Isolation and identification of highly pathogenic avian influenza virus subtype H5N1 in Peafowl (Pavo cristatus)

Mahmoud Moussa Ismail1,2, Owais Ahmed Khan1,4, Giovanni Cattoli1, and Huaguang Lu4
1Central Veterinary Diagnostic Laboratory, P.O.Box, 15831, Riyadh 11454, Kingdom of Saudi Arabia
2Department of Veterinary Biotyine, K. U. Leuven, Belgium
3OIE/FAO and National Reference Laboratory for Newcastle Disease and Avian Influenza, Istituto Zooprofylattico Sperimentale delle Venezie, Viale dell’Universita’, 10, 35020 Legnaro, Padova, Italy.
4Animal Diagnostic Laboratory, Pennsylvania State University, University Park, PA 16802, USA

ABSTRACT

An outbreak of highly pathogenic avian influenza (H5N1) virus subtype H5N1 was first diagnosed in a “backyard” flock of peafowl raised in a palace premises in the Kingdom of Saudi Arabia (KSA) in December 2007. The flock consisted of 40 peafowl birds and their ages ranged from 3 to 5 years old. The birds suffered from depression, anorexia, and white diarrhea. Four dead birds were submitted for HPAI diagnosis at the Central Veterinary Diagnostic Laboratory (CVDL) in Riyadh. Brain and liver tissues, tracheal and cloacal swabs were taken from the dead birds and processed for real-time RT-PCR test and virus isolation in specific-pathogen-free (SPF) embryonating chicken eggs (ECE). The virus isolates were characterized as H5N1 HPAI5 virus by sequencing analysis. Phylogenetic comparison revealed that the H5N1 viruses isolated from peafowl belong to the genetic clade 2.2 according to the WHO nomenclature. The peafowl H5N1 virus falls into 2.2.2 sublineage and cluster with the H5N1 viruses isolated from poultry in Saudi Arabia in 2007 – 2008.

RESULTS AND DISCUSSION

Peafowl H5N1 infection. Peafowl birds experienced mainly whitish diarrhea, depression, anorexia, disorder, and convulsions prior to death. Paled head skin and nasal discharge were also observed. Feathers around the vent were wet and soiled. Initial etiological diagnosis of the H5N1 HPAI infection in peafowl caused 10% mortality (4 deaths out of 40 birds) in one day. Actual mortality remains unknown because the 36 live birds were disposed by standard procedures once the diagnosis of H5N1 was confirmed. Necropsy examination of the dead birds revealed organ hemorrhagic lesions on proventricular mucosa membrane and splenomegaly.

Virus isolation and RT-PCR. The inoculated ECE were examined and embryo mortality was recorded (Table 1). Embryo deaths occurred as early as 18 hours post-inoculation. This early embryo death was proven to be specific by HA and HI testing. The harvested CAF was tested for HA activity and confirmed as avian influenza virus by means of HI and HI tests. Results confirmed that the isolate was H5N1 AIV positive. This peafowl H5N1 isolate sequencing data was submitted to GenBank with the accession number of A-peacock/Saudi Arabia/3489-74/VIR08/2007 (LaB Id No.1201) (H5N1). By RT-PCR, the peafowl brain tissue yielded a strong positive reaction (ct-values of 15.05 for type A matrix and 14.85 for H5). The early embryo mortality and the low RT-PCR cycles prompted us to conduct HA test on original tissue homogenates. As a result, supernatant of brain homogenate was H5N1 AIV positive. This peafowl H5N1 isolate was sequenced to confirm that it was a highly pathogenic avian influenza virus (AIV) subtype H5N1.

Gene sequencing. The amino acid sequence of peacock H5N1 virus revealed a cleavage site characteristic of HPAI (PGQERRKKRGFL) in the HA molecule. In the NA molecule, mutations related to antiviral drugs resistance were not detected. Phylogenetic analysis showed that the virus belongs to the genetic clade 2.2 according to the WHO nomenclature. The analyzed isolates fall into 2.2.2 sublineage and cluster together and with the H5N1 viruses isolated from domestic poultry in KSA in 2007 (Fig 1). The similarity ranged from 99.3% to 100% for NA gene and from 99.4% to 100% for HA gene. In a previous study, the nucleotide (nt) sequences of the entire genome of the viruses isolated from wild birds were tested by the two step rRT-PCR following the same standard procedures once the diagnosis of H5N1 was confirmed. Necropsy examination of the dead birds revealed organ hemorrhagic lesions on proventricular mucosa membrane and splenomegaly.

MATERIALS AND METHODS

Case history. During the HPAI outbreaks in KSA during 2007–2008, a small flock of peafowl, that was raised in the “backyards” of a palace premises, suffered major clinical signs of depression, anorexia and white diarrhea. The case was at once reported to the Avian influenza emergency office under the Ministry of Agriculture. The flock consisted of 40 peafowl and their ages ranged from 3 to 5 years old. Clinical examination was performed at the site by state veterinarians. Four fresh dead birds were submitted for HPAI diagnosis at the Central Veterinary Diagnostic Laboratory (CVDL) in Riyadh.

Sample collection and processing. Pooled cloacal and tracheal swabs, liver and brain tissues were collected from the dead birds. The virus isolates and tissue homogenates were clarified at 3000g for 30 min and then supernatants were treated with multi-antibiotic medium and then sonicated. Feathers around the vent were collected from the dead birds. The swab samples and tissue homogenates were clarified at 3000g for 30 min and then supernatants were treated with multi-antibiotic medium and then sonicated. Organ hemorrhagic lesions on provetricular mucosa membrane and splenomegaly were also observed. Feathers around the vent were wet and soiled. Initial etiological diagnosis of the H5N1 HPAI infection in peafowl caused 10% mortality (4 deaths out of 40 birds) in one day. Actual mortality remains unknown because the 36 live birds were disposed by standard procedures once the diagnosis of H5N1 was confirmed. Necropsy examination of the dead birds revealed organ hemorrhagic lesions on proventricular mucosa membrane and splenomegaly.

REFERENCES