We investigated the infectivity and transmissibility of the human seasonal H3N2, pandemic (pdm) H1N1 (2009) and B influenza viruses in dogs. Dogs inoculated with human seasonal H3N2 and pdm H1N1 influenza viruses exhibited nasal shedding and were seroconverted against the viruses; this did not occur in the influenza B virus-inoculated dogs. Transmission of human H3N2 virus between dogs was demonstrated by observing nasal shedding and seroconversion in naïve dogs after contact with inoculated dogs. The seroprevalence study offered evidence of human H3N2 infection occurring in dogs since 2008. Furthermore, serological evidence of pdm H1N1 influenza virus infection alone and in combination with canine H3N2 virus was found in the serum samples collected from field dogs during 2010 and 2011. Our results suggest that dogs may be hosts for human seasonal H3N2 and pdm H1N1 influenza viruses.

Influenza virus is a clinically and economically important virus that can infect diverse species and phyla, including humans, pigs, horses and fowl (Wright et al., 2001). Studies of the epidemiology and pathogenesis of influenza have largely overlooked the role of dogs in disease transmission. Furthermore, the contribution of influenza to respiratory diseases in companion animals such as dogs and cats has not previously been considered important in practice (Beeler, 2009; Harder & Vahlenkamp, 2010). There has also been no evidence for sustained transmission of human seasonal influenza viruses in dogs, although outbreaks of equine origin canine influenza H3N8 viruses have been observed in racing greyhounds in the USA (Crawford et al., 2005). Avian origin canine influenza H3N2 viruses have been reported in South Korea and China (Li et al., 2010; Song et al., 2008; Song et al., 2009). Thus, dogs can be infected by several types of influenza virus.

As pets, dogs live in close contact with people and there are many opportunities for contact with the seasonal epizootic influenza virus. This could pose an important public health concern, because pre-existing canine influenza virus may recombine or reassort with human seasonal viruses and give rise to novel viruses that could in turn lead to unique pandemics. In this study, we investigated the virological characteristics and pathology of beagle dogs inoculated with human seasonal influenza viruses (H3N2, pdmH1N1 and influenza B).

All viruses were obtained from the Korea Centers for Disease Control and Prevention or from Green Cross Veterinary Products Company. The influenza viral strains were: (1) pdm H1N1 (A/California/04/2009), (2) human H3N2 (A/Brisbane/10/2007) and (3) human influenza B virus (B/Brisbane/60/2008). Stock viruses were propagated once in 10-day-old embryonated chicken eggs maintained at 37 °C for 48 h.
Fig. 1. Daily estimated body temperature (right-hand panels) and nasal shedding of the viruses (left-hand panels) in pdm H1N1-inoculated and pdm H1N1-contact dogs (a), seasonal H3N2-inoculated and seasonal H3N2-contact dogs (b), influenza B-inoculated and influenza B-contact dogs (c). In the viral titre plots, each bar represents data from a separate dog. Body temperatures were converted to percentage values by using the pre-inoculation body temperature as a denominator. Each symbol represents the mean value of the temperatures from three dogs, and each error bar indicates the standard deviation. DC, Direct contact, IN, intranasally inoculated, NC, negative control. Viral titres are expressed as $\log_{10}\text{EID}_{50}\text{ ml}^{-1}$. 

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Twenty-one 9-week-old beagle puppies, which were serologically negative for influenza virus, were used. The animals were randomly assigned to one of the following experimental groups (three dogs per group): pdm H1N1-inoculated, pdm H1N1-contact, human H3N2-inoculated, human H3N2-contact, influenza B-inoculated, influenza B-contact and a negative control group.

Dogs were anaesthetized by intramuscular injection of Zoletil (VIRBAC, 10 mg kg$^{-1}$) and then inoculated intranasally with a viral load of $10^{6.5}$ EID$_{50}$ in 2 ml sterile PBS (1 ml per nostril). After inoculation, the dogs were housed in a cage designated to the experimental group to which it belonged. Dogs assigned to the viral contact groups were introduced to their respective cages 24 h after those in the inoculation groups were inoculated, i.e. the H1N1-contact group was introduced to H1N1-inoculated dogs after a period of 24 h.

We monitored clinical signs and rectal temperature every day for 10 consecutive days post-inoculation (days p.i.) or days post-contact (dpc). Nasal swabs for viral titration were collected by applying moistened cotton wads to both nostrils. Viral shedding was quantified by real-time PCR as described previously (Lee et al., 2012; Song et al., 2011). The log EID$_{50}$ ml$^{-1}$ was calculated from the real-time PCR results, using the regression curve of ct values in serially diluted viruses. Blood samples for serological assessment were collected 10 days p.i. Serum antibodies from experimental inoculated groups were assessed by a haemagglutination inhibition (HI) assay (Infl & Manu, 2002; Lerdsamran et al., 2011) coupled with commercially available competitive nucleoprotein (NP) ELISA kits (Bionote). All experiments with animals and viruses were conducted in biosafety level 2-plus facilities at the Korea Research Institute of Bioscience and Biotechnology. All procedures involving animals were approved by the Institutional Animal Care and Use Committee.

The pdm H1N1 virus was only detected between 1 and 8 days p.i. in the nasal swabs of two of three dogs in the H1N1-inoculated group (Fig. 1a); no nasal shedding of pdm H1N1 virus was found in the nasal swabs of dogs in the pdm H1N1-contact group. All dogs in the pdm H1N1-inoculated group were seroconverted at 10 days p.i., showing average 640 HI mean titers (Table 1). No seroconversion was detected in the pdm H1N1-contact group.

Between 3 and 10 days p.i., we detected human H3N2 virus in dogs in the human H3N2-inoculated group, although the shedding period differed for each dog (Fig. 1b). The nasal swabs of dogs in the seasonal H3N2-contact group tested positive for H3N2 virus between 4 and 9 dpc (Fig. 1b). At 10 days p.i. or dpc, all dogs in the human H3N2-inoculated and contact groups were seroconverted, showing average 43.4 and 35 HI titres, respectively (Table 1).

We did not detect influenza B virus in the influenza B-inoculated or contact groups (Fig. 1c). No seroconversion was found (Table 1).

Evidence suggests dogs are susceptible to certain strains of influenza viruses, including pdm H1N1 (Lin et al., 2012), and concern for their role in interspecies transmission of influenza to humans and/or other species has been growing. Because dogs have unique behavioural characteristics, their role in the genesis of novel influenza pandemics should not be ignored – especially when considering that in many countries dogs are considered part of the family and that approximately 50% of dog owners allow their dog(s) to sleep with them on their bed (Chomel & Sun, 2011). Moreover, recent reports of avian origin canine H3N2 influenza virus showed that dogs can have receptors for both avian and human influenza viruses, SA$_{2,6}$-gal and SA$_{2,3}$-gal, respectively (Song et al., 2008). In the present study, we inoculated or contact-exposed dogs to human-infecting pdm H1N1, human H3N2 and influenza B virus to assess the infectivity of these viruses in dogs.

Dogs could not be infected with influenza B virus, and we found no serological or virological changes in dogs inoculated or exposed to this viral strain. Influenza B virus infection was previously detected in seals (Bodewes et al., 2013), but our results suggest it is unlikely that dogs are a natural host for this virus.

Consistent with previous reports (Lin et al., 2012), dogs in this study could be infected with pdm H1N1 influenza virus (viral shedding and seroconversion), but they do not

<table>
<thead>
<tr>
<th>Table 1. Canine serological responses</th>
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<tr>
<td>Positive rate of NP ELISA*</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Pdm H1N1 inoculated</td>
</tr>
<tr>
<td>Pdm H1N1 contact</td>
</tr>
<tr>
<td>Human H3N2 inoculated</td>
</tr>
<tr>
<td>Human H3N2 contact</td>
</tr>
<tr>
<td>Influenza B inoculated</td>
</tr>
<tr>
<td>Influenza B contact</td>
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*Samples were classified as positive if the percentage inhibition (PI) value was $\geq 50$ and negative if the PI value was $< 50$. –, Not performed.
seem to transmit the virus to other dogs. Human H3N2 virus (A/BR/10/07 [H3N2]), on the other hand, infected dogs and was transmitted between them, causing nasal viral shedding. The dogs inoculated with H3N2 virus as well as those in contact with the virus were all seropositive at 10 days p.i. or dpc. This is evidence of human seasonal H3N2 influenza virus infection and transmission in dogs and suggests the possibility of transmission of the human H3N2 influenza virus between humans and dogs.

Our results clearly show that pdm H1N1 and human H3N2 infect dogs, but there are differences in their infectious efficiency. Seasonal H3N2 virus was more infectious in dogs than the other viruses used in this study. To test the hypothesis that human H3N2 virus infection might be common in dogs, canine sera collected between 2007 and 2011 were tested for HI titres of H3N2 virus (Table 2). We found H3N2 seropositive canine sera in samples collected from 2008 onwards. Although the geometric mean titre (GMT) HI titre was not high, these results suggest seasonal H3N2 can infect dogs. In the cross-reactivity test with three canine sera which were seropositive for human H3N2 virus (data not shown), the human H3N2 virus-positive sera were negative for canine H3N2 (<10). Seropositive samples against canine H3N2, pdm H1N1 and influenza B viruses were also found in the historical canine sera. Of particular interest was the finding of co-positivity for canine H3N2 and pdm H1N1 in sera dated from 2010. This indicates that dogs can be dually or sequentially infected with these viruses, making them possible hosts for the generation of a new influenza virus. This premise is supported by reports of a novel reassortant canine H3N1 influenza virus derived from pdm H1N1 and canine H3N2 (Song et al., 2012). Therefore, dogs would be victims of reverse zoonoses of pdm H1N1 and vectors for novel viral variants (e.g., a novel reassortant human/canine H3N2).

Dogs, in general, have closer contact with humans than pigs do, even sharing a bed with their owners (Chomel & Sun, 2011). Dogs are also infected by avian influenza viruses in poultry markets (Su et al., 2014). Our findings show that dogs may be hosts for human influenza viruses (pdm H1N1 and human H3N2) and may be co-infected with canine H3N2 virus. It is clear that the interaction between dogs and their owners could be an important interface for the recombination and transmission of zoonotic influenza A viruses. This premise is supported by the report of a 13-year-old pet dog from New York that became ill after its owner was diagnosed with pandemic H1N1 during the 2009 H1N1 pandemic, indicating probable human-to-animal transmission (Keenliside, 2013).

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### References


### Table 2. Seroprevalence of seasonal H3N2, canine H3N2, influenza B and pdm H1N1 influenza viruses in dogs from 2007 to 2011

<table>
<thead>
<tr>
<th>Year</th>
<th>HI GMT*</th>
<th>Chick RBC</th>
<th>Turkey RBC</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>A/Brisbane/10/07 (H3N2)</td>
<td>A/canine/Korea/GCVP01/2007 (H3N2)</td>
</tr>
<tr>
<td>2007</td>
<td>&lt;10 (0/44)</td>
<td>&lt;10 (0/44)</td>
<td>40 (1/44)</td>
</tr>
<tr>
<td>2008</td>
<td>10 (21/103)</td>
<td>&lt;10 (0/103)</td>
<td>10 (2/103)</td>
</tr>
<tr>
<td>2009</td>
<td>19 (21/223)</td>
<td>67 (8/223)</td>
<td>10 (1/223)</td>
</tr>
<tr>
<td>2010</td>
<td>11 (7/272)</td>
<td>61 (25/272) †</td>
<td>40 (1/272)</td>
</tr>
<tr>
<td>2011</td>
<td>10 (1/73)</td>
<td>48 (12/73) ‡ 8</td>
<td>&lt;10 (0/73)</td>
</tr>
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*The results are the GMTs of positive sera (>10). The numbers of positive samples are indicated in parentheses (number of positive samples/total number of samples).

†Eleven serum samples were co-seropositive against A/canine/Korea/GCVP01/2007 (H3N2) and A/California/04/09 (H1N1).

‡Nine serum samples were co-seropositive against A/canine/Korea/GCVP01/2007 (H3N2) and A/California/04/09 (H1N1). HI, Haemagglutination inhibition; RBC, red blood cells.


