SEVERE OUTBREAK OF POX IN CAPTIVE HOUBARA BUSTARDS (*CHLAMYDOTIS UNDULATA UNDULATA*) IN MOROCCO

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KEYWORDS

Birds, Houbara Bustard, avian pox

ABSTRACT

A severe outbreak of avian pox in a private collection of houbara bustards in Morocco is reported. In a flock consisting of 1100 juvenile houbara bustards and 420 breeder birds, 483 (43.9%) juvenile and 8 (1.9%) breeder birds succumbed to pox between August and December 2004. Gross pathology revealed multiple diphtheroid lesions irregularly distributed across the oropharyngeal mucosa (wet pox) as well as broncho-pneumonia, pancreatitis and hepatomegaly (systemic pox). Four weeks after the first fatalities occurred, several birds also developed the cutaneous form of pox (dry pox). Histopathology revealed proliferative dermatitis, diphtheroid-necrotizing pharyngitis, proliferative broncho-pneumonia, and granulative pancreatitis with intracytoplasmatic eosinophilic inclusions (Bollinger bodies) in these organs as well as in renal tubular cells. Electron microscopy from both crusts and virus isolates demonstrated typical avipox virions. A CPE virus appeared on CEF-cells and also in 11 day-old embryonated eggs from pooled organ samples from 20 investigated houbara bustards.

Although biosecurity measures were immediately implemented, the disease spread through all juvenile holding pens. Crowding appeared to favour disease spread as only a few fatalities occurred in single housed birds. During this outbreak all 3 forms of pox were encountered, with diphtheroid and systemic forms occurring first, followed by the cutaneous form. The authors recommend prophylactic vaccination with a novel houbara bustard pox vaccine.

INTRODUCTION

Poxviruses are the largest and most complex of all viruses. Avian poxviruses are only distantly related at antigenic level to other poxvirus genera, and under natural conditions produce a disease only in avians. Although the genus avipoxvirus (APV) is divided into 10 defined species, many avian pox isolates are not clearly classified (Mayr 1993). All APVs are morphologically similar, but do exhibit varied host specifity, which is important in controlling cross-species infections.

Avian pox is a common viral disease of domestic and free-living birds, which occurs worldwide. The disease has been reported in more than 70 species representing 20 families. A severe outbreak was recently reported in 15-week-old layer pullets (Anonymous 2004) which had developed the cutaneous “dry pox” and the oropharyngeal fibronecrotic “wet pox” or diphtheroid pox form.

Avian pox is a relatively slow-spreading viral disease that is characterized by the development of 3 different forms. The virus may cause cutaneous, diphtheroid or systemic lesions. The cutaneous and the diphtheroid or wet forms have been described in houbara bustards by Samour et al. (1996) and Wernery et al. (2007) and the systemic form has also been reported, causing severe losses (Bailey et al. 2000). APV-infections have also been reported in other bustard species, with Seidel (1995) previously describing the diphtheroid and cutaneous forms in the great bustard (Otis tarda).

The avipoxvirus is spread by biting insect vectors or by direct contact with infected birds or their fomites. Poxviruses cannot pass intact skin and must enter the body through abrasions or cuts. In most cases, mosquitoes serve as mechanical vectors for APV, passing it from bird to bird during each successive feeding. The skin form rarely develops into a fatal disease but the diphtheroid and systemic forms are often associated with fatalities, commonly associated with the involvement of secondary opportunistic pathogens.

MATERIAL AND METHODS

The pox outbreak occurred in the Houbara Bustard Breeding Centre in Agadir, Morocco in August 2004. The flock consisted of 1100 juvenile houbara bustards aged between 2 and 7 months, and 420 breeder birds. The juveniles were housed in groups according to their age in different outside holding pens and locations within a perimeter of 18,000sq meters. The breeding flock was also housed within this perimeter, yet within single cages.

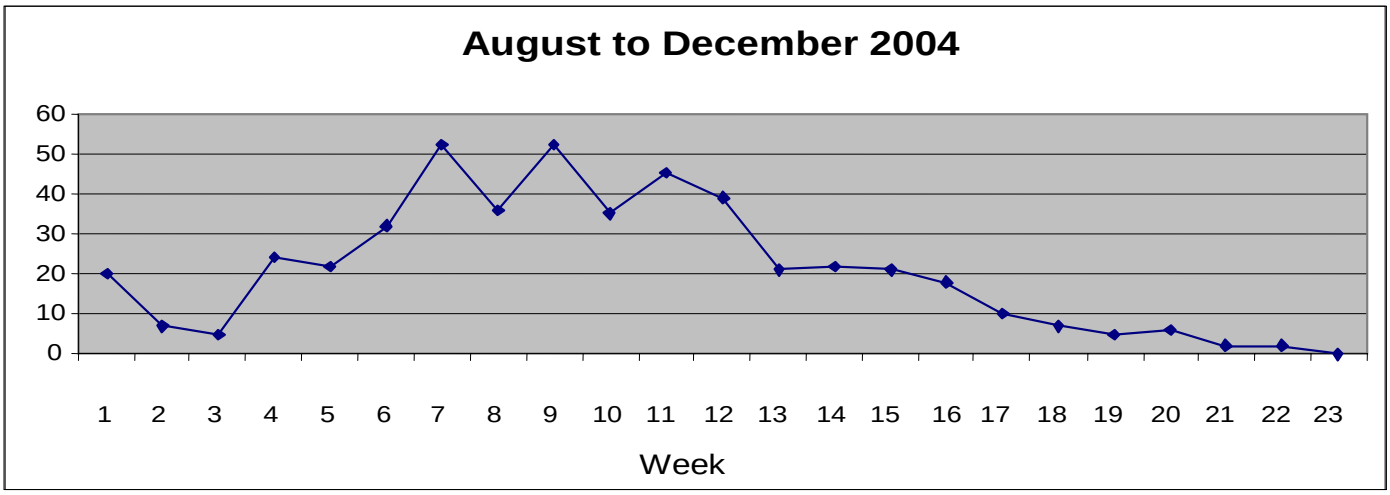
At the beginning of August 2004, five 3-month-old birds were found dead in one pen, and by the end of December, 483 juvenile and 8 breeder birds had succumbed to the disease. Out of 483 juvenile birds 92 developed the cutaneous form, 94 the diphtheroid form and 297 the systemic form. Breeder birds only developed the systemic form. Most of the birds were necropsied at the Agadir Houbara Breeding Centre, and 18 juveniles and 2 adults were examined at the Central Veterinary Research Laboratory (CVRL) in Dubai using routine virological, bacteriological, mycological, pathological and parasitological test procedures.

Dried crusts from thigh and periorbital lesions of 2 houbara bustards were sent to the Institute for Medical Microbiology, Infectious and Epidemic Diseases in Munich, Germany for electron microscopic examination. Samples of all internal organs, including brain and pharynx, of each of the 20 necropsied birds were pooled, cut into small pieces, and tested for viral growth according to Kaaden et al. (1996). In brief, the samples were placed into a mortar containing 10ml of Minimum Essential Medium (MEM) with antibiotics. The suspension was ground with a pestle and sand for 5min, and centrifuged at 1600g for 15min. One millilitre of supernatant was added to 4ml of MEM and filtered through a bacterial 0.45µm membrane filter. One millilitre of the filtrate was then applied onto a chicken embryo fibroblast primary cell culture (CEF). The isolated strains were dispatched to the Avian Virology in Weybridge, England for identification. Upon receipt of the isolated viral agents, samples were passaged in 9 to 10-day-old SPF chicken eggs inoculated by allantoic and chorio-allantoic membrane routes, chick embryo liver (CEL) and chick embryo fibroblast (CEF) cultures.

RESULTS

The pox outbreak lasted 5 months during which 43.9% of the total number of juvenile (Table 1) and 1,9% of the adult houbara bustards died.

Table 1: Weekly mortality (number) of juvenile houbara bustards caused by pox



Gross pathology revealed multiple yellow/cream-coloured, raised necrotic lesions, 4-8mm in diameter, irregularly distributed across the oropharyngeal mucosa yet primarily at the base of the tongue (Figure 1). Thirty days following the onset of disease, the oropharyngeal lesions had developed into necrotic, moist ulcers. Additionally, the houbara bustards had a focal broncho-pneumonia with greyish areas (Figure 2), a swollen, haemorrhagic and enlarged pancreas and hepatomegaly. Gross pathological details of 20 houbara bustards

Figure 1: Diphtheroid pharyngitis in a houbara bustard with wet pox .

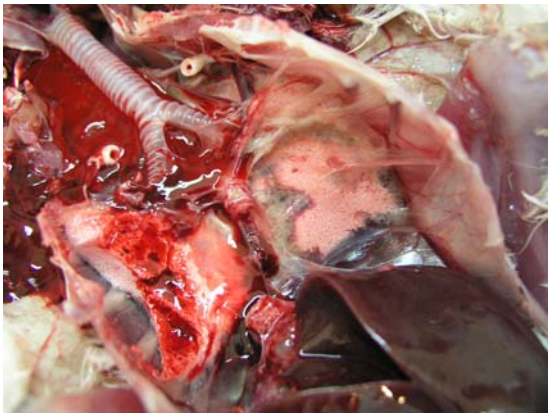


Figure 2: Focal broncho-pneumonia (greyish areas) in a houbara bustard with systemic pox.

necropsied at CVRL in Dubai are shown in Table 2.

Histopathological examinations revealed the following abnormalities: diphtheroid-necrotizing inflammation of the oropharyngeal area; proliferative broncho-pneumonia with cells containing eosinophilic masses; granulative pancreatitis with large necrotic areas and numerous cells containing intracytoplasmatic eosinophilic inclusions (Bollinger bodies, Figure 3); marked interstitial hepatitis with accumulation of heterophils. Numerous intracytoplasmatic eosinophilic inclusions were also found in renal tubular cells (Figure 4).

Table 2: Necropsy findings in 20 houbara bustards, which died from internal pox (diphtheroid and systemic)

Lesions	No	%
Oropharyngeal lesions	11	55
Hepatomegaly	15	75
Pericarditis	7	35
Abdominal congestion	4	20
Pancreatitis	12	60
Splenomegaly	8	40
Focal areas of pneumonia	6	30
Airsacculitis	5	25
Pulmonary congestion	6	30

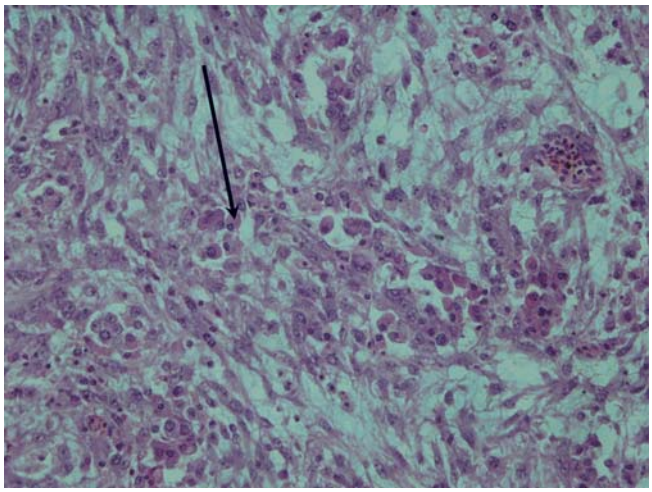


Figure 3: Photomicrograph of the pancreas with granulative pancreatitis showing cells containing intracytoplasmatic eosinophilic inclusions (HE-stain).

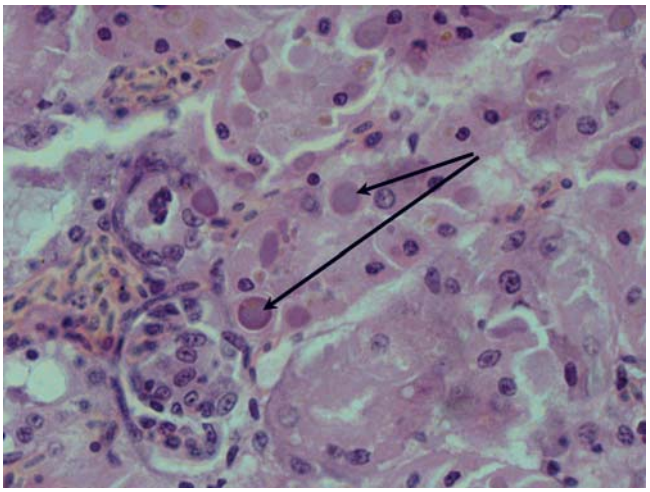


Figure 4: Photomicrograph of numerous renal tubular cells containing Bollinger bodies (HE-stain).

Four weeks following the original fatalities, 92 birds also developed the cutaneous pox form characterised by the appearance of vesicles, ulcers and crusts around the periorbital area (Figure 5), the inner side of the thighs and the underside of the wings where no or only few feathers were present.

At the beginning of the outbreak, Trichomonas sp. was microscopically detected in oropharyngeal samples. Following treatment with Metronidazol and Ronidazole, the birds became negative for Trichomonas sp. but the lesions remained. Furthermore, several pathogenic bacterial species were cultured from the pharyngeal lesions and various internal organs. These included Pseudomonas aeruginosa, Clostridium perfringens, Staphylococcus sp., Escherichia coli and Salmonella sp. Also Aspergillus fumigatus was isolated from 15 airsacs and lungs of 123 necropsied birds. Coprological investigations failed to reveal any parasites or eggs.

Crusts from the thigh and periorbital areas, which were examined electron microscopically, demonstrated typical avipox virions. From all 20 houbara bustards necropsied at CVRL, a CPE virus appeared on CEF from pooled organ samples 5 days following inoculation. Ten viral strains were sent to the Avian Virology Department in Weybridge, England and identified by electron microscopy as poxvirus. No CPE was observed on the inoculated CEL cultures, however, an agent producing CPE was isolated on CEF cells and also in 11-day-old embryonated eggs. The agent resulted in the development of a solid oedematous thick chorio-allantoic membrane around the inoculation site with pronounced injection of vessels (Figure 6).

DISCUSSION

Out of 1100 juvenile houbara bustards 483 (43,9%) died within 5 months from avian pox, and 8 adult breeding birds out of 420 (1,9%) also succumbed to the disease. Out of 483 juvenile birds 92 (19%) birds developed the cutaneous form, 94 (19,5%) the diphtheroid form and 297 (61,5%) the systemic form. Breeder birds only developed the systemic form.

The juvenile birds were housed in holding pens comprising of 15 to 20 birds per pen. The adult birds were housed in single cages. Although birds from the pen where the first fatalities occurred were immediately isolated in clinical wards and biosecurity measures put

into place, the disease spread through all juvenile holding pens. It is very obvious that crowding favoured the spread of the virus either through close contact and/or vectors such as mosquitoes, as there were only few fatalities in single housed birds. Prior to the outbreak it had rained for several days. Although it was not possible to trace the source of the virus, it is possible that transmission occurred via arthropod vectors and wild birds, in particular pigeons, which had indirect contact with the houbara bustards through ceiling netting.

Figure 5: Ulcerative lesions on the periorbital area caused by poxvirus

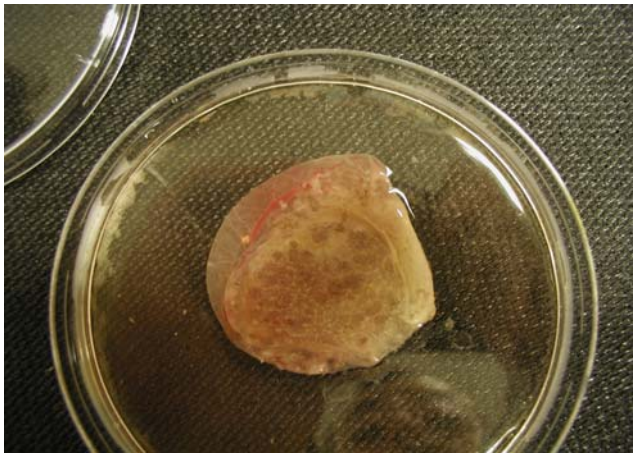


Figure 6: A pock-lesion caused by the systemic pox virus on the chorio-allantoic membrane of an 11 day-old chicken embryo

On this houbara bustard-breeding farm all 3 forms of pox were encountered. It is worthwhile mentioning that only 4 weeks after the first fatalities occurred through systemic pox, the cutaneous pox form appeared. The cutaneous form, which is characterized by the appearance of nodular lesions on various parts of unfeathered skin, probably results from infection by biting arthropods. In contrast to the greater anticipated likelihood of the cutaneous form appearing first, the diphtheroid and the systemic forms occurred first in this outbreak. The diphtheroid form is characterized by lesions on the mucous membranes of the mouth, nares, pharynx, larynx, oesophagus and trachea, and probably results from aerosol-derived infection. Tracheal lesions can result in breathing difficulties, with symptoms resembling those of infectious laryngotracheitis (ILT), trichomonosis or vitamin A deficiency (Wernery et al. 2004). The systemic or septicaemic form of the disease occurs in the absence of cutaneous lesions and is therefore difficult to diagnose unless a detailed necropsy is performed. In this outbreak, the diphtheroid and systemic forms of the disease were always observed together. It is believed that the diphtheroid form is probably caused by aerosol infection, which may subsequently develop into the septicaemic form.

The categorisation of APVs has largely been based upon the species of birds, which they infect, with some strains infecting only one avian species and others infecting several species. The antigenic relationship between poxviruses isolated from various avian species including houbara bustards, falcons and stone curlew remains undetermined. Consequently, it is unknown if commercially available pox vaccines derived from other species may afford protection to houbara bustards. Ostrowski et al. (1996) demonstrated in a cross-protection experiment that canaries vaccinated with a commercial canary poxvirus vaccine remained healthy when challenged with a cutaneous houbara bustard poxvirus strain isolated from an outbreak. Wernery et al. (2007) established a novel houbara bustard attenuated pox vaccine, which is currently used in Moroccan and Dubai-birds. Future work on houbara pox strains isolated by CVRL will be subjected to genetic sequencing and by phylogeny to determine the antigenic relationship to each other and to other avian poxvirus.

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