



## CASE REPORT

# Concurrent avian pox and Newcastle disease infection in a Houbara bustard (*Chlamydotis undulata*)

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## SUMMARY

A case of acute respiratory disease was observed on a 3-month-old Houbara bustard (*Chlamydotis undulata*) at the National Wildlife Research Center (NWRC) breeding unit. Newcastle disease (ND) and avian pox viruses were isolated from the lungs, spleen and trachea of the affected bird. A heavy secondary bacterial infection was also noticed. The clinical and pathological findings observed in this case are described. It is the first description of the isolation of ND virus from a Houbara bustard. The epidemiology of the disease in the breeding flock is discussed.

Newcastle disease (ND) and poxvirus infections are worldwide in distribution, and birds of all ages are susceptible. According to Kaleta & Baldauf (1988), in addition to domestic avian species, natural or experimental ND infection has been demonstrated in at least 236 species from 27 orders. The host spectrum of ND is broad, but the clinical expression of the infection varies among groups of birds and with virus strains. Avian pox infection has been reported in approximately 60 species of wild birds representing about 20 families (Kirmse, 1967). The Houbara bustard (*Chlamydotis undulata*) has been classified as "vulnerable", by the IUCN (World Conservation Monitoring Centre, 1992). At the NWRC of Taif, Saudi Arabia, captive breeding of Houbara bustard was initiated in 1986, with the aim of restoring wild populations. This paper reports a case of concurrent avian pox and ND infection in a captive born juvenile Houbara bustard. It also describes the first isolation of NDV from a Houbara bustard.

In April 1989, the Houbara bustard flock comprised 53, 104 and 22 birds of respectively 3, 2, and 1 year of age. Additionally, there were 36 birds of unknown age. The sex ratio was 1:1. Birds were housed in individual cages. Each cage measured 6 × 4 × 2.3 m, and consisted of an 80 cm high block wall, on top of which was fixed a tubular metal frame covered with a 5 × 5 cm square mesh to

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form a cage. The breeding unit was not isolated from potential infectious agent carriers (insects, passerines and rodents).

During July 1989, a 3-month-old houbara chick displayed symptoms of a sudden disease. Clinical signs included very poor appetite, depression, ruffled feathers, dehydration and slight ocular and buccal discharges. Pseudomembraneous lesions, and bleeding erosions in the buccal cavity were observed. Antibiotic therapy with tylosin (35 mg/kg, Tylan 50 Injectable, Elanco) and an oral rehydration with normal saline solution were carried out. The bird died within 2 days of first developing signs.

Postmortem examination was performed within 2 h of death. Fat deposits on abdominal organs and in the thoraco-abdominal cavity were consistent. Twenty-one diphterical lesions were counted on the mucous membranes of buccal cavity, and 14 ulcers on the mucous membranes of the oesophagus. Thoraco-abdominal organs were generally congested. The most striking lesions were found in the upper and lower respiratory tract, in liver and spleen. The proximal trachea was severely congested, and diphteric lesions and haemorrhagic spots were present. Lungs were highly congested with areas of hepatization in the right lobe. Liver was swollen, congested and friable containing multifocal pale spots of necrosis. Similar lesions were observed in the spleen.

Specimens were collected from various organs, fixed in 10% neutral-buffered formalin, processed according to standard techniques and stained with haematoxylin and eosin. Swabs were taken from heart, lung, kidney, liver, spleen, trachea, duodenum, pancreas, caecum and oesophagus. They were inoculated on to blood agar and MacConkey agar, and incubated aerobically for 72 h. Bacteria were identified based upon growth characteristics on various media and biochemical profiles. Poxvirus isolation were attempted from buccal cavity mucous membranes, trachea and oesophagus tissues. Submitted samples for virology (lungs, spleen, brain and liver) were finely minced and added to a buffered lactose peptone solution (pH 7.4) containing penicillin (100 IU/ml) and streptomycin (100 mg/ml). They were frozen at  $-30^{\circ}\text{C}$ . Samples were inoculated onto the chorio-allantoic membrane (CAM) of 10-day-old embryonated chicken eggs.

Histology sections were examined. The heart was hyperaemic and haemorrhages were present. A slight degeneration of the myocardium was noticed. The kidneys showed a gutty hyaline degeneration of the tubuli, and large swollen glomerula with thickened homogenized walls. The lungs were highly congested, with focal solidification, and infiltrating mononuclear inflammatory cells. Phlebitis with thrombi were seen. The liver was congested. Slight perivascular infiltration of mainly mononuclear cells with disseminated necrosis of hepatocytes and increased numbers of intrasinusoidal cells was observed. The spleen showed a severe depletion of lymphocytes, and a necrosis of reticulum cells. The oesophagus showed focal necrosis infiltrated with mononuclear inflammatory cells within the epithelium. A typical ballooned degeneration of the epithelium of oesophagus and trachea was noticed, but no clear-cut Bollinger bodies. No lesions were visible in the digestive tract and the pancreas.

CAM inoculated with the tracheal sample presented proliferative focal pock

lesions. Pox virus isolation was confirmed by electron microscopy. A 10% suspension of infected CAM was prepared in Hank's balanced salt solution, blended and centrifuged at 200 *g* for 30 min, and the supernatant centrifuged at 35,000 *g* for 20 min. The resulting pellet was resuspended in distilled water, negatively stained in 2% phosphotungstic acid, and examined with an electron microscope. Numerous pox virions were observed.

The embryonated chicken eggs died 24 to 36 h after inoculation with lung and spleen samples, the embryos exhibiting marked congestion. The allantoic fluids were harvested and tested in standard hemagglutination inhibition (HI) test with antisera against six representative strains of paramyxoviruses. The isolate was identified as NDV by the haemagglutination-inhibition test using inactivated monospecific anti-NDV serum. Haemagglutination titrations and HI tests were performed as described by Cottral (1978).

Concurrent bacterial infection was present. Organisms isolated included *Pseudomonas aeruginosa* strain P3 from liver, heart, kidney and oesophagus; *Escherichia coli* serotype O78K80 from heart, kidney, caecal contents and spleen; D B-haemolytic *Streptococcus* sp. from kidneys, heart and oesophagus. *P. aeruginosa* was fully sensitive to neomycin and polymixin B. No bacteria were isolated from trachea, lung, duodenum and pancreas.

The pox disease described in the postmortem examination and confirmed by virus isolation was not the characteristic pox disease seen in many bird species (Gerlach, 1986). Histopathological involvements of classical cutaneous or diphteroid forms of the disease are loco-regional and rarely generalised (Gerlach, 1986). The same author described a septicaemic form of the disease, characterised by acute onset of general symptoms. In the genera *Amazona* and *Ara* this form can cause diphteroid enteritis and/or necrosis of the myocardium with or without cutaneous or diphteroid forms. No enteritis was observed, and only a slight non-specific degeneration of the myocardium was noticed in the dead bustard. Histological findings of the diphteric lesions observed in the present case differ, may be in part due to the high density of glands in the oesophagus and crop of Houbara bustard. An acute course of disease with sudden death was not typical of an avian pox infection, also associated histopathological findings were not usual for poxvirus disease and led us to suspect a combined aetiology.

The histopathology of NDV infections is as varied as the clinical signs and gross lesions (Alexander, 1991). Hyperaemia and haemorrhages found in the blood vessels of many organs, regressive changes in the lymphopoietic system, consisting of disappearance of lymphoid tissue in the spleen were observed in the present case. These pathological findings have been described in ND affecting poultry (Alexander, 1991). *Enterobacteriaceae* do not belong to the natural flora of the Houbara bustard (Greth *et al.*, 1990). Additionally, no bacteria were isolated from lungs and trachea. This allows us to assume that they were only secondary infectious agents, probably precipitating death.

Avian pox has always occurred in the breeding unit as a mild, latent cutaneous infection. Expression in adults was mostly in the form of nodules on the legs or on the unfeathered parts of the head. In November 1989, we received at the

Center 48 adult and juvenile Houbara bustards freshly caught from the wild in Pakistan. Two adult birds developed symptoms affecting their central nervous system, inducing paralysis of legs, wings and torticollis. One bird died after 6 days and the other was killed. No respiratory symptoms were observed prior to the sudden onset of neurological signs. Although ND was strongly suspected, no virological isolations were carried out. These events motivated NDV isolation attempts on sentinel birds in the captive breeding unit. The virus was isolated from faeces of three asymptomatic juvenile and adult sentinel Houbara bustards. Concurrently, serological examination of sentinel bustards in the breeding unit showed that the virus propagated in the captive flock.

The probable source of the virus infection on the Houbara bustard breeding center might have been diseased broiler farms some 10 km distant where ND was endemic. Fomite transmission by visitors or by wild birds was assumed.

No ICPI or monoclonal antibody examination were carried out on the isolated NDV. Susceptibility of the Houbara bustard for ND is unknown. Nevertheless, a combined infection with pox virus and ND virus has led to an acute and fatal respiratory disease in a juvenile bustard. Since this episode, a general vaccination programme against ND was introduced. Further studies will show if Houbara bustards are natural reservoirs for the virus.

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## RESUME

**Infection combinée des virus de la maladie de Newcastle et virus variolique aviaire chez une jeune outarde houbara (*Chlamydotis undulata*)**

Un cas de maladie respiratoire aigüe observé au Centre National de Recherches pour la Faune Sauvage (NWRC) de Taïf, chez une outarde houbara âgée de 3 mois, est décrit. Le virus de la maladie de Newcastle ainsi qu'un virus variolique aviaire ont été isolés à partir du poumon, de la trachée et de la rate de cet oiseau. Une surinfection bactérienne fut également notée. Les symptômes cliniques ainsi que les principaux résultats de laboratoire sont précisés. Il s'agit du premier cas d'isolement du virus de la maladie de Newcastle chez l'outarde houbara. L'épidémiologie de la maladie de Newcastle dans l'élevage est décrite.

## ZUSAMMENFASSUNG

**Gleichzeitige Geflügelpocken- und Newcastle-Infektion bei einer Kragentrappe (*Chlamydotis undulata*)**

Ein Fall von akuter Atemkrankheit wurde bei einer 3 Monate alten Kragentrappe (*Chlamydotis undulata*) in der Zuchtstation des Nationalen Wildtierforschungszentrums in Saudi-Arabien beobachtet. Newcastle Disease (ND)- und Vogelpocken-Viren wurden aus den Lungen, der Milz und Trachea des erkrankten Tieres isoliert. Außerdem wurde eine schwere Sekundärinfektion festgestellt. Die bei diesem Fall ermittelten klinischen und pathologischen Befunde werden beschrieben. Dies ist die erste Beschreibung der Isolierung von ND-Virus aus einer Kragentrappe. Die Epidemiologie der Erkrankung in der Zuchtherde wird diskutiert.

## RESUMEN

**Infección simultánea con el virus viruela aviar y de la enfermedad de Newcastle en una avutarda (*Clamydotis undulata*)**

Se observó un proceso agudo respiratorio en una avutarda (*Clamydotis undulata*) de 3 meses de edad alojada en la unidad reproductora del National Wildlife Research Center (NWRC). Se aislaron virus pox y de la enfermedad de Newcastle de los pulmones, bazo y tráquea de dicha ave. También se observó una infección bacteriana secundaria intensa. Se describen los hallazgos clínicos y anatomopatológicos. Esta es la primera descripción del aislamiento del virus de la enfermedad de Newcastle en una avutarda. Se discute la epidemiología de la enfermedad en el grupo de aves reproductoras.