newcastle disease is one of the virulent infectious diseases which affects the poultry industry and causes significant economic losses. Free movement of migratory aquatic birds makes this population to be considered as an extremely important vector for virus dissemination. In this study, we investigated the presence of NDV in aquatic asymptomatic birds from Khozestan province considered as one of the entry portal of NDV into Iran. It has been suggested that nonvirulent ND viruses maintained in different wild bird species could cause NDV outbreaks in poultry; we considered it important to search for the presence of NDV in these reservoirs. This study is intended to give general concepts for surveillance of the NDV in aquatic birds.

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## 1. Background

Wild aquatic birds are recognized reservoirs of NDVs belonging to the family of Paramyxoviridae and may act as a vehicle for transference to poultry, causing outbreaks of disease. Newcastle disease virus (NDV), has been isolated from a variety of species of domestic, cage birds and wild around the world. Major signs of the disease include respiratory distress, circulatory disturbances, diarrhea and central nervous system impairment. NDV belongs to the Avulavirus genus of the Paramyxovirine subfamily, Paramyxoviridae family, and is designated as avian paramyxovirus type 1 (APMV-1), and is serotyped from one to nine APMVs serotypes (1, 2). Strains of ND virus have been grouped into five pathotypes on the basis of the clinical signs seen in infected chickens; Viscerotropic velogenic: a highly pathogenic form in which haemorrhagic intestinal lesions, Neurotropic velogenic: a form that presents with high mortality, usually following respiratory and nervous signs, Mesogenic: a form that presents with respiratory signs, occasional nervous signs, Lentogenic or respiratory: a form that presents with mild or sub-clinical respiratory infection and Asymptomatic enteric: a form that usually consists of a subclinical enteric infection (2-4).

The clinical signs seen in infected birds are dependent on factors such as: host species, age of host, and the virus, infection with other organisms, environmental stress and immune status. In some circumstances infection with the extremely virulent viruses may result in sudden death with few clinical signs. (5, 6).

Lancaster (4) and Alexander (6) reviewed the modes of spread of NDV in Great Britain they have stated 11 outbreaks confirmed in chickens and turkeys because of migratory birds. As other scientists mentioned, intestinal infection by fecal-oral route would be the main cause of NDV infections (7, 8).

Chicken meat is important not only for Iranian economy, but it is also a source of affordable, high-quality protein for the population. The emergence of diseases

such as Newcastle disease (ND), which is highly contagious with great lethal and epidemic potential and affects both domestic poultry and wild birds, may have a very negative impact not only on the economy of a country due to trade restrictions and embargoes, but also on the life quality of a large part of the population. Poultry can be infected with NDV by direct or indirect contact with wild birds including resident and migratory wild birds, which may be natural reservoirs or carriers of the ND virus, since many migratory birds come from regions where NDV is endemic. Therefore, we conducted a serological surveillance of NDV in different regions of Khuzestan province.

## 2. Objectives

The primary objective of this study was to determine whether the aquatic birds of Khuzestan province were infected with NDV.

## 3. Materials and Methods

In winter, 24 Muscovy ducks from Shoshtar, 20 geese from Dezful, and 51 geese from Khorramshahr (Aquatic birds of Khuzestan province) were bled, and birds' sera were collected and used in HI assay, and antibody titer to NDV was determined. Beta procedure of hemagglutination inhibition (HI) assay was performed in U-bottomed 96-well microtiter plates with 0.5% chicken erythrocytes.

Hemagglutination test was performed according to standard procedure (9) and infective allantoic fluid by NDV was used as reference Antigen. The HI assay was performed using 96 'U'-well microtiter plates, doubling dilution in PBS, 0.5% v/v red blood cells (RBC), and 4 HA units of NDV antigen.

## 4. Results

All serum samples tested for antibodies against hemagglutinin by HI assay had positive results, and all of these birds had high levels of antibody titer to the NDV.

**Table 1.** Results of Detection of Antibody to NDV in Aquatic Birds in Khuzestan Province by HI Assay.

HI assay	Status			
	Shooshtar Muscovy ducks	Khorramshahr geese	Dezful geese	Total
Positive	24	51	20	95
Negative	0.00	0.00	0.00	0.00
Total	24	51	20	95

**Table 2.** Mean±SD. Deviation of Antibody Titer to NDV in Aquatic Birds in Khuzestan Province.

HI assay	Status		
	Shooshtar Muscovy ducks	Khorramshahr geese	Dezful geese
Mean of antibody titer± SD.	27.67 ± 1.1	28.47 ± 1.6	27.1 ± 1.2

The 95 serum samples had all positive results by HI (titer > 24).

## 5. DISCUSSION

Wild aquatic birds are considered as reservoir hosts for Newcastle disease viruses (NDVs), and may act as vectors for transferring these viruses to poultry, causing outbreaks of disease. Avian paramyxoviruses type 1 or Newcastle disease viruses (NDV) are frequently recovered from wild birds and such isolates are most frequently of low virulence (9). Velogenic NDV are usually recovered from poultry and only occasionally from wild birds. ND and avian influenza (AI) A viruses are pathogens which can be disseminated throughout the world by migratory birds. Many efforts have been made to control the introduction of these viruses into new environments. As with AI most NDVs isolated from wild birds are nonpathogenic, causing no clinical diseases. Recent studies suggested that the velogenic viruses arise from avirulent strains originating from wild birds (10). These findings indicate that it is important to survey for NDVs in aquatic birds. In a number of outbreaks over the last 10 years, wild birds have been implicated in the introduction of virulent NDV into poultry. Wild bird populations are pools of NDV, usually of low virulence for poultry, and direct or indirect contact of this bird with poultry populations may result in transition of NDV. (11).

Detection of NDV antibodies in poultry sera by a variety of methods including single radial immunodiffusion, single radial hemolysis, agar gel precipitin, VN in chick embryos, and plaque neutralization (12). Enzyme-linked immunosorbent assays (ELISAs), have become famous, especially as part of flock screening procedures. Correlation between ELISA and HI assay is high. In the present study the NDV was detected in the sera of all aquatic birds in Khozestan province by HI assay and all of examined birds had positive results, and they had high levels of ND antibody titer which are believed to be the reservoirs for NDV. This result indicates that prior exposure to the virus and primary introduction in poultry populations may occur by direct or indirect contact with these wild birds.

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## Authors' Contribution

All authors participated in the present study equally.

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