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Short Communication

Influenza A Virus Infection in Domestic Ferrets

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ABSTRACT: Ferrets are animals that are known to be susceptible to influenza A virus (IAV) infection. To evaluate the risk of IAV transmission from diseased ferrets to humans, a survey was performed to detect specific antibodies against the H1, H3, H5, and H7 subtypes of IAV. Using enzyme-linked immunosorbent assay for hemagglutinin proteins, we found a high positive rate for the H1 (24.1%) and H3 (5.2%) subtypes. The results were confirmed by a virus neutralization test for representative antibody-positive serum samples. We also detected hemagglutinin and neuraminidase genes in two ferrets showing acute respiratory disease and whose owner was diagnosed with IAV infection; a human H1N1pdm virus was isolated from one of these ferrets. Our findings suggest that attention should be paid to IAV infection from humans to ferrets and vice versa.

Influenza A viruses (IAVs) are segmented, negative-sense, single-stranded RNA viruses of the family Orthomyxoviridae. They infect a wide range of host species along with humans (1). Domestic animals, such as dogs and cats, which live in relatively closer contact with humans than other animals, are susceptible to IAVs and can be an accidental source of IAV infection in humans (2–4). Therefore, epidemiological surveys of IAV infection among pet animals are needed to assess the risk of IAV transmission from animals. Ferrets are highly susceptible to IAV infection, and infected ferrets exhibit sneezing, nasal discharge, and high fever and are an experimentally useful model of IAV infection (5). However, no epidemiological survey on IAV in pet ferrets has been performed to date.

We established an enzyme-linked immunosorbent assay (ELISA) using extracts from HEK-293T cells transfected with plasmids expressing the hemagglutinin (HA) of A/California/04/2009 (H1N1), A/duck/Mongolia/301/2001 (H3N2), A/chicken/Yamaguchi/7/2004 (H5N1), and A/seal/Massachusetts/1/1980 (H7N7) to examine the seroprevalence of IAV infections in domestic ferrets. In a previous study, we used a similar method to successfully establish an ELISA for detection of antibodies against hepatitis E virus infection in numerous mammalian species (6). In the present study, ELISA was performed using 79 serum and/or plasma samples collected from pet ferrets in animal hospitals throughout Japan between August 2012 and January 2018. Although the samples were collected for the diagnosis of ferret coronavirus infection, many of them were collected regardless of symptoms (7,8). Before the virus neutralization (VN) test, all sera were treated with receptor-destroying enzyme to remove nonspecific inhibition. The results of the ELISA indicated that 19 of 79 (24.1%) and 4 of 77 (5.2%) ferrets had antibodies against HA(H1) and HA(H3), respectively. In contrast, no ferrets had antibodies against HA(H5) or HA(H7) (Fig. 1).

To confirm IAV infection in representative ELISA-positive ferrets, we performed a VN test to detect antibodies against the former seasonal H1N1 virus (A/Kawasaki/UTK4/09), seasonal H3N2 virus (A/Kawasaki/UTK20/08), and H1N1pdm virus (A/Osaka/369/09) using MDCK cells (Table 1). Due to the limited volume of serum samples, not all samples were used in the VN test. All nine HA(H1)-positive ferrets possessed high levels of VN antibodies against H1N1pdm virus but not against the former seasonal H1N1 virus. None of the seven HA(H1)-negative ferrets had VN antibodies against the H1N1pdm virus or the former seasonal H1N1 virus. One of the two HA(H3)-

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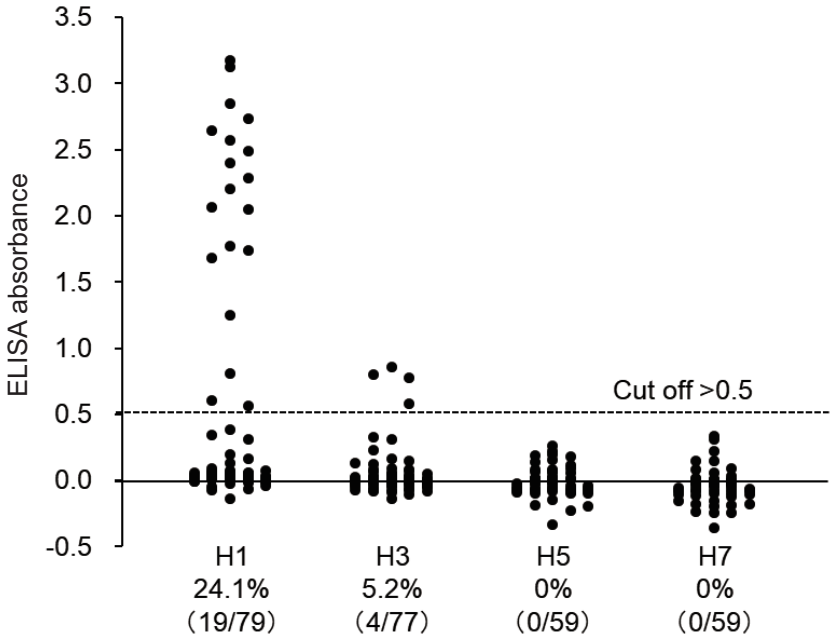

Fig. 1. Seroprevalence of influenza A virus infection in domestic ferrets. ELISA was performed using serum samples (diluted 1:100) from pet ferrets. Each dot represents a single sample that has been examined. In the ELISA, an absorbance value >0.5 was tentatively defined as seropositive for each HA subtype. ELISA, enzyme-linked immunosorbent assay; HA, hemagglutinin.

Table 1. The results of the virus-neutralization (VN) test and enzyme-linked immunosorbent assay (ELISA) using representative sera

Sample ID	ELISA absorbance		VN titer		
	H1	H3	H1N1pdm	H1N1	H3N2
1	**2.29**	− 0.02	**1:512**	<1:8	<1:8
2	**1.74**	0.05	**1:32**	<1:8	<1:8
5	**2.40**	**0.86**	**1:512**	<1:8	**1:64**
7	**3.13**	0.23	**1:1024**	<1:8	**1:32**
13	**1.68**	0.15	**1:512**	<1:8	<1:8
32	**2.07**	− 0.01	**1:512**	<1:8	<1:8
33	**0.81**	0.01	**1:128**	<1:8	<1:8
34	**1.77**	0.00	**1:128**	<1:8	<1:8
41	**2.85**	− 0.09	**1:512**	<1:8	<1:8
3	0.05	**0.78**	<1:8	<1:8	<1:8
4	− 0.00	− 0.06	<1:8	<1:8	<1:8
6	0.02	− 0.04	<1:8	<1:8	<1:8
10	0.03	− 0.02	<1:8	<1:8	<1:8
17	0.02	0.33	<1:8	<1:8	<1:8
18	0.05	0.03	<1:8	<1:8	**1:64**
35	− 0.07	− 0.08	<1:8	<1:8	<1:8

Bold letters indicate positive results.

positive ferrets had VN antibodies against the seasonal H3N2 virus. Interestingly, two HA(H3)-negative ferrets were positive for VN antibodies against the seasonal H3N2 virus.

We collected clinical samples from two ferrets that exhibited acute respiratory disease in an animal hospital in Tokyo, Japan, in 2019. They had been kept in their owner's house as pets, and their owner was diagnosed with influenza at a hospital just 1 day before the ferrets exhibited symptoms. One ferret exhibited sneezing and nasal discharge and recovered from the disease 6 days after the onset of the first clinical sign. The other ferret exhibited not only sneezing and nasal discharge but also diarrhea, lethargy, anorexia, and open-mouth breathing. Reverse transcription polymerase chain reaction was performed with nasal discharge samples from the two ferrets (9), and the complete sequences of the HA and neuraminidase (NA) genes were determined. Both

the HA and NA nucleotide sequences were identical between the two ferrets and highly homologous to those of H1pdm and N1, respectively. Phylogenetic analysis indicated that these HA genes were classified into clade 6B.1 (data not shown).

Virus isolation was also performed using AX-4 cells (10), and IAV A/ferret/Tokyo/358/2019 was isolated from the nasal swab of the ferret that recovered earlier. The HA and NA nucleotide sequences of the isolate were 100% identical to those of the clinical sample (DDBJ accession numbers: LC635746 for HA and LC635747 for NA).

In this study, the sequence of the influenza virus that infected the owner of the diseased pet ferrets could not be obtained. However, these ferrets had been kept in the owner's house and did not go outside, and their clinical symptoms were observed immediately after the development of the owner's influenza infection. Moreover, the two pet ferrets developed respiratory symptoms simultaneously, and the obtained sequences were identical, indicating that they were infected from the same source. Therefore, although there is no clear evidence for direct transmission from the owner to the ferrets, epidemiological evidence indicates that the ferrets were infected by their owner.

IAV was isolated from the nasal swabs of infected ferrets, which suggests that contact with IAV-infected ferrets may be associated with a risk of IAV infection in veterinarians and animal handlers in veterinary hospitals, pet shops, and breeding farms. We observed that 24.1% of ferrets were seropositive for HA(H1) and had VN antibodies against H1N1pdm virus (Fig. 1 and Table 1). Therefore, pet ferrets may be infected with the H1N1pdm virus at a relatively high rate. Two HA(H3)-negative ferrets were positive for VN antibodies against the seasonal H3N2 virus. This result may have been influenced by differences in antigenicity among H3 subtypes.

We found that approximately 20% of pet ferrets had IAV infection. This finding indicates that reverse zoonotic infection of IAV from humans to animals may frequently occur, and IAV infection from diseased animals to humans should be surveyed for further risk assessment.

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Conflict of interest None to declare.

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