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Natural A(H1N1)pdm09 influenza virus infection case in a pet ferret in Taiwan

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Abstract

Ferrets have demonstrated high susceptibility to the influenza virus. This study discusses a natural 2009 pandemic influenza A (H1N1) (A(H1N1)pdm09) virus infection in a pet ferret (*Mustela putorius furo*) identified in Taiwan in 2013. The ferret was in close contact with family members who had recently experienced an influenza-like illness (ILI). The ferret nasal swab showed positive results for influenza A virus using one-step RT-PCR. The virus was isolated and the phylogenetic analysis indicated that all of the eight segmented genes were closely related to the human A(H1N1)pdm09 virus lineage isolated in Taiwan. This study may provide a perspective view on natural influenza A virus transmission from the local human population into pet ferrets.

Key Words: A(H1N1)pdm09, ferret, Taiwan

A pandemic influenza A (H1N1) (A(H1N1)pdm09) virus first identified in the United States emerged during the spring of 2009 and spread globally. It caused severe human illness requiring hospitalization, including pneumonia and death⁹⁾.

Influenza induced rhinitis in ferrets was first reported in 1933. Ferrets were demonstrated to have high susceptibility to type A and B human influenza viruses and the ability to transmit virus mutually^{1,13)}. Ferrets inoculated with the 2009 pandemic influenza A (H1N1) (A(H1N1)pdm09) exhibited the clinical symptoms of

lethargy, sneezing, nasal discharge, anorexia and weight loss¹¹⁾. Natural A(H1N1)pdm09 virus infection in pet ferrets has been reported in 2009 and 2010 in the USA^{2,14)}. However, the current condition of pet ferrets, including the virus evolution, is rarely known in other countries.

An eight-year-old neutered female ferret attending National Taiwan University Veterinary Hospital (NTUVH) in July 2013 showed panting, severe coughing (especially at night), with open-mouth breathing all day long, lack of appetite, poor activity and low body temperature (36.6°C).

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The ferret had a history of adenocarcinoma in the left adrenal gland with unilateral adrenalectomy performed 4 years ago. Swelling in multiple lymph nodes was noted since November 2012. Lymphoma was suspected due to the monoclonal gammopathy in the serum protein electrophoresis (SPEP) test. However, further biopsy was declined by the owner. Two family members in the household who had close contact with this ferret presented influenza-like illness (ILI) symptoms before the ferret's visit at NTUVH. The ILI signs of the owners included fever, coughing and nasal discharge. The ferret became sick three days after the onset of the owner's clinical signs. Chest radiographs showed a moderate to severe increase in bronchial markings and tissue opacity in the left and right lungs (Fig. 1). Pleural effusion bacterial culture was negative. The ferret was treated with subcutaneous fluid, antibiotics and airway nebulization. The nasal discharge was eliminated after two days of therapy, but the ferret still exhibited lethargy and dyspnea (oxygen saturation < 80%). Lung sounds from the ferret could not be heard through auscultation. The ultrasound image showed pulmonary consolidation. Euthanasia was executed on the third day of hospitalization. Necropsy was not performed because of the owner's disagreement. PCR detection of the ferret nasal swab showed negative results for canine distemper virus and feline coronavirus but a positive result for the influenza A virus. The nasal swab sample was then submitted to virus isolation.

The ferret's nasal swab sample was dissolved in 1 mL Viral Transported Medium (Creative Microbiologicals, Taipei, Taiwan). Influenza A virus Matrix (M) gene was detected using One Step RT-PCR Kit (QIAGEN, Hilden, Germany) with primer set M52C/M253R⁴⁾. One aliquot of nasal swab sample solution was inoculated into the allantoic sac of 10-day-old specific-pathogen-free chicken eggs (Animal health research institute, Taipei, Taiwan) which were incubated for 48 hours at 35°C. Allantoic fluid was harvested

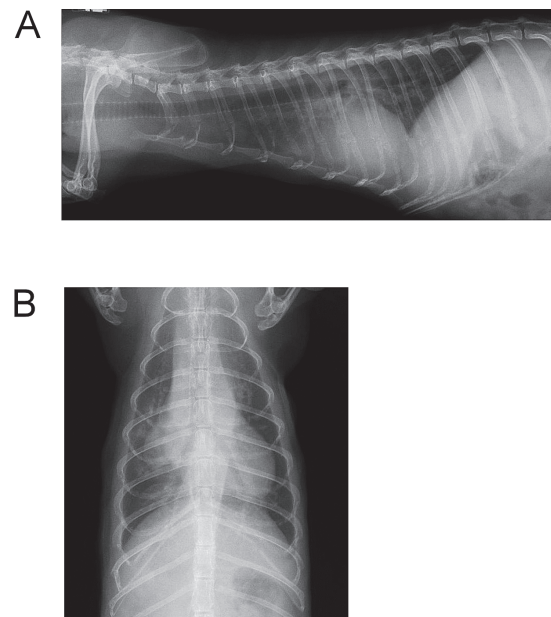


Fig. 1. Chest radiographs of the ferret with confirmed A/ferret/Taiwan/E01/2013 infection. (A) Lateral view. (B) Dorsal ventral view. Alveolar pattern with a moderate to severe diffusive increase in bronchial markings is shown. Increased tissue opacity is observed in both left and right lung lobes in the dorsal ventral view.

to conduct influenza A virus subtyping. The entire eight segmented genes of the influenza A virus were amplified using the universal primer set described by Hoffmann *et al.*⁸⁾. The amplified products were purified from agarose gels (Amresco, solon, OH) using QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany), cloned into pGEM-T Easy vector (Promega Corp, Madison, WI, USA) and then transferred into Arrow 58sec DH5α competent cell (Arrowtec, Taipei, Taiwan). Plasmids were sent for automated sequencing (Tri-l Biotech, Inc., Taipei, Taiwan).

The sequence identity of eight segmented genes was conducted using the nucleotide Basic Local Alignment Search Tool (BLAST) in the GenBank of the National Center for Biotechnology Information (NCBI). Virus genes representative of the relevant major lineages from different hosts were used in this analysis. Complete gene sequences were downloaded from GenBank. Sequence alignments and phylogenetic analysis were performed using Mega 6

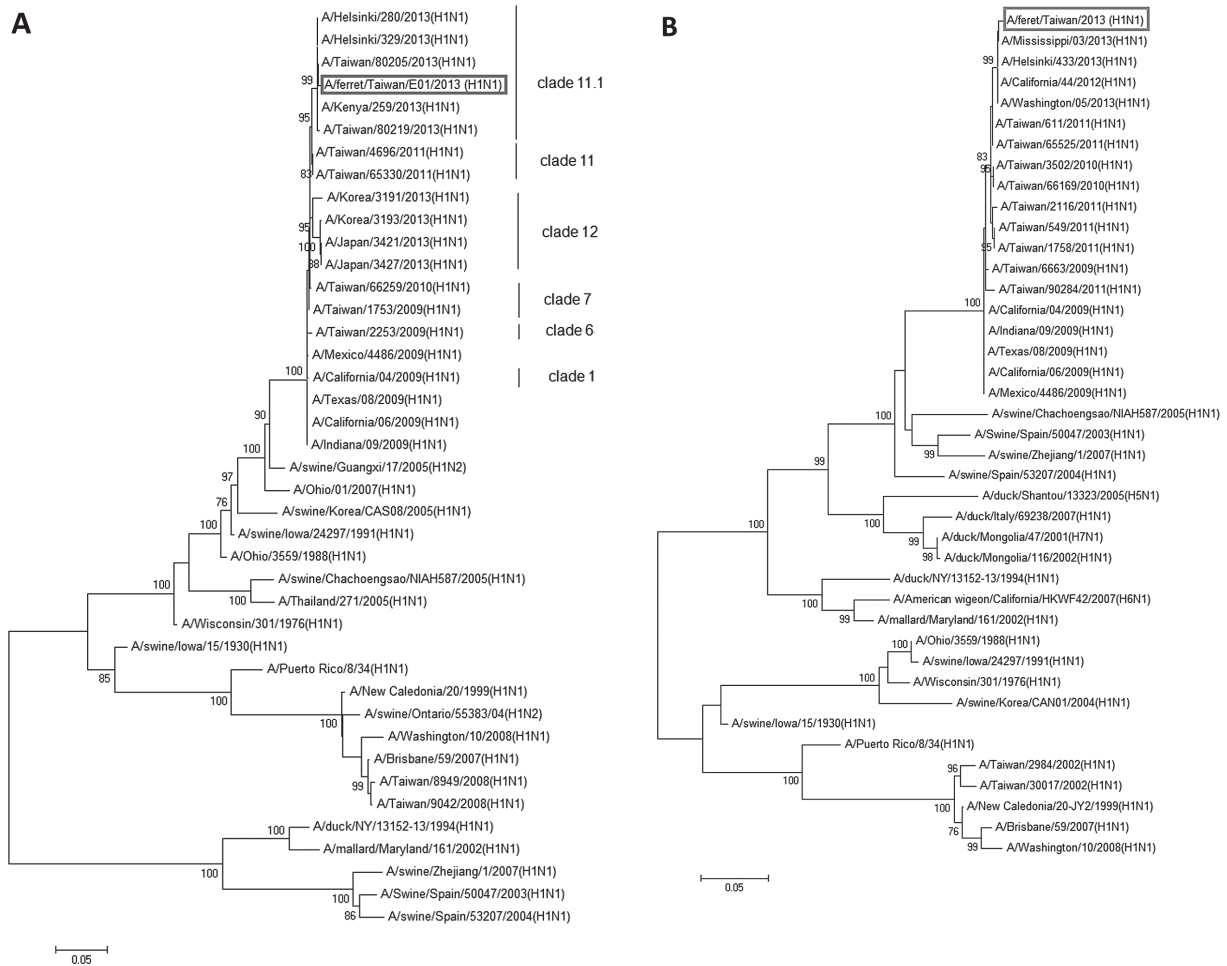


Fig. 2. Phylogenetic trees of A/ferret/Taiwan/E01/2013 with other influenza A virus lineages. Maximum likelihood using bootstrap analysis was conducted with 1,000 replications. Only viruses with bootstrap values greater than 75% are presented. The complete genes of Ferret/Taiwan/2013 are clustered with A(H1N1)pdm09 viruses. (A) HA gene. (B) NA gene. Scale bar indicates nucleotide substitutions per site.

software¹⁵). Sequences were aligned using the Clustal W method and Phylogenetic trees were constructed using maximum likelihood with bootstrap analysis with 1,000 replications.

Both the ferret nasal swab sample and the virus isolate were positive for M gene detection using one-step RT-PCR. This virus was designated as A/ferret/Taiwan/E01/2013 (abbreviated as Ferret/Taiwan/2013). The eight segmented gene sequences from Ferret/Taiwan/2013 were submitted to GenBank under the accession numbers KJ702009-KJ702016. All gene segments from Ferret/Taiwan/2013 had the highest nucleotide sequence similarity to H1N1 viruses. Eight segmented genes fell into the same A(H1N1)

pdm09 virus cluster. Ferret/Taiwan/2013 was most closely related to the A(H1N1)pdm09 virus lineage isolated in Taiwan. Its hemagglutinin (HA) and M genes were most closely related to the A/Taiwan/80205/2013 strain. The phylogenetic trees of HA and NA genes are shown in Fig. 2.

A prevalence peak occurred in November 2009 for the A(H1N1)pdm09 virus epidemic situation in Taiwan, but comprehensively declined in January 2010 after a mass vaccination program. However, A(H1N1)pdm09 still recurred during Taiwan's 2010–11 influenza seasons after WHO announced that the world shifted into the post-pandemic period on August 10, 2010^{3,7,10}. Based on previous studies A(H1N1)pdm09

viruses have begun to evolve and diversify into at least 7 clades (clade 1 to 7) with various spatial and geographic patterns¹²⁾. The Clade 7 virus strain was the predominantly imported strain in August 2009 in Taiwan. The phylogenetic analysis of the HA gene from Ferret/Taiwan/2013 revealed that it belonged to clade 11.1, a branch of the previous predominant clade 11 circulating from 2010 to 2011 in Taiwan. Ferret/Taiwan/2013 held the HA amino acid characteristics of clade 11 (D114N, S202T, S468N) and the additional substitution features of clade 11.1(V251I, K300E)¹⁷⁾. This indicated that Ferret/Taiwan/2013 possessed features specific to A(H1N1)pdm09 viruses circulating in Taiwan.

Because of severe lethargy and dyspnea, euthanasia was finally executed on the third day of hospitalization. Due to lack of necropsy, it was impossible to confirm the neoplasia diagnosis and explore the relationship between A(H1N1)pdm09 infection and neoplasia. According to previous studies most cases of A(H1N1)pdm09 infection were mild, but patients with an underlying disease, immunosuppression, pregnancy, cancer or chronic diseases may have greater incidence to acquire influenza infection or the infection severity would be aggravated due to immune system disruption. Serious clinical presentations, such as acute respiratory distress syndrome and death may develop^{5,6,16)}. The suspected neoplasia and old age could be negative impacts on immunity in this ferret, which aggravated the disease severity.

Whether the family members were the influenza A virus sources for the ferret could not be proven directly. However, based on the RT-PCR, virus isolation and phylogenetic analysis results, this ferret did have A(H1N1)pdm09 virus infection. Ferret/Taiwan/2013 (H1N1) was the first isolate identified from a pet ferret bearing the characteristics of human influenza viruses circulating in Taiwan. Influenza in pet ferrets is not a routine diagnosis item for veterinary practitioners in Taiwan. Further surveillance on influenza infections in pet ferrets and attention

to the virus transmission from the human population would be necessary.

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