Influenza virus causes acute respiratory disease in multiple mammalian species, including humans, horses, birds, pigs, cats, and ferrets, and has recently been identified as an emerging pathogen in dogs.\(^1\,2\) Canine influenza H3N8 is believed to have arisen from an equine strain of the virus that was initially transmitted from horses to dogs and then established itself as a canine pathogen. The virus has been identified in stored, frozen hematologic samples from dogs dating back to 1999\(^3\,5\,10\,11\), however, sustained dog-to-dog transmission of CIV H3N8 was not recognized in the United States until 2004, when an outbreak of respiratory disease occurred in racing Greyhounds in Florida.\(^1\,3\,5\) Since that time, viral exposure has been identified in dogs in at least 30 states and is considered enzootic in certain areas, including New York and Pennsylvania.\(^3\,6\)

Canine influenza is highly contagious, and the virus is transmitted via contact with respiratory secretions from infected dogs, direct contact with dogs, or contact with contaminated fomites or humans. The H3N8 virus can remain viable for as long as 2 days on surfaces, 4 days in water at room temperature, and up to 24 hours on clothing or 12 hours on hands.\(^2\)

**Objective**—To determine the seroprevalence of antibody against canine influenza virus H3N8 in a group of pet dogs that participate in flyball in Pennsylvania.

**Design**—Serologic survey.

**Animals**—Dogs attending a flyball tournament in Downingtown, Pa, from November 13 to 14, 2009.

**Procedures**—Blood samples were collected from dogs following owner consent. Medical, travel, and activity history of the dogs for the previous 10.5 months was obtained from owners. Serum was harvested and submitted to Cornell University Diagnostic Laboratory for measurement of antibody against canine influenza virus H3N8 via hemagglutination inhibition testing.

**Results**—Serum samples were obtained from 100 of 256 dogs participating in the flyball event. Although 3 of the 100 (3%) samples had positive results for antibody against canine influenza, none of the associated dogs had respiratory signs of infection (eg, coughing, sneezing, or nasal or ocular discharge) in the 10.5 months prior to testing. Eleven dogs had a history of respiratory signs, but none of those dogs had antibody against canine influenza H3N8. In addition, none of the study dogs had been vaccinated against canine influenza H3N8.

**Conclusions and Clinical Relevance**—Although canine influenza is considered enzootic in certain areas of the country (eg, Pennsylvania or New York), this study identified a low seroprevalence in dogs considered at high risk for infection given their life conditions and geographic origins. More research is warranted to elucidate the prevalence of exposure to the H3N8 virus in competitive sporting dogs and determine whether vaccination is warranted in such dogs. (J Am Vet Med Assoc 2011;238:726–730)
pet population, epidemiological data are limited, but results of 1 study\(^2\) suggest that < 0.5% of dogs have serum antibody against CIV H3N8. Other studies\(^3\) revealed that 3% to 50% of dogs tested had antibodies against CIV H3N8, but these dogs all had signs consistent with infectious tracheobronchitis. Epidemiological data for other at-risk populations are limited. We have identified a population of dogs (ie, those that compete in flyball) that regularly involves high-density, short-term, direct contact among members. Such dogs may be more representative of the average pet dog than dogs from a racing kennel or shelter yet have more risk factors for CIV H3N8 than an average pet dog, which is usually exposed to a more limited number of dogs. Our focus has been on dogs competing in the Northeastern United States, where several states (eg, New York and Pennsylvania) have been identified as enzootic for CIV H3N8 infection.\(^3\)\(^6\)\(^9\) The nature of the competition results in the dogs being in close contact with other dogs, handlers, and equipment, providing an opportunity for spread of disease.

Flyball is a popular, competitive canine team sport, with 400 active flyball clubs and > 6,500 competing dogs registered in the North American Flyball Association.\(^1\) In the 10.3 months prior to the present study, there were 324 tournaments in North America.\(^1\)\(^4\) The competition consists of 2 racing lanes consisting of 4 jumps and a spring-loaded box. Two teams of 4 dogs each race in relay. The first dog on each team races over the jumps, retrieves a ball from the box, and returns over the jumps as the next dog starts down the lane and so on until all 4 dogs have successfully completed the course. Competitions usually last 1 to 2 days, and teams typically travel throughout multiple states in a geographic area. The degree of direct (eg, close crating or racing) and indirect (eg, ball sharing or shared handlers) interaction among dogs is high. In the Northeast, tournaments are held in indoor arenas, further increasing contact between dogs.

The purpose of the study reported here was to determine the point prevalence of serum antibody against CIV H3N8 in a population of pet dogs competing in a flyball tournament in Pennsylvania. The hypothesis was that given the dogs’ travel history, origins from reportedly enzootic areas, and high rate of interaction with other dogs, the prevalence of exposure to CIV H3N8 would be greater than the 0.5% reported in pet dogs\(^1\) but less than the 70% to 100% reported for shelter dogs\(^1\) or racing Greyhounds.\(^3\) We also sought to determine whether there was an association between seropositivity for antibody against CIV H3N8 (or having clinical signs of respiratory illness) and dog age, sex, and body weight; number of tournaments completed; history of international travel; or living with other dogs.

### Materials and Methods

**Animals**—Any dog attending the flyball tournament in Downingtown, Pa, on the weekend of November 13 to 14, 2009, was eligible for inclusion; however, participation was voluntary. Owners were asked to complete a questionnaire regarding dog living conditions, travel history, health history for the previous 10.5 months (from January through November 2009), and vaccination status. In addition, detailed information about dog participation in flyball tournaments, including dates and locations, was obtained. The study was designed as a seroprevalence survey, and the associated protocol was reviewed and approved by the University of Pennsylvania Institution Animal Care and Use Committee. Owner consent was obtained for all samples collected.

**Sample collection and serologic testing**—A blood sample (1.5 to 3.0 mL) was collected by a veterinarian or trained veterinary student from each dog via a jugular, cephalic, or saphenous vein by use of a 1-inch, 22-gauge needle on a 3-mL syringe.* Choice of vein was made on the basis of dog size and collector preference. Samples were then placed in serum separator tubes\(^6\) and centrifuged\(^7\) for 5 minutes at 2,500 rpm within 15 minutes after collection. Serum samples were stored at 4°C immediately after centrifugation until submission to the Animal Health Diagnostic Laboratory at Cornell University for hemagglutination inhibition analysis of antibody against CIV H3N8.\(^3\)\(^9\) All samples were shipped overnight in an insulated, sealed container with cold packs. An antibody titer > 16 was considered a positive result. Positive test results could have resulted from exposure, vaccination, or maternal transfer of antibodies. Titers between 8 and 16 were considered to represent a nonspecific response or acute phase of infection. Dogs with titers < 8 were considered unexposed to CIV H3N8.

**Statistical analysis**—Histograms of data were visually evaluated, and interval data were tested for normality by use of the Kolmogorov-Smirnov test.\(^4\) Because distributions were nonnormal, median values, IQRs, and complete ranges were used to summarize the data. The Pearson $\chi^2$ test and Fisher exact test were used to identify associations between putative risk factors and CIV H3N8 seropositivity or clinical signs of respiratory illness (outcomes). Values of $P < 0.05$ were considered significant. A 1-sided Fisher exact test was used when independent variables (ie, number of tournaments completed, whether the dog had traveled internationally, and whether the dog lived with other dogs) were hypothesized to have a unidirectional association with an outcome. For ease of analysis, independent variables were arbitrarily dichotomized, with the median value as the cutoff. Seroprevalence of CIV H3N8 and the associated 95% confidence interval were calculated, and the study seroprevalence was compared with historical data from an Ontario study\(^10\) by means of $\chi^2$ analysis.\(^7\)

### Results

**Animals**—Two-hundred fifty-six dogs on 29 teams were registered to participate in the flyball competition; 101 dogs belonging to 60 handlers from 22 teams participated in the study (39% of dogs representing 76% of teams). Because participation was voluntary, no data were collected from handlers that chose not to participate. Blood samples and data were obtained from an additional 4 dogs that attended the tournament but were not registered participants or affiliated with any team. Data were collected from 1 additional participating dog, but serum was not available for testing. Data from these additional 5 dogs were not included in the analysis or results.
Only data from the 100 competing dogs with serum samples available were included in the calculation of summary statistics. Twenty-five pure breeds and various mixed breeds were represented among the study dogs; mixed (27/100; 27%) and Border Collie (20/100; 20%) were the most common breeds. The median age was 5.0 years (IQR, 2.9 to 7.7 years; range, 0.6 to 16.7 years). There were 57 females (8 sexually intact and 49 spayed) and 43 males (7 sexually intact and 36 castrated). The median body weight was 15 kg (33.0 lb; IQR, 8.6 to 18.6 kg [19.0 to 41.0 lb]; range, 3.6 to 31.8 kg [8.0 to 70.0 lb]).

Dogs participated in a median of 8.5 flyball tournaments in the previous year (IQR, 6.0 to 11.0; range, 1 to 31). Thirty-two dogs had competed in >10 tournaments in 2009; 4 (4%) dogs had competed in >15 tournaments. Forty-one (41%) dogs routinely took part in other group dog activities (eg, obedience or agility competitions), and 17 (17%) dogs had been boarded at a kennel in the last year.

The 100 dogs resided in 8 US states, of which 2 (Pennsylvania and New York) are reportedly enzootic for CIV H3N8.\(^6\) State distribution was as follows: Pennsylvania, 57; Maryland, 13; New York, 10; Virginia, 7; New Jersey, 6; Connecticut, 3; Massachusetts, 2; and Missouri, 2. The median number of states visited by the dogs in 2009 was 3 (IQR, 1 to 4; range, 0 to 15), for a total of 30 states. Nine (9%) dogs had traveled internationally in 2009; 4 (4%) dogs had competed in flyball tournaments. Twenty-five pure breeds and various mixed breeds were represented among the study dogs; mixed (27/100; 27%) and Border Collie (20/100; 20%) were the most common breeds. The median age was 5.0 years (IQR, 2.9 to 7.7 years; range, 0.6 to 16.7 years). There were 57 females (8 sexually intact and 49 spayed) and 43 males (7 sexually intact and 36 castrated). The median body weight was 15 kg (33.0 lb; IQR, 8.6 to 18.6 kg [19.0 to 41.0 lb]; range, 3.6 to 31.8 kg [8.0 to 70.0 lb]).

Eleven of 100 (11%) dogs had had at least 1 clinical sign consistent with influenza virus infection since January 2009: sneezing (n = 8 dogs), coughing (7), ocularonasal discharge (5), fever (1), and decreased appetite (1).

**Serologic testing**—Of the 100 samples analyzed, 3 (3%; 95% confidence interval, 0.6% to 8.5%) were seropositive for CIV H3N8 (titers of 256, 128, and 64); the remainder had titers <8 and were thus classified as seronegative. Dogs with positive results all resided in Virginia and included a 5-year-old spayed female mixed-breed dog and 9-year-old castrated male mixed-breed dog from the same flyball team as well as a 3-year-old spayed female Cocker Spaniel–Poodle cross (cockapoo) with a history of international travel. None of the seropositive dogs reportedly had clinical signs consistent with CIV H3N8 infection in the 10.5 months prior to testing.

**Risk factors for seropositivity**—Dogs that lived with other dogs were at an increased risk of having positive serologic results for antibody against CIV H3N8, compared with dogs that lived without other dogs (P = 0.026). Seropositivity was not associated with age, sex, breed, body weight, international travel, or number of tournaments completed (Table 1). The seroprevalence of 3% was not significantly different than that reported for pet dogs\(^15\) (P = 0.166).

Clinical signs were not associated with presence of seropositivity or age, sex, breed, body weight, or international travel (Table 1). However, clinical signs were significantly associated with the number of tournaments completed in the past 10.5 months.

Table 1.—Results of risk factor analysis for CIV H3N8 seropositivity and having at least 1 clinical sign of influenza in dogs that attended a flyball tournament in Pennsylvania in November 2009.

<table>
<thead>
<tr>
<th>Factor</th>
<th>No. of seropositive dogs</th>
<th>No. of seronegative dogs</th>
<th>P value</th>
<th>No. of dogs with signs</th>
<th>No. of dogs without signs</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (n = 100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5 y</td>
<td>2</td>
<td>50</td>
<td>0.606</td>
<td>5</td>
<td>43</td>
<td>0.858</td>
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<tr>
<td>≥ 5 y</td>
<td>47</td>
<td></td>
<td></td>
<td>6</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Sex (n = 100)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>42</td>
<td>0.731</td>
<td>5</td>
<td>38</td>
<td>0.882</td>
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<tr>
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<td>2</td>
<td>55</td>
<td></td>
<td>6</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Body weight (n = 100)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 15.5 kg</td>
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<td>50</td>
<td>0.534</td>
<td>5</td>
<td>46</td>
<td>0.697</td>
</tr>
<tr>
<td>≥ 15.5 kg</td>
<td>2</td>
<td>47</td>
<td></td>
<td>6</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>No. of tournaments in 2009 (n = 98)</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 10</td>
<td>3</td>
<td>62</td>
<td>0.210</td>
<td>4</td>
<td>61</td>
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<tr>
<td>≥ 10</td>
<td>0</td>
<td>33</td>
<td></td>
<td>7</td>
<td>26</td>
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</tr>
<tr>
<td>International travel (n = 100)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
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<td>8</td>
<td>0.135</td>
<td>0</td>
<td>9</td>
<td>0.269</td>
</tr>
<tr>
<td>No</td>
<td>2</td>
<td>89</td>
<td></td>
<td>11</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>No. of dogs in home (n = 100)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>89</td>
<td>0.026</td>
<td>10</td>
<td>80</td>
<td>0.915</td>
</tr>
<tr>
<td>&gt; 1</td>
<td>2</td>
<td>89</td>
<td></td>
<td>1</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

— = Not applicable.

A serum antibody titer >16 was considered a positive result.
The 32 dogs that had competed in > 10 tournaments in 2009 were more likely to have had at least 1 clinical sign of respiratory disease than were other dogs (P = 0.032). One dog with a history of clinical signs had traveled to Canada in the past year. None of the dogs with clinical signs had positive serologic results.

**Discussion**

In dogs housed in animal shelters, the rate of exposure to CIV H3N8 can be quite high (> 70%). A study involving shelter dogs in Philadelphia revealed that 15% of dogs had positive serologic test results within ≤ 7 days and 71% had positive results > 7 days after arrival in the shelter. Antibody response is not detectable until at least 7 days after exposure to the virus. Because of the lag in antibody production after exposure, the aforementioned findings indicate that most dogs were unexposed to CIV H3N8 prior to arrival at the shelter and that exposure to the virus occurred at the shelter. In contrast, a cross-sectional study of pet dogs in 9 veterinary practices in Ontario found that only 1 of 225 (0.4%) dogs tested were seropositive for antibodies against CIV H3N8. It has been suggested that in addition to shelters, dog shows represent a high risk of CIV H3N8 exposure; however, no seroprevalence studies have been performed in any dog show venues.

A flyball tournament was chosen for the present study of CIV H3N8 seroprevalence because dogs that participate in these competitions congregate in fairly large numbers (approx 250 dogs/event) and have extensive histories of travel to many regions, in which CIV H3N8 may be enzootic. The overall seroprevalence of 3% in our study did not differ from that in a general pet dog population and was lower than that in racing Greyhounds and shelter dogs. These findings were unexpected because the study dogs had several characteristics (eg, frequent close contact with multiple dogs, frequent travel, and contact with potentially contaminated fomites) that are considered to put them at an increased risk relative to most other pet dogs.

Most of the dogs in the present study appeared not to have been exposed to the virus. This is consistent with findings of the study involving owned pet dogs in Ontario but drastically different from the surveillance study performed at a Philadelphia animal shelter. Earlier epidemiological studies of racing Greyhounds showed that up to 100% had antibody against CIV H3N8, with 80% having clinical signs of disease. The exact cause of this difference in exposure status has yet to be determined and requires further investigation.

Among dogs at the flyball tournament, 11 were reported by owners to have had clinical signs that could have been consistent with CIV H3N8 infection. None of the dogs in which clinical signs were noticed were seropositive for CIV H3N8 infection. It is likely that these dogs contracted infectious tracheobronchitis or some other respiratory infection. Conversely, of the 3 dogs that had positive serologic test results, none had clinical signs consistent with influenza infection in the last year, although there was an association between respiratory signs and the number of competitions. These findings suggest that if CIV H3N8 were introduced into a similar group of dogs, a large number of those dogs would be susceptible to infection and might spread the virus.

Limitations of the study reported here include the fact that only 1 measurement was made at 1 time point in 1 geographic area, a small number of dogs underwent serologic testing, and only 3 dogs were seropositive for CIV H3N8 infection. The small sample size and low number of seropositive dogs limited the statistical power for detecting risk factors for seropositivity. Only 39% of the dogs and 76% of the teams that were registered to compete in the tournament were included. Reasons for the lack of participation were not determined; however, it may be that owners perceived blood collection as stressful, potentially interfering with their dogs’ racing performance. Conversely, handlers that were worried about CIV H3N8 infection may have been more likely to participate, so it is unknown whether the effects of selection bias were toward or against accepting the null hypothesis. Given that none of the dogs with previous respiratory signs were seropositive, such a bias likely would not have resulted in an artificially high seroprevalence. Prevalence may have been underestimated if dogs had been exposed to the virus but had not yet seroconverted or if the duration of natural immunity to CIV H3N8 infection is brief and serum antibody titers had already waned below detectable concentrations in the study dogs. Finally, these results may not be generalizable to other competitive sports or dogs competing in flyball in other geographic regions.

Although the present study was focused on flyball dogs in the Northeastern United States, many of the dogs were also exposed to other dogs in other competitive venues and in training classes. The presence or absence of clinical signs during the previous 10.5 months was determined on the basis of owner reports, which may have been subject to recall bias. Additionally, the dogs with positive serologic results may have had clinical signs of influenza > 10.5 months prior to testing.

The overall prevalence of CIV H3N8 exposure in dogs participating in a flyball competition was lower than anticipated. These findings could be interpreted in 2 ways. First, the dogs may have lacked exposure to animal shelters or other environments where CIV H3N8 has been detected. In this scenario, vaccination against CIV H3N8 would not be considered a high priority. On the other hand, because the study dogs did not have antibody against CIV H3N8, they did not have any acquired immunity to the disease. If CIV H3N8 were introduced into the group of dogs, given the course of viral shedding and the close contact of these dogs with each other, then the virus could spread rapidly. In such a scenario, vaccination could be seen as a potential means to limit disease spread and severity. The expected clinical signs and existence of innate immunity in healthy, athletic dogs is unknown. Of the 3 seropositive dogs, none had clinical signs consistent with influenza infection and none of the dogs with compatible clinical signs had positive serologic test results. Therefore, we cannot provide any clear recommendations regarding vaccination against CIV H3N8 in dogs that compete in flyball tournaments. Additional studies to evaluate the prevalence of CIV H3N8 in this population and others are needed and will help to deter-
mine whether the exposure rate is changing and whether seroconversion to CIV H3N8 infection is associated with clinical signs of influenza.

References

**Radiographic quantitative assessment of cranial tibial subluxation before and after tibial plateau leveling osteotomy**

*Stanley E. Kim et al*

**Objective**—To determine the influence of stifle joint flexion angle, cranial cruciate ligament (CrCL) integrity, tibial plateau leveling osteotomy (TPLO), and cranial tibial subluxation on the distance between the location of origin and insertion of the CrCL (CrCL_o) in dogs.

**Sample**—4 pairs of pelvic limbs from adult dog cadavers weighing 23 to 34 kg.

**Procedures**—Mediolateral projection radiographs of each stifle joint were obtained with the joint flexed at 90°, 105°, 120°, 135°, and 150°. Radiopaque markers were then placed at the sites of origin and insertion of the CrCL. Afterward, radiography was repeated in the same manner, before and after CrCL transection, with and without TPLO. Following CrCL transection, radiographs were obtained before and after inducing overt cranial tibial subluxation. Interobserver variation in measuring the CrCL_o without fiduciary markers was assessed. The effect of CrCL integrity, cranial tibial subluxation, flexion angle, and TPLO on CrCL_o was also determined.

**Results**—Interobserver agreement was strong, with an intraclass correlation coefficient of 0.859. The CrCL was significantly shorter (< 1 mm) at 90° of flexion; otherwise, flexion angle had no effect on CrCL_o. Cranial tibial subluxation caused a 25% to 40% increase in CrCL_o. No effect of TPLO on CrCL_o was found, regardless of CrCL integrity, forced stifle joint subluxation, or flexion angle.

**Conclusions and Clinical Relevance**—Overt cranial tibial subluxation in CrCL-deficient stifle joints can be detected on mediolateral projection radiographs by comparing CrCL_o on neutral and stressed joint radiographs at joint angles between 105° and 150°, regardless of whether a TPLO has been performed. (*Am J Vet Res* 2011;72:410–418)