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First detection of highly pathogenic avian influenza virus H5N1 in common kestrel falcon (*Falco tinnunculus*) in Egypt

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Abstract

Highly pathogenic avian influenza virus (HPAIV) poses threats to animal and human health worldwide. A common kestrel *(Falco tinnunculus)* was submitted to Avian and Rabbit Medicine Department, Zagazig University, Egypt. It exhibited torticollis, incoordination, and inability to stand. Conjunctivitis and crust formation were seen. Postmortem findings revealed congestion in internal organs and greenish content in gizzard. No avian pox virus was detected in cutaneous lesions neither in histopathological sections nor in PCR. The presence of HPAIV H5N1 was confirmed by both virus isolation and RT-PCR. The mean death time of virus in chicken embryo is 36.2 hours. Influenza A virus antigen was detected in renal glomerular tufts and neurons of the submucosal plexus of the intestine of falcon by IHC. This paper describes the isolation of HPAIV H5N1 in falcon with systemic infection suggests that common kestrel falcon can be susceptible to HPAIV and could be a source of infection to other birds and human.

Introduction

Influenza A viruses, members of the family Orthomyxoviridae, pose a threat to animal and human health[27)]. Wild birds are deemed the natural reservoirs for avian influenza virus (AIV). Their migratory nature may help in dissemination of AIV across countries. Since predatory birds commonly feed on avian carcasses and diseased avian prey, they are at increased risk for highly pathogenic avian influenza (HPAI) virus infection[5,28)]. In falconry, birds of prey are also regularly kept in captivity and come in close contact with humans consequently they may pose a risk of transmitting the virus to humans or to other captive avian species, including poultry[13)].

Formerly, HPAI viruses were rarely found in predatory birds and were restricted to only a few isolated cases. In 2000, the HPAIV subtype H7 was isolated from a Peregrine falcon in the United Arab Emirates[16)] and Saker falcon in Italy[14)] Serial natural infections with H5N1 in 2004, 2006 and 2008 were recorded in Hong Kong[6,22,23)]. Other countries reported HPAI cases

in different predatory species, such as Hodgson's hawk eagles in Belgium[29], saker falcons in Saudi Arabia[26]. Furthermore, 20 confirmed cases of H5N1 infection in February 2007 were reported in falcons at a Zoo and a farm in Southern Kuwait[25]. Later on, falcons that came into contact with houbara bustards were infected with AIV in Saudi Arabia[11]. Previous experimental studies have demonstrated that falcons deemed to be highly susceptible to HPAI subtype H5N1[2,9,13]. Frequent falcon infections with HPAI viruses demonstrate the increased susceptibility to these pathogens. This paper describes the isolation, lesions and pantropism of influenza A virus subtype H5N1 from a common kestrel, Egypt.

Materials and methods

History and clinical examination: In March, 2013, a falcon had dropped at the yard of Faculty of Veterinary Medicine, Zagazig University, Egypt and was submitted to the Department of Avian and Rabbit Medicine. Clinical examination was carried out.

DNA extraction and PCR amplification: Cutaneous lesions were used for DNA extraction following the manufacturer's instructions (GeneJET™ genomic DNA Purification Kit, Fermentas). The APV specific PCR was performed using a primer pair as described before[12]. The amplification reaction was carried out under the following temperature profile: 2 min at 95°C (initial denaturation) followed by 35 cycles of 30 s at 95°C (denaturation), 30 s at 55°C (annealing) and 1 min at 72°C (extension), and cycle of final extension at 72°C for 10 min. Then, the amplified PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide.

Virus isolation and identification: Samples of trachea, lung, brain, liver, kidney and intestinal tissues were collected for virus isolation and histopathological examination. The isolation was carried out into 10 days old embryonated chicken eggs (ECE) via allantoic sac[19]. The collected allantoic fluid was examined for hemagglutinating activity. The infected allantoic fluid was tested for the presence of AIV and Newcastle disease virus (NDV) by RT-PCR. The PCR was performed with a set of primers specific for H5and N1 gene segments as well as NDV as described elsewhere [18,20].

Histopathology and immunohistochemistry (IHC): The specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5μm and stained with hematoxylin and eosin (HE)[24]. Consecutive paraffin sections were stained by IHC to detect the AIV antigen in tissues[21]. A monoclonal antibody (ATCC HB-65 influenza A hybridoma cell line) was used as the primary antibody. Specific antigen-antibody reactions were detected by 3,3´diaminobenzidine tetrahydrochloride using the Dako EnVision system (DakoCytomation Inc. Carpinteria, CA, USA) and counterstained with hematoxylin. Positive reactions appeared as brown precipitate localized at the site of binding.

Results

Clinical and postmortem findings:

The falcon exhibited torticollis, incoordination, inability to move, anorexia and conjunctivitis with crust formation on eyelids (Fig 1A). The bird was kept under observation however, greenish dropping was observed in the second day. The falcon soon was found dead despite supportive care; shortly the necropsy was carried out. Postmortem examination revealed extradural hemorrhage in skull with severe congestion in the meningeal blood vessels (Fig. 1B) besides, congestion in the internal organs (larynx, trachea, lungs, liver and intestine). Fibrinous exudates on inner aspects of keel bone, slightly turbid air sacs, and greenish content in gizzard were seen.

Virus isolation and identification:

The crust was taken for pox virus detection; no avian pox virus was detected in cutaneous

lesions neither in histopathological sections nor in PCR. The infected ECEs with intestinal tissues revealed embryo mortality within 48 hours post inoculation. The virus was also isolated from brain and lung tissues. The isolated virus was identified as H5N1 AIV by RT-PCR. NDV infection was excluded in HA-positive allantoic fluids by negative results of RT-PCR. The AIV isolate was tested for pathogenicity in chicken embryos and the mean death time (MDT) of 36.2hours±4.8 (Mean ± Standard Error) was recorded.

Histopathology and IHC:

Histologically, the liver proved focal aggregations of leukocytes in the portal areas with moderate congestion of the blood vessels. The cerebral cortex showed vacuolation few necrotic neurons and congested blood vessels. Severe hemorrhage and organized thrombus was observed in the meningeal blood vessels and submeningeal space (Fig. 1C). Immunohistochemical analysis of the Influenza viral nucleoprotein showed positive moderate immunolabeling in the renal glomerular tufts (Fig 1D) and neurons of the submucosal plexus of the intestine (Fig. 1E).

Discussion

Highly pathogenic avian influenza virus subtype H5N1 was reported in poultry in Egypt since 2006[1]. Remerging outbreaks of HPAIV subtype H5N1 poses a major challenge to animal and public health. Eventually, a disruption of AIV transmission between and within avian collections considered as an important tool for disease control, particularly in populations of rare species as demonstrated by many species of predatory birds[8,13]. The susceptibility of some terrestrial wild birds to HPAI H5N1 virus infection[3] increases the risk of virus transmission to falcons[17]. In the present study, HPAIV H5N1 was isolated and identified from brain, lung and intestinal tissues of a common kestrel (*Falco tinnunculus*), Egypt during 2013.

Falco tinnunculus is a prevalent kestrel species, found throughout Africa, Asia and Europe. Its range spans from Great Britain to China and as far south as South Africa. In Europe, *Falco tinnunculus* is migratory and winters in southern Europe and sub-saharan Africa[10,30]. It was previously reported that the pronounced clinical signs may develop in falcons because of the stress situations and concurrent diseases. These conditions may immunocompromise the birds, leading to increased vulnerability, and affecting the severity of disease[13].

In our study, greenish dropping and neurological signs accompanied with brain lesions were observed. These findings were consistent with the findings of Hall et al.[9] who recorded that kestrels infected with HPAIV H5N1 showed severe neurological signs which have not been previously reported in HPAIV H5N1 infected falcons suggesting continuous evolution and increased pathogenicity of HPAI in falcons. In contrast to the record of Bröjer et al.[4] who mentioned that detection of virus nucleoprotein in peripheral nervous system is not well documented in birds with natural H5N1 infection, the virus nucleoprotein was detected in the submucosal plexus of the intestine of common kestrel. The aforesaid results of severe neurological dysfunction observed in kestrel species besides virus isolation from brain tissues and IHC suggest the viral neurotropism.

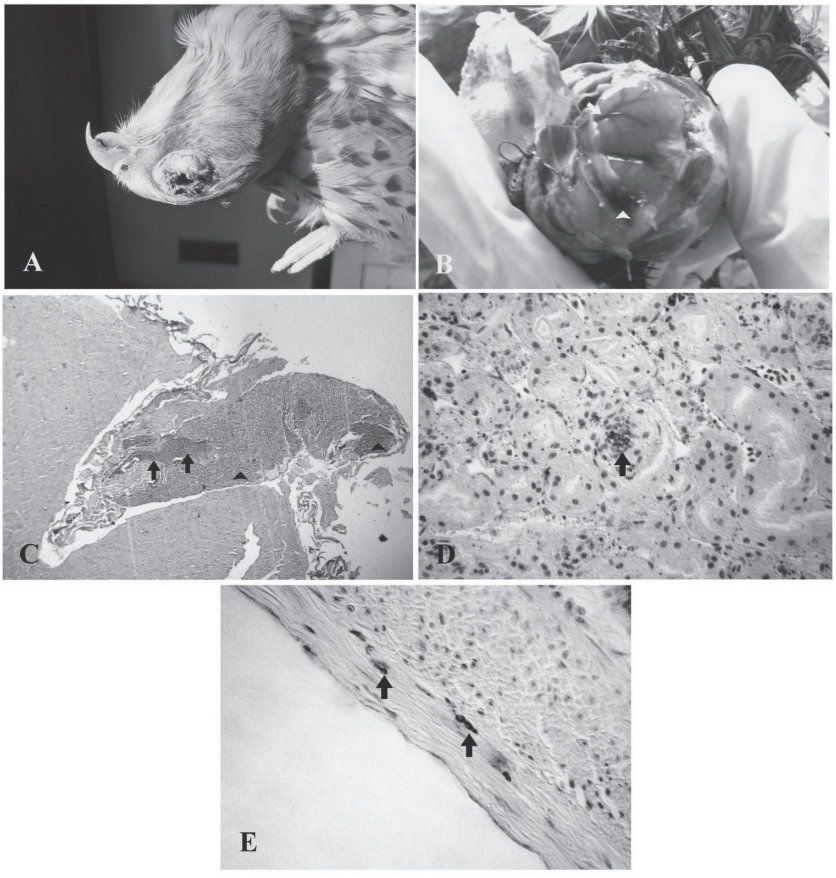

Fig. 1. Falcon infected with HPAIV subtype H5N1 showing; (A) Torticollis and incoordination with crust formation on the eyelids; (B) Skull, showing severe extradural hemorrhage (arrowhead) and severely congested meningeal blood vessels (arrow); (C) Brain, showing severe hemorrhage (arrow head) and organized thrombus in the sub-meningeal space (arrow) HE X100; (D) Kidney, showing positive Immunolabeling of influenza A virus in the glomerular tuft (arrow) IHC X400; (E) Intestine, showing positive Immunolabeling of influenza A virus antigen in neurons of the submucosal plexus (arrows) IHC X400.

The HPAI H5N1 viruses have been isolated from a tree sparrow, a feral pigeon, and a peregrine falcon[7] and pigeons in Egypt[15]. The infection of ostriches, sparrows, pigeons, falcons, and wild ducks with HPAIV increases the likelihood of human infections. To the best of our knowledge, this study is the first to report H5N1 HPAI virus infection in *Falco tinnunculus*, Egypt. Since the HPAIV was isolated from common kestrel with systemic infection, the study of AIV infections in falcon with special attention to its tropism and full genome sequencing in addition to investigation of role of these birds in the ecology of influenza viruses become a must. It is meaningful to carry out a continuing surveillance and monitoring raptor species for HPAIV infections.

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