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Authors: Peter M. DiGeronimo, Kyle Van Why, Harris Glass, Edward J. Dubovi, and La'Toya V. Latney

Source: Journal of Wildlife Diseases, 55(1) : 227-230

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/2018-03-078

DOI: 10.7589/2018-03-078

*Journal of Wildlife Diseases*, 55(1), 2019, pp. 227–230


# Serosurvey for Influenza Virus Subtypes H3N8 and H3N2 Antibodies in Free-Ranging Canids in Pennsylvania, USA

**Peter M. DiGeronimo,[1,4] Kyle Van Why,[2] Harris Glass,[2] Edward J. Dubovi,[3] and La'Toya V. Latney[1]** [1]Department of Clinical Sciences and Advanced Medicine, University of Pennsylvania School of Veterinary Medicine, 3900 Delancey Street, Philadelphia, Pennsylvania 19104, USA; [2]Wildlife Services, US Department of Agriculture Animal and Plant Health Inspection, PO Box 60827, Harrisburg, Pennsylvania 17106, USA; [3]Department of Population Medicine and Diagnostic Sciences, Cornell University School of Veterinary Medicine, 240 Farrier Road, Ithaca, New York 14853, USA; [4]Corresponding author (email: pmdigeronimo@gmail.com)

ABSTRACT: Canine influenza virus (CIV) subtypes H3N8 and H3N2 are endemic among domestic dog (*Canis lupus familiaris*) populations in the northeastern US. Infection of free-ranging carnivores with influenza virus has been sporadically reported. Generalist mesocarnivores that exploit anthropogenic, peri-urban habitats share a wide interface with domestic dogs that allows for the transmission of infectious disease. To investigate the potential exposure of free-ranging canids to CIV in Pennsylvania, US, serum samples were obtained from freshly killed coyotes (*Canis latrans*, *n*=67), grey foxes (*Urocyon cinereoargenteus*, *n*=8), and red foxes (*Vulpes vulpes*, *n*=5) from 24 counties. Animals were harvested during the January–February 2017 hunting season. We failed to detect antibodies to CIV subtypes H3N2 and H3N8 by using hemagglutination inhibition assays validated for domestic dogs. Results suggest CIV was not endemic in free-ranging canid populations in Pennsylvania or that prevalence was too low to be detected by our limited sample size.

*Key words:* Canine influenza virus, *Canis latrans*, coyote, fox, hemagglutination inhibition, *Urocyon cinereoargenteus*, *Vulpes vulpes*.

Influenza A virus is a disease of global concern known to infect domestic and free-ranging birds and mammals across diverse genera (Beeler 2009). A diversity of subtypes, high mutation rates, and the potential for reassortment in coinfected hosts makes this virus particularly challenging to monitor, prevent, and control when outbreaks occur in domestic species. When novel subtypes are transmissible between individuals, they can become endemic in populations of even novel host species (Reperant et al. 2009).

In 2004, equine influenza virus subtype H3N8 was found to cause disease and be transmissible between domestic dogs (*Canis lupus familiaris*), and within several years, this strain, known as canine influenza virus (CIV), was endemic in domestic dog populations in the northeast US (Harder and Vahlenkamp 2010; Rivailler et al. 2010). In 2016, an epidemic of CIV subtype H3N2, previously documented only in dogs in southeast Asia (Song et al. 2008), occurred among domestic dogs in the Chicago area (Newbury et al. 2016). Experimental and naturally occurring influenza virus infection has been reported in domestic dogs (*Canis lupus familiaris*), cats (*Felis silvestris catus*), ferrets (*Mustela putorius furo*), captive tigers (*Panthera tigris*), and several free-ranging mustelids and viverrids (Zitzow et al. 2002; Keawcharoen et al. 2004; Thanawongnuwech et al. 2005; Roberton et al. 2006; Thiry et al. 2007). However, the potential role of free-ranging canids in the epidemiology of an influenza virus has not been studied.

Across anthropogenic, peridomestic landscapes, generalist mesocarnivores, such as coyotes and foxes, are exposed to free-ranging wildlife and domestic animals (Kowalski et al. 2015). Sympatry of free-ranging and domestic canids can potentiate the transmission of infectious diseases between these populations and in the northeast US has allowed for hybridization between coyotes (*Canis latrans*) and domestic dogs (Monzón et al. 2014). Infectious diseases of domestic dogs, including canine parvovirus, canine distemper virus, and canine adenovirus, require direct or indirect contact for transmission and are endemic in some free-ranging coyote (Chitwood et al. 2015), grey fox (*Urocyon cinereoargenteus*; Davidson et al. 1992), and red fox (*Vulpes vulpes*; Little et al. 1998) populations. Since the emergence of CIV subtypes

TABLE 1. The numbers of adult, subadult, and juvenile coyotes (*Canis latrans*), grey foxes (*Urocyon cinereoargenteus*), and red foxes (*Vulpes vulpes*) harvested and sampled in 2017 across 24 counties in Pennsylvania, USA, for detection of canine influenza virus subtypes H3N8 and H3N2 antibodies by hemagglutination inhibition assay.

| Species | Adult | | Subadult | | Juvenile | | Total |
|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female | |
| Coyote (*C. latrans*) | 17 | 15 | 9 | 10 | 11 | 5 | 67 |
| Grey fox (*U. cinereoargenteus*) | 3 | 5 | 0 | 0 | 0 | 0 | 8 |
| Red fox (*V. vulpes*) | 2 | 0 | 1 | 1 | 0 | 1 | 5 |

H3N8 and H3N2 in the domestic dog population of the northeastern US, little is known of its possible impact on free-ranging populations of potential canid hosts. To investigate potential exposure of free-ranging canids to CIV subtypes H3N8 and H3N2, we tested coyotes, grey foxes, and red foxes for antibodies to these CIV subtypes.

Blood samples were obtained from the cardiac ventricles of freshly killed animals harvested by recreational hunters during the coyote- and fox-hunting season (January through February) from 24 counties in Pennsylvania, US. Only fresh cadavers with minimal gross evidence of autolysis were sampled. The sex and age class (juvenile, subadult, or adult) were recorded for each individual. Age class was estimated on the basis of body size and gross evaluation of tooth wear (Landon et al. 1998). Serum was collected following centrifugation and stored at 4 C for several days prior to freezing at –80 C until analysis. All federal, state, and local permits were secured prior to field work, and the project was approved by the Pennsylvania Game Commission (Special Use Permit 39894).

For each sample, serum was incubated with receptor-destroying enzyme (Denka Seiken Co., Tokyo, Japan) in a 1:3 (v/v) ratio for 16 h at 37 C prior to heat inactivation for 60 min at 56 C, and hemagglutination inhibition (HI) assays were performed (Crawford et al. 2005; Anderson et al. 2013). Briefly, at room temperature, four hemagglutinating units of CIV virus subtypes H3N8 and H3N2 (A/canine/FL/43/2004 and A/canine/IL/11613/2015, respectively) were added to an equal volume of serially diluted serum in 96-well plastic plates and incubated for 30 min prior to the addition of an equal volume of 0.5% (v/v) of turkey erythrocytes and incubation for an additional 30 min. The end point antibody titer was defined as the reciprocal of the highest dilution of serum that completely inhibited hemagglutination. A negative control included sera from specific-pathogen-free dogs from a research facility, and a positive control included sera from dogs with confirmed H3N8 and H3N2 CIV infections.

Eighty serum samples were obtained from 67 coyotes, five red foxes, and eight grey foxes harvested across 24 counties in Pennsylvania (Table 1). One sample was collected from each animal. We failed to detect circulating specific antibodies to CIV subtypes H3N8 and H3N2 (titers $<8$) in any sample by HI assay. Positive and negative control sera performed as expected. On the basis of the results of our sample, the mean true prevalence of CIV infection among coyotes was estimated to be $<0.01\%$ (SD$\pm$0.041, 95% confidence interval –0.00972 to 0.00992 when simulated over 10,000 iterations (Rogan and Gladen 1978) by using EpiTools software (Sergeant 2016). True prevalence among grey and red foxes was not estimated due to the limited sample size for these species. Sensitivity and specificity of the HI assay for coyote and fox sera were assumed to be 99.6% and 94.6%, respectively, the same as those for dog serum (Anderson et al. 2012).

Our results did not yield evidence of endemic CIV in free-ranging canids in Pennsylvania. This study assumed that coyote and fox are susceptible to CIV due to their close

phylogenetic relationship to domestic dogs, coyote and fox infected with CIV mount a specific immune response to the virus, antibodies could persist to the time of sample collection, and these antibodies are detectable by an assay validated for a closely related species. Although the HI assay is highly sensitive and specific for detecting CIV antibodies in dog samples (Anderson et al. 2012), it has not been validated for other canids.

Despite a wide potential interface between free-ranging canid and domestic dog populations, the low prevalence of CIV in dog populations may mitigate risk of exposure of free-ranging canids to CIV. Further studies are required to obtain samples representative of coyote and fox populations in Pennsylvania. Opportunistic sampling was limited to 24 of 67 of the state's counties and biased toward those individuals selected by hunters. Because hunting takes place outside of human centers, our sample may have also been biased toward individuals less likely to have contact with domestic dogs. Furthermore, in dogs, CIV transmission is facilitated by communal housing (Holt et al. 2010; Newbury et al. 2016). Therefore, transient contact between individual free-ranging canids and dogs also may limit the risk of CIV transmission.

Morbidity and mortality due to CIV vary by subtype among host species, with novel subtypes often able to expand host range and exhibit high virulence in naïve species and populations (Maines et al. 2005). Because the mortality of coyotes and foxes due to CIV is unknown, death of free-ranging canids that contract CIV prior to transmission of the disease to conspecifics and to the time of sample collection must also be considered. We conclude that free-ranging canids harvested in Pennsylvania were either unexposed to CIV subtypes H3N8 and H3N2 or that true prevalence in the population is too low to be detected by our small sample size.

We thank Rebecca Revay for her assistance in this research. The use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

## LITERATURE CITED

Anderson TC, Crawford PC, Dubovi EJ, Gibbs EPJ, Hernandez JA. 2013. Prevalence of and exposure factors for seropositivity to H3N8 canine influenza virus in dogs with influenza-like illness in the United States. *J Am Vet Med Assoc* 242:209–216.

Anderson TC, Crawford PC, Katz JM, Dubovi EJ, Landolt G, Gibbs EPJ. 2012. Diagnostic performance of the canine influenza A virus subtype H3N8 hemagglutination inhibition assay. *J Vet Diagn Invest* 24:499–508.

Beeler E. 2009. Influenza in dogs and cats. *Vet Clin N Am Small Anim Pract* 39:251–264.

Chitwood MC, Swingen MB, Lashley MA, Flowers JR, Palamar MB, Apperson CS, Olfenbuttel C, Moorman CE, DePerno CS. 2015. Parasitology and serology of free-ranging coyotes (*Canis latrans*) in North Carolina, USA. *J Wildl Dis* 51:664–669.

Crawford PC, Dubovi EJ, Castleman WL, Stephenson I, Gibbs EPJ, Chen L, Smith C, Hill RC, Ferro P, Pompey J, et al. 2005. Transmission of equine influenza virus to dogs. *Science* 310:482–485.

Davidson WR, Nettles VF, Hayes LE, Howerth EW, Couvillion CE. 1992. Diseases diagnosed in gray foxes (*Urocyon cinereoargenteus*) from the southeastern United States. *J Wildl Dis* 28:28–33.

Harder TC, Vahlenkamp TW. 2010. Influenza virus infections in dogs and cats. *Vet Immunol Immunopathol* 134:54–60.

Holt DE, Moyer MR, Brown DC. 2010. Serologic prevalence of antibodies against canine influenza virus (H3N8) in dogs in a metropolitan animal shelter. *J Am Vet Med Assoc* 237:71–73.

Keawcharoen J, Oraveerakul K, Kuiken T, Fouchier RAM, Amonsin A, Payungporn S, Noppornpanth S, Wattanodorn S, Theamboonlers A, Tantilertcharoen R, et al. 2004. Avian influenza H5N1 in tigers and leopards. *Emerg Infect Dis* 10:2189–2191.

Kowalski B, Watson F, Garza C, Delgado B. 2015. Effects of landscape covariates on the distribution and detection probabilities of mammalian carnivores. *J Mammal* 96:511–521.

Landon DB, Waite CA, Peterson RO, Mech LD. 1998. Evaluation of age determination techniques for gray wolves. *J Wildl Manag* 62:674–682.

Little SE, Davidson WR, Howerth EW, Rakich PM, Nettles VF. 1998. Diseases diagnosed in red foxes from the southeastern United States. *J Wildl Dis* 34: 620–624.

Maines TR, Lu XH, Erb SM, Edwards L, Guarner J, Greer PW, Nguyen DC, Szretter KJ, Chen LM, Thawatsupha P, et al. 2005. Avian influenza (H5N1) viruses isolated from humans in Asia in 2004 exhibit increased virulence in mammals. *J Virol* 79:11788–11800.

Monzón J, Kays R, Dykhuizen DE. 2014. Assessment of coyote-wolf-dog admixture using ancestry-informative diagnostic SNPs. *Mol Ecol* 23:182–197.

Newbury S, Godhardt-Cooper J, Poulsen KP, Cigel F, Balanoff L, Toohey-Kurth K. 2016. Prolonged intermittent virus shedding during an outbreak of canine influenza A H3N2 virus infection in dogs in three Chicago area shelters: 16 cases (March to May 2015). *J Am Vet Med Assoc* 248:1022–1026.

Reperant LA, Rimmelzwaan GF, Kuiken T. 2009. Avian influenza viruses in mammals. *Rev Sci Tech* 28:137–159.

Rivailler P, Perry IA, Jang Y, Davis CT, Chen LM, Dubovi EJ, Donis RO. 2010. Evolution of canine and equine influenza (H3N8) viruses co-circulating between 2005 and 2008. *Virology* 408:71–79.

Roberton SI, Bell DJ, Smith GJD, Nicholls JM, Chan KH, Nguyen DT, Tran PQ, Streicher U, Poon LLM, Chen H, et al. 2006. Avian influenza H5N1 in viverrids: Implications for wildlife health and conservation. *Proc Biol Sci* 273:1729–1732.

Rogan WJ, Gladen B. 1978. Estimating prevalence from the results of a screening test. *Am J Epidemiol* 107: 71–76.

Sergeant E. 2016. *Epitools epidemiological calculators*. Ausvet Pty Ltd. http://epitools.ausvet.com.au. Accessed February 2018.

Song D, Kang B, Lee C, Jung K, Ha G, Kang D, Park S, Park B, Oh J. 2008. Transmission of avian influenza virus (H3N2) to dogs. *Emerg Infect Dis* 13:741–746.

Thanawongnuwech R, Amonsin A, Tantilertcharoen R, Damrongwatanapokin S, Theamboonlers A, Payungporn S, Nanthapornphiphat K, Ratanamungklanon S, Tunak E, Songserm T, et al. 2005. Probable tiger-to-tiger transmission of avian influenza H5N1. *Emerg Infect Dis* 11:699–701.

Thiry E, Zicola A, Addie D, Egberink H, Hartmann K, Lutz H, Poulet H, Horzinek MC. 2007. Highly pathogenic avian influenza H5N1 virus in cats and other carnivores. *Vet Microbiol* 122:25–31.

Zitzow LA, Rowe T, Morken T, Shieh WJ, Zaki S, Katz JM. 2002. Pathogenesis of avian influenza A (H5N1) viruses in ferrets. *J Virol* 76:4420–4429.

*Submitted for publication 17 March 2018.*
*Accepted 17 May 2018.*