



Vaccination with an inactivated canine influenza H3N2 virus vaccine is safe and elicits an immune response in cats

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Abstract

Objectives The aim of this study was to evaluate safety and seroconversion when an inactivated H3N2 canine influenza virus (CIV) vaccine was administered to cats.

Methods Twenty 7–8-week-old seronegative cats were randomly assigned to two groups of 10 animals each. Cats in treatment group T01 were subcutaneously administered two doses of an adjuvanted placebo 3 weeks apart to serve as non-immunized controls. Cats in treatment group T02 were subcutaneously administered with two doses of H3N2 CIV vaccine at 3 weeks apart. All animals were actively monitored for 5 days after each injection for local and systemic reactions. Tympanic temperatures were recorded the day before and 5 days after each vaccination. Blood samples for serology were collected prior to each vaccination (days –1 and 20), and 7 and 14 days post-second vaccination.

Results Minor vocalization was observed in both control and vaccinated animals after the first and second dose administration. The only injection site reaction observed was mild swelling in one control cat, which resolved within 24 h. Transient fevers (39.5–39.7°C) that resolved within 24 h post-injection were observed in both treatment groups (T01 = 3/10 and T02 = 5/10). All vaccinated, but no control, animals successfully seroconverted within 14 days of second vaccination, with H3N2 CIV-specific hemagglutination inhibition (HAI) titers ranging from 32 to 128.

Conclusions and relevance Cats vaccinated subcutaneously with an inactivated H3N2 CIV vaccine had similar rates of adverse events post-vaccination as the control group. Increased HAI titers provided evidence of post-vaccination seroconversion with the H3N2 CIV-vaccinated group.

Keywords: Shelter cats; canine influenza virus; H3N2; inactivated H3N2 CIV vaccine; H3N2 CIV-specific HAI titers

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Introduction

Influenza A viruses of Orthomyxoviridae family are responsible for highly contagious, acute respiratory disease in a wide range of vertebrate hosts, including birds and domesticated animals, as well as humans.¹ Recent epidemiological studies have indicated exposure of dogs to the H3N2 canine influenza virus (CIV) subtype is more prevalent in the US. In 2015, an epidemic of unusual respiratory disease occurred in Chicago, IL,^{2,3} and phylogenetic analysis indicated that the outbreak strain was homologous to the H3N2 CIV subtype previously reported in Korea and Southern China.^{4,5} This influenza A virus was believed to have evolved from an avian influenza virus.² Since 2015, outbreaks of H3N2 CIV have been reported with increasing frequency across the US

(<https://www.dogflu.com/outbreak-map>). Avoiding contact with infected dogs and contaminated inanimate objects, and prophylactic vaccination are key strategies to minimize exposure and infection risk, but they need to be

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widely practiced to be effective.⁶ The first commercially available inactivated H3N2 CIV vaccine was introduced with a conditional license in the USA in 2015.

Although dogs are most at risk from H3N2 CIV epidemics, there is potential risk for cross-species transmission.⁷ Canine H3N2 has a relatively broad host range and infection in ferrets, guinea pigs and cats has been demonstrated following experimental challenge.⁸ Infected cats in South Korean outbreaks presented with fever, tachypnea, sneezing, coughing, dyspnea and lethargy, and there were some fatalities.^{9,10} In March 2016, the cases of dog-to-cat transmission of H3N2 CIV in the US were reported in Northwest Indiana.² This emerging infectious disease appeared to be self-limiting and did not seem to cause life-threatening illness in cats; infected cats manifested clinical signs similar to those seen in dogs. Transmission between cats was confirmed by laboratory findings and routine isolation and quarantine procedures were used to halt further spread. This study aimed to test the hypothesis that vaccination with an inactivated H3N2 CIV vaccine is safe and would stimulate a humoral immune response in cats.

Materials and methods

The Institutional Animal Care and Use Committee of Zoetis reviewed and approved this study (Animal Use Protocol number: KZ-3172d-2016-06-dac). Twenty clinically healthy, 7–8-week-old male and female domestic shorthair, specific-pathogen-free cats were enrolled in the study. These animals were acclimated for at least 7 days prior to enrollment in the study and tested for H3N2 CIV-specific hemagglutination inhibition (HAI) titers. The seronegative cats with HAI titers <8.0 were blocked by date of birth and dam, and were randomly assigned to two treatment groups of 10 animals each and housed in two rooms in a biosafety level 2 isolation facility. Cats in treatment group T01 were administered subcutaneously with 1.0 ml doses of an adjuvanted placebo (0.9% sodium chloride plus 5% aluminum hydroxide) on days 0 and 21, to serve as non-immunized controls. Cats in treatment group T02 were vaccinated subcutaneously with 1.0 ml doses of a commercial inactivated H3N2 CIV vaccine (Zoetis) on days 0 and 21. First and second doses were administered to respective cats in the right rear and left rear legs, respectively. Cats were monitored for approximately 30 mins immediately after each injection, and were examined daily for at least 5 days after each injection for clinical signs of local and systemic reactions.

Blood samples for serology were collected via venipuncture from all animals prior to the first and second vaccination (days –1 and 20, respectively), and at weekly intervals post-second vaccination (days 27 and 34). HAI assays were performed as described elsewhere, with minor modifications.¹¹ Technicians performing the animal observations and assays were masked to the identity of treatment group assignments. The data obtained in this

study were subjected to biometric analyses using SAS Version 9.4, with HAI titers as the primary variable, and tympanic temperatures and adverse reactions as secondary variables. Geometric mean HAI titers and 90% confidence interval (CI) were calculated for both treatment groups at each sample collection point during the study.

Results

Vocalization during initial dose administration was observed in 4/10 (40%) and 2/10 (20%) cats in the control and vaccinated groups, respectively. Vocalization was also observed in 3/10 (30%) control and 2/10 (20%) vaccinated cats after the second dose administration. Two vaccinated animals licked the injection site following first vaccination and another cat showed mild stinging after the second vaccination. One of the control cats showed mild swelling at the injection site that resolved within 24 h. No injection site reactions were observed in cats vaccinated with the inactivated H3N2 CIV vaccine. Following vaccination, there were no immediate systemic reactions observed in either treatment groups. Pyrexia (39.5–39.7°C) of 24 h duration was observed in 5/10 (50%) and 3/10 (30%) control and vaccinated cats, respectively, after the initial injection. One cat in each group also had a transient fever after the second injection (Table 1).

H3N2 CIV-specific HAI titers are presented in Table 2. All cats were seronegative prior to day 0 with HAI titers approximately 4.0. At day 20, 2/10 vaccinated cats (20%) developed four-fold increases in titers when compared with day –1 (range 16–32). Two weeks post-second vaccination (on day 34), titers had risen at least four-fold in all vaccinated cats vs day –1 (range 32–128). Seroconversion was not demonstrated in any cats in the T02 group during the study period.

Discussion

Although H3N2 CIV subtype is commonly associated with dogs, it has been sporadically isolated from shelter cats during outbreaks of respiratory disease in South Korea^{9,10} and the USA.² There is evidence that in a recent US outbreak, viral transmission occurred not only from dogs to cats, but also among cats.¹² This study provided evidence of safety and immunogenicity in cats following immunization with an inactivated H3N2 CIV vaccine.

During the study period, all cats remained healthy and none exhibited clinical signs suggestive of illness caused by influenza virus. The vaccine appeared to be well tolerated during both vaccinations with a low number of reported episodes of immediate local reactions that included vocalization, licking the injection site and stinging. These adverse events were minor, comparable to the control group and seen to last for brief time periods. No injection site reactions were observed in cats that received the inactivated H3N2 CIV vaccine during the study period. The only injection site reaction occurred in a control animal, and it was most likely associated with the

Table 1 Distribution of immediate local reactions and transient fever within each treatment group

Vaccination	Adverse event	Control cats (treatment group T01; n = 10)	Vaccinated cats (treatment group T02; n = 10)
First dose	Vocalization	4 (40)	2 (20)
	Injection site reaction	1 (10)	–
	Injection site licking	–	2 (20)
	Stinging	–	–
	Transient fever (39.5–39.7°C)	5 (50)	3 (30)
Second dose	Vocalization	3 (30)	2 (20)
	Injection site reaction	–	–
	Injection site licking	–	–
	Stinging	–	1 (10)
	Transient fever (39.5–39.7°C)	1 (10)	1 (10)

Data are n (%); TO = treatment group

Table 2 Hemagglutination inhibition (HAI) titers in sera of cats vaccinated with an inactivated H3N2 canine influenza virus vaccine

Group	Status	Study day	Geometric mean HAI titers (90% CI)	HAI titer range
T01	Control (n = 10)	–1	4.00 (2.91–5.50)	4.00
		20	4.00 (2.91–5.50)	4.00
		27	4.00 (2.91–5.50)	4.00
		34	4.00 (2.91–5.50)	4.00
T02	Vaccinated (n = 10)	–1	4.00 (2.91–5.50)	4.00
		20	7.46 (5.43–10.27)	4.00–32.00
		27	29.86 (21.71–41.06)	16.00–64.00
		34	73.52 (53.46–101.11)	32.00–128.00

CI = confidence interval; HAI = hemagglutination inhibition; TO = treatment group

adjuvant. Transient fever was observed in 5/10 and 3/10 control and vaccinated cats after the initial injection, respectively, and one cat in each treatment group after the second injection. These findings are consistent with the safety data reported in an earlier study of dogs vaccinated with an inactivated H3N2 CIV vaccine.¹³

All vaccinated cats demonstrated seroconversion with at least a four-fold increase in H3N2 antibody titers 14 days post-second vaccination with an inactivated H3N2 CIV vaccine (Table 2). These findings are consistent with other studies demonstrating an induction of HAI antibody titers following immunization with H3N2 CIV vaccine.^{13,14} Nonetheless, controlled experimental challenge with a virulent H3N2 CIV strain is necessary to test the protective efficacy of this inactivated H3N2 CIV vaccine in cats.

Conclusions

In this study, the subcutaneous administration of two doses of an inactivated H3N2 CIV vaccine was safe, and was associated with minor reactions that were similar to those observed in sham-vaccinated cats. Humoral immune responses to vaccination were demonstrated in all vaccinated cats, with four-fold titer increases over the

5 week study period. Feline vaccine challenge studies are required to investigate the protection provided against H3N2 CIV infection by this vaccine in cats.

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Conflict of interest All authors are employed by Zoetis and canine influenza vaccine, H3N2, killed virus is a product of the company with a business and/or financial interest.

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