Animal Health

Canine Influenza (H3N8) in the Rocky Mountain Region

Carlie M. Koonce, Undergraduate
Kathleen J. Austin, Research Scientist
E. Lee Belden, Professor
Brenda M. Alexander, Assistant Professor
Departments of Animal and Veterinary Sciences
University of Wyoming, Laramie

Abstract

Canine influenza (H3N8) is new respiratory pathogen of dogs. Since emerging in racing greyhounds in 2004, serologic evidence indicates virus circulation in the pet dog population. Although estimates of prevalence among dogs in Colorado and Wyoming varies in the literature, recent syndromic serosurveillance reports 30% and 38% prevalence in dogs showing clinical signs of acute respiratory disease in Wyoming and Colorado, respectively. In the present study, we surveyed convalescent dogs in northern Colorado and southern Wyoming for influenza A antibody to determine disease prevalence in these states. Serologic testing of 175 dogs found 32% of institutionalized dogs in Colorado and 34% of dogs in Wyoming were seropositive for influenza A antibody. There was no significant difference between prevalence among institutionalized dogs in Colorado and Wyoming, but significantly more institutionalized dogs than non-institutionalized dogs were seropositive in Wyoming, suggesting a greater risk of exposure in this population. Continued surveillance of canine influenza should monitor virus infection in a larger population of dogs to determine if prevalence among dogs with acute respiratory disease accurately reflects rate of exposure and infection in the total dog population.

Introduction

Canine influenza is newly emerging, highly contagious respiratory infection of dogs caused by a novel virus closely related to contemporary equine influenza A virus subtype H3N8 [1,2,3]. Molecular analyses show that these viruses share greater than 96% nucleotide sequence identity, suggesting a recent transmission from horses to dogs [1]. Amino acid sequencing of the canine influenza virus (CIV) H3 hemagglutinin, however, shows conserved substitutions among canine isolates differentiating them from contemporary equine influenza strains, suggesting sustained horizontal transmission within the canine population [1].

Canine influenza virus (CIV) H3N8 was first identified in the canine population in January 2004 following an outbreak of respiratory disease in 22 greyhounds at a Florida racetrack [1,2,3]. From January to May 2005, respiratory disease outbreaks occurred in at least 11 states (Florida, Texas, Arkansas, West Virginia, Kansas, Iowa, Colorado, Rhode Island, Wisconsin, and Massachusetts), representing more than 20,000 dogs [2,4]. Sera collected from greyhounds in 3 of these states showed that 100% of dogs were seropositive for CIV antibodies [3]. Subsequent isolation of 4 closely related influenza A subtype H3N8 viruses from dogs in different geographic locations over a 25-month period suggested not only widespread and sustained horizontal transmission within the racing population, but also a significant risk of infection in the pet dog population [1].

The first evidence of CIV infection in the non-racing canine population came from serologic testing of 70 dogs with acute respiratory disease in shelters, boarding kennels, and veterinary clinics in Florida and New York in 2004 and 2005 [1]. Ninety-seven percent of these dogs were seropositive for CIV antibody [3]. Subsequent syndromic surveillance data has demonstrated CIV in the pet population in 27 states and Washington, D.C., with 15% of dogs exhibiting comparable clinical symptoms testing positive since 2005 [4,5]. Although the literature reports the disease as epizootic with prevalence near 60% in the Rocky Mountain region [4], the current surveillance report of seroprevalence is 30% and 38% for Wyoming and Colorado, respectively [5].

Although syndromic surveillance is useful for defining risk groups for CIV and tracking virus activity [4] it does not account for subclinical infection—accounting for 20% of exposed, infected dogs—or cases of prior exposure with full recovery. The purpose of this study was to serosurvey the convalescent canine population in northern Colorado and southern Wyoming for presence of influenza A antibody in order to determine the prevalence of CIV among dogs in these states. Serologic testing of institutionalized and non-institutionalized dogs was also analyzed to identify risk of exposure in sheltered animals compared to the pet dog population.

Materials and Methods

Specimen Collection

Convalescent phase serum samples were obtained from 146 dogs from Colorado and Wyoming in April and May 2009 by licensed veterinarians [n_1=93 institutionalized dogs from Larimer County, Colorado; n_2=31 institutionalized dogs from Laramie County, Wyoming; n_3=22 non-institutionalized dogs from Albany County, Wyoming]. Whole blood was stored at 2-8°C and centrifuged at 2550 rpm for 10 minutes within 96 hours of collection. Twenty-nine archival canine serum samples from December 2008 to March 2009 were obtained from the Wyoming State Veterinary Laboratory (Albany County, Wyoming). All sera were stored at -20°C until analysis.

Influenza A Nucleoprotein Antibody Assay

Sera were assayed in duplicate for influenza A nucleoprotein (NP) antibody using an influenza A NP antibody inhibitor ELISA (Virusys Corporation, Sykesville, MD). Sera absorbance values were determined using a plate reader and 450 nm filter.

Data Analysis

A NP Reduction Index (NPRI) was calculated for each sample using mean absorbance levels for the diluent and negative controls: NPRI = 1-[(sample absorbance – mean diluent control absorbance)/(mean negative control absorbance – mean diluent control absorbance)]. NPRI > 30 indicated the presence of influenza A NP antibody; NPRI < 20 indicated the absence of influenza A NP antibody; NPRI ≥ 20 but ≤ 30 represented an equivocal result, suggestive of the presence of influenza A NP antibody.

All data were analyzed by use of *SAS*. Significance was based on the Chi-squared test of the null hypothesis that resulted in a value of $P \leq 0.05$.

Results

Sera of 34 dogs (19%) had a NPRI >30, indicating the presence of influenza A NP antibody. Of these, 15 were institutionalized dogs from Larimer County, CO; 10 were institutionalized dogs from Laramie County, WY; 1 was a non-institutionalized dog from Albany County, WY; 8 were archival sera samples from the Wyoming State Veterinary Laboratory. Sera of 24 dogs (14%) had a NPRI between 20 and 30 indicating an equivocal result, suggestive of the presence of influenza A NP antibody (Table 1).

Table 1—Seroprevalence of influenza A NP antibody by county and state based on NP Reduction Index (NPRI)

Source	Positive NPRI >30 No. of Dogs % of Pop.	Probable 20≤ NPRI ≤30 No. of Dogs % of Pop.	Total Population Sampled
Larimer County, CO	15 16%	15 16%	93
Laramie County, WY	10 32%	8 26%	31
Albany County, WY	1 4.5%	0 0%	22
WSVL Archival Sera (WY)	8 27%	1 3%	29

Of the 34 dogs testing positive for viral antibody, 15 were from Colorado (16%) and the remaining 19 were from Wyoming (33%), P=0.0351 (Table 2). Twenty-five dogs were institutionalized at the time of sampling, representing 20% of the total institutionalized population from both states, P=0.0094. When positive and probable dogs were considered together as "positive" for CIV antibody, 32% and 34% of dogs were positive in Colorado and Wyoming, respectively, P=0.7912 (Table 3). Thirty-nine percent of institutionalized dogs in both states were positive or probable, compared to only 20% of non-institutionalized dogs, P=0.0147 (Table 3). Fifty-eight percent of Wyoming institutionalized dogs were positive or probable for CIV antibody, compared to only 32% of Colorado institutionalized dogs, P=0.0106 (Table 4).

Table 2—Seroprevalence of influenza A NP antibody by state of origin based on NP Reduction Index (NPRI)

NPRI	State		*P* value
	Colorado	Wyoming	
Positive (NPRI>30)	16%	32%	0.04
Probable (20≤NPRI≤30)	16%	26%	
Negative (NPRI<20)	68%	42%	

Table 4—Percentage of institutionalized dogs testing positive or probable (NPRI ≥20) for influenza A NP antibody by state of origin

	State		
	Colorado	Wyoming	*P* value*=
% NPRI ≥ 20	32%	58%	0.01
Total Population	93	31	

Thirty-three percent of all dogs sampled were positive or probable (NPRI ≥ 20) for influenza A NP antibody (Table 5). The Animal Health Diagnostic Center at Cornell University College of Veterinary Medicine reports seroprevalence of CIV antibody in 30% and 38% of syndromic dogs from Wyoming and Colorado, respectively, and a national average of 15% (representing dogs in 27 states and Washington, D.C.) (Table 5).

Table 5—Percentage of dogs testing positive or probable (NPRI ≥ 20) for influenza A NP antibody by state compared to seroprevalence in syndromic dogs reported by Cornell University

				Cornell Sero survey Data		
	Colorado	Wyoming	Total Population	Colorado	Wyoming	National Average*
% NPRI ≥20	32%	34%	33%	38%	30%	15%

*As of October 2008

Discussion

Analysis of seroprevalence of influenza A NP antibody in convalescent phase dogs showed no significant difference between the percentage of exposed institutionalized dogs in Colorado and Wyoming when positive and probable cases are considered together. However, significantly more institutionalized dogs in Wyoming were seropositive for viral antibody than non-institutionalized dogs, indicating that shelter status at the time of sampling had some influence on risk of exposure. Although the medical history, including history of acute respiratory disease, in all dogs was unknown, these data suggest that dogs in animal shelters are more likely to have a history of infection than dogs in the pet dog population. This was expected, given the increased prevalence of infectious diseases among animals in shelters.

These data also support prevalence data reported by Cornell University's syndromic serosurvey: although only 80% of dogs infected with CIV will show clinical signs, prevalence among dogs with acute respiratory disease varies only slightly from prevalence among convalescent dogs, suggesting a strong correlation between rate of clinical disease and rate of exposure in the pet dog population. Continued surveillance should monitor prevalence in a larger population of both syndromic and convalescent dogs in order to establish whether this trend accurately describes canine influenza virus infection in the Rocky Mountain dog population.

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