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Seroprevalence of dogs in Hong Kong to human and canine influenza viruses

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ABSTRACT

As a unique mammalian host for influenza A viruses, dogs support the transmission of canine influenza viruses (CIVs) of H3N8 and H3N2 subtypes and are susceptible to infection by avian and human influenza viruses. A cross-sectional serological study was performed to assess the exposure history of dogs in Hong Kong to CIV and human influenza viruses. Among 555 companion dogs sampled in 2015–2017, 1.3 per cent and 9.5 per cent showed hemagglutination inhibition (HI) antibody titre to CIV of H3N8 or H3N2 subtypes and to A(H1N1)pdm09 human influenza viruses, respectively. Among 182 shelter dogs sampled in 2017–2018, none showed HI titre to CIV and 1.1 per cent reacted to H3N2 human influenza virus. There was a poor correlation between ELISA and HI test results. The higher seropositive rates to human influenza viruses suggests that the contact dynamics of dogs under urban settings may affect the exposure risk to human influenza viruses and CIVs.

INTRODUCTION

Dogs have been identified as an important host for influenza A viruses in the past two decades as they support sustained transmission of the equine-origin H3N8 and avian-origin H3N2 canine influenza viruses (CIVs). The H3N8 CIV was first isolated in Florida racetrack in 2004 but serological evidence suggested that the viruses may have been introduced into dogs in the USA since 1999.[1 2] The avian-origin H3N2 CIV was first isolated from dogs in South Korea in 2007 and was subsequently reported in China and Thailand.[3–6] Retrospective serology studies suggest that the avian-origin H3N2 CIVs has become enzootic in dogs in Asia since 2005.[7] In 2015, the H3N2 CIV was introduced to North America through rehoming rescued dogs from meat markets in South Korea and caused substantial outbreaks across the USA.[8]

In addition, serological evidences showed that dogs are susceptible for avian and human influenza virus infection without sustained dog-to-dog transmission.[9–11] Infections by human influenza viruses including H1N1 and H3N2 seasonal influenza viruses and A(H1N1)pdm09 virus are of most concerns at regions where H3N2 CIV is enzootic in dogs as this may lead to generation of novel reassortant viruses.[12] Of note, reassortment between avian-origin H3N2 CIVs and A(H1N1)pdm09 viruses has generated a novel H3N1 influenza virus in dogs.[12] Recently, swine-origin H1N1 viruses and their reassortants with the avian-origin H3N2 CIVs were isolated from dogs in Southern China, which further implies the complex ecology and genetic diversity of CIV in this region.[13]

Hong Kong is located in proximity to the 'epicentre' of influenza virus with a high human population density.[14] Approximately 7.1 per cent of the 2.3 million households in Hong Kong kept dogs as companion animal according to a survey conducted in 2010. Bidirectional interspecies transmission of CIV or human influenza may occur under the close contact between companion dogs and humans. In addition, recent studies have identified the importance of shelters and kennels in supporting CIV circulation among dogs.[15 16] Here, the authors report a cross-sectional serological analysis to assess the exposure history of companion and shelter dogs in Hong Kong to CIV and human influenza viruses.

MATERIALS AND METHODS

Sera collection from companion and shelter dogs in Hong Kong

A total of 555 sera were collected from canine patients during procurement of blood for diagnostic or health screening by eight veterinary clinics located at Hong Kong Island, Kowloon, and New Territories from December 2015 to May 2016 with consents provided by the owners. A total of 182 sera were collected from canine patients for health screening from two shelters located at Hong Kong Island and New Territories from June 2017 to February 2018. The health condition for the companion dogs were not fully recorded while 9/182 shelter dogs were reported to show respiratory symptoms while the blood sample was collected. The

Committee on the Use of Live Animals in Teaching and Research (CULATR) has been consulted and concluded that animal ethics approval to be waived as no living vertebrate animal was directly involved in the study.

Detection of influenza A nucleoprotein-specific antibody by competitive ELISA

Antibody against influenza nucleoprotein (NP) protein in canine sera was detected in duplicate using the ID Screen Influenza A Antibody Competition ELISA kit (ID.vet, Grabels, France) according to the protocol provided by the manufacturer.

Hemagglutination inhibition assay

Canine influenza viruses A/canine/New York/dog23/2009 (H3N8) (Canine/H3N8) and A/canine/Hong Kong/10005/2018 (H3N2) (Canine/H3N2) as well as human influenza viruses A/California/07/2009 (A(H1N1)pdm09) (CA07/H1N1), A/Perth/16/2009 (H3N2) (Perth16/H3N2),A/Switzerland/9715293/2013 (H3N2) (CH9715293/H3N2) were used for hemagglutination inhibition (HI) assay. An Eurasia avian-like H1N1 swine influenza virus A/swine/Hong Kong/NS4848/2011(H1N1) (EAsw/H1N1) was also included as its HA protein is highly homologous (99 per cent amino acid identity) to the novel swine-origin H1N1 CIVs reported in Southern China.[13] Canine sera were treated with receptor destroying enzyme (RDE) (Denka Seiken, Tokyo, Japan) and HI assay was performed according to the WHO protocol.[17] Briefly, one volume of canine serum sample was treated with three volumes of RDE for 18–20 hours at 37°C, followed by heat inactivation at 56°C for 30 minutes, then six volumes of PBS was added to each sample. The final dilution of RDE-treated sample is 1:10, which was the highest serum concentration used in the HI assay to prepare twofold serial dilutions in 96-well microtitre plates at the volume of 25 µl per well. Viruses and antigens were diluted to four hemagglutination units (HAU) in 25 µl and were allowed to react with diluted sera at room temperature for 30 minutes. Afterwards, 50 µl of 0.5 per cent Turkey red blood cells (RBCs) was added to all wells and incubated at room temperature for 30 minutes; turkey RBC has been shown to react well in detecting antibody against A/canine/Florida/2004(H3N8) virus from canine patients.[18] HI titre was recorded as the reciprocal of the last dilution of serum that completely inhibits hemagglutination.

Statistical analysis

A paired t-test was used to compare the differences in HI titres and age. McNemar's test was applied to examine the agreement between the ELISA and HI assay test results. Cohen's kappa coefficient (κ) was used to estimate inter-rater reliability between the ELISA and HI tests. Calculation of McNemar's P value and Cohen's κ are performed using the GraphPad software.

RESULTS

To investigate the exposure history of companion and shelter dogs to influenza A viruses, sera were first tested using the commercial ELISA kit. Antibodies against NP were detected from 11/555 (2.0 per cent) sera collected from companion dogs, and a further 4/555 (0.7 per cent) sera showed borderline reactive range. No sera (0/182) collected from the shelter dogs was tested positive by the ELISA test.

To assess specific exposure history of the companion dogs to canine and human influenza viruses, HI assay was applied using HI titre ≥1:20 as the cut-off value. Among 555 companion dogs, 6/555 (1.1 per cent) (median HI titre=40) and 3/555 (0.5 per cent) (median HI titre=80) sera reacted to Canine/H3N2 and Canine/H3N8 viruses, respectively; specifically, two of these sera showed cross-reactivity to both CIVs (figure 1A and table 1). Overall, a total of 7/555 (1.3 per cent) companion dogs had exposure history to CIV. A higher seropositive rate was noted for A(H1N1)pdm09 human influenza viruses, as 53/555 (9.5 per cent) (median HI titre=40) sera reacted to CA07/H1N1. None of the sera reacted to Perth16/H3N2 or CH9715293/H3N2 influenza viruses but six samples showed low HI titre=10 to Perth16/H3N2 virus (figure 1A). In addition, 13/555 (2.3 per cent) sera showed HI titre ≥1:20 against the EAsw/H1N1 virus (median HI titre=40), which is highly homologous to the swine-origin H1N1 CIV detected in Southern China (figure 1a). Among these, 9/13 also cross-reacted with the CA07/H1N1 virus (figure 1a and table 1). Table 1 summarised the cross-reactivity of the canine sera.

To evaluate if the age distribution was comparable between seropositive and seronegative dogs, one of the clinics was selected for further analyses. Among 150 sera samples collected from this clinic, 14 samples showed positive antibody response and 136 samples showed no antibody response to human influenza or canine influenza viruses. The median age of the seropositive dogs and seronegative dogs were 108.5 months (range 2–172 months) and 112 months (range 8–216 months), respectively (P=0.93, t-test).

Interestingly, a different seroprevalence profile was noted among the 182 shelter dogs. None of the sera reacted to CIV (Canine/H3N8 and Canine/H3N2) or human influenza viruses (CA07/H1N1 and CH9715293/H3N2) at HI titre ≥20. Only two sera reacted to human influenza virus Perth16/H3N2 (2/182) (HI titres: 80 and 80, respectively) (figure 1b). In addition, none of the sera reacted to the EAsw/H1N1 virus that is highly homologous to the swine-origin H1N1 CIV detected in Southern China. Overall, shelter dogs showed limited exposure to CIV or human influenza viruses.

McNemar's test shows the ELISA results were significantly different from HI test results (table 2) (P<0.0001); sera showed doubtful results by ELISA test were considered as negative in the contingency table. In addition, Cohen's κ of 0.17 (95 per cent CI=0.05 to 0.28) suggested poor agreement between the ELISA and HI assay test results.

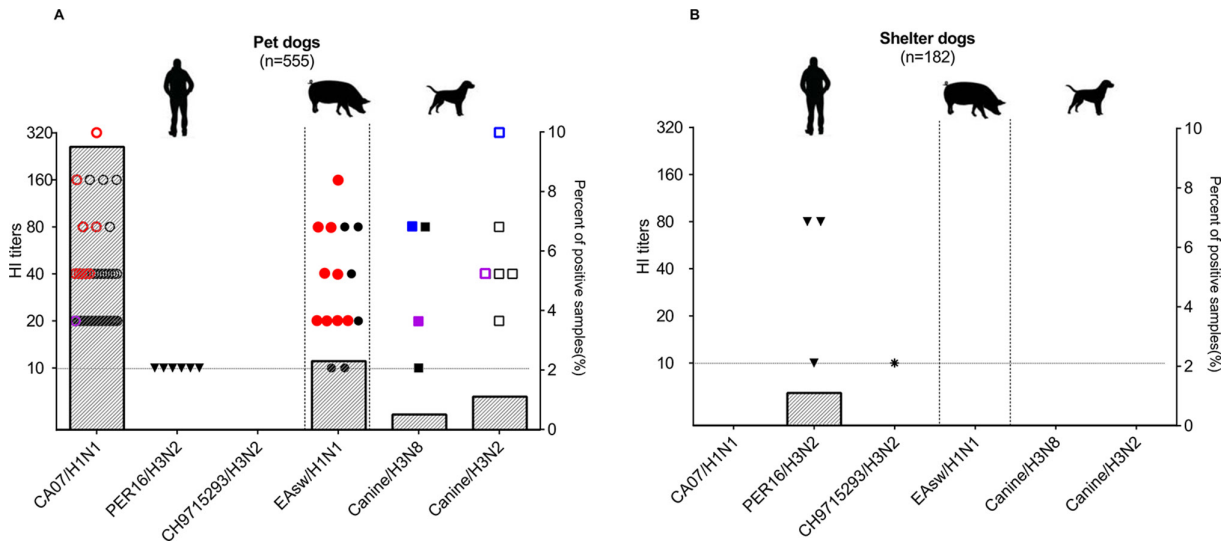

Figure 1 Seroprevalence and antibody titres of (a) companion and (b) shelter dog to influenza viruses of different host origin. Hemagglutination inhibition (HI) titres (left y axis) against human influenza viruses (CA07/H1N1 (open circles), Perth16/H3N2 (solid triangle), CH9715293/H3N2 (star)) and canine influenza viruses (Canine/H3N8 (solid square), Canine/H3N2 (open square), EAsw/H1N1 (solid circle)) are shown. Only the serum with no less than HI titres of 20 is considered as positive. Seroprevalence (%) (right y axis) are shown in columns. Sera showing cross-reactivity between CA07/H1N1 and EAsw/H1N1 are highlighted in red. Sera showing cross-reactivity between Canine/H3N8 and Canine/H3N2 are highlighted in blue. One serum showing cross-reactivity to CA07/H1N1, Canine/H3N8 and Canine/H3N2 viruses is highlighted in purple. The HI detection limit (HI=10) is shown by the horizontal dotted line.

DISCUSSION

Among the diverse reservoir hosts for influenza A viruses, dogs are in close contact with humans especially under urban cities settings. While high seropositive rate (range from 6.7 per cent to 10.7 per cent) to Canine/H3N2 virus have been reported from dogs in the inland cities of China, such as Shenzhen, Guangzhou, Shanghai and Beijing,[19] the results showed that the companion and shelter dogs in Hong Kong showed limited exposure history to Canine/H3N8 (3/737, 0.4 per cent) or Canine/H3N2 (6/737, 0.8 per cent) viruses from 2015 to 2018. In contrast, a higher proportion of dogs (55/737, 7.5 per cent) in Hong Kong have been exposed to human influenza viruses. Human contact dynamics probably contributed to the differences in seroprevalence to human influenza viruses the authors observed between the companion dogs (53/555, 9.5 per cent) and the shelter dogs (2/182, 1.1 per cent). The seropositive rate of companion dogs to human influenza viruses in Hong Kong were higher than that reported in companion dogs in Northern China (2.7 per cent for A(H1N1)pdm09 and

Table 1 Detection frequency of cross-reactive HI titre against canine, human or swine influenza viruses

	H1		H3	
Sample ID	CA07 /H1N1	EAsw /H1N1	Canine /H3N8	Canine /H3N2
HVV 7 extra	<10	<10	80	320
HVV 34 extra	80	40	<10	<10
PEV-5	320	160	<10	<10
PEV-47	40	20	<10	<10
PEV-165	80	80	<10	<10
PEV-187	40	20	<10	<10
PHA-2	40	40	<10	<10
PHA-20	20	<10	20	40
SAP-50	40	80	<10	<10
SAP-67	160	20	<10	<10
SAP-125	20	<10	<10	40
SAP-135	40	20	<10	<10

*HI assay was determined using RDE-treated sera and 0.5 per cent Turkey red blood cells. All samples were performed in duplicate and the mean HI titres were shown.
HI, hemagglutination inhibition.

Table 2 Detection frequency of anti-influenza antibody in canine sera by competitive ELISA assay and HI assay

	HI assay*		
	Positive	Negative	Total
ELISA†			
Positive	7	4	11
Negative	57	669	726
Total	64	673	737

*Serum with HI titre ≥20 to any of the canine (Canine/H3N8, Canine/H3N2), human (CA07/H1N1, Perth16/H3N2, CH9715293/H3N2) or Eurasian swine (EAsw/H1N1) influenza viruses by HI assay was counted. Serum cross-reacted against multiple influenza viruses was counted once.
†Doubtful results by ELISA test were considered as negative in the contingency table.
HI, hemagglutination inhibition.

H3N2 viruses, HI titre ≥32) but lower than that reported in companion dogs (20.5 per cent for A(H1N1)pdm09, HI titre ≥32) in Southern China.[9 11] Taken together, the results suggest that humans may serve as the major source of exposure to influenza virus for dogs in a densely populated city. As well-connected dog population has been proposed to sustain CIV transmission in dogs,[16] the results suggest the likelihood of large-scale canine influenza outbreaks in Hong Kong may be low considering the contact dynamics of dogs in Hong Kong.

We identified our sera that were specifically reacted to the EAsw/H1N1 virus without cross-reacting with the CA07/H1N1 virus. The EAsw/H1N1 virus was antigenically similar to the swine-origin CIV that were recently isolated from Southern China. The result highlights the necessity to further investigate the potential source of exposure and to continue monitoring influenza at the human-animal interface, which should include the companion animals.

CONCLUSION

Companion and shelter dogs sampled between 2015 and 2018 in Hong Kong showed lower seroprevalence to CIV of H3N2 or H3N8 subtype (7/737, 0.9 per cent) than to human influenza viruses of A(H1N1) pdm09 or H3N2 subtypes (55/737, 7.5 per cent). Our results suggest that the contact dynamics of dogs in a dense and highly populated urban environment as Hong Kong may affect the exposure risk to human and canine influenza viruses. The high exposure frequency of dogs to human influenza viruses highlights the importance of monitoring the influenza interspecies transmission between humans and the companion animals.

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