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Epidemiology and Ecology of H3N8 Canine Influenza Viruses in US Shelter Dogs

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Background: H3N8 canine influenza virus (CIV) infection might contribute to increased duration of shelter stay for dogs. Greater understanding of factors contributing to CIV within shelters could help veterinarians identify control measures for CIV.

Objectives: To assess community to shelter dog CIV transmission, estimate true prevalence of CIV, and determine risk factors associated with CIV in humane shelters.

Animals: 5,160 dogs upon intake or discharge from 6 US humane shelters, December 2009 through January 2012.

Methods: A cross-sectional study was performed with prospective convenience sampling of 40 dogs from each shelter monthly. Nasal swabs and serum samples were collected. Hemagglutination inhibition and real-time reverse transcriptase-polymerase chain reaction assays were performed for each nasal and serum sample. True prevalence was estimated by stochastic latent class analysis. Logistic regression was used to identify risk factors associated with CIV shedding and seropositivity.

Results: Nasal swabs were positive from 4.4% of New York (NY), 4.7% of Colorado (CO), 3.2% of South Carolina, 1.2% of Florida, and 0% of California and Texas shelter dogs sampled. Seropositivity was the highest in the CO shelter dogs at 10%, and NY at 8.5%. Other shelters had 0% seropositivity. Information-theoretic analyses suggested that CIV shedding was associated with region, month, and year (model weight = 0.95) and comingling/cohousing (model weight = 0.92).

Conclusions and Clinical Importance: Community dogs are a likely source of CIV introduction into humane shelters and once CIV has become established, dog-to-dog transmission maintains the virus within a shelter.

Key words: Akaike; Canine infectious respiratory disease; Humane shelter; Information-theoretic approach.

Canine influenza virus (CIV) (H3N8) was first detected in Florida (FL) racing greyhounds in 2004 and quickly spread into New York (NY) and FL shelter dog populations.[1] The transmission of influenza A viruses is thought to be by direct contact, deposition of infectious droplets (≥5 μm) on nasal or oral mucosa, or inhalation of infectious particles (<5 μm).[2] Recent CIV seroprevalence studies suggest that an increased risk of CIV infection exists for dogs housed in closed-air communal environments,[3,4] where promotion of efficient virus transmission by any of the aforementioned routes of infection is possible. Indeed, humane shelters have consistently reported high rates of CIV in dogs exhibiting signs of canine infectious respiratory disease (CIRD). In a NY and FL humane shelter study conducted shortly after CIV emergence, up to 97% of dogs were seropositive for CIV.[1] Similarly, dogs from humane shelters in Colorado (CO) were infected with CIV in 5 of 5 humane shelters reporting CIRD outbreaks in 2006 and 2007.[5] More recently, in Pennsylvania, 42% of dogs with and without clinical signs of CIRD were CIV seropositive.[6]

Abbreviations:

AICc	small sample size-corrected Akaike Information Criterion
CIRD	canine infectious respiratory disease
CIV	canine influenza virus
HI	hemagglutination inhibition
rRT-PCR	real time reverse transcriptase-polymerase chain reaction

Besides respiratory illness signaled by fever (>103°F), nasal/ocular discharge, nonproductive, persistent cough, anorexia, lethargy, and weight loss,[7] CIV infection in dogs might account for significant delays between intake into a shelter and discharge.[8] Indeed, one study found that the risk of respiratory disease increases 3% with every day a dog spends in a humane shelter.[9] Considering the threat CIV poses to health of dogs and adaptability, persistence of the virus within humane shelters is worrisome, especially as CIV transmission dynamics are virtually unknown. Studies conducted in nonshelter dog populations find very little CIV seropositivity in household, racing sled, and flyball tournament dogs,[3,10,11] suggesting CIV infection to be relatively lower in dogs from the nonshelter community. In contrast, CIV is routinely detected in humane shelters throughout the United States, especially in CO and NY, where surveillance studies have been recently performed.[4,5,12,13]

The discrepancy between CIV in community versus shelter dogs leads to more questions than answers. Particularly, what role do facility-entering dogs play in

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CIV introduction? What factors contribute to the establishment of CIV within humane shelters once the virus has been introduced? Furthermore, and perhaps the most significant biological question, are humane shelters centers for CIV amplification? Given these questions and the unknown transmission dynamics of CIV, greater knowledge of true CIV prevalence in shelter dogs, as well as a greater understanding of the epidemiologic and ecologic factors contributing to CIV persistence within humane shelters, would help shelter veterinarians and personnel identify strategies for CIV prevention. To this end, we compared CIV shedding and seroprevalence in shelter dogs upon shelter intake and discharge and evaluated factors contributing to CIV infection status in US humane shelters.

Materials and Methods

Animals

Before initiation, all studies described were reviewed and approved for conduct by the Colorado State University Institutional Animal Care and Use Committee. For these cross-sectional studies, we utilized a prospective convenience sampling method. The number of nasal swabs and serum samples required for estimation of infection rates and seroprevalence was 4,680[14] among 6 participating humane shelters in California (CA), CO, FL, NY, South Carolina (SC), and Texas (TX). Shelters were a variety of open admission, nonprofit with animal control contract, and municipal and were chosen based on expected CIV prevalence. The CA, TX, and SC shelters were expected to have relatively lower CIV prevalence, as no H3N8 CIV outbreaks had previously been reported in these facilities. In contrast, the CO, FL, and NY humane shelters were expected to have relatively higher CIV prevalence because of previous reports of CIV. The annual intake of dogs at each shelter varied from 750 to 800 (NY) to 5,000–7,000 (CA and FL) to over 10,000 (CO, SC, and TX) and all the shelters except CA and TX accepted dogs transferred from out of state. In most of the facilities (CA, CO, SC, and TX), dogs were allowed some contact with other dogs, including using common runs, playing together outside, and sharing housing.

Personnel at each shelter collected blood and nasal swab samples from 20 admitted and 20 discharged dogs each month. Generally, sampling was performed on designated day(s) and collections from all dogs admitted to and discharged from the humane shelter continued until the monthly goal was met. All dogs, irrespective of age, breed, sex, and vaccination or health status, were included, unless the primary caretaker determined that blood collection would be unsafe. Discharged dogs were dogs that had resided at the shelter for at least 7 days and included dogs that were adopted out into the community, as well as dogs that were scheduled by the humane shelter for euthanasia. Between December 2009 and January 2012, over 5,160 samples were collected from the 6 shelters.

Clinical Samples

Paired nasal swab and serum samples were collected from dogs (although occasional single blood or nasal swab samples were also processed). Briefly, a sterile polyester-tipped swab was inserted deep into one of the nostrils and rotated to collect respiratory secretions. The swab samples were immediately placed in 1 mL of viral transport medium (phosphate-buffered saline, 0.5% bovine serum albumin, 2,000 U/mL potassium penicillin G, 4 mg/mL streptomycin, 16 μg/mL gentamicin, and 100 U/mL nystatin) and stored at 4°C. Additionally, ~2 mL of blood was drawn from either the cephalic, saphenous, or jugular veins, placed in an additive-free tube, and stored at 4°C. Once all samples were collected for the month, they were shipped to the laboratory at Colorado State University on ice overnight. At the laboratory, samples were immediately processed for further analysis.

Hemagglutination Inhibition (HI) Assay

In accordance with procedures recommended by the World Organization for Animal Health (OIE) and from previous studies,[3,15] sera from blood samples were used to determine CIV H3N8 antibody titers by HI assay. Briefly, 1 volume of serum was incubated at 37°C for 20 hours with 3 volumes of *Vibrio cholera* receptor destroying enzyme.[a] After a deactivation period at 56°C for 60 minutes, 25 μL of treated serum was diluted in duplicate with phosphate-buffered saline across a 96-well plate. Four hemagglutinin units of a clinical isolate, A/canine/CO/224986/2006 (H3N8) (GenBank #HQ917678), were incubated with the diluted sera and positive (sera from A/canine/WY/86033/07-experimentally infected dogs) and negative (phosphate-buffered saline) controls for 45–60 minutes. To develop the assays, 0.5% (v/v) chicken red blood cells were added to each well and allowed to agglutinate for 25–30 minutes. HI antibody titers were determined as the reciprocal of the highest dilution causing complete agglutination inhibition. Seropositivity was defined as a HI antibody titer equal to or >1 : 16 in accordance with the OIE guidelines.[16]

Real Time Reverse Transcriptase-Polymerase Chain Reaction (rRT-PCR)

Viral ribonucleic acid was extracted from 200 μL of nasal swab viral transport medium and eluted in a final volume of 60 μL RNase-free water using an automated ribonucleic acid isolation system[b] before storage at −80°C. The one-step rRT-PCR assay was performed as previously described, with matrix gene copy numbers of over 1,000 considered positive for CIV shedding.[5] Briefly, a 5 μL-aliquot of RNA template was mixed with 20 μL of mixture containing iScript One-Step RT-PCR Kit reagents,[c] 200 nmol each of the forward (5′ GAA CAC CGA TCT TGA GGC ACT C 3′) and reverse (5′ GGC ATT TTG GAC AAA GCG TCT AC 3′) primers to amplify 144 bp of the influenza A virus matrix gene, and 80 nmol of probe. Water and viral transport medium were used as negative controls, whereas the positive control consisted of 10 TCID50 of CIV isolate A/canine/CO/224986/2006 (H3N8). For consistency, the epMotion Ep5070p[d] automated system was used to load 96 well plates before amplification and detection by Mastercycler Realplex[d] using previously described conditions.[5]

Prevalence Estimates

As the use of a single diagnostic testing method can bias estimates of the prevalence of a disease in a population, true prevalence was estimated by stochastic latent class analysis, as described by Branscum et al[17] using Gibbs sampling,[18] and implemented in WinBUGS statistical software.[19] Assumptions for this method include constant sensitivity and specificity of the diagnostic test used and that only 1 population was sampled. For prior distributions, the rRT-PCR mode was set at 0.96 for sensitivity and 0.74 for specificity at a 0.95 probability interval, based on previous studies.[5,20] Because each shelter is located in a distinct geographic region constituting a unique

population, true prevalence was determined separately for each shelter.

Logistic Regression Model Development and Analysis

Using ProcLOGISTIC as implemented in SAS v9.3[21] for logistic regression analyses, we evaluated possible risk factors for CIV shedding and seropositivity associated with the probability of being CIV-positive, as indicated by a positive nasal swab on rRT-PCR and positive serum sample on HI assay, respectively. As temporal, spatial, and seasonal patterns have been observed with human influenza A virus infections,[22,23] explanatory variables of interest included number of days in the shelter before sampling, geographic region of the shelter, study year, and sampling month. Additional covariates included to evaluate the hypotheses of interest were sample type (intake/discharge), receipt of CIV vaccination upon admission, and allowance of comingling or cohousing. Additional data collected but not included in the analysis because of lack of variability among the shelters included length of quarantine upon admission, number of dogs admitted to the shelter annually, whether admitted dogs were transferred from out of state, isolation of apparently sick dogs, and adherence to an all-in/all-out room protocol. The specific shelter itself was not considered because of collinearity with other explanatory variables.

One emerging thought regarding the endemic nature of H3N8 CIV in some shelters is that CIV is being propagated within and among regional shelters with intra- and intershelter movement of CIV-infected dogs.[13] Therefore, 2 sets of hypotheses were proposed to explain CIV shedding and seropositivity risk in shelter dogs, which led to 4 separate analyses: Analysis 1 – temporal-spatial effects on CIV-shedding; Analysis 2 – temporal-spatial effects on CIV seropositivity; Analysis 3 – within-shelter dog-interaction effect on CIV-shedding; and Analysis 4 – within-shelter dog-interaction effect on CIV seropositivity. Analyses 3 and 4 included only a subset of the data because the data on comingling and cohousing from shelters in some regions lacked variability. In these last 2 analyses, data were included from SC, which allowed comingling of dogs outside during playtime, and FL, where only littermates were occasionally allowed to be co-housed but no other comingling of animals was practiced.

Analyses 1 and 2 each consisted of a model set that included 14 a priori models (including an intercept-only model as a model of no effect) to assess the associations between the probability of CIV shedding (rRT-PCR positive = 1, negative = 0; Analysis 1) or seropositivity (HI assay positive = 1, negative = 0; Analysis 2) and the temporal covariates study year (year 1 = 1, year 2 = 2), sampling month (1–12, corresponding to month of the year), geographic region (west [CO] = 1, east [NY] = 2, southeast US [FL and SC] = 3; because CA and TX had one and no positives, respectively, they were excluded from the analyses), vaccination status (no = 0, yes = 1), and days in the shelter before sample collection (continuous variable), as well as additive effects and interactions among these variables.

Considering previous studies which suggest CIV transmission among dogs is because of direct contact,[24,25] the model sets for Analyses 3 and 4 each consisted of 9 a priori models (including an intercept-only model) used to assess associations between CIV shedding (rRT-PCR positive = 1, negative = 0; Analysis 3) and seropositivity (HI assay positive = 1, negative = 0; Analysis 4) and within-shelter dog interactions such as comingling/cohousing (no = 0, yes = 1), days in the shelter (continuous), and sampling month (1–12, corresponding to month of the year), plus additive effects and interactions between comingling/cohousing and days and month.

Model Selection and Multimodel Inference

We used an information-theoretic approach[26] using Akaike's Information Criterion corrected for small sample sizes (AICc[27,28]) for model selection and multimodel inference. Models were ranked using their AICc values where the model with the lowest AICc was considered the best model in the set. We also calculated the AICc differences (ΔAICc) between each of the models in the set (i) and the top-ranked model. Akaike weights for each model were calculated to reflect the probability that each model was the top model given the data set. Additionally, we report beta estimates, standard errors, and 95% profile likelihood confidence limits for variables of interest from the top-ranking model for each analysis. Finally, we include maximum rescaled R^2 values[21] as the portion of variance explained by each model.

Results

Geographic Distribution

A total of 5,161 serum and 5,182 nasal swab samples were collected from the 6 humane shelters (Table 1). Samples per shelter ranged from ~700 (SC) to ~1,000 (CO) and all shelters except CA and TX submitted more intake samples than discharge samples. As expected, the NY and CO shelters had the highest numbers of CIV nasal shedding and each had continuous months where CIV shedding was detected (Fig 1). For example, the CO shelter had detectable virus for 10 continuous months (June 2010 to March 2011), whereas the NY shelter had CIV shedding in samples for 9 continuous months (February 2011 to December 2011). In the case of CO, there was no more detectable CIV after March 2011, when the virus appears to have disappeared. In contrast, the FL humane shelter had much lower than expected CIV nasal shedding, given the fact that CIV first emerged in the state and previous FL shelter studies showed relatively higher prevalence.[1] On the other hand, the humane shelter in SC had more than expected CIV nasal shedding, which can be attributed to a CIV outbreak during the first months of sample collection (Fig 1). After these initial CIV-positive nasal swabs, only 2 CIV-positive serum samples were collected from SC throughout the remainder of the study. Both CA and TX had no CIV-seropositive samples and CA had only 1 dog shedding CIV upon admission into the shelter (April 2010) throughout the entire study. True prevalence estimates (Table 2) corroborate other reports suggesting CIV is endemic in CO and NY[4,5,12,13] with prevalence estimates of 2.7 and 2.5% for these shelters, respectively. The CIV-positive nasal swabs collected from SC in the first 2 months of the study may explain the higher CIV-shedding prevalence estimates for the SC shelter (1.9%) compared to the FL shelter (0.7%). The 2 shelters with 1 or no CIV-shedding dogs (TX and CA) had the lowest estimates for CIV prevalence (0.12–0.16%).

Community-Shelter Dog Transmission

Differences between intake and discharge CIV-shedding rates depended on the humane shelter sampled

Table 1. Numbers of canine influenza virus positives and total submitted nasal swabs and serum samples from admitted and discharged shelter dogs, December 2009 to January 2012.

	Nasal Swabs				Serum Samples			
	Admitted		Discharged		Admitted		Discharged	
Shelter	Positives	Total	Positives	Total	Positives	Total	Positives	Total
CA	1	411	0	443	0	420	0	442
CO	8	573	41	462	64	564	45	460
FL	7	575	5	376	0	574	4	374
NY	34	498	4	368	28	489	44	363
SC	16	419	7	288	0	419	3	287
TX	0	299	0	470	0	298	0	471
Overall	66	2,775	57	2,407	92	2,764	96	2,397

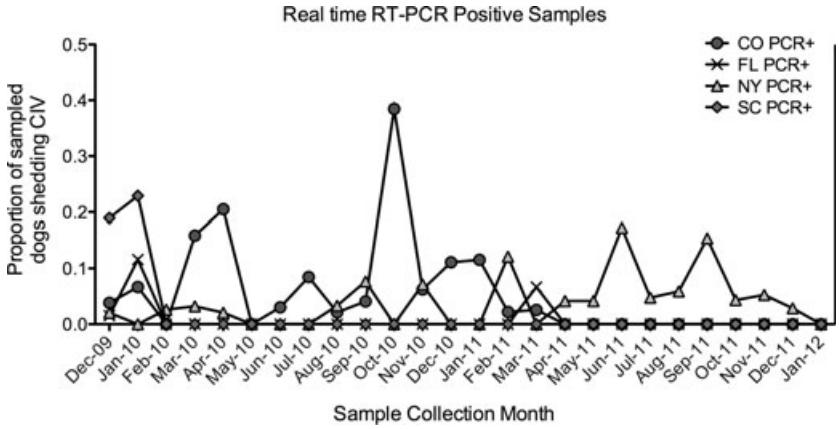

Fig 1. Proportion of dogs sampled each month of the study that were shedding canine influenza virus (CIV) detected by real time reverse transcriptase-polymerase chain reaction (RT-PCR) (PCR+).

Table 2. Median estimates (95% credible intervals) of true prevalence based on the proportion of canine influenza virus nasal shedding in US shelter dogs.

Shelter	Crude Prevalence Estimate	True Prevalence Estimate
CA	0.001	0.002 (0.0–0.009)
CO	0.047	0.027 (0.020–0.085)
FL	0.013	0.007 (0.0–0.027)
NY	0.044	0.025 (0.020–0.082)
SC	0.032	0.019 (0.010–0.062)
TX	0.000	0.001 (0.0–0.007)

(Fig 2). For CO shelter dogs, there were 6 times more dogs (8.9%) leaving the facility shedding CIV than dogs that entered (1.4%). In contrast, NY dogs were more likely to be shedding virus upon intake (6.9%) rather than upon discharge (1.1%). In the other 2 shelters (FL and SC) where CIV was detected in nasal swab samples, intake and discharge dogs had similar rates of shedding (ranging from 1.2 to 3.8%). Overall, among all the shelters, 66 of 2,768 (2.4%) dogs sampled were shedding CIV when admitted into the shelters and 57 of 2,418 (2.4%) dogs sampled were discharged while shedding the virus. These numbers included dogs from all 4 positive shelters, plus the 1 positive dog at the CA shelter. A total of 92 dogs were seropositive upon intake (of 2,765 – 3.3%) and 97 dogs (of 2,408 – 4.0%) were seropositive upon discharge. Three hundred twenty-five dogs were reported to have received at least 1 dose of the CIV vaccine while residing in the shelter. Of these, 125 were sampled on discharge. Only five of the 125 vaccinated discharged dogs were seropositive (3 dogs that received 2 vaccine doses and 2 dogs that received only 1 vaccine dose). Additionally, 51 dogs received both the initial CIV vaccination and a booster 7–14 days later while residing in the shelter. All but one of these were seronegative upon discharge, while three were shedding CIV upon discharge.

Risk Factor Analyses

After excluding dogs from the CA and TX shelters, any samples with missing information, and 148 duplicate samples (dogs that had been sampled both on intake and discharge; we randomly selected one of the replicates), we used a total of 3,407 nasal swab and 3,385 serum samples in Analyses 1 and 2, respectively, and a total of 1,608 nasal swab and 1,604 serum sam-

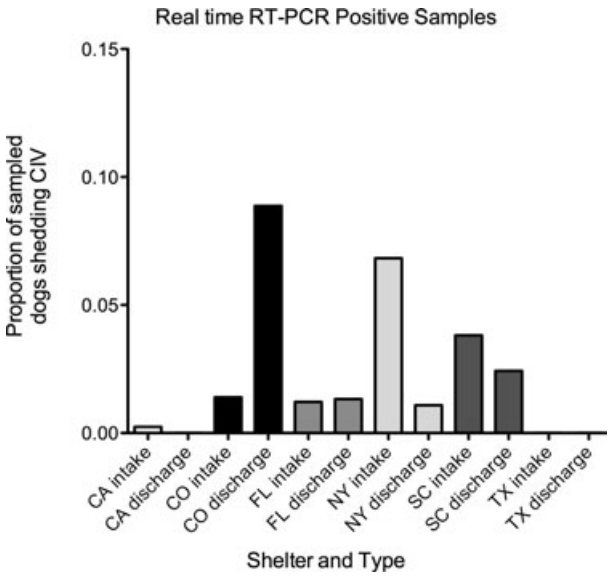

Fig 2. Proportion of dogs sampled at intake and discharge shedding canine influenza virus (CIV) in US humane shelters. Note that there were no CIV-shedding dogs on intake or discharge at the TX shelter.

ples in Analyses 3 and 4, respectively. Models were ranked by their ΔAIC_c value, with the top-ranked models having a ΔAIC_c of zero. The top model from each analysis is listed in Table 3. Beta estimates, standard errors, and 95% profile likelihood confidence limits for variables from these top-ranked models are in Table 4.

Analysis 1. The model incorporating the year x region interaction was ranked the highest for the temporal-spatial analyses, carrying a model weight of 0.93 (Table 3). This model indicates that CIV shedding was highly associated with both the region in which the shelter was located and the study year of sampling, where the region with the most dogs shedding CIV was different for each study year. The next best model (ie, next highest-ranked model) for CIV nasal shedding included all of the variables of interest (vaccination status, region, days in shelter, year of study, and month) and had model weight of 0.04 (data not shown) indicating little support for this model. The adjusted R^2 values for both of the top 2 models were low (0.04).

Analysis 2. For CIV seropositivity in US shelter dogs, the highest-ranking model included vaccination status, region, days in shelter, and year of study, and had the highest model weight of 0.42 (Table 3). In this analysis, the top 4 models had a cumulative model weight of 0.95 and included a combination of the variables from the highest-ranked model (data not shown). All four of the models carrying nonzero model weight included days in shelter as a covariate, suggesting that the probability of being CIV seropositive increases with days in shelter.

Analysis 3. In the analysis examining association between CIV-shedding and within-shelter interactions, the month × comingling/cohousing model was the highest-ranking model with a model weight of 0.92 and an R^2 of 0.07 (Table 3). The probability of CIV shedding was higher for shelters that allowed dogs to interact during certain months of the year than those that limited dog interactions.

Analysis 4. The top-ranked model examining association of days in the shelter with seropositivity had a model weight of 0.53 (Table 3). Combined with the next 3 top models, which included an additive or interactive combination of days in shelter, comingling/cohousing, and month, the cumulative Akaike model weight was 0.95 (data not shown). These models had slightly higher R^2 values (0.10) than models in Analyses 1–3 (ranging from 0.4 to 0.9).

Discussion

Canine influenza virus continues to be a threat to the health and welfare of shelter dogs. The studies described evaluate the CIV status of community dogs entering humane shelters and shelter dogs being discharged back out into the community. The intake and discharge data from each shelter suggest that the transmission dynamics between the community and shelter dog populations vary among individual shelters. The humane shelters with consistently positive nasal swabs (CO and NY) released CIV-shedding dogs back into the community. It is also clear, however, that CIV is entering humane shelters from community dogs. All of the shelters, except TX, had at least 1 intake dog that was shedding CIV. It is possible that the dogs admitted while shedding virus were previous shelter dogs or shelter dogs being transferred from other shelters. However, as the dogs in our study were randomly sampled, it is likely that some of the dogs that tested positive were dogs entering the facility directly from the community. Several epidemiologic and ecologic factors that contribute to H3N8 CIV infection once the virus has been introduced into the shelter have been identified and are discussed below.

Temporal-Spatial Patterns

Temporal-spatial patterns for influenza A viruses have been previously described.[22,23,29] From studies that have sequenced H3N8 CIV isolates from dogs residing in CO and NY shelters,[5,12,13,30] it is clear that CIV is present within the regions where the CO and NY humane shelters are located. What is less apparent is to what extent CIV affects the other regions in our study. Although there was no CIV detected in any of the dogs sampled at the TX shelter, PCR-positive CIV cases were reported in TX near the end of our sampling period.[31] Additionally, CIV was last confirmed in FL dogs by the Cornell Diagnostic Laboratory in 2008[12,31] and a CIV isolate was sequenced from a dog in CA in 2006.[12] Therefore, CIV has, at one time, been present in all the regions we sampled.

It is, therefore, likely that CIV, instead of being a pathogen persisting at low levels in endemic shelters,

Table 3. Top-ranking models for each analysis examining factors contributing to canine influenza virus (CIV) shedding or seropositivity in US shelter dogs. The intercept-only model, representing a model of no effect, is included with each top-ranked model for comparison. R^2 values are maximum rescaled R^2 values. Two times the maximized log-likelihood (−2 log(L)), the number of parameters in each model (K), and the small sample size-corrected AIC values (AICc) are shown for the top-ranked model and the intercept-only model for each analysis. The model with the lowest AICc value was assumed to be the best model in the set; thus, other models were ranked by their AICc differences (ΔAIC_c) relative to this top model. Akaike weights (w_i), or model probabilities, are estimates of the probability that model i is the best model given the data and the model set.

Model (i)	R^2	−2 log(L)	K	AICc	ΔAIC_c	w_i
Analysis 1: temporal-spatial factors contributing to CIV-shedding						
Study year and region interaction	0.0400	960.36	4	968.37	0.00	0.93
Intercept-only	—	998.75	1	1,000.75	32.35	0.00
Analysis 2: temporal-spatial factors contributing to CIV seropositivity						
Vaccination status, region, days in shelter, and study year	0.0900	1,304.10	5	1,314.12	0.00	0.42
Intercept-only	—	1,412.30	1	1,414.30	100.18	0.00
Analysis 3: within-shelter dog interactions contributing to CIV-shedding						
Month of sampling and comingling/cohousing interaction	0.0700	308.53	4	316.55	0.00	0.92
Intercept-only	—	329.51	1	331.51	14.96	0.00
Analysis 4: within-shelter dog interactions contributing to CIV seropositivity						
Days in shelter	0.1039	80.93	2	84.94	0.00	0.53
Intercept-only	—	90.05	1	92.05	7.12	0.02

Table 4. Beta estimates and 95% CI for variables from top-ranked models.

Variable of Interest	Beta Estimate	Standard Error	Profile Likelihood 95% CI
Analysis 1: temporal-spatial factors contributing to CIV-shedding			
Year	0.562	0.136	0.315, 0.855
Region	−0.053	0.136	−0.302, 0.239
Year and region interaction	−0.449	0.136	−0.741, −0.201
Analysis 2: temporal-spatial factors contributing to CIV seropositivity			
Vaccination status	0.029	0.157	−0.264, 0.353
Region	−0.734	0.082	−0.897, −0.574
Days in shelter	0.018	0.003	0.013, 0.024
Year	0.174	0.088	0.005, 0.350
Analysis 3: within-shelter dog interactions contributing to CIV-shedding			
Month of sampling	−0.206	0.077	−0.384, −0.074
Comingling/cohousing	0.260	0.310	−0.347, 0.881
Month of sampling and comingling/cohousing interaction	−0.192	0.077	−0.370, −0.060
Analysis 4: within-shelter dog interactions contributing to CIV seropositivity			
Days in shelter	0.046	0.012	0.019, 0.069

actually falls more in line with an ebb and flow pattern where outbreaks occur at 1 shelter in 1 region and wane before CIV emerges in an entirely different shelter in another region. For example, South Carolina submitted all of their positive nasal swab samples during a 2-month period in study year one (December 2009 through January 2010), while the majority (9/12) of FL's positive swab samples were collected during those same 2 months. Similarly, this was also observed in the CO and NY shelters. The CO shelter sampled 90% (44/49) of their CIV-shedding dogs during the first year and submitted the last positive nasal swab in March 2011; in the subsequent 10 months until the study ended, there were no reports of CIV in this shelter. The opposite is true for the NY shelter. Few shedding dogs were sampled in study year one, while positive swabs gradually increased over time (in fact, 25 of the 36 total positive samples for NY were collected after January 2011). Interestingly, H3N8 CIV isolated and sequenced from the CO and NY shelters during the study period appear to be genetically different at key antigenic sites.[30] These findings suggest that H3N8 CIV is the circulating influenza A virus in these shelters and that H3N8 CIV may be undergoing selection pressures.[30]

Within-Shelter Interactions

Direct contact among dogs and the number of days in shelter appear to be the top factors contributing to CIV shedding and seropositivity, respectively. During months when CIV-shedding was detected in the Southeast region, separating dogs (as done at the FL shelter) appeared to be associated with lower CIV shedding compared to the shelter within the same region that allowed comingling/cohousing (as practiced at the SC shelter). The CIV-positive sample sizes were small in Analyses 3 and 4 with only 34 CIV-shedding and 7 CIV-seropositive dogs. However, despite the small

sample size, direct contact is the likely route of H3N8 CIV transmission. Therefore, minimizing within-shelter interactions among dogs will indeed limit CIV infections.

For association with H3N8 CIV seropositivity, the number of days in the shelter is particularly interesting, as it has been previously associated with the occurrence of CIV and other respiratory infections in shelter dogs.[6,9] In Analysis 4, evaluating the within-shelter dog-interaction effect on CIV seropositivity, days in the shelter was common among all models with nonzero weight. Thus, in shelters where dogs are allowed to interact for longer periods of time, there appears to be a higher risk of becoming CIV seropositive. It is important to note that seropositivity indicates either exposure to the virus or that the animal has been vaccinated. As CIV antibodies in experimentally infected and sentinel dogs have been noted between days 7 and 12 postchallenge,[25] a duration of shelter stay over 7 days allows time for seroconversion if infected upon, or before, admittance. This likely accounts for a number of the seropositive discharged dogs, especially in the NY shelter where dogs infected upon intake would have seroconverted if in the shelter for 7 days or longer.

CIV Vaccination Status

It is difficult to assess the significance of the lack of seroconversion of known CIV vaccinates in this study, as most of the dogs either received only 1 vaccine before the serum sample was taken or the time interval between the second vaccination and the serum sample collection was not sufficient to elicit an antibody response. Furthermore, it is possible that two of the 3 vaccinated dogs shedding CIV upon discharge may have shed more CIV had they not been vaccinated, as the virus detected in their nasal swab samples was close to the negative threshold point (1,000 matrix gene copies) for rRT-PCR.[5,12,13] Although vaccination status appears in the top model for the analysis examining temporal-spatial factors contributing to CIV seropositivity (Analysis 2), it is in combination with other temporal-spatial variables. The models that contained only vaccine status carried low Akaike weights. This suggests that receiving the vaccine alone is not associated with increased or decreased risk of CIV. Thus, especially as dogs might not reside in the shelter long enough to receive the recommended CIV vaccine booster and as CIV isolates are known to cause subclinical infection in experimental dogs,[25] further research on the potential of the CIV vaccine to reduce CIV infection in shelters is warranted.

Model Limitations

Though several risk factors identified here have been reported elsewhere,[6,24] other variables not included in our analyses are likely contributors to CIV infection dynamics within shelters. The low R^2 values and model selection uncertainty in Analyses 2 and 4 (no single model carried a weight >0.53) suggest that some other factors are likely in play. A number of variables could not be included in our analysis, including quarantine, isolation, and all-in/all-out practices, as well as the effect of accepting dogs transferred from out of state and the size of the shelter. For example, NY was the only shelter that quarantined their animals for 7 days or more. Coincidentally, NY was also the only shelter that saw a 6-fold decrease in CIV-shedding dogs upon discharge compared to intake. It should also be noted that NY was the smallest shelter in the study (750–800 annual dog intake). On the other hand, CO, which was one of the largest participating shelters (11,000 annual dog intake), had a quarantine period of only 24–48 hours during the study period and had a 6-fold increase of CIV-shedding dogs upon discharge compared to intake. The effects of shelter size and quarantine practices could not be included here because of lack of intra- and intershelter variability. However, these possible effects should be taken into account during the study design process of any future studies.

Conclusions

Our results indicate that community dogs are a source of CIV introduction into humane shelters and that once CIV has become established within the shelter, dog-to-dog transmission maintains CIV. As CIV is a disease of space and time, continued modeling using an information-theoretic approach that allows for multimodel inference could be helpful for predicting future CIV outbreaks, and should include other risk factors which were unable to be evaluated in these analyses. Ultimately, this report will aid the process of identifying preventative and control measures for CIV in humane shelters, and thus reduce the risk of CIV within one of the US' most vulnerable dog populations.

Footnotes

[a] Denka Seiken Co, Tokyo, Japan
[b] Qiagen Qiaxtractor, Hilden, Germany
[c] BioRad, Hercules, CA
[d] Eppendorf, Hamburg, Germany

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