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BRIEF REPORT

Seroprevalence of seasonal and pandemic influenza A viruses in domestic cats

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Abstract Infection of domestic cats with pandemic H1N1 influenza virus has recently been documented. We conducted a seroprevalence survey and found that 17 of 78 (21.8%) cats sampled during the 2009–2010 influenza season had antibody titers $\geq$40 against the novel H1N1 strain by hemagglutinin-inhibition assay, compared to only 1 of 39 (2.6%) sampled in 2008 prior to emergence of the pandemic ($p = 0.006$). Seroprevalance of seasonal H1N1 (41.9%) and H3N2 (25.6%) viruses was similarly high. These data reflecting past infection of household cats raise the possibility that they may act as a vector of influenza transmission within households.

Influenza A viruses (IAVs) are medically important zoonoses of humans. Although the progenitor strains of all IAVs are thought to derive from the avian reservoir, infections have been documented in multiple species, including domestic poultry, pigs, dogs, cats, ferrets, foxes, horses, and sea mammals [1–3]. Although some of these hosts have been implicated as intermediates for transmission to susceptible humans, the extent of risk for other species is not known. In 2009 a novel H1N1 subtype IAV emerged from swine and caused the first influenza pandemic since 1968 [4]. In the course of this pandemic, severe infections of domestic house cats were documented by direct detection of viral RNA in the respiratory tract [5, 6]. Since influenza is not considered to be a common etiologic agent of respiratory disease in cats [7], we undertook a seroprevalence study to estimate how common such infections might be.

A convenience sampling of blood was collected from multiple veterinary practices across a diverse geographic footprint, including clinics in Memphis and Nashville (Tennessee), New Orleans (Louisiana), Madison (Wisconsin), St. Paul (Minnesota), and Cleveland and Toledo (Ohio) (Fig. 1). Sera and/or plasma were shipped to a central laboratory at St. Jude Children's Research Hospital (SJCRH) for analysis. Sera were treated by a standard protocol to destroy nonspecific serum inhibitors [8]. Briefly, 100 µl of serum was diluted 1:10 by addition of 300 µl *Vibrio cholerae* Ogawa type 588 receptor-destroying enzyme (Seiken, Tokyo, Japan) for 18 h at 37°C, 300 µl 2.5% (w/v) sodium citrate for 30 min at 56°C, and 300 µl phosphate-buffered saline. Reciprocals of the titers of serum needed to inhibit hemagglutination of 0.5% chicken red blood cells or neutralize infectious virus in Madin–Darby canine kidney cells were determined as described [9]. High-growth reassortant influenza viruses (gift of Richard Webby, SJCRH) expressing the surface proteins of A/California/7/09 (pandemic H1N1),

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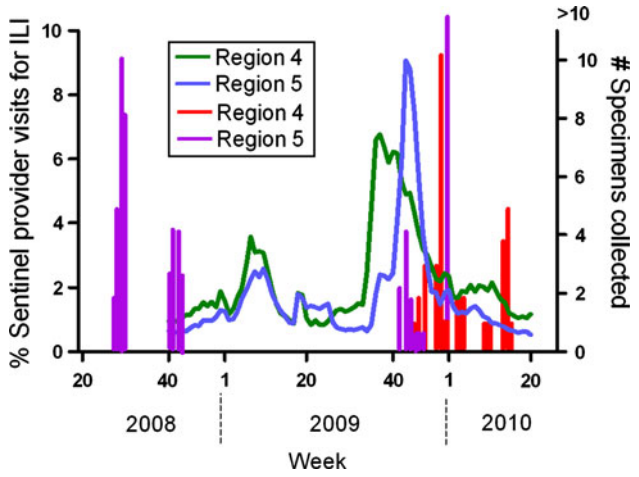

Fig. 1 Timing of specimen collection in relation to the pandemic H1N1 wave. The weekly percentage of visits for influenza-like illness based on total sentinel provider patient visits, derived from data published by the United States Influenza Sentinel Provider Surveillance Network [16], are graphed (*continuous lines*) for Regions 4 (the *South*) and 5 (the *Midwest*) from week 40 of 2008 to week 20 of 2010 (earlier data are not shown, as the geographic definition of regions had changed). This is compared to the timing of specimen collection in each region (*vertical bars*). For convenience, the 27 specimens collected in week 50 of 2009 are shown as ">10" in the figure

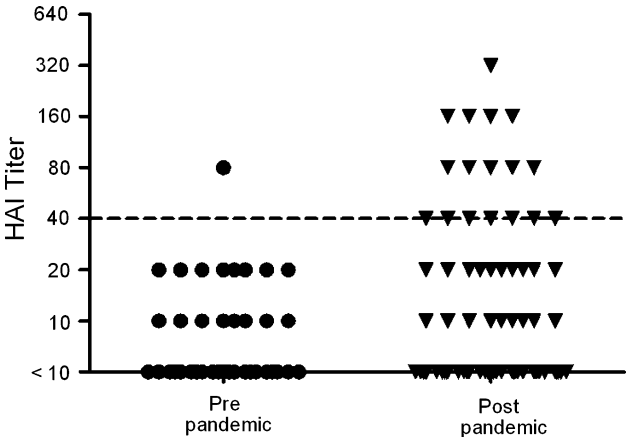

Fig. 2 Hemagglutinin-inhibition titers of cats against pandemic H1N1 influenza virus. HAI titers from sera collected from domestic cats 7/08 to 10/08 prior to emergence of the novel H1N1 virus (Pre-pandemic) are compared to those collected 12/09–4/10, after circulation of the pandemic strain began (Post-pandemic). The *dotted line* represents the minimum theoretical titer necessary to protect adult humans that is traditionally used to calculate seroprevalence. Significantly more cats are seropositive post-pandemic than pre-pandemic using this breakpoint ($p = 0.006$ by χ^2 test)

A/Brisbane/59/07 (seasonal H1N1), and A/Brisbane/10/07 (H3N2) were grown in eggs for use in these assays.

In the pre-pandemic period, there was little evidence in cats for antibodies reactive against the novel H1N1 strain. Only 1 of 39 (2.6%) samples had a titer ≥40 (Fig. 2). During the pandemic, however, 17 of 78 (21.8%) of cats were found to be seropositive. Compared to the earlier period, this was a statistically significant difference ($p = 0.006$ by χ^2 test). Some regional differences were evident despite the small sample size, as post-pandemic specimens from the South (Tennessee 4 of 13, Louisiana 10 of 27; total 14/40 = 35.0%) were more often positive than those from the Midwest (Wisconsin 3/32, Minnesota 0/6; total 3/38 = 7.9%). This disparity may be due to the relative timing of the major pandemic wave in each region; the peak of visits for influenza-like illness in the Midwest was later than in the South, and some of the sera were collected while the pandemic wave was ongoing (Fig. 1). This may have influenced our ability to detect seroconversion, as detectable antibodies might not have developed if the infection was recent. An alternate explanation is that cat-to-cat transmission is involved, and these findings stem from decreased contact in colder climes compared to the south. Since the numbers from each site were small, however, it is also possible that this represents sampling bias.

Because seasonal viruses circulate annually and the age and potential exposure history of the cats was not known, we could not analyze serology results for the seasonal H1N1 and H3N2 strains in the same manner. Thus, sera from all subjects are described together. The seroprevalence by HAI against H3N2 influenza was 25.6% (Table 1). A functional assessment of these antibodies showed that 22% of cats with HAI titers ≥40 also had microneutralization (MN) titers ≥40 (range 40–640). Seroprevalence against seasonal H1N1 influenza was higher than that against H3N2, at 41.9% ($p = 0.009$ by χ^2 test). However, fewer of these subjects had MN titers ≥ 40 (2.0%; titer = 40). The significance of the poor neutralization activity of these cat antibodies and the dissociation between HAI and MN results is not clear at this time. This may represent differences in serum inhibitors that contribute to neutralization in cats compared to humans, or may simply reflect differences in biology, with cats being less likely to mount a neutralizing response to influenza virus.

It has been appreciated for more than 40 years that cats can be infected with IAVs [10]. Experimental infections of domestic cats have been demonstrated to be possible for human, swine, and avian influenza viruses from IAV subtypes H2N2, H3N2, H5N1, H7N3, and H7N3 and for influenza B viruses [2, 10–14]. Natural infection has been documented by direct virus detection only for H5N1 and the novel pandemic H1N1 [5, 6, 14], but transmission under controlled conditions has been shown, both cat-to-cat and human-to-cat, with an H3N2 virus [10].

Few seroprevalence studies have been attempted in domestic cats. Paniker and Nair [10] surveyed household cats immediately after the 1968 pandemic and found that 6

Table 1 Seroprevalence in cats against seasonal influenza A viruses

	H1N1 (A/Brisbane/59/07)	H3N2 (A/Brisbane/10/07)
Number ≥40/total (%)	49/117 (41.9%)	30/117 (25.6%)
Geometric mean titer (95% confidence intervals)	23.9 (15.6–36.5)	15.3 (11.2–20.9)
Range of titers	<10–640	<10–160

of 28 (21.4%) had titers ≥40 by HAI to the circulating H3N2 strain, a similar proportion as seen in our data. Kawano et al. sampled multiple species in Japan, including cats, for antibody to influenza B and C viruses but found no positive samples among the 52 animals tested. Meenan et al. [15] tested sera collected immediately prior to the 1957 pandemic for evidence of infection to assess the hypothesis that cats or other domestic animals had been the source of the virus incursion into humans. Although 4 of the 39 sera tested were positive, the authors attributed this to non-specific reactivity, since the specimens had not been treated with receptor-destroying enzyme to eliminate serum inhibitors. Although this issue of nonspecific inhibition has been a concern for interpretation of previous studies [10, 11], the low prevalence of positive samples in the pre-pandemic compared to the post-pandemic seen in our study suggests that our results are due to the presence of antibody and not other components of serum.

These seroprevalence data, coupled with experimental data suggesting susceptibility to a broad range of viruses, implicate influenza as a common respiratory infection of cats. The clinical presentation of the cats documented to be infected with pandemic H1N1 by PCR thus far has been severe, with 2 of 3 cats dying [5, 6]. While conducting this study, we attempted to isolate virus from 16 cats presenting to our veterinary clinics with influenza-like illness, but throat swabs from all of these animals were negative by PCR for pandemic H1N1. The high seroprevalence and infrequent reporting of confirmed disease to this point suggest either that most infections are non-severe or that influenza is not suspected in sick cats. Prospective studies to detect influenza viruses in cats and determine the true incidence of infection by direct virus identification are needed.

In conclusion, we show in this report that past infection of domestic cats with both seasonal and pandemic IAVs is surprisingly common. This has implications for veterinary practice that should be pursued. In addition, a role for household pets as vectors for transmission within families should be considered. Similar seroprevalence studies in other regions should be conducted, and prospective natural history and incidence studies should be done.

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