Cross-Reactivity to Field Isolates of Canine Influenza Virus by a Killed Canine Influenza Virus (H3N8, Iowa05) Vaccine

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ABSTRACT

Objectives

To evaluate the hemagglutination inhibition assay (HAI) reactivity of serum derived from dogs vaccinated with a killed, monovalent canine influenza virus (CIV) vaccine, Iowa05 strain (H3N8), against other more recent field isolates of CIV.

Animals

Purpose-bred beagles approximately 8 weeks old.

Procedure

Sera from vaccinated dogs were used for HAI titer determination. Dogs in two studies were vaccinated using a killed CIV or placebo vaccine with a 1 mL dose administered subcutaneously, and another 1 mL dose administered approximately 3 weeks later. Two weeks after the second vaccination, dogs in the first study were challenged with heterologous CIV strain. Serum samples were collected before first vaccination on day 0, and before challenge on day 35. Serum HAI titers were determined for the vaccine strain, the challenge strains, and several more recently isolated strains of CIV.

Results

The HAI titers of the vaccine and challenge strains were significantly higher in treated dogs (n=24) than in controls (n=24, $P<0.0001$). All vaccinated dogs demonstrated detectable HAI titers to all strains, with 6- to 29-fold titer increases. Overall, 93.5% amino acid identity and >97% homology was observed for the HA isolates, and 95.2% amino acid identity and 98% homology for the NA isolates compared with the consensus sequences. Even the most genetically diverse CIV strain induced mean titers that were equivalent to or greater than the titers induced by the vaccine strain.

Conclusions

A killed CIV, H3N8, Iowa05 vaccine demonstrated cross-reactivity against several heterologous CIV challenge strains, as well as more recent CIV field isolates.

INTRODUCTION

Canine influenza virus (CIV) causes a highly contagious respiratory infection in dogs. The first recognized outbreak occurred in racing greyhounds in Florida in 2004.[1,2] Between June and August of that year, 14 tracks in 6 states reported outbreaks. Investigators isolated a novel influenza A, subtype H3N8 virus from sick dogs.[2] The virus shared extensive homology with the H3N8 equine influenza virus, which has existed in horses for more than 40 years.[3] Because few dogs have developed natural immunity, susceptibility to CIV remains high. Since the original outbreaks, CIV has been isolated from clinical cases in over 30 states, and the virus continues to circulate (Animal Health Diagnostic Center, Cornell College of Veterinary Medicine, Ithaca, New York, ahdc.vet.cornell/news/civ.cfm). The purpose of this study was to evaluate the cross-reactivity of a killed CIV, Iowa05 vaccine containing an aluminum hydroxide adjuvant against more recent CIV strains that have emerged since the vaccine strain was isolated in 2005.

MATERIALS AND METHODS

Study Design

In a clinical safety and efficacy study, 48 dogs were randomly assigned to receive either the test vaccine containing CIV A/Ca/Iowa/13528/05 (Iowa05) or a placebo. All dogs received a total of two 1.0 mL doses of vaccine or placebo, administered subcutaneously 3 weeks apart on days 0 and 21. Serum samples were collected on days 0 and 21 before vaccine administration, on day 35 before challenge with aerosolized CIV A/Ca/CO/6723-10/08 egg p3, and at necropsy on days 39 or 46. This study is explained more fully in a companion article (in press).

Seventy-two dogs in a second study were randomly assigned to receive the test vaccine containing CIV A/Ca/Iowa/13528/05 or placebo. All dogs received a total of two 1.0 mL doses of vaccine or placebo, administered subcutaneously 3 weeks apart on days 0 and 21. Serum samples for HAI determination were collected from each dog on study days 0 and 21, before vaccine or placebo administration, and on day 35, before intranasal challenge with CIV A/Ca/CO/8880-06.

Gene Sequencing

The HA and NA genes for 5 CIV strains were sequenced at Pfizer Animal Health following reverse transcriptase-PCR amplification. The strains, received from Cornell University (Ithaca, NY), were isolated from clinical cases in Florida (A/Ca/FL61156-2/07 MDCK p2), Colorado (A/Ca/CO6723-10/08 egg p3), Pennsylvania (A/Ca/PA/33929/07 MDCK p3), and New York (A/Ca/NY135407-1/08 and A/Ca/NY/105447-1/08 egg p2). Nucleotide sequencing was performed using a standard fluorescence-based cycle sequencing protocol. The amino acid sequence was derived from the translated nucleotide sequence and aligned using VectorNTI 9.0 software (Carlsbad, CA). To enhance the quality of the alignment, the amino acid sequences were trimmed slightly at the amino- and carboxy-terminal ends to adjust the alignment to the sequence that had the least amount of sequence data available. The HA and NA sequences from CIV A/Ca/Florida 43/03, and the HA sequences from CIV A/Ca/Iowa/13628/05 and A/Ca/Texas/1/04, obtained from the National Center for Biotechnology Information database, were included in the alignment. The sequence results were used to construct dendograms.

Serology

Hemagglutination inhibition assays were performed in a Pfizer Animal Health, Laboratory Sciences-Assay Development Laboratory. Serological response to vaccination was measured by HAI using the CIV H3N8, Iowa05 vaccine strain antigen (A/Ca/Iowa/13528/05), two challenge strains (A/Ca/CO/6723-10/08 egg p3 and A/Ca/CO/8880-06, both isolated in Colorado), and against three additional field isolates, including A/Ca/FL/61156-2/207 isolated in Florida, A/Ca/NY/15407-1/08 isolated in New York, and A/Ca/PA/33929/07 isolated in Pennsylvania.

Sera from the dogs were heat inactivated for 30 minutes at 56°C. Nonspecific promoters/inhibitors of hemagglutination were removed by treating 200 µL heat-inactivated serum with 1 mL 25% Kaolin/Delbecco phosphate buffered saline (DPBS) and 0.4 mL of 2% chicken red blood cells for 20 to 25 minutes at room temperature with rotation. Samples were centrifuged at 1,500 rpms for 15 minutes in an Eppendorf Microfuge 18 centrifuge (Hamburg, Germany). The supernatant was transferred to a separate sterile 1.5 mL polypropylene tube. This represented a 1:8 dilution of the serum. Twenty-five µL DPBS that contained 8-16 HA units/50 µL of BEI-inactivated influenza antigen were added to the wells containing 2-fold dilutions of adsorbed sera in polystyrene round-bottomed plates. Plates were tapped lightly and incubated for 50 minutes at room temperature. Following incubation, 50 µL of a 0.4% chicken red blood cell suspension were added to all wells. Plates were tapped gently, the bottoms of the plates wiped with a static-free cloth, and plates incubated at room temperature for 2 hours. The HA concentration was confirmed by back-titration of the antigen. The HAI titer was recorded as the last dilution demonstrating 100% inhibition of hemagglutination.

Statistical Analyses

Antibody titers were logarithm transformed before statistical analysis. The transformed values were analyzed with a general linear mixed model with repeated measure. The model included the fixed effects of treatment, time point, and treatment-by-time interaction, and the random effects of room, block within room, treatment-by-block with room interaction, and residual. Pairwise treatment comparisons were made between the vaccine and placebo groups at each time. The treatment least square means (LSMs) at each time and 95% confidence intervals (CIs) were back-transformed to obtain the geometric means and their CIs. Minimums and maximums were calculated for each treatment and group.

RESULTS

Gene Sequencing

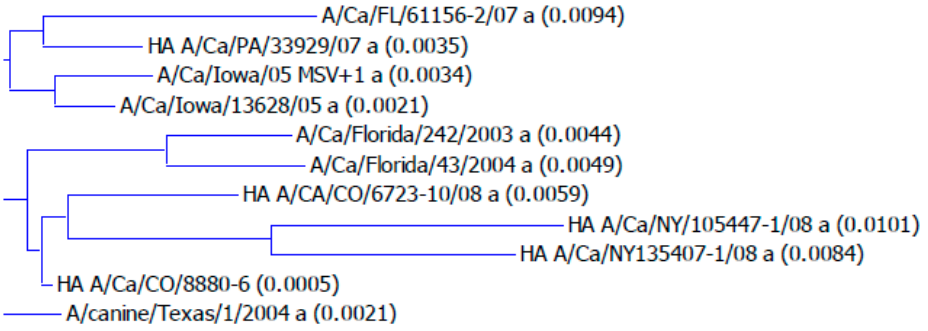
Overall, 93.5% amino acid identity was observed based on the consensus sequence from the HA translated sequences of 11 canine influenza, H3N8 isolates, and >97% homology was noted between the amino acid sequences from each isolate and the consensus sequence (Figure 1). The greatest amount of divergence correlated with the more recent isolates, A/Ca/NY/105447-1/08 and A/CA/NY135407-1/08. When comparing the Iowa05 test vaccine strain to the other strains, the greatest divergence was also noted between it and the New

Table 1. *Hemagglutination Inhibition Assay Titers (HAI/25 µL Serum) against Canine Influenza Virus (CIV) Strains in Dogs Vaccinated with CIV Iowa05 Vaccine*

		HAI GMT (Range)	
Study	CIV Strain	Day 0*	Day 35 Post-Vaccination
1	A/Ca/Iowa/13528/05	<8	75 (32-256)
1	A/Ca/CO/8880/06	<8	27.3 (<8-64)
1	A/Ca/FL61156-2/07	<8	64 (32-256)
1	A/Ca/PA/33929/07	<8	45.7 (16-128)
1	A/Ca/NY135407-1/08	<8	118.2 (32-256)
2	A/Ca/CO/6723-10/08	<8	53.8 (16-256)
2	A/Ca/Iowa/13528/05	<8	46.6 (16-256)

**4.0 was used for values of <8 to calculate geometric means.*

***Figure 1**. Dendogram of Canine Influenza H3N8 HA Sequences*



York strains. The NA sequence was slightly more conserved than the HA sequence, with 95.2% amino acid identity observed based on the consensus sequence from the NA translated sequences of 9 canine influenza, H3N8 isolates (Figure 2).

Serology

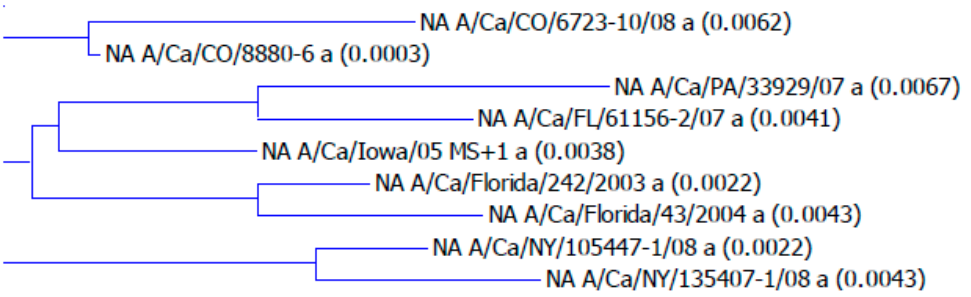
In the first study, all dogs had HAI titers against CIV Iowa05 on day 0 of <8.0 units/25 µL, indicating that none of the dogs had been exposed to CIV before vaccination. Additionally, all dogs in the placebo group had undetectable test vaccine titers on days 21, 35, and 39, with one exception on day 21. All 24 dogs in the test vaccine group had detectable titers to the vaccine strain on day 35 before challenge. In this group, back-transformed LSM HAI values for vaccine antigen (Iowa05) and the heterologous challenge (A/Ca/CO/6723-10/08) strains on day 35 were 46.6 and 53.8, respectively. These values were significantly higher (P<0.0001) than values of 4.0 for both CIV strains for dogs in the placebo group on days 21, 35, 39, and 46.

All dogs vaccinated with the Iowa05 test vaccine demonstrated detectable HAI titers on day 35 to the additional strains tested (Table 1). Geometric mean titer values ranged from 27.3 HAI/25 µL serum for a Colorado strain to 118.3 HAI/25 µL serum for a New York strain. These results represented a 6- to 29-fold increase in titer. Seroconversion is defined as at least a 4-fold titer increase.

DISCUSSION

Vaccinated dogs successfully seroconverted with HAI titers to heterologous CIV

***Figure 2.** Dendogram of Canine Influenza H3N8 NA Sequences*



strains that were more than 6- to 29-fold higher than in unvaccinated dogs. The Iowa05 CIV vaccine strain induced desirable titer increases even against the most genetically diverse strains tested. For example, the HAI titer against NY/135407 antigen (118.3 units/25 μL, range 32-256 units/25 μL) using sera from dogs vaccinated with the Iowa05 strain was equivalent to or greater than the homologous HAI titer using the Iowa05 antigen (46.6 units/25 μL, range 16-256 units/25 μL).

Canine influenza virus continues to evolve, as evidenced by the HA and NA divergences identified in gene sequencing of strains isolated since the first CIV outbreaks in 2004. The Iowa05 vaccine produced 6- to 29-fold serum titer increases against all the strains tested, and titers were significantly higher in treated dogs than in controls. HAI reactivity to more recent isolates was similar to homologous antigen, which indicates the likelihood for cross-protection. In clinical safety and efficacy studies, the vaccine also reduced the incidence and severity of lung lesions, and the incidence of clinical coughing and viral shedding (see companion article, in press).

Mortality following CIV infections is relatively low, yet CIV can damage respiratory epithelium within as little as 4 days. This initial tissue damage opens the way for secondary pathogens, which are usually only mildly pathogenic, to colonize and exert their pathogenic influence. It is also likely that CIV plays a role in more widespread canine infectious respiratory disease complex (CIRD). The availability of an effective CIV vaccine may not only help control CIV, but also help minimize the opportunistic infections that frequently follow viral respiratory infections.

CONCLUSIONS

A killed CIV, H3N8, Iowa05 vaccine demonstrated cross-reactivity against several heterologous CIV challenge strains, as well as more recent CIV field isolates.

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REFERENCES

1. American Veterinary Medical Association. Canine Influenza Virus Backgrounder. Available at http://www.avma.org/public_health/influenza/canine_bgnd.asp. Accessed on Oct. 10, 2010.
2. Payungpom S, Crawford PC, Kouo TS, et al. Influenza A virus (H3N8) in dogs with respiratory disease, Florida. *Emerging Infect Dis*. 2008;14(6):902-908.
3. Centers for Disease Control and Prevention. Key Facts about Canine Influenza (Dog Flu). Available at http://www.cdc.gov/flu/canine/. Accessed on Oct. 10, 2010.