Newcastle Disease in Captive Falcons in the Middle East: a Review of Clinical and Pathological Findings:

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Abstract: Newcastle disease is an important viral disease of falcons in the Middle East. Two different clinical presentations producing distinct clinical symptoms and pathologic lesions have been identified in affected falcons, denominated as neurotropic velogenic and viscerotropic velogenic forms. Humoral response after vaccination using commercially available oil-emulsion inactivated poultry vaccines has been observed for up to 9 months in vaccinated falcons. Public awareness programs are needed to promote annual vaccinations at falcon medical facilities in the region to prevent ND in falcons.
Introduction

The Paramyxovirinae subfamily comprises five genera: Rubulavirus, Respirovirus, Morbillivirus, Henipavirus, and Avulavirus. The genus Avulavirus currently comprises 11 unique serotypes of avian paramyxoviruses classified as APMV-1 to APMV-11 according to the antigenic affinity in serological assays. The last two strains of the virus were recently isolated. Thus a strain, classified as APMV-10, was isolated from Adelie penguins (*Pygoscelis adeliae*) from King George Island in the Antarctic region and from rockhopper penguins (*Eudyptes chrysocome*) in the Falkland Islands. More recently, an additional strain, denominated APMV-11, was isolated from the common snipe (*Gallinago gallinago*).

Newcastle disease (ND) is an infectious and highly contagious disease produced by APMV-1, a pleomorphic, roughly spherical enveloped virus with one large molecule of single stranded RNA. Two glycoproteins of the projections on the viral surface possess both hemagglutinin and neuraminidase activities. Avian PMV-1 can remain infectious in the bone marrow and muscles of slaughtered domestic poultry for up to 6 months at -20°C and it may survive in dried feces for several years. This serotype is known to infect at least 241 avian species belonging to 27 orders, including different falcon species and falcon hybrids. Newcastle disease viruses form the serogroup APMV-1, but cross reaction with other serotypes, in particular APMV-3, has been reported. In an early study, the binding pattern of a selected group of monoclonal antibodies was used to position APMV-1 isolates into specific antigenic groups. More recently, phylogenetic analyses of a partial nucleotide sequence of the fusion protein gene classified all APMV-1 isolates into 6 lineage groups. Isolates originated from falcons from the Kingdom of
Saudi Arabia (KSA) and the United Arab Emirates (UAE) have been positioned in lineage 4, sublineages 4a and 4c. The highly variable virulence of the different strains is dictated by the amino acid sequence of the F and HN viral proteins and the type of proteases present in the host for cleavage of the protein precursor. The virulence of the strains is measured as a neuropathic index (NI), established by intracerebral inoculation of day-old chicks.

While APMV-1 produces severe and very often fatal disease in some bird species, only mild clinical signs are seen in others. In the domestic fowl, APMV-1 strains have been classified according to the type and severity of the clinical symptoms and pathologic lesions produced. This includes viscerotrophic velogenic, neurotropic velogenic, mesogenic, lentogenic, and asymptomatic enteric forms. It has been suggested that this classification format cannot be used for any other species because the viral strains vary in virulence and clinical symptoms produced in the different avian hosts. The incubation period of APMV-1 is between 3 to 28 days depending on the susceptibility of the host, the virulence of the infecting strain, and the dose of the virus to which the bird is exposed. Mortality rates in domesticated, and in some non-domesticated species infected with velogenic strains, can reach up to 100% within two weeks.

In the Middle East, ND is an important viral disease among different falcon species and falcon hybrids used in the traditional sport of falconry. Falcon species affected includes gyr falcons (*Falco rusticolus*), saker falcons (*Falco cherrug*), peregrine falcons (*Falco peregrinus*), and lanner falcons (*Falco biarmicus*) as well as hybrid falcons.
including mainly gyr-saker falcons and gyr-peregrine falcons. All species appear to be equally susceptible. Newcastle disease was first diagnosed and confirmed through virus isolation in falcons in the Middle East in 1992. Veterinary hospitals in the UAE reported the deaths of more than 150 falcons during 1990 – 1995. During two falconry seasons (September to March) at a falcon medical facility in the KSA, 64 of 4,516 falcons (1.4%) presented for examination were diagnosed with ND infection. The incidence of ND in falcons in the Middle East appears to vary from country to country and from year to year. Similar observations have been made with other viral diseases in the region, in particular with avian pox infections. This could be associated with variations in falconry practices from country to country and fluctuating illegal imports of domesticated and non-domesticated avian species from countries such as Pakistan, Iran, and Iraq. Observations made throughout the years suggest that ND in falcons in the Middle East may also be seasonal in occurrence as it is more often seen at the end of summer (September – October). This could be related to seasonal changes in environmental conditions or simply reflect the time of the year when the number of falcons is higher or when falcons are more often handled for the sport of falconry, as this coincides with the beginning of the training season. In domesticated species, no seasonal peak of ND has ever been described, even though high environmental temperatures has been suggested as possible triggering factor of the disease. Avian PMV-1 is shed from infected birds in all secretions but mainly in respiratory excretions and in feces. Falcons are commonly infected directly with APMV-1 through feeding on infected domesticated pigeons, coturnix quail, and, more rarely, poultry, or indirectly by being housed in close proximity to infected birds or by fomites. Aerosolized
fecal dust and contaminated substrate is considered a potential indirect exposure to APMV-1. The incidence of ND disease amongst falcons and falcon hybrids in the region could also be attributed to the widely used and deeply-rooted traditional falconry practice of using domestic pigeons during training practices.

In this report, the clinical and pathological findings associated with ND in falcons in the Middle East are reviewed, and a classification format of the disease based on the predominant clinical symptoms and pathologic lesions observed in falcons is proposed. In addition, results of vaccination trials using commercially available oil-emulsion inactivated vaccines conducted in the region are also presented.
Clinical Findings in Falcons with Newcastle Disease

Clinical Signs

Based on clinical signs at admission, two different clinical presentations producing distinct clinical symptoms and pathologic lesions can be identified in affected falcons, denominated as the neurotropic velogenic and the viscerotropic velogenic forms. In domesticated species, the neurotropic velogenic form produces respiratory and nervous signs such as leg and wing paresis, torticollis, circling, and tremors. Clinical signs associated with the neurotropic velogenic form of ND in falcons vary in type and severity but at an early stage include general nonspecific signs such as reduced to total absence of interactive and preening activity, loss of appetite, shredding and flicking of the food, regurgitation, and metallic-green colored urates. Signs progress rapidly to more specific central nervous system (CNS) symptoms such as dysphagia, tongue paresia, salivation, amaurosis, unilateral or bilateral paresia or paralysis of the third eyelid, hyperesthesia, clonic spasms, ataxia, unilateral or bilateral progressive leg paralysis, head tremors, fits and convulsions, or death. One of the most common clinical presentations is characterized by a cluster of CNS symptoms comprising hyperesthesia, clonic spasms, ataxia, and head tremors. In most cases, the symptoms develop and progress in a course of 12 to 24 hrs. In acute cases, the clinical signs can be present for 5 to 8 days before death, while in more chronic cases, in particular those with only leg paralysis, birds can survive for 12 to 15 days, and sometimes longer, with supportive therapy.
In domesticated species, the viscerotropic velogenic form of ND causes periorbital swelling and bloody diarrhea. Clinical signs associated with the viscerotropic velogenic form of ND in falcons also vary in type and severity but at an early stage commonly include general nonspecific clinical signs similar to those described above for the neurotropic velogenic form. These signs are followed by moderate to severe depression, mucoid-hemorrhagic diarrhea, or constant distressed vocalization, and death. In general, the symptoms develop and progress rapidly in 24 to 48 hrs. In acute cases, the clinical signs are present 3 to 5 days before death.

At one falcon veterinary facility, a total of 4,516 falcons were presented for examination over two 7-month periods between September 2002 to March 2003 and September 2003 to March 2004, corresponding to two falconry seasons. Of these, 64 (1.4%) falcons were suspected of ND infection. At the time of examination, 45 (70.3%) falcons had clinical signs of the viscerotropic velogenic form while 19 (29.6%) falcons had clinical signs of the neurotropic velogenic form. Newcastle disease was confirmed by virus isolation in 32 falcons submitted for necropsy.

Birds affected with the neurotropic velogenic form usually do not show any significant radiographic or endoscopic changes. However, radiographs obtained in 12 of 30 falcons (40%) affected with ND viscerotrophic velogenic form revealed a severely distended ventriculus and a distinctly inflamed ventricular wall. In some of these cases, inflammation of the intestinal tract was also evident. Endoscopic examination of the upper gastrointestinal tract of falcons affected with the viscerotropic form often shows
petechial hemorrhages across the isthmus of the proventriculus and across the ventriculus.\textsuperscript{21}

**Clinical Pathologic Findings**

In 57 falcons affected with APMV-1, hematologic test results revealed absolute heterophilia (84.2 ± 0.9 %). Total white cell count was within reference ranges.\textsuperscript{22} In most cases, the heterophils showed signs of toxicity (grades 3 and 4) including mild to moderate basophilic cytoplasm, moderate to severe loss of granulation and moderate loss of lobulation, or a left shift. These nonspecific hematologic responses appear consistent with a peracute to acute viral infection. In 4 falcons, a moderate to severe leukopenia (1.8 ± 0.45 x 10^9/l) and severe monocytosis (31.33 ± 8.3 %) was present,\textsuperscript{22} consistent with a more chronic course (> 5 days). The collection of antemortem samples to confirm the diagnosis is possible but in most cases impractical because of the rapid course of the disease. However, when an antemortem diagnosis of APMV-1 infection in birds is required, the diagnosis can be confirmed by virus isolation and, to a lesser extent, serologic testing.\textsuperscript{6,8,10,13}

**Postmortem Findings**

No pathognomonic gross postmortem findings appear to be associated with APMV-1 infection in avian species.\textsuperscript{6} In poultry, some authors describe the presence of typical petechial hemorrhagic lesions in the proventriculus and the ventriculus,\textsuperscript{9} while others consider the occurrence of such hemorrhagic lesions most significant in the small intestine.\textsuperscript{6}
In one study, 32 of 64 falcons suspected of ND infection were submitted for necropsy. Postmortem findings of falcons with viscerotropic velogenic ND infection, and in which radiographs revealed a distended ventriculus and inflamed ventricular wall, revealed moderate to severe petechial hemorrhages mainly on the isthmus of the proventriculus and across the ventriculus. Microscopic lesions were not considered specific or are of any diagnostic importance.

Postmortem findings in falcons with neurotropic velogenic ND included edema, hyperemia, and necrosis in the brain, heart, liver, pancreas, and kidneys. Non-septic encephalomyelitis was seen in some cases while mild to severe demyelinization in the brain was also a common pathologic finding.

**Preventative Vaccination**

Vaccination against APMV-1 is an integral component of preventative medicine programs of falcons maintained in captivity. Live vaccines have been used in falcons in addition to other non-domesticated avian species, however cell-mediated immunity alone may not provide adequate protection against virulent strains of APMV-1. Inactivated oil-emulsion ND vaccines have been used successfully in the poultry industry offering long-term immunity without undesirable effects.

**Vaccination trials**
In 2003, a vaccination trial was conducted in a group of 15 unvaccinated adult female saker falcons (*Falco cherrug*) using a commercially available oil-emulsion inactivated ND vaccine (Imopest, Merial, Lyon, France). All birds tested negative for the presence of APMV-1 antibodies before the vaccine trial. A total dose of 0.3ml/kg SC of the vaccine was administered to all falcons. Blood samples were collected from all falcons at 0, 7, 30, 60, 90, 120 and 180 days after vaccination for serologic testing by ELISA (Newcastle Disease Virus Antibody Test, Svanovir NDV-Ab, Svanova Biotech AB, Uppsala, Sweden) and expressed in percentage of inhibition (PI). Falcons vaccinated with the inactivated ND vaccine showed a PI of 74 ± 2.1 (mean ± SD) at day 7 after vaccination, and by day 30 the PI had reached 89.8 ± 0.3. The PI value was maintained at 89.7 ± 1.0 by day 180 after vaccination. Additional sampling after vaccination was not possible because all falcons participating in the study were used in the sport of falconry at the end of the vaccine trial.

A second vaccine trial was done in a group of 38 juvenile (6 to 10 weeks) gyr-peregrine hybrid (*Falco rusticolus – Falco peregrinus*) falcons by using a commercially-available oil-emulsion inactivated vaccine (Talovac 105 ND, Lohmann Animal Health, Cuxhaven, Germany). The birds were maintained indoors before vaccination. Antibody response was monitored for up to 45 days after vaccination. In addition, the yolks of 15 infertile eggs laid by falcons vaccinated with the same vaccine 5 months previously was also tested. One group of vaccinated falcons was tested at 14 - 29 days, while the second group was tested at 30 - 45 days after vaccination. The mean PI value of vaccinated falcons at 14 - 29 days after vaccination was 54.5 ± 13.0, while the mean PI value at 30 -
45 days was 67.0 ± 13.8. Two falcons failed to seroconvert. All eggs showed positive results with a mean PI value of 84 ± 10.4, demonstrating vertically transmitted immunity in the gyr-peregrine hybrid falcons.²⁶

The results of these vaccine trials indicate that the use of commercial oil-emulsion inactivated APMV-1 vaccines have a role to play in preventative medicine programs in captive falcons. No adverse reaction to this type of vaccine was observed in any of the falcons participating in the described studies.²⁶ Vaccination is recommended as soon as falcons are imported into a region or as soon as they finish the molt and start training. This is common in all countries in the region where falconry is practiced and is usually done between mid-August to mid-September.¹⁵

**Discussion**

The number of falcons affected with the viscerotropic velogenic form of ND appears to be higher than those affected with the neurotropic velogenic form.¹⁷ The clinical symptoms associated with the viscerotropic velogenic form of ND may not be easily recognized by attending clinicians and could represent a challenge in establishing a correct diagnosis. In domestic species, the viscerotropic velogenic form of ND causes periorbital swelling and bloody diarrhea,¹⁰ while the neurotropic velogenic form produces respiratory and neurologic signs including leg and wing paresis, torticollis, circling, and tremors.¹⁰ Periorbital swellings typically are not observed in affected falcons with the viscerotropic velogenic form of ND nor are respiratory signs observed in falcon affected with the neurotropic velogenic form of ND.¹⁷
The radiographic observations of ventricular dilatation and thickening of the ventricular wall are very seldom mentioned in the literature.\textsuperscript{17} Similarly, the occurrence of petechial hemorrhages in the proventriculus and ventriculus are common findings in poultry affected with ND; however, but this finding has been very seldom described in falcons.\textsuperscript{17,21}

Results of the vaccine trials conducted in the region indicate that most participating falcons develop and maintain an adequate level of immunity up to 180 days after vaccination. A high humoral response (PI = 74 ± 2.1) was observed in the group of saker falcons vaccinated against ND at day 7 after vaccination.\textsuperscript{17} Slightly lower, but a positive humoral response nevertheless (PI = 54.5 ± 13.0), was detected in 3 gyr-peregrine hybrid falcons 14 days after vaccination.\textsuperscript{26} Why the humoral response was different in the two trials is not known, but factors such as species differences in humoral response or slower vaccine absorption due to the diluents used in the two different vaccines used in the trials may have influenced response. The results of the vaccination trials are nevertheless comparable to the 6 to 10 day period required in poultry to obtain detectable levels of serum antibodies.\textsuperscript{6} Further vaccine trials using commercially-available inactivated vaccines and follow-up of the humoral response in vaccinated falcons for a full calendar year are essential to determine efficacy. Interestingly, adult female falcons vaccinated against ND are able to lay eggs that exhibit high levels (PI, 84.0 ± 10.4) of detectable antibodies.\textsuperscript{26} This is a relevant finding with practical application to captive breeding programs in the region.
Extensive public awareness campaigns, using graphic and photographic pedagogic exhibits displayed at the reception areas of falcon medical facilities, together with pictorial and informative bilingual brochures promoting annual vaccination against ND would ensure that falcons are adequately protected against this relatively common and fatal disease in the region.

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