DOMESTIC CATS AS HOSTS FOR HUMAN INFLUENZA A AND B VIRUSES IN BRAZIL

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ABSTRACT

Studies on the host range of influenza viruses have been of great importance to prove the role of some animals that were already considered as unlikely links, in the virus transmission chain. This study aimed to investigate the circulation of the influenza virus in cats in Brazil. Domestic cats, assisted at the clinic of the Faculty of Veterinary Medicine at the University of São Paulo, were grouped according to gender and age (young and adult). Serum samples were collected and analyzed for antibodies to influenza A and B viruses by the haemagglutination inhibition (HI) test using the corresponding antigens from the circulating viruses in Brazil. Twenty per cent of the cats aged between 6 and 20 years old responded with high antibody titers (≥ 640 HIU/25 µL) against human influenza A (H1N1) virus. Lower percentages of the animals in the same age group, 11% and 8%, presented the same high titers in response to human influenza A (H3N2) and B virus samples respectively. When the animals were classified by gender, 17 % of males and 8% of females showed a poor antibody response against the influenza A (H1N1) virus (titers of ≤ 20 HIU/25 µL). For the first time in Brazil, protective antibody titers against human influenza viruses revealed the presence of virus infection possibly transmitted to the domestic cats by man. In conclusion, our results show that domestic cats, like other mammals, may play a role in interspecies transmission and spread of the influenza virus.

INTRODUCTION

The influenza viruses belong to the family Orthomyxoviridae, presenting RNA as genome and exhibiting hemagglutinin (H) and neuraminidase (N) as glycoproteins inserted in their lipoproteic envelope. Their genera A and B has been causal agents of human and animal infections, the hemagglutinin and neuraminidase permitting to classify the influenza A virus samples for 16 (H1 to H16) and 9 (N1 to N9) subtypes, respectively (Taubenberger & Morens 2010). The first study on the influenza A virus infection in cats and dogs dates back to the 1970s and was stimulated by concerns about interspecies transmission and the emergence of new influenza pandemics (Paniker & Nair 1970). Based on their findings that domestic cats were susceptible to the influenza viruses, they conducted another study with animals including domestic cats (Paniker & Nair 1972). Serological analysis and experimental infection in cats indicated that they could directly transfer the virus to another susceptible host. Their results suggest that, as cats are in close contact with humans, they may act as vectors for the spread of the influenza viruses.
Not long ago, genetic recombinations among subtypes H1, H2, H3 of the influenza A virus in species, such as birds and mammals, were a major concern, but, according to recent studies, avian influenza A virus from subtype H5 seems to adapt in mammals through recombination with influenza A virus in pigs. Thus, avian influenza viruses could be transmitted to susceptible hosts like pigs, horses and humans (Webster et al. 1997).

Other studies have reported that cats are the most susceptible to influenza viruses, including the subtype H5N1, which has caused several deaths among felines such as cats, tigers and leopards, previously considered to be resistant to influenza virus infection. Within recent years, it has been observed that feeding on infected birds is the most likely route of influenza infection of felines; moreover, ingestion of non-cooked chicken has been reported to cause fatal influenza in carnivores (Van Riel et al. 2007, Vahlenkamp et al. 2008).

In Germany and Austria, avian influenza virus infections in cats have been reported which confirm their susceptibility to infections and their ability to transmit the virus to other species, mainly dogs and humans (Marshall et al. 2008, Song et al. 2009).

The present work aimed to investigate exposure of cats to human influenza viruses, and their role in the virus epidemiology and to supply data to the scarce literature on the incidence in Brazil of influenza viruses in felines.

MATERIAL AND METHODS

Animals.

In order to detect the presence of influenza viruses in cats and evaluate their serum response to the viruses, 42 domestic cats, assisted at the clinic of the Faculty of Veterinary Medicine at the University of São Paulo, were grouped according to gender (19 male cats and 23 female cats) and age (7 cats aged between <1 and 5, 18 aged between 6 and 10, 16 aged between 11 and 20 and 1 over 20 years old). The protocol of the experiments was certified by the Ethic Committee in the use of animals of the School of Veterinary and Animal Science of the University of São Paulo nr. 2021/2010.

Serum.

Blood serum samples were taken, inactivated by heat treatment at 56°C for 30 min and then treated with Kaolin at 20% and erythrocytes at 50% according to Mancini et al. 2004.
**Serology.**

The haemagglutination inhibition (HI) test was performed according to Mancini et al. (2004) using 4 HAU/25 µl (4 haemagglutination units/25 µl) of viruses, with determination of the haemagglutination inhibition titers (expressed as haemagglutination inhibition units per 25 µl = HIU/25 µL) of the sera.

**Antigens.**

Antigens of the influenza viruses from types A/SP/2/95 ≅ A/Beijing/353/89 (H3N2), A/SP/1/91 ≅ A/Singapore/6/86 (H1N1) and type B/SP/1/91 ≅ B/Panama/45/90 (B) were used. Influenza virus samples were grown in MDCK (Madin Darby canine kidney) cell cultures (Mancini et al. 2004).

**RESULTS**

The analysis of cat sera against human influenza A (H1N1) viruses by haemagglutination inhibition test showed that 20% of cats aged between 6 and 20 years old responded with high antibody titers (≥ 640 HIU/25 µL). Lower percentages of the animals in the same age group, 11% and 8%, presented the same high titers in response to human influenza A (H3N2) and B viruses, respectively. The only cat over 20 years old showed high titers against both influenza A (H3N2) and B viruses. Ten percent (10%) of the younger cats (between <1 to 5 years old) responded to influenza A and B viruses with titers ranging from ≥20 to 80 HIU/25 µL (Tables 1, 2 and 3).

**Table 1.** Antibody response in cats against to influenza A/SP/2/95 ≅ A/Beijing/353/89 (H3N2) analyzed by haemagglutination inhibition test.

<table>
<thead>
<tr>
<th>Number of cats</th>
<th>Age/group</th>
<th>HI Antibody level (HIU/25 µL)</th>
<th>&lt;20</th>
<th>20 to 8</th>
<th>&gt;80 to 160</th>
<th>&gt;160 to 320</th>
<th>≥640</th>
</tr>
</thead>
<tbody>
<tr>
<td>07</td>
<td>&lt;1 to 5yr</td>
<td>0%</td>
<td>9%</td>
<td>4%</td>
<td>2%</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>6 to 10yr</td>
<td>7%</td>
<td>4%</td>
<td>7%</td>
<td>10%</td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>11 to 20yr</td>
<td>7%</td>
<td>7%</td>
<td>9%</td>
<td>14%</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>01</td>
<td>&gt;20yr</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>14%</td>
<td>20%</td>
<td>20%</td>
<td>26%</td>
<td>20%</td>
<td></td>
</tr>
</tbody>
</table>

_yr= years old_
Table 2. Antibody response in cats against to influenza A/SP/1/91 ≅ A/Singapore/6/86 (H1N1) analyzed by haemagglutination inhibition test.

<table>
<thead>
<tr>
<th>Number of cats</th>
<th>Age/ group</th>
<th>HI Antibody level (HIU/25 µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≤20</td>
</tr>
<tr>
<td>07</td>
<td>&lt;1 to 5 yr</td>
<td>2%</td>
</tr>
<tr>
<td>18</td>
<td>6 to 10 yr</td>
<td>2%</td>
</tr>
<tr>
<td>16</td>
<td>11 to 20 yr</td>
<td>7%</td>
</tr>
<tr>
<td>01</td>
<td>&gt;20 yr</td>
<td>0%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>11%</td>
</tr>
</tbody>
</table>

yr = years old

When the animals were classified by gender, it was observed that 8% of the females presented low antibody titers (≤ 20 HIU/25 µL) to the influenza A (H1N1) virus, while a higher percentage of males (17%) responded with low titers against the same virus sample. As for the influenza A (H3N2) virus, more female cats (21%) showed lower antibody titers than male cats (5%); however, poor antibody response (≤ 20 HIU/25 µL) against influenza B virus was observed more in males (24%) than in females (8%). Protective titers (≥40 HIU/25 µL) against influenza A (H1N1), A (H3N2) and B viruses were detected in 70% of these cats, female or male (Fig. 1 and Fig. 2).
Figure 1. Percentage of haemagglutination inhibition titers (HIU/25 µl) against influenza A (H1N1), A (H3N2) and B viruses in female cats.

Figure 2. Percentage of haemagglutination inhibition titers (HIU/25 µl) against influenza A (H1N1), A (H3N2) and B viruses in male cats.
DISCUSSION AND CONCLUSION

Klopfleisch et al. (2007) have investigated the infection of cats with human influenza A (H3N2) and A (H2N2) viruses via horizontal interspecies transmission. Also, they have reported the death of felines, such as domestic cats and tigers from a zoo, infected with avian influenza A (H5N1) virus after ingestion of chicken carcasses infected with the viruses. Natural and experimental infections investigated by Marshall et al. (2008) have shown that cats are susceptible to avian influenza A (H5N1) virus and that they can be severely and fatally affected. According to Neumann & Kawaoka (2006), the factors that determine the interspecies transmission and pathogenicity of influenza viruses are still poorly understood; however, the HA protein plays an important role in overcoming the interspecies barrier and in virulence of the avian influenza viruses.

Viral attachment to the host cell is critical for the tissue and species specificities of virus infections. Thus, the pattern of viral attachment (PVA) to either the human or animal respiratory tract determines the extent of influenza virus infectivity (Van Riel et al. 2007).

Since 1996, influenza A (H7N7), (H5N1) and (H9N2) viruses have been transmitted from birds to humans, but have apparently failed to spread in the human population. Influenza virus transmission between humans and other animals has also been demonstrated (Alexander & Brown 2000, Webster et al. 1997).

In pioneering studies on influenza infection in cats, based on previous findings that mammalians, such as horses and pigs, and birds participate in the virus transmission, Paniker and Nair (1970 and 1972) suggested that domestic cats could act as vectors in the transmission chain. Serological analysis showed that adult cats and kittens responded against the influenza A2/Hong Kong/68 (H3N2) virus sample, presenting an average of antibody titers of $\geq 80$ HIU/25 µL (Paniker & Nair 1970, Paniker & Nair 1972).

Vahlenkamp et al. (2008) have successfully infected cats with the influenza A H5N1 virus and reproduced the disease. Diseased cats excreted the virus not only via the respiratory tract, but also via the digestive one. Also, serological results obtained in the haemagglutination inhibition (HI) test showed that cats responded with high antibody titers at 14 days post-infection.

The first cases of lethal respiratory disease associated with pandemic (H1N1) 2009 influenza virus infection in domestic cats have recently been reported in Oregon (USA). The most likely sources of infections were the people in the household with influenza-like illness or confirmed pandemic (H1N1) 2009 influenza (Löhr et al. 2010).
Therefore, based on our data and results from another research groups, it can be concluded that domestic cats in close contact with humans can be infected by human influenza viruses. Likewise, other felines can acquire the virus through contact with infected wild birds. However, there is no evidence of the transmission of the avian influenza A (H5N1) virus from infected cats to humans. Even so, cats may play a considerable role in the evolution and adaptation of the virus to mammals, including humans, as already considered (Paniker & Nair 1970, Paniker & Nair 1972, Klopfleish et al. 2007, Marshall et al. 2008, Vahlenkamp et al. 2008, Löhr et al. 2010). In our study, for the first time in Brazil, it is revealed the presence of influenza virus infection possibly transmitted to the domestic cats by man.

Experimental infection studies carried out by Song et al. (2008) suggest potential for direct transmission of avian influenza A (H3N2) virus from poultry to dogs. Their results provide evidence that dogs may play a role in interspecies transmission and spread of the influenza viruses.

In order to prevent influenza virus infection and interspecies transmission, influenza vaccination is strongly recommended for cats as well as dogs. Our next study will focus on domestic dogs and their likely role in the ecology of the influenza virus in the environment.

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