HHS Public Access

Author manuscript

Prev Vet Med. Author manuscript; available in PMC 2022 October 16.

Published in final edited form as:

Prev Vet Med. ; 191: 105349. doi:10.1016/j.prevetmed.2021.105349.

Evidence of Influenza infection in dogs and cats in central Chile

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Summary

As companion animals, dogs and cats live in close contact with humans, generating the possibility of interspecies pathogen transmission events. Equine origin H3N8 and avian origin H5N1 influenza virus have been reported in dogs and cats respectively since 2004 with outbreaks associated with different strains recorded for both species in Asia and North America. To date, there have been no reports of influenza viruses from companion animals in South America. To fill this gap in knowledge, we performed active epidemiological surveillance in shelters that received abandoned animals, backyard production systems and veterinary clinics between May 2017 and January 2019 to estimate the burden of influenza infection in cats and dogs in the central region of Chile. Blood samples, oropharyngeal swabs or both were collected for influenza A virus detection by RT-qPCR, NP-ELISA, and hemagglutination inhibition assay. Logistic regression models were performed to assess the association between NP-ELISA-positivity and variables including sex and animal origin. The percentage of ELISA-positive samples was 43.5% (95% CI: 37.0 to 50.1) and 23.3% (95% CI: 10.6 to 42.7) for dogs and cats, respectively. No association was found between NP-ELISA results and sex or animal origin for either dogs or cats. Two ELISA positive samples showed hemagglutination inhibition titers against pandemic H1N1 influenza. One dog sample tested positive by RT-qPCR, indicating an overall RT-qPCR positivity in dogs of 1.1% (95% CI: 0.05 to 6.7). None of the tested cat samples were positive by this assay.

Keywords

cats; Chile; dogs; Influenza A; serology; ELISA; RT-qPCR

Conflict of interest

the authors declare that they have no conflict of interest.

Introduction:

Influenza A viruses (IAV) can infect a wide range of species, including humans, pigs, horses, seals, birds, cats, and dogs (Kawaoka et al., 1989; Beeler, 2009; Anthony et al., 2012; Yoon et al., 2014). Dogs and cats live in close contact with humans, generating the possibility of zoonotic and reverse-zoonotic transmission events (Chomel, 2014). Initial reports of influenza in dogs came from racing greyhounds infected with equine H3N8 influenza virus in 2004. The disease rapidly spread to companion dogs and has been considered endemic in the United States the late 2000's (Payungporn et al., 2008). Moreover, an avian origin H3N2 influenza virus affecting dogs in 2007 infected and transmitted between dogs and has since become endemic in China and Korea (Song et al., 2008; Song et al., 2009) and in the US since 2015 (Voorhees et al., 2017). While other subtypes have also been detected in dogs, including H5N1 (Songserm et al., 2006b; Dundon et al., 2010), pandemic H1N1 Influenza (pdmH1N1) (Dundon et al., 2010) and human-canine reassortant H3N2 (Moon et al., 2015), none of these strains have established themselves in dog populations, nor have caused major zoonotic events. While dogs are relatively permissive to different strains of Influenza virus, cats seem less prone to infection. To date, only avian origin H5N1, H5N6, H7N2 and pdmH1N1 have been identified in cats. While pdmH1N1 (Sponseller et al., 2010; Fiorentini et al., 2011) and avian origin H5Nx viral infections (Songserm et al., 2006a; Yu et al., 2015) is sporadic and has not lead to sustained transmission in cats, low pathogenic avian influenza H7N2 was identified as the causative agent of an outbreak of zoonotic influenza in a cat shelter in the US that also infected the treating veterinarian and subsequently spread to other areas of the country (Belser et al., 2017; Hatta et al., 2018). Although avian and equine origin IAV appear to play a major role in the genesis of canine and feline IAV infections, the potential for companion animals to serve as reservoirs of zoonotic infections has not been established to date. This could be due to specific host barrier that preclude natural transmission of human-origin Influenza viruses in dogs (Cauldwell et al., 2014) or could simply be due to a lack of systemic Influenza surveillance in dogs that could detect these inter-species transmission events, similarly to a situation that could be occurring in cats (Borland et al., 2020). While mounting literature highlights infections in dogs and cats in Asia and North America, little information is available in Latin America, where only Mexico and Argentina have reported surveillance results. In Mexico, one study detected antibodies to pdmH1N1 and H3N8 influenza in samples obtained from 113 household dogs by hemagglutination inhibition assay (HAI) but did not detect viral RNA in swabs (Ramírez-Martínez et al., 2013). In Argentina, HAI performed on sera obtained from 161 dogs belonging to both households and shelters did not detect any antibodies against equine H3N8 IAV (Bratanich et al., 2011). Thus, the aim of this study was to identify evidence of IAV infection in dogs and cats through active surveillance in central Chile.

Material and Methods:

Ethics statement:

All sampling activities and animal experiments were approved by the Institutional Animal Care and Use Committee (CICUA) of the University of Chile.

Sampling:

A cross-sectional study was performed using convenience sampling method from dogs and cats at veterinary clinics located in the city of Santiago and animal shelters and small rural backyard production farms (BPS) across the Metropolitan and Valparaíso regions of central Chile between May 2017 and January 2019. Samples were obtained by trained veterinarians and included blood samples, oropharyngeal swabs, or both. Oropharyngeal swabs (OP) were stored in 1 mL universal transport media (Copan, Italy) and transported to the laboratory at 4 °C and stored at −80 °C until analysis. For serological analysis, up to 1 mL (cats) and 5ml (dogs) blood was obtained from the cephalic or jugular vein and centrifuged at 4000g for 15 minutes for sera separation and stored at −20 °C until analysis.

Serology

A competitive Influenza A nucleoprotein ELISA (NP-ELISA) was performed to detect overall anti-NP antibodies in sera (Virusys Corporation, USA) following manufacturer's instructions. Sensibility and specificity information of the ELISA test were not available from the vendor due to the "for research only" characteristic of the kit. ELISA plates were read at 450 nm using a Sunrise absorbance microplate reader (TECAN, Switzerland). ELISA positive samples were further tested by HAI to screen for the presence of antibodies against the following Influenza A strains: A/California/7/09 (pdmH1N1), A/Indiana/Can/1177-17-1/2017 (avian origin H3N2) and A/canine/Florida /14/2006 (equine origin H3N8). Briefly, 25 μL of each serum sample was treated with a receptor destroying enzyme (RDE) (Denka Seiken, Co., Japan) and then serially diluted 2-fold in 25 μL of PBS in duplicate in a 96-well v-bottom plate. Twenty-five μL of a solution containing 4 hemagglutinin units of each virus were added and the plate was incubated at room temperature for 15 min before adding 50 μL of 0.5% chicken red blood cells diluted in PBS. The plate was stored at 4°C for 30 min before analysis.

Molecular analysis:

To detect the presence of Influenza A in OP swabs, viral RNA was extracted using the MagMax viral RNA isolation kit (Thermofisher, USA), following manufacturer's instructions. Viral RNA was screened by real time RT-PCR (RT-qPCR) using an Mx3000p Thermocycler (Agilent, USA) with the TaqMan Fast virus Master Mix (Thermofisher, USA) using primers and probes to specifically targeting the M influenza A matrix gene, following WHO guidelines (Control and Prevention, 2009). Samples with a cycle threshold value below 38 were considered positives (Shu et al., 2011).

Data Analysis

A logistic regression model was built for each species to assess the association between NP-ELISA test results as the outcome variable and sex and animal origin (veterinary clinics, animal shelters or BPS) as independent variables. Significance level was set at 5% and InfoStat statistical software was used (Di Rienzo et al., 2011).

Results:

Serologic and molecular analysis:

A total of 282 animals (248 dogs and 34 cats) were screened. One hundred and seven samples were obtained from BPS's (101 dogs and 6 cats), 126 from animal shelters (111 dogs and 15 cats) and 49 from veterinary clinics (36 dogs and 13 cats). From 282 blood samples collected, 260 samples (230 dogs and 30 cats) yielded sufficient sera for ELISA analysis. Forty-three point five percent (95% CI: 37.0 to 50.1) (n = 100 positive samples) dogs and 23.3% (95% CI: 10.6 to 42.7) (n = 7 positive samples) cats were positive, respectively (Figure 1). No association was found between ELISA-positivity and sex or animal origin for both dogs and cats (Table 1).

None of the NP-ELISA positive sera reacted against the canine H3Nx viruses by HAI; however, sera of a 7-year old male shelter dog and a 1-year old male cat sampled at a veterinary clinic showed 1:20 inhibition titers against pdmH1N1. The HAI test showed that specific influenza antibodies against pdmH1H1 were present in 0.43% (95% CI: 0.02 to 2,8) (1 in 230) and 3.3% (95% CI: 0.2 to 19.1) (1 in 30) of sera obtained from dogs and cats, respectively.

Finally, a total of 131 nasal swabs were collected from dogs (n = 93) and cats (n = 38). One dog sample tested positive by RT-qPCR, with a Ct value of 36.1, indicating an overall RT-qPCR positivity in dogs of 1.1% (95% CI: 0.05 to 6.7). The sample belonged to a 4-year-old backyard farm dog without clinical signs. None of the tested cat samples were positive. Regrettably, attempts to isolate the RT-qPCR positive sample by blind-passaging through embryonated eggs or MDCK cells were unsuccessful, nor could the sample be subtyped.

Discussion:

Our results show that IAV is circulating in dogs and cats in both urban and rural settings in Central Chile and are similar to studies in pet cats in North America in 2009, where the seroprevalence was 21.8% and 25.6% against pdmH1N1 and H3N2 viruses, respectively (McCullers et al., 2011). We were unable to establish any significant association between seropositivity and sex or animal origin. This is similar to other studies showing only circumstantial or no association to possible risk factors associated with seropositive dog samples (Barrell et al., 2010; Ramírez-Martínez et al., 2013). Surprisingly, overall seropositivity for dogs as measured by NP-ELISA was up to two times higher than farmed dogs and eight times higher than pet dogs in a study performed in China in 2011 (Su et al., 2013). The high seropositivity as measured by NP-ELISA coupled with the absence of equally robust HAI results in our study, could therefore be due to strain mismatch in the HAI assay to a not yet identified influenza strain circulating in dogs and cats in Chile.

Ideally, subtype and sequence information would have provided valuable information for both phylogenetic analysis and for the implementation of subtype specific neutralization or ELISA assays. Regrettably, we were not able to grow the PCR positive sample in neither cells nor eggs. This was most certainly due to the high cycle threshold value

(36.1) of the RT-qPCR positive sample, thus making subsequent subtyping and sequencing efforts fruitless. Another drawback of this study is the lack of both specificity and sensibility information of the NP-ELISA assay. While this information is available for many commercial ELISA test kits, we are confident of the quality of the assay mainly due to years of successful quality assurance and control inspections at our laboratory using this kit as well as peer-reviewed publications that back the use of this assay (Karlsson et al., 2012; Richt et al., 2012; Ahmed et al., 2015; Jang et al., 2018).

Our results indicate that past infections in dogs and cats by influenza viruses in Chile are common, and that influenza viruses are actively circulating, at least, in dogs. While little attention has been given to companion animals as hosts of the disease in the region, these results indicate that perhaps symptomatic animals have been to date misdiagnosed due to a false perception of absence of this disease in the country or due to the lack of available diagnostic tools for clinicians. Further prospective studies should therefore be conducted in dogs and cats to determine the true prevalence of this disease in these species in the region.

Acknowledgments:

We would like to thank pet owners, animal shelter supervisors and veterinary clinics who voluntarily agreed to have their animals sampled.

Funding:

This work was supported by National Institute of Allergy and Infectious Diseases, contract HHSN272201400006C.

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Impacts:

- Influenza A virus is circulating in dogs and cats in Chile.
- The lack of data on influenza in dogs and cats in Chile could be due to a lack of diagnostic tools available to clinicians.
- Surveillance of Influenza A virus infections in dogs and cats is important given the potential for emergence of novel influenza viruses with pandemic potential, particularly in an understudied region like South America.

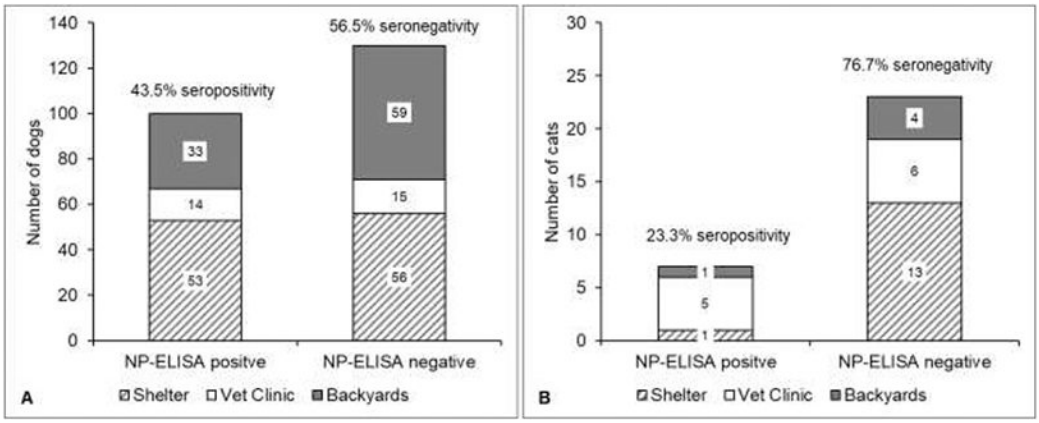

Figure 1:

NP-ELISA results for dogs (A) and cats (B) by animal origin. Samples obtained from shelter animals in gray/white parallel lines; samples obtained at veterinary clinics in white and samples obtained at backyard production systems in gray. Overall seropositivity/seronegativity indicated for each specie.

Table 1:

Logistic regression models for the association between NP-ELISA test results and independent variables (sex and animal origin) for dogs and cats.

Predictor	Estimate	SE	OR	95% CI	*P*-value
Logistic model for dogs					
Intercept	−0.80	0.27			
Sex (Ref: female)					
Male	0.39	0.28	1.47	0.85-2.56	0.171
Animal origin (Ref: BPS[a])					
Shelter	0.48	0.29	1.61	0.91-2.86	0.102
Vet Clinic	0.47	0.43	1.61	0.69-3.75	0.274
Logistic model for cats					
Intercept	−1.04	1.18			
Sex (Ref: female)					
Male	−1.08	1.27	0.34	0.03-4.10	0.394
Animal origin (Ref: BPS[a])					
Shelter	−1.31	1.55	0.27	0.01-5.65	0.399
Vet Clinic	1.74	1.49	5.72	0.31-106.71	0.243

[a]Backyard production system; SE: Standard Error; OR: Odd Ratio; CI: Confidence interval