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Current and emerging diseases in a captive flock of adult houbara bustards (Chlamydotis macqueenii).

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ABSTRACT

Houbara bustards are traditional game birds of Arab falconers. In response to the marked population decline of Chlamydotis macqueenii in Saudi Arabia, a captive-breeding programme was initiated in 1986 at the National Wildlife Research Center, Taif, Saudi Arabia, with the purposes of reintroducing this species into its former habitat. The project has benefited from continuous veterinary support since its beginning. We present the morbidity and mortality results observed in the NWRC flock between 2000 and 2003, and discuss the emergence of two new sources of mortality, i.e. mycobacteriosis and neoplasia.

Each year, 12.6% of the flock required a veterinary intervention for diagnosis and treatment or necropsy. The mean annual mortality rate reached 6.6%. Trauma affected 7.9% of the flock, resulting in death/euthanasia of 3.6% of the flock. Every year, 4.7% of the flock required veterinary assistance for non traumatic disorders including infection, anorexia, old-age impairments or metabolic disorders. Mycobacteriosis and neoplasia were together responsible for the death of 0.8% of the flock in 2003. We report two clinical cases of mycobacteriosis consisting of peri-orbital necrotico-granulomatous nodular lesions combined with pulmonary, intestinal and hepatic lesions containing acid-fast bacilli. A Mycobacterium avium complex specimen differing from Mycobacterium avium paratuberculosis was identified. The extent to which the flock is affected by the disease is unknown, but PCR-RFLP screening of contact-birds so far failed to detect new cases. Neoplasia comprised sarcoma, carcinoma, and lymphoproliferative disease. Several affected birds were directly related or siblings. The etiology of these neoplasia remains to be elucidated.

INTRODUCTION

Houbara bustards belong to the genus Chlamydotis and are traditional game birds of Arab falconers. Two species are currently recognized with Chlamydotis macqueenii and Chlamydotis undulata which comprised two subspecies Chlamydotis undulata undulata and Chlamydotis undulata fuertaventurae (Broders et al, 2003). Inhabiting arid plains and semi-deserts, the Asian houbara bustards C. macqueenii ranges from the Gobi desert to the Nile Valley whilst the African houbara bustards C. u. undulata ranges from North Africa to the Nile Valley. C. u. fuertaventurae inhabits the Canary Islands.

Adult houbara bustards are medium-sized bustard of slender appearance, measuring 55-65cm and weighing 900g to 2600g. They achieve adult size by two to six months respectively for females and males and can breed from the first spring, i.e. before one year-old. Life-span of wild houbara bustards is unknown. The oldest captive houbara bustards of the NWRC flock were 22 year-old at the end of 2004.
In response to marked population declines of *C. macqueenii* in Arabia, a captive-breeding programme was initiated in 1986 at the National Wildlife Research Center (NWRC), Taif, Saudi Arabia, with the purposes of reintroducing this species into its former habitat. The project has benefited from continuous veterinary support since its beginning.

The causes of mortality in the NWRC houbara captive flock were documented by Ostrowski & Combreau (1995) for the period between 1989 and 1994. Most the deaths were trauma-related. Among the infectious pathologies, chlamydiosis (Greth *et al.*, 1993a & 1993b), Newcastle disease and avian poxvirus disease were sources of mortality (Ostrowski *et al.*, 1995a & 1995b). In response, the NWRC Veterinary Department attempted to eradicate chlamydiosis in the NWRC flock by means of therapeutic and sanitary measures (Ostrowski *et al.*, 1996a), and developed a vaccination programme against Newcastle disease virus (Ostrowski *et al.*, 1998) and avian poxvirus disease (Ostrowski *et al.*, 1996b). Herein, we present the morbidity and mortality results observed in the NWRC flock between 2000 and 2003, and discuss the emergence of two new sources of mortality, i.e. mycobacteriosis and neoplasia.

**MATERIALS & METHOD**

**Birds**
The NWRC flock consists of 600 (in year 2000) to 1000 (from year 2003) adult houbara, individually monitored, including mainly Asian houbara bustards *C. macqueenii*, with some African houbara bustards *C. u. undulata* and some hybrids *C. macqueenii* x *C. u. undulata* obtained by artificial insemination according to Saint Jalme *et al.* (1994). Herein, «adult» refers to bustards that were not hatched during the year. The average age of the flock varied year to year between four and five years depending of the annual input of new young adults. At the NWRC, breeders lived in individual outdoor cages in two breeding units whilst discarded bustards were usually housed in outdoor collective pens located in other area away from the breeding units. The floor of each facility was covered with sand with no vegetation. Bustards fed mainly on dry pellets and water, with a supplement of live mealworms and fresh alfalfa leaves for the breeders. Every bustard is currently vaccinated against Newcastle disease virus and avian poxvirus disease and regularly dewormed using fenbendazole and niclosamide.

**Veterinary monitoring**
The flock was monitored daily and keepers report any abnormalities to the veterinarian who will act immediately and do the necessary investigations. In addition, as a routine, we examined every houbara once a year during the annual vaccination campaign. Every death was subjected to a necroscopic examination performed by veterinarians, and, when necessary, to histopathological examination as well as to bacteriological investigations. Herein, medical case refers to any (single or repeated) veterinary intervention for diagnosis and treatment of one sickness event or to necropsy. We recorded once findings from chronic pathology like lameness or permanent injuries such as healed fractures. We did not include in this report sperm abnormalities detected during the routine sperm analyses for insemination purposes. Gastro-intestinal parasitic infections currently affecting the flock were not regarded as medical cases.
Examination of faecal material for mycobacterial DNA

Fresh faecal samples were collected in sterile containers from 25 houbara bustards which had direct contact with the first individual dead of mycobacteriosis at the NWRC. In addition, cloacal swabs were collected in sterile containers from 50 other bustards accommodated in collective pens. Samples were preserved frozen at -70°C until submission to King Khalid Wildlife Research Centre (KKWRC) laboratories for molecular investigations. DNA was extracted from the faecal samples using QIAGEN faecal extraction kit (QIAGEN GmbH, Hilden, Germany). Extracted DNA was subjected to Polymerase Chain Reaction (PCR) using primers that amplify the 65 kDa heat shock protein gene (hsp65) according to Shinnick (1987) and Telenti et al (1993). PCR results were visualised after stained agarose gels and photographed. The PCR products were then digested using two restriction enzymes (HaeIII and BstEII). Depending on the restriction sites, different mycobacteria are diagnosed (Telenti et al, 1993).

RESULTS

Birds that succumbed before any veterinary intervention constituted nearly one third of the medical cases in the present study. Every year, 12.6% [9.1%-15%] of the adult bustards required a veterinary intervention for diagnosis and treatment or necropsy. When the rate of veterinary interventions increased, this was related to an increasing of the trauma cases. The mean annual mortality rate reached 6.6% [5.9%-7.5%] with traumatic injuries being the main cause of death (Fig.1, Fig.2 & Fig.3). They affected on average 7.9% [4.5%-10.7%] of the flock and are responsible for the death or euthanasia of 3.6% [2.4%-4.6%] of the flock. The majority of the trauma cases (60%) involved breeders. Traumatic lesions concerned mainly the rachis (44.4%) and the leg to foot (30.2%), and less often the skin (14.3%), the wing (7.1%) or internal organs (4%) (Fig.4). As ecchymoses in the calvarium were rarely indicative of head trauma, but were most frequently agonal pooling of blood within the skull (Ostrowski & Combreau, 1995), such postmortem findings of this category have not been included in this report. When such finding was not associated with other significant lesions, it was regarded as inconclusive necropsy. The “inconclusive necropsy” category also comprised post-mortem findings from decomposed houbara bustards.
Fig. 1. Prevalence of the pathologies in the NWRC flock

![Prevalence of Pathologies](image)

Birds not presented for sickness >87.3%

Fig. 2. Distribution of the medical cases according to pathology

![Distribution of Medical Cases](image)

Trauma 63.8%

Skin Cyst 0.3%

Anorexia 6.3%

Old age related pathology 4.3%

Dead birds with not conclusive necropsy 6.1%

Infection 8.1%

Metabolic disorder 3.8%

Neoplasia 2.5%

Genital Pathology 1.5%

Cardiopathy 1.0%

Ocular pathology 2.5%
Non traumatic pathology corresponded to 46.2% of the medical cases (Fig. 2). Every year, 4.7% [4.2%-5.8%] of the bustards required veterinary investigations for non traumatic pathology. Few birds presented metabolic disorders (0.4% [0.2%-0.8%] of the bustards annually). In four years, we recorded fatty liver syndrome (four cases), hypothermia after heavy rainfall in old debilitated birds (three cases), toxemia not related to digestive paralysis (three cases), visceral goutte (one case) and calcium metabolism disorder (one case).
Infection (excluding neoplasia) constituted 8.1% of the medical cases. They comprised buccal infection, leg infection, pericardio-peritonitis, peritonitis-hepatitis-splenitis, cardio-pulmonary infection, septicemia, or mycobacteriosis. We did not observe any signs of Newcastle or avian poxvirus diseases. Annual bird mortality rate due to infection constituted 0.75% [0.3%-1.2%] and they were mostly showing lesions associated with cardio-pulmonary infections. Such birds died before any veterinary intervention was possible. Infection due to *Mycobacterium* spp. appeared to be a new source of mortality. Two cases have been diagnosed and confirmed by demonstration of acid-fast bacilli both microscopically after Ziehl Neelsen (ZN) staining and histopathologically. Affected birds were males aged four and five years and both were hybrid houbara bustards.

- The first case displayed a supra-orbital subcutaneous 2cm³ cyst whilst the bird was in a very good body condition. After excision of the mass under anesthesia, histological examination revealed a granulomatous lesion composed of macrophages and giant multinucleated cells. Macrophages formed a palisade around small necrotic foci. Despite this peri-orbital lesion being evocative of a mycobacteriosis, ZN staining did not reveal acid-fast bacilli. The bird died six months later whilst emaciated. Gross lesions comprised ascites, hepatomegaly with numerous grayish-white ulcerative nodules of various sizes (Fig. 5) and digestive grayish-white nodular lesions. Histological examination revealed interstitial pneumonia, granulomatous and necrotic pleurisy with giant multinucleated cells, intestinal granulomatous lesions with giant multinucleated cells and severe lesions of multifocal necrotic granulomatous hepatitis with giant multinucleated cells. Histological examination revealed acid-fast bacilli in different organs. The detection of antibodies against *Mycobacterium* species in this case was unsuccessful. The detection of mycobacterial DNA through the Polymerase Chain Reaction (PCR), Restriction Fragment Length Polymorphism (RFLP) technique in formalin fixed liver and lung was negative. Results of DNA amplification of the hsp65 gene of the faecal samples from the 25 houbara bustards having had contact with the dead houbara gave positive PCR product in six cases (24%). Subsequent digestion of the PCR product resulted in a digestion pattern that is different from that detected in *M. avium* or any of the known *Mycobacterium* spp. One of the houbara bustard showing a positive PCR/RFLP faecal result was euthanised for lymphoproliferative disease and PCR/RFLP analysis of the organs and neoplasia was negative. Right now, none of the 24 other contact-birds displayed any suspected lesion.

- The second case presented an infra-orbital subcutaneous 2cm³ mass (Fig. 6) whilst in a good body condition. As soon as detected, the houbara bustard was euthanised and sampled. Histopathological examination of serial slides showed acid-fast bacilli in the liver, lungs and the digestive tract. The infra-orbital lesion consisted of a large subcutaneous granuloma which comprised macrophages and numerous giant multinucleated cells containing acid-fast bacilli, with several small necrotic foci and some calcifications. A *Mycobacterium avium* complex specimen different from *Mycobacterium avium paratuberculosis* was identified through bacteriological cultures and Coetsier *et al.* (2000) duplex PCR-RFLP protocol from the infra-orbital mass samples. The second hybrid houbara bustard presenting mycobacteriosis lesions was living in a collective pen where some houbara bustards had had contact with the first hybrid houbara bustard dead of mycobacteriosis. One year after the discovery of this second tuberculous case, none of the other houbara bustards housed in this pen showed suspected lesions. Two additional cases of granulomatous necrotic lesions were encountered in eight and nine years old female Asian houbara bustard (*C. macqueenii*), histopathological examination, however, did not reveal any acid-fast bacilli.
Results of DNA amplification of the hsp65 gene of the cloacal swabs collected from 50 houbara bustards accommodated in collective pens, gave positive PCR product in 16 cases (32%). Subsequent digestion of the PCR product resulted in a digestion pattern that is different from that detected in *M. avium* or any of the known *Mycobacterium* spp.

Fig.5. Mycobacteriosis liver lesions consisted of hepatomegaly with numerous grayish-white ulcerative nodules of various sizes (© Olivier Couppey, NWRC Photolibrary)

Fig.6. Mycobacteriosis infra-orbital lesions consisted of a 2cm³ granuloma with a necrotic core (© Olivier Couppey, NWRC Photolibrary)
The second new source of mortality consisted of neoplasia (Table 1). Between 2001 and 2003, clinical examination and necropsy revealed ten cases of neoplasia affecting mainly young houbara bustards (Table 1 & Table 2). Neoplasia consisted of liver lymphosarcoma, lymphoproliferative disease affecting at least liver, lung, spleen, kidney, subcutaneous sarcoma (including few differentiated sarcoma, wing composite osteosarcoma or hemangiosarcoma) with pulmonary metastasis. Several affected birds were directly related or siblings (Table 2).

Table 1. Number of neoplasia observed every year

<table>
<thead>
<tr>
<th>Period</th>
<th>Number of neoplasia</th>
<th>Size of the flock</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989-1994</td>
<td>3</td>
<td>250 to 428</td>
<td>unknown</td>
</tr>
<tr>
<td>1995</td>
<td>0</td>
<td>410</td>
<td>0</td>
</tr>
<tr>
<td>1996</td>
<td>0</td>
<td>398</td>
<td>0</td>
</tr>
<tr>
<td>1997</td>
<td>0</td>
<td>259</td>
<td>0</td>
</tr>
<tr>
<td>1998</td>
<td>0</td>
<td>313</td>
<td>0</td>
</tr>
<tr>
<td>1999</td>
<td>0</td>
<td>500</td>
<td>0</td>
</tr>
<tr>
<td>2000</td>
<td>0</td>
<td>571</td>
<td>0</td>
</tr>
<tr>
<td>2001</td>
<td>1</td>
<td>614</td>
<td>0.16%</td>
</tr>
<tr>
<td>2002</td>
<td>2</td>
<td>760</td>
<td>0.26%</td>
</tr>
<tr>
<td>2003</td>
<td>7</td>
<td>1050</td>
<td>0.67%</td>
</tr>
</tbody>
</table>
### Table 2. Neoplasia cases in captive-born houbara bustards at the NWRC from 2001

(■ siblings through the sir, ◇ siblings through the dam, ● ○ directly correlated).

*The last six neoplasia cases were diagnosed in 2004.*

<table>
<thead>
<tr>
<th>Bird Identification</th>
<th>Species</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Neoplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>NWRC98041 ■</td>
<td><em>C. macqueenii</em></td>
<td>♀</td>
<td>3</td>
<td>evolving lymphosarcoma of the right hepatic lobe</td>
</tr>
<tr>
<td>NWRC00075 Hybrid houbara bustard</td>
<td>♀</td>
<td>2</td>
<td>lymphoproliferative disease (tumoral infiltration of the lung, liver, kidney and spleen by lymphoid cells)</td>
<td></td>
</tr>
<tr>
<td>NWRC99037 ■</td>
<td><em>C. macqueenii</em></td>
<td>♂</td>
<td>3</td>
<td>metatarsal skin carcinoma</td>
</tr>
<tr>
<td>NWRC01190</td>
<td><em>C. macqueenii</em></td>
<td>♀</td>
<td>2</td>
<td>lymphoproliferative disease (tumoral infiltration of the liver, kidney, spleen and ovary by lymphoid cells)</td>
</tr>
<tr>
<td>NWRC99047</td>
<td><em>C. macqueenii</em></td>
<td>♀</td>
<td>4</td>
<td>lymphoproliferative disease (tumoral infiltration of the tongue, pharynx, oesophage, spleen, caecum, rectum and subcutaneous cervical lesions by lymphoid cells)</td>
</tr>
<tr>
<td>NWRC93222 ●●</td>
<td><em>C. macqueenii</em></td>
<td>♀</td>
<td>10</td>
<td>coccygian epidermoid carcinoma</td>
</tr>
<tr>
<td>NWRC01133 ■</td>
<td><em>C. macqueenii</em></td>
<td>♂</td>
<td>2</td>
<td>wing osteosarcoma, hemangiosarcoma with pulmonary metastasis</td>
</tr>
<tr>
<td>NWRC92034 ○</td>
<td><em>C. macqueenii</em></td>
<td>♂</td>
<td>11</td>
<td>breast deep sub-cutaneous few differentiated sarcoma</td>
</tr>
<tr>
<td>NWRC95032 ■</td>
<td><em>C. macqueenii</em></td>
<td>♀</td>
<td>10</td>
<td>wing (radius-ulna) osteosarcoma</td>
</tr>
<tr>
<td>NWRC93126</td>
<td><em>C. macqueenii</em></td>
<td>♀</td>
<td>10</td>
<td>wing (radius-ulna) composite osteosarcoma</td>
</tr>
<tr>
<td>NWRC01190 ●</td>
<td><em>C. macqueenii</em></td>
<td>♀</td>
<td>3</td>
<td>lymphoproliferative disease (tumoral infiltration of the liver, spleen, ovary, kidney by lymphoid cells)</td>
</tr>
<tr>
<td>NWRC87104 ○</td>
<td><em>C. macqueenii</em></td>
<td>♀</td>
<td>17</td>
<td>cholangiocarcinoma with ascites</td>
</tr>
<tr>
<td>NWRC02181</td>
<td><em>C. macqueenii</em></td>
<td>♂</td>
<td>2</td>
<td>myelo-lymphoproliferative disease (with leukemic manifestations and tumoral infiltration of the liver, spleen) with ascites</td>
</tr>
<tr>
<td>NWRC91028</td>
<td><em>C. macqueenii</em></td>
<td>♀</td>
<td>13</td>
<td>breast rhabdomyosarcoma with pulmonary metastasis</td>
</tr>
<tr>
<td>NWRC91022</td>
<td><em>C. macqueenii</em></td>
<td>♂</td>
<td>13</td>
<td>bilateral breast polymorphic sarcoma</td>
</tr>
<tr>
<td>NWRC02097</td>
<td><em>C. macqueenii</em></td>
<td>♀</td>
<td>2</td>
<td>lymphoproliferative disease (tumoral infiltration of the liver, spleen by lymphoid cells) with hydro-pericardium</td>
</tr>
</tbody>
</table>
DISCUSSION

The process of houbara bustards production of the NWRC is based on artificial insemination and artificial incubation. Van Heezik and Ostrowski (2001) reported two main mortality peaks during the houbara bustards production at the NWRC between 1992 and 1999. The first peak occurs during the incubation with 47% of the fertile eggs which do not hatch. The second peak of mortality occurs by six months of age and is followed by a steady slow attrition, resulting in a survival of 72.5% of the hatchlings by the end of the first year. This means that only 38 juveniles were obtained from 100 fertile eggs during this period at the NWRC. In comparison to the mortality during the first year of development of the houbara bustards, a mean annual mortality of about 6.6% in the adult flock could appear as minor mortality. However, this 6.6% mean annual mortality rate in the adult houbara bustards flock results in a reduction of the initial flock size of 50% after 10 years of life in captivity at the NWRC.

A high prevalence of morbidity or deaths that were related to trauma, were also reported in the captive bustards collection of the National Avian Research Center (Al Ain, United Arab Emirates) (Bailey et al. 1996a; Bailey et al. 1996b) as well as previously at the National Wildlife Research Center (Ostrowski & Combreau, 1995). Houbara bustards are long-leg birds displaying a large wing’s span. Capture and handling are required for regular insemination of the females and for the annual vaccination of the flock. Inappropriate capture or wrong handling result in trauma. Presence of air-borne or terrestrial predators around the cages or disturbance by visitors result in panicking reaction and birds tend to jump and can run on the walls of their cage/enclosure. Certain designs of cages also facilitate trauma when scared houbara bustards bit the rigid walls of their enclosure. To prevent trauma would require a global approach including developing tameness in the houbara from early age and reduction of any potential danger for the houbara like inappropriate human behaviours and practices as well as wrong cage design.

Comparing the causes of mortality at the NWRC during the previous years is not possible because young and adult houbara were mixed in Ostrowski & Combreau’s (1995) report. Despite attempts having been undertaken to eradicate chlamydiosis in the 1990’s, several post-mortem lesions could evoke chlamydiosis lesions described by Greth et al. (1993a). In contrast, the Newcastle disease and the avian poxvirus disease did not occur anymore at the NWRC since the implementation of vaccination using an avian poxvirus canary strain (Ostrowski et al., 1996b), and against the Newcastle disease virus which is endemic in the country.

Mycobacteriosis is a new source of mortality at the NWRC. Avian mycobacteriosis is a world-wide disease reported widely in pet birds, free-living birds, captive birds and poultry. This disease is diagnosed far more frequently in adult birds than in young birds. Slow progression of the disease from the time of inoculation when the bird was immature, is common in avian mycobacterial infections (Tell et al., 2001).

Avian mycobacteriosis can be caused by the Mycobacterium avium complex which comprises two species M. intracellulare and M. avium including M. avium avium, M. avium paratuberculosis and M. avium sylvaticum (Tell et al., 2001). The M. avium complex specimen found in the hybrid houbara bustard differs from M. avium paratuberculosis. Within the M. avium complex, several serovars (i.e. intimately related mycobacteria presenting a common set of antigens) infecting birds have been distinguished (Pavlas, 1997),
but serovars and virulence in hens was not definitely correlated (Schroder & Naumann, 1994). Surprisingly, the mycobacteriosis lesions confirmed by acid-fast bacilli presence affected only hybrid male houbara bustards. Further investigations are required to determine the virulence and pathogenicity of the \textit{M. avium} complex specimen found in the hybrid houbara bustard as well as the sensitivity of non-hybrid houbara bustards (i.e. \textit{C. macqueenii} or \textit{C. undulata}) and of other avian species inhabiting the areas where houbara bustards produced at the NWRC are re-introduced.

The oral-faecal route is the most common mode of transmission in naturally-occurring cases of mycobacteriosis in birds, although aerosol contamination is possible (Tell \textit{et al}, 2001). Hejlicek & Treml (1995) categorized different fowls according to their resistance to mycobacteriosis experimental inoculation. Rooks, pigeons and turtle-doves are very resistant and can play an important role in the epidemiology of avian mycobacteriosis as a source of mycobacteria because of their capability to survive despite shedding mycobacteria in their environment. In contrast, quail, domestic fowl, sparrow and pheasant seem very sensitive to experimental inoculation. Guinea fowl and turkey seem to be less sensitive to experimental infection (Hejlicek & Treml, 1995; Tell \textit{et al}, 2001 and 2003a). Despite the NWRC open-air houbara captive-breeding structures being located in an arid protected area, irrigated trees plantation attracts a large population of uncontrolled wild-bird population. The abundant wild bird species inhabiting the NWRC can play a significant role in the epidemiology of mycobacteriosis in Houbara bustards.

Post-mortem diagnosis of mycobacteriosis is based on histopathological examination whilst bacteriological culture and/or molecular assays are required for the mycobacterial species identification. Mycobacteriosis in the two hybrid houbara bustards comprised peri-ocular lesions in addition to gastro-intestinal, hepatic and pulmonary lesions. Ocular mycobacterial granulomatous lesions are not very common but widely reported in other avian species (Tell \textit{et al} (2001). Ante-mortem diagnosis of avian mycobacteriosis can be carried out by different means depending on their efficiency. Regular physical examination of every houbara bustard of the flock did not reveal any new suspected lesion in the NWRC flock. Detecting circulating antibodies against \textit{Mycobacterium} spp. by means of serological tests is possible, but it failed with the serum of the first hybrid houbara dead of mycobacteriosis. Ante-mortem diagnosis of mycobacteriosis could also be based on the cytokines which are produced during the activation of macrophages during the process of the disease. However, the interpretation of these cytokines is difficult in avian species (Tell \textit{et al}, 2001). Bacteriological culture and molecular assays are methods that directly detect the infecting organism. Molecular techniques are quite sensitive and less time consuming compared to culture methods but it is highly dependant on the quality of the DNA obtained (Tell \textit{et al} 2003b). Results obtained from molecular assays indicated that the positive amplification of hsp65 gene from some of the faecal swabs of birds at NWRC is indicative of mycobacteria in the flock. RFLP results following the PCR indicated the DNA detected does not belong to \textit{M. avium} and it belongs to an unidentified \textit{Mycobacterium} spp. Further work is needed in order to determine the identity of the \textit{Mycobacterium} sp. we are dealing with.

Neoplasia has only rarely been reported in bustards. Bailey \textit{et al} (2004) described some neoplasia from other \textit{Otididae} birds like a sertoli cell tumour in a white-bellied bustard and three other cases in houbara bustards. The panel of neoplasia cases at the NWRC ranged widely. All are malign neoplasia and affected sexually mature houbara. Females were more frequently affected by neoplasia than male houbara bustards despite both genders being equally represented in the NWRC flock.
At the NWRC, the lymphoproliferative disease was the most frequent neoplasia. It affected two/three year-old houbara bustards, what is very young in comparison with a life-span of more than 20 years in captivity. According to Latimer (1994), lymphoid neoplasia is the most common form of hemolymphatic neoplasia occurring in domestic, captive or free-ranging birds. It may originate from the peripheral lymphoid tissues as lymphosarcoma or in the bone marrow as leukemia. Lymphosarcoma usually presents as a disseminated multisystemic disease that can involve all tissues of the body. It is very rare to find a lymphosarcoma localized in a single, localized neoplasia. Both kinds of lymphoproliferative disease described by Latimer (1994) exist in the NWRC flock. Bailey et al (2004) reported three cases of lymphoid leucosis in houbara bustards with lesions comprising ascites and extensive hepatomegaly and whitish nodules in the spleen and kidney similarly to some lymphoproliferative disease cases recorded at the NWRC. In contrast, this report is the first one of lymphoproliferative disease consisting of tumoral infiltration of the tongue, pharynx, oesophagus, spleen, caecum, rectum and subcutaneous cervical lesions by lymphoid cells in houbara bustards.

Cholangiocarcioma is the most frequent hepatic neoplasia reported in the free-ranging birds (Latimer, 1994). This neoplasia which was not yet reported in houbara bustards, was detected in the NWRC houbara flock.

The bones neoplasia are the most frequently encountered type of neoplasia following lymphoproliferative disease at NWRC. They consisted of osteosarcoma and hemangiosarcoma affecting mainly the radio-ulnary wing segment. Hemangiosarcomas may arise singly or multicentric pattern affecting skin, liver, lung, spleen, muscle, mesentery, kidney, heart, oviduct, synovium or bone where they can exhibit osteolysis as found in the NWRC cases. They can metastasize to distant tissues including lung, liver, or myocardium. Osteosarcomas usually originate from proximal or distal portion of long bones, and less frequently from ribs, phalanges, cranium, orbit and coccyx (Latimer, 1994).

The skin carcinoma found on the metatarsus of a three-year-old male houbara at NWRC seemed similar to the squamous carcinoma case on the back of a houbara bustard reported by Bailey et al (2004).

The etiology of avian neoplasia is not fully elucidated. Beside neoplasia of unknown etiology, Calneck (1991) classified the transmissible neoplasia of poultry into four categories:

- Marek disease herpes-virus-related;
- Leucosis/sarcoma groups closely related to RNA Retroviridae resulting in leucosis, sarcomas and other connective tissue tumors;
- Reticulo-endotheliosis-virus-related neoplasia (REV) resulting in lymphoid leucosis or reticulo-endotheliosis;
- Lymphoproliferative disease affecting turkeys and related to another retrovirus that is distinct from both REV and leucosis/sarcomas groups.

Among the virus-induced-neoplasia, a virus can induce neoplasia of different histological nature (Calneck, 1991). After a diagnosis based on lesions of the houbara neoplasia, viral isolation or identification through molecular assays, are required to confirm any viral etiology. Ostrowski & Combreau (1995) were the first ones to report three cases of generalized neoplasia associated with reticulo-endotheliosis virus affecting the NWRC flock between 1989 and 1994. Bailey et al (2004) undertook a viral isolation attempt on frozen organs from a houbara bustard dead with lymphoproliferative disease, but a viral etiology
was not demonstrated. Further investigations are required to elucidate the etiology of the neoplasia affecting in the NWRC houbara from 2001.

The NWRC captive-breeding programme was initiated with the purposes of reintroducing Asian houbara bustards into its former habitat. Houbara bustards reintroduced into a wild environment may pose a significant disease risk to free-ranging populations of either the same or different species (Ostrowski & Combreau, 1996). Despite Bailey et al (1996a & 1996b) reported several medical problems in illegally imported houbara bustards that were confiscated by the authorities, very little is known about the medical status of wild houbara bustards in their natural environment. The presence of mycobacteriosis and of possibly contagious neoplasia in the NWRC breeding flock designated for production of houbara bustards to be re-introduced in the wild, rises the risk of introduction of both diseases to the free-ranging species as well as to the poultry industry.

CONCLUSION

Trauma-related pathology is the main cause of morbidity and mortality in the NWRC adult houbara captive flock. Vaccination against Newcastle disease virus and avian poxvirus resulted in the disappearance of both diseases in the flock, whilst chamydiosis has not been totally eradicated. Complementary investigations should go further to clarify the pathogenicity of the mycobacteriosis in non-hybrid houbara bustards and in other avian species inhabiting the protected areas where NWRC houbara are re-introduced. Similarly, the origin of the neoplasia should be elucidated despite epidemiology and lesions being evocative of a viral process. These investigations are essential for assessing the extent of both diseases in the NWRC captive flock as well as for managing the risk of transmission of both diseases to free-ranging species as well as to the poultry industry, following houbara bustard reintroduction.

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LITERATURE CITED


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