An Outbreak of Low Pathogenic Avian Influenza in a Mixed-Species Aviculture Unit in Dubai in 2005

Jo Kent a,b, *, Tom Bailey b, Christudas Silvanose b, Sean McKeown c, Ulrich Wernery d, Joerg Kinne d, Ruth Manvell e

a 53 Henver Road, Newquay, Cornwall, TR7 3DQ, United Kingdom
b The Dubai Falcon Hospital, PO Box 23919, Dubai, United Arab Emirates
c H.E. Sheikh Butti Maktoum’s Wildlife Center, PO Box 7237, Dubai, United Arab Emirates
d Central Veterinary Research Laboratory, PO Box 597, Dubai, United Arab Emirates
e Central Veterinary Laboratory, New Haw, Weybridge, Surrey, United Kingdom

The family Orthomyxoviridae is divided into types A, B, and C. Type A is the only type of veterinary importance, with avian influenza virus (AIV) of recent concern to human and bird health [1]. Type A AIV subtypes are categorized according to the surface antigens hemagglutinin (H) and neuraminidase (N) [2]. To date, 16 H (H1–H16) and 9 N (N1–N9) subtypes have been identified [3]. Further classification is determined by pathogenicity, with the Organization on Infectious Epizootics (OIE) classifying the virus into high or low pathogenic AIV (HPAIV and LPAIV, respectively) [4]. All HPAIV isolated to date has been H5 or H7 subtype; thus, these are of the greatest concern for the health of birds and human beings [5,6].

In the United Arab Emirates (UAE), 34 AIV cases have been isolated (Table 1). Infections with LPAIV H9N2 were diagnosed in farmed chickens and Houbara Bustards (Chlamydotis undulata), and an HPAIV H7N3 strain was isolated from a Peregrine Falcon (Falco peregrinus) [7,8]. To the authors’ knowledge, no clinical cases of AIV in the Stone Curlew (Burhinus oedicnemius), White-Bellied Bustard (Eupodotis senegalensis), or Blacksmith Plover (Anitibyx armatus) have been previously reported in the literature.

* Corresponding author. 53 Henver Road, Newquay, Cornwall, TR7 3DQ, United Kingdom.
E-mail address: jokent_@hotmail.com (J. Kent).
Case report

Birds in a mixed-species aviculture unit at a private zoologic collection in Dubai showed varying degrees of respiratory disease from May 31 to June 22, 2005, and 4 birds died during this period. The aviculture unit contained 33 birds of six species, including the White-Bellied Bustard (*E. senegalensis*) (n = 12), Spotted Thick-Knee (*Burhinus capensis dodsoni*) (n = 9), Blacksmith Plover (*A. armatus*) (n = 4), quail (*Coturnix coturnix*) (n = 1), Fulvous Whistling Duck (*Dendrocygna bicolor*) (n = 2), Red-Billed Whistling Duck (*Dendrocygna autumnalis*) (n = 4), Mandarin Duck (*Aix galericulata*) (n = 7), Carolina Duck (*Aix sponsa*) (n = 5), Ringed Teal (*Callonetta leucophrys*) (n = 3), Greater Flamingo (*Phoenicopterus ruber rubeus*) (n = 3), and Caribbean Flamingo (*Phoenicopterus ruber ruber*) (n = 4). All birds had been hatched and were housed in the unit, with the exception of the plovers and ducks, which were occasionally housed in open aviaries during the day and returned to the indoor unit at night. Birds housed in the unit were not vaccinated.

Initially, clinical signs presented in four birds as a reduction in food consumption and general lethargy. These four clinically affected birds exhibited varying degrees of tachypnea and dyspnea, ranging from mild respiratory signs to open-mouthed breathing and collapse (Figs. 1 and 2). Ocular and nasal discharge was obvious in two cases (White-Bellied Bustard and Stone Curlew). Physical condition was lost rapidly, and all four birds died by disease or euthanasia. The presentation of each clinical case is summarized in Table 2. A further four birds (three White-Bellied Bustards and one Stone Curlew [cases 5–8]) displayed nonfatal disease with lethargy, inappetence, serous ocular and nasal discharge, and dyspnea.

Clinical samples were collected during examination of the first four affected birds (cases 2 and 4; see Table 2; Table 3). Saline-moistened swabs were obtained from the oropharynx and cloaca and examined under a light microscope for helminth and protozoan parasites. Conjunctival, oropharyngeal, and cloacal swabs were submitted to the Dubai Falcon Hospital for diagnostic testing. The results of these tests are presented in Table 2. Additionally, blood samples were collected for serological testing for avian influenza virus (H7N3, H7N1, H9N2, H9N1, and H9N2 strains) and other respiratory pathogens. The results of these tests are also presented in Table 2.

Table 1

<table>
<thead>
<tr>
<th>Avian species</th>
<th>Number</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peregrine Falcon</td>
<td>1</td>
<td>H7N3</td>
</tr>
<tr>
<td>Chicken</td>
<td>7</td>
<td>H9N2</td>
</tr>
<tr>
<td>Houbara Bustard</td>
<td>3</td>
<td>H9N2</td>
</tr>
<tr>
<td>Blacksmith Plover</td>
<td>7</td>
<td>H7N1</td>
</tr>
<tr>
<td>Quail</td>
<td>7</td>
<td>H9N2</td>
</tr>
<tr>
<td>Sudanese Bustard</td>
<td>1</td>
<td>H9N2</td>
</tr>
<tr>
<td>White-Bellied Bustard</td>
<td>4</td>
<td>H9N2</td>
</tr>
<tr>
<td>Stone Curlew</td>
<td>2</td>
<td>H9N2</td>
</tr>
<tr>
<td>Blacksmith Plover</td>
<td>1</td>
<td>H9N2</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td></td>
</tr>
</tbody>
</table>
(DFH) for aerobic bacteria and fungal culture. Cytology preparations from ocular discharges were stained with a modified Giemsa stain for microscopic examination (Neat stain; Midlantic Biomedica, Paulsboro, USA). Tracheal swabs were collected into MycoVeto transport media for mycoplasma culture at the Central Veterinary Research Laboratory (CVRL) in Dubai. Virus isolation was conducted on clinical samples from case 2. Swabs were placed in virologic transport media (minimum essential medium; GIBCO Life Technologies, Scotland). Virus isolation was conducted at the CVRL on chicken embryo fibroblasts, and virus characterization was conducted at the Veterinary Laboratories Agency in the United Kingdom. The results of these clinicopathologic investigations are summarized in Table 3.

Fig. 1. Stone Curlew (case 4) in severe respiratory distress.

Fig. 2. Lung of Stone Curlew (case 4) with catarrhal to suppurative bronchopneumonia.
Bacteriology, cytology, and parasitology findings other than those summarized in Table 3 were unremarkable. Hematology values were measured and found to be within normal ranges [9], with the exception of a moderate leucocytosis with heterophilia and an increased number of reactive lymphocytes in case 4.

The four dead birds were submitted for postmortem examination at the CVRL. Tissue samples from the liver, lung, brain, and kidney and feces were investigated for bacteria, viruses, fungus, and parasites using routine methods. Findings of postmortem gross examination, histopathologic examination, bacteriology (including culture for mycoplasma), mycology, and parasitology are summarized in Table 4. Choanal swabs were collected into *Chlamydophila* transport media (Ideia; Dako Diagnostics, Ely, United Kingdom), and infection with *Chlamydophila* spp was ruled out in all cases by antigen detection ELISA.

Pooled oropharyngeal-cloacal swabs from cases 1 through 4 were screened for AIV antigen using Directigen Flu-A antigen-capture ELISA (Becton-Dickinson, New Jersey), and whole-virus isolation was performed on postmortem tissues at the CVRL. Influenza virus type A was isolated in tissues from all four dead birds. A summary of these results is presented
in Table 4. To confirm the subtype of the AIV isolates, serotype-specific influenza viral antibody was identified in serum samples by hemagglutination inhibition tests at the Central Veterinary Laboratory (New Haw, Weybridge, Surrey, United Kingdom). The influenza virus was confirmed as subtype H9N2 in two of these cases (cases 2 and 4). Subtyping was not attempted in cases 1 and 3.

A diagnosis of AIV subtype H9N2 was made based on the clinical signs and isolation of the virus from clinical and pathologic specimens. Bacterial and fungal isolates, including *Streptococcus bovis*, *Candida albicans*, *Escherichia coli*, *Enterococcus* sp, *Streptococcus* sp, and *Acinetobacter baumanii*, were considered to be secondary opportunistic bacteria and fungus, which were unlikely to have been the primary cause of disease or mortality.

Symptomatic treatment with marbofloxacin (10 mg/kg administered per os every 12 hours) and meloxicam (0.2 mg/kg administered intramuscularly every 24 hours) was initiated in case 2. In addition, the bird was nebulized with a quaternary ammonium and biguanidine disinfectant (F10; Health and Hygiene Ltd, South Africa) twice a day for 30 minutes. An improvement in clinical signs was seen over the first 2 days of treatment,
but this was followed 1 day later by a rapid worsening of respiratory signs and death. Acute onset of signs and sudden death precluded treatment in two cases (cases 1 and 3). Case 4 presented with such severe respiratory distress that the bird was euthanized immediately on humane grounds.

Four birds (cases 5–8) subsequently affected with mild respiratory signs were treated with a 5-day course of oseltamivir phosphate (10 mg/kg administered per os every 12 hours). The dose of 10 mg/kg every 12 hours was calculated from the recommended human dose (7 mg/kg every 24 hours) using metabolic scaling methods described by Sedgewick [10]. All four birds recovered fully within the 5 days of treatment and showed no further clinical signs. The affected rearing unit was fogged (with the birds contained inside) twice daily for 2 weeks, using F10 antiseptic at a dose rate of 1 part per 25 parts.

After the confirmation of AIV in cases 1 through 4, the remaining birds in the rearing unit were screened for AIV antigen. Serology for antibodies against AIV was performed at the CVL, and pooled oropharyngeal and cloacal swabs were submitted to the CVRL for Directigen Flu-A antigen-capture ELISA. Swabs from cases 5 through 8 were found to be positive by Directigen Flu-A antigen-capture ELISA. The same swabs, repeated after 5 days of administration of oseltamivir phosphate to these four cases, were negative for influenza viral antigen. Antibodies titers specific for AIV H9N2 were measured from the serum of healthy and clinically affected birds before treatment with oseltamivir phosphate. Titers to H9N2 above the negative control level were detected in six of six White-Bellied Bustards, seven of nine Stone Curlews, and five of six quail tested.

Quail randomly selected from an intensive breeding and rearing unit located 20 m from the affected rearing room were also screened for AIV. AIV antigen was detected by Directigen Flu-A antigen-capture ELISA in a pooled sample from six quail. Postmortem examination of five killed quail showed congestion of the liver and lung, with focal acute catarrhal pneumonia. AIV was isolated from the liver, lung, and brain of these five birds. Serum from the same five killed birds revealed high positive titers for antibody against AIV H9N2. All quail in the unit were apparently healthy and were used as a food source for hatchling, juvenile, and adult birds in a falcon-breeding project. There had been no reports of clinical abnormalities in the falcons, which were fed from this source on a daily basis.

Discussion

Influenza A has recently been implicated as the cause of mass avian mortalities worldwide and as the potential etiology for major pandemics among human populations [11]. Therefore, the isolation of an H9N2 AIV subtype in the UAE was a cause for concern. The method used for AIV antigen detection in this outbreak, Directigen Flu-A antigen-capture ELISA, was
developed to detect influenza A virus in mammals but has been applied in infections in avian species [7,12]. Studies have demonstrated a variety of sensitivities and specificities of this test compared with cell cultures and direct fluorescent antibody tests. The reported ranges of sensitivities, from 78% to 100%, and specificities, from 95% to 100% [12–14], are possibly attributable to differing bird species as well as to specimen collection and handling methods [4]. Despite these limitations, Directigen Flu-A antigen-capture ELISA remains a valuable test for the rapid diagnosis of influenza in birds.

Large congregations of waterfowl are thought to form a major reservoir of AIV, and their migrations have been shown to contribute to the carriage of the virus around the world [4]. The aviculture unit reported here was situated adjacent to lakes that accommodate large numbers of pinioned and free-flying waterfowl. Thus, the area could be considered to be one of high risk for AIV infection because of the proximity of resident avian species potentially carrying AIV. Once contaminated, the survival time of AIV in muddy waters is unknown, but such water bodies should also be considered a possible source of infection. No wild waterfowl on these lakes were identified as being sick or to have died as a result of AIV infection. Difficulties with capture of free-living waterfowl precluded the sampling and testing of these species for evidence of infection during the outbreak of AIV in the adjacent rearing unit.

One possible route of AIV infection into the rearing unit was from birds that were occasionally housed outdoors and brought into the rearing unit at night. Sudden death occurred in one Blacksmith Plover (case 3) that had been housed outdoors and moved back into the rearing unit several days before the first presentation of clinical problems in the group. Additionally, eggs from plovers and ducks laid and collected outdoors and hatched in the aviculture unit may have been contaminated by infected feces. Similarly, quail eggs laid in the adjacent quail-breeding unit were frequently moved into the rearing unit for hatching. Adult quail in the breeding group were found to be asymptomatic carriers of AIV, and eggs may have been infected or contaminated with AIV. Other possible modes of indirect transmission of AIV include contaminated fomites and material carried by personnel working in the rearing unit or contaminated water supplies [5]. Unfortunately, identification of the source of this outbreak was never established.

Four birds showing respiratory disease were treated with oseltamivir phosphate at a dose of 10 mg/kg administered every 12 hours. This dose was calculated by metabolic scaling, extrapolating from the dose recommended for human treatment. Four influenza antigen-positive birds with mild clinical signs recovered after treatment. Meijer and colleagues [15] used oseltamivir phosphate at a dose rate of 120 mg/kg administered every 12 hours in chickens and found it was effective in reducing the transmission, morbidity, and mortality of HPAIV. Further studies are needed to determine the doses necessary to achieve therapeutic levels in different avian species.
From the small numbers of birds involved, we are unable draw any statistically supported conclusions about relative susceptibility of species to infection with AIV, the resulting pathologic findings, and the response to antiviral treatment. It may be of significance to note, however, that antibodies against AIV H9N2 were detected in serum from six of six apparently healthy killed quail in a production unit of the same site. These quail were used to feed adult and juvenile falcons at a captive breeding facility on the same site, and no clinical problems were reported from these birds. Quail belong to the order Galliformes, the group of birds that have been identified as most susceptible to the pathologic changes associated with AIV [6], thus highlighting the low pathogenicity of this strain of H9N2 virus. Quail in the breeding and rearing unit were housed indoors; thus, infection of these birds was thought to have occurred indirectly via the movement of infected material on personnel or fomites.

Summary

Although the AIV responsible for the outbreak reported here was identified as being a low pathogenic strain and most birds within the until remained healthy, the severity of disease seen in the four clinical cases serves to highlight the potential significance of AIV to bird populations worldwide. The use of oseltamivir phosphate seemed to be successful in eliminating AIV from mildly affected birds, and further studies in this area may reveal useful dose ranges for its future use.

Acknowledgments

The authors thank His Highness Sheikh Hamdan bin Rashid al Maktoum for his continued support of the DFH. They also thank Humaid Obaid al Muhari, the staff of the DFH and CVL, and S. Joseph and R. Wernery from the CVRL for their technical support and observations.

References


