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Tier 1 Study of the Effect of SinoFresh Nasal Spray  
On H1N1 Infection in the Cotton Rat

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## **Background and Objectives**

Viral infections of the nasal passages, sinuses, and upper respiratory tract are a major health issue worldwide. Generally, the most common such infections are colds and flu and such infections are the most common infectious disease in most adults (Garibaldi, 1985) and among children as well, with children sometimes getting 6 to 12 colds annually (Simasek and Blandino, 2007). In the U.S. colds and flus lead to well over 100-million physician visits annually (Garibaldi, 1985) with some 114,000 hospitalizations annually in the U.S. for flu (Hunt, 2009). In the U.S. flu and pneumonia are the sixth leading cause of death (Hunt, 2009).

Colds are produced by rhinoviral species (members of the picornavirus family) or, less commonly, coronaviruses. Flu is caused by influenza viruses (members of the orthomyxovirus family). Aside from so-called "seasonal flu", whose season in the U.S. typically starts in October or November and peaks from December through March, there are also episodic flus such as the recent "swine flu" associated with the H1N1 variant of influenza A virus<sup>1</sup> (Hunt, 2009).

The nasal passages play a significant role in the infection pathway for both cold and flu viruses.

In the case of cold viruses entry is usually through the nose but can also occur from the eyes via the tear ducts which provide passage for the virus to the nasopharynx. In the adenoid area cold viruses attach to a receptor (ICAM-1) on the cells lining the nasopharynx (Gwaltney and Hayden, 2007). Viral replication in these cells and the immune system's efforts to eliminate the infection results in the symptoms of a cold.

For influenza viruses the nasal passage are also important although the infection involves the lungs as well. In particular the nasal passage and nasopharynx have been suggested to be a predominate site for influenza virus inoculation (Scull *et al.*, 2009). These tissues can be exposed to flu viruses when virus-containing aerosols (generally 10 microns or less MMAD) or from contaminated surfaces (animate and inanimate) by transfer on fingers to the nose (Bitko *et al.*, 2007; Nicholls *et al.*, 2007; Thompson *et al.*, 2006).

Bases on the above-summarized natural history of cold and flu infection, killing of cold or flu viruses in the nose before they can attach to cellular receptors could be a rational approach to potentially reducing the severity or duration of infection and/or reducing the risk that exposure will result in frank infection.

Previous studies conducted by SinoFresh Health Care established that pre-incubation of adenovirus and of respiratory syncytial virus (RSV) with SinoFresh nasal spray leads to reduction or elimination in the infectivity of these viruses (SinoFresh, 2005).

This present study was conducted as a first tier assessment of the following:

- (a) The ability of SinoFresh nasal spray to affect the incidence, severity or duration influenza infection in the cotton rat using influenza strain H1N1 (New Caledonia);
- (b) The effect of treatment on virus production in the nose and lungs; and,
- (c) The effect of treatment on the histology of the olfactory and lung epithelial tissue.

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<sup>1</sup> Of the three major types of influenza viruses (A, B and C) influenza A is usually associated with more severe symptoms than are the other two.

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In this study the index of infection and severity of infection used was core temperature. Body weight was also recorded in this study but since there was no treatment control<sup>2</sup> this measure could not be used to follow the course of infection specific weight loss<sup>3</sup>.

Viral titer in lung and nasal tissue at the typical peak times for viral expression in the cotton rat following infection<sup>4</sup> was assessed by fixing tissue samples and examining for viral particles by electron microscopy.

As noted, in the present study the histopathology of the olfactory epithelium and lung epithelium from rats in the infectivity phase and in the viral shedding phase of the study was collected.

The cotton rat (*Sigmodon hispidus*) is a well established model for the study of human viral infection and pathology (Murphy *et al.*, 1981; Ottolini *et al.*, 2005; Eichelberger, 2007). With regard to influenza these investigators have studied both parainfluenza virus (Murphy *et al.*, 1981) and influenza (type A) virus (Ottolini *et al.*, 2005). Other types of viruses have been studied in this model, including Hanta virus and adenoviruses. Eichelberger (2007) has recently reviewed the field.

An advantage of the cotton rat model for studying influenza infection is that this model does not require viral adaptation (Ottolini *et al.*, 2005). Therefore, data obtained in the cotton rat is more directly translatable to the human situation than data obtained with “adapted” viruses. In the cotton rat model the course of viral infection can be followed by a number of measures inclusive of depression of core temperature, loss of body weight, increased respiration rate and signs of respiratory distress, and viral titer in lung and nasal tissues.

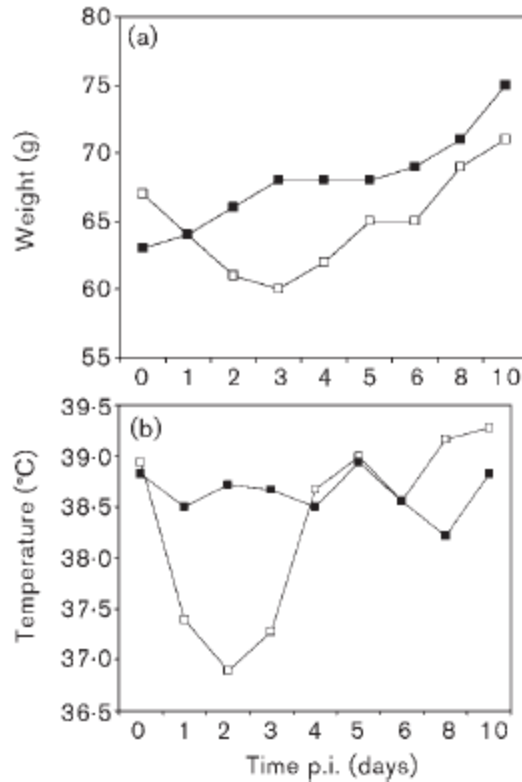
Ottolini *et al.*, (2005) have reported the time course of core temperature depression and body weight depression in the cotton rat following infection by nasal instillation of influenza A / Wuhan / 395 / 95 (see Figure 1, next page, which is reproduced from Ottolini *et al.*, 2005). As can be seen, core temperature depression in their study was maximal in the period 1 – 3 days post-infection (peaking at day 2) and returned to normal (baseline) at day 4 post infection. Body weight depression began at day 1 post-infection and did not return to baseline levels until 7 to 8 days post-infection. These data reflect the effects of infection with this viral strain and the time course and magnitude of changes in core temperature and body weight loss may differ for other strains of influenza virus or other viral species.

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<sup>2</sup> The study control was an untreated group of animals. The treated groups received 3 applications per day of SinoFresh nasal spray daily for 14 days. The effect of such treatment on food and water consumption was not controlled and, as such, changes in body weight could not be used to follow the comparative course of infection in this study.

<sup>3</sup> Treatment related weight loss occurs in animal studies in which nasally instilled liquid agents are used. This is due to movement of the test article from the nasal passage to the oropharynx and thence to the oral cavity and causation there of taste effects which can put animals off their feed until the animals acclimate to the effect.

<sup>4</sup> These are 1 day post-infection for lung titer and 4 days post-infection for nasal titer. These times are based on data developed with influenza A strains other than H1N1 and may differ slightly for H1N1.



**Fig. 1.** Cotton rats infected with influenza virus experience weight loss and decreased body temperature. Serial measurement of weight (a) and temperature (b) were made on days 0–10 after intranasal infection with A/Wuhan/395/95 (□), and in age-matched uninfected control animals (■).

In the present study, since a different influenza virus strain was used than that used by Ottolini *et al.* the time course and degree of core temperature depression in SinoFresh treated animals was compared to that for untreated control animals. Two treatment groups were used: one pre-treated with SinoFresh at 15 minutes prior to intra-nasal instillation of the virus suspension followed by three times daily (t.i.d.) intra-nasal application of SinoFresh and another which did not receive the first SinoFresh treatment until 15 minutes until after instillation of the virus suspension.

For severity of infection the study endpoints was whether there was a statistically significant difference between either of the SinoFresh treated groups and the untreated control group with respect to degree of core temperature depression. For incidence of clinically significant infection the endpoint in this present study was the number of animals in each group which exceeded a 1% decrement in core temperature compared to baseline. For duration of infection the endpoint in this study was the time course for core temperature depression.

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### **Procedures**

#### ***Infectivity Phase –***

##### Study Groups

All groups consisted of 10 young (6 – 8 weeks age) female cotton rats (*S. hispidus*). There were three study groups designated as follows:

- A Infected morning of Day 0 / Untreated post-infection
- B Infected morning of Day 0 / SinoFresh treated 15min post-infection then twice again the day of infection (midday and late afternoon) and then t.i.d daily (morning, midday and afternoon) Days 1 - 14 post-infection.
- C Infected / SinoFresh treated 15min prior to infection then twice again the day of infection (midday and late afternoon) and then t.i.d daily (morning, midday and afternoon) Days 1 - 14 post-infection.

##### Schedule of Study Procedures

Study Day	Procedures
Day 0	<p>Divide 30 young female cotton rats (<i>S.hispidus</i> 6-8 weeks old) into three groups of 10 animals (A, B and C as above defined). Prebleed and eartag all animals. Measure core temperature and weight of all animals.</p> <p>15 minutes prior to infection treat group C with 0.01ml (10 micro-L) of SinoFresh intranasally (T = -15 min)</p> <p>Infect all animals (Groups A, B and C) intranasally with 0.1mL of Influenza/a/new caledonia H1N1 (T = 0)</p> <p>15 minutes after infection treat group B with 0.01ml (10 micro-L) of SinoFresh intranasally (T = +15 min)</p> <p>3 hrs after infection treat groups B and C with SinoFresh as indicated above (10 micro-L). (T = +3 hrs)</p> <p>6 hrs after infection treat groups B and C with SinoFresh as indicated above (10 micro-L). (T = +6 hrs)</p>
Days 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13	<p>Measure weight, temperature and general health for all animals.</p> <p>Treat groups B and C (10 micro-L SinoFresh t.i.d, AM, Noon, and PM).</p>
Day 14	<p>Sacrifice all animals, perform gross necropsy, remove the olfactory epithelium (OE) of 5 animals from each group for histopathology (H&amp;E).</p>

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### ***Virus Shedding Phase –***

#### Study Groups

All groups consisted of 8 young (6 – 8 weeks age) female cotton rats (*S. hispidus*). There were three study groups designated as follows:

- A Infected morning of Day 0 / Untreated post-infection
- B Infected morning of Day 0 / SinoFresh treated 15min post-infection then twice again the day of infection (midday and late afternoon) and then t.i.d daily (morning, midday and afternoon) Days 1 - 4 post-infection.

#### Schedule of Study Procedures

Study Day	Procedures
Day 0	<p>Divide 16 young female cotton rats (<i>S. hispidus</i> 6-8 weeks old) into two groups of 8 animals (A and B as above defined). Prebleed and eartag all animals. Measure core temperature and weight of all animals.</p> <p>Infect all animals (Groups A, B and C) intranasally with 0.1mL of Influenza/a/new caledonia H1N1 (T = 0)</p> <p>15 minutes after infection treat group B with 0.01ml (10 micro-L) of SinoFresh intranasally (T = +15 min)</p> <p>3 hrs after infection treat groups B and C with SinoFresh as indicated above (10 micro-L). (T = +3 hrs)</p> <p>6 hrs after infection treat groups B and C with SinoFresh as indicated above (10 micro-L). (T = +6 hrs)</p>
Day 1	<p>Sacrifice 4 animals from each group. Remove the nose and lungs for viral titer. Treat remaining animals in group B (AM, Noon, and PM).</p>
Days 2 and 3	<p>Measure weight, temperature and general health for all animals. Treat group B (10 micro-L SinoFresh t.i.d, AM, Noon, and PM).</p>
Day 4	<p>Sacrifice all animals and remove the nose for viral titers. Remove the lung en bloc and bisect for viral titers and histopathology (H&amp;E).</p>

Viral titers were determined by electron microscopic determination of viral particles in tissue sections. This method quantitates viral particles but cannot determined whether these are viable or not.

**Histopathology Phase -**

Nasal and pulmonary tissue was collected as above described for the infectivity and viral shedding phases. Tissue was fixed and paraffin embedded, sectioned and the prepared and stained with hematoxylin – eosin stain (H&E) and examined microscopically.

**Results**

**Infectivity Phase –**

The group mean data for core temperature changes are shown in Table 1 (below).

**Table 1: Group Mean Core Temperature Depression  
(as percent of baseline core temperature)**

<b>Days Post Infection</b>	<b>Group A (untreated)</b>	<b>Group B (1<sup>st</sup> treatment 15 min post-inf)</b>	<b>Group C (1<sup>st</sup> treatment 15 min pre-inf)</b>
1	- 1.09	- 1.74	- 1.03
2	- 2.35	- 2.22	- <b>1.53*</b>
3	- 1.33	- 1.49	- <b>0.55*</b>
4	- 0.91	- 0.67	- <b>0.05*</b>
5	- 1.66	+ <b>1.37*</b>	- <b>0.90*</b>
6	- 0.74	+ <b>1.73*</b>	+ <b>1.41*</b>
7	- 1.64	+ <b>0.56*</b>	+ <b>0.11*</b>
8	0.00	- <b>1.09^</b>	- 0.31
9	+ 0.21	- <b>1.61^</b>	- <b>1.35^</b>
10	- 0.75	- 0.52	- 0.57
11	- 1.38	- <b>0.43*</b>	- <b>0.60*</b>
12	- 0.53	+ <b>1.00*</b>	+ <b>0.62*</b>
13	- 1.30	+ <b>1.30*</b>	+ <b>1.23*</b>
14	- 0.55	- 0.87	- 0.52

\* Statistically less drop than for untreated control (p < 0.05, N = 10)

^ Statistically greater drop than for untreated control (p < 0.05, N = 10)

The data in Table 1 show that Group C is significantly less affected by viral infection during the first few peak days of symptoms than are Groups A or B. They also show that animals in both Groups B and C return to baseline or somewhat higher core temperature more rapidly than animals in Group A. Interestingly, there appears to be a second period of core temperature depression in the untreated control animals (Group A) which starts at day 10 and lasts through day 13 / 14. This second wave of core temperature depression is not seen in the SinoFresh treated animals.

The time course and degree of group mean core temperature depression is represented graphically in Figures 2 and 3 (at the end of this report). Fig 2 is for the absolute core temperature (normalized to the baseline Group A value). Figure 3 is for percent change from

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baseline for each of the groups.

In both figures, core temperature drops in all groups with a maximum decrease occurring in the period 1 – 3 days after infection (and a peak drop at day 2). This is consistent with the study of Ottolini *et al.* (2005). However core temperature for Group A (untreated) out through Day 7 and did not return to baseline until Day 8. From Day 8 through 14 the time averaged core temperatures were depressed in Group A.

In contrast, Groups B and C (SinoFresh treated) re-established baseline core temperatures by Day 4 and then remained (time averaged) at baseline for the remaining study period of observation.

Group C (pre-treated with SinoFresh) had a significantly smaller depression of core temperature at Day 2 compared to Group A (untreated) and Group B (15 minutes post-treated)<sup>5</sup>. Compared to Group B, Group C also had consistently higher normalized core temperature on all study days (Figure 2) and appeared, as noted above, to not undergo a second wave of core temperature depression which was seen in Group A. There were no notable differences between Group B and Group C in percent core temperature relative to baseline for the period from Day 5 through the end of the study period.

Figure 4 shows the group means average percent change in core temperature for the different time periods (Day 0 – 4, Day 5 – 9, and Day 10 – 14). As is shown the degree of core temperature depression was significantly greater ( $p < 0.05$ ) for Groups A and B compared to Group C for the Day 0 – Day 4 period. For the other two periods the group means core temperature was within 0.2% of baseline for Groups B and C but was depressed to a significantly greater extent (0.74% for D5-D9 and 0.69% for D10-D14).

Table 2, below, provides incidence data for animals in each group showing a 1% or greater drop in core temperature relative to baseline.

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<sup>5</sup> See Table 1

**Table 2: Group Incidence of Animals with Core Temperature Depression  $\geq$  1% Relative to Baseline**

<b>Days Post Infection</b>	<b>Group A (untreated)</b>	<b>Group B (1<sup>st</sup> treatment 15 min post-inf)</b>	<b>Group C (1<sup>st</sup> treatment 15 min pre-inf)</b>
1	7/10 (70%)	8/10 (80%)	4/10 (40%)
2	10/10 (100%)	10/10 (100%)	<b>6/10 (40%)*</b>
3	7/10 (70%)	7/10 (70%)	<b>2/10 (20%)*</b>
4	4/10 (40%)	3/10 (30%)	<b>1/10 (10%)*</b>
5	9/10 (90%)	<b>0/10 (0%)*</b>	<b>0/10 (0%)*</b>
6	5/10 (50%)	<b>0/10 (0%)*</b>	<b>0/10 (0%)*</b>
7	8/10 (80%)	<b>1/10 (10%)*</b>	<b>4/10 (40%)*</b>
8	2/10 (20%)	4/10 (40%)	2/10 (20%)
9	1/10 (10%)	<b>8/10 (80%)*^</b>	<b>7/10 (70%)*^</b>
10	4/10 (40%)	3/10 (30%)	4/10 (40%)
11	9/10 (90%)	<b>2/10 (20%)*</b>	<b>4/10 (40%)*</b>
12	4/10 (40%)	<b>0/10 (0%)*</b>	<b>0/10 (0%)*</b>
13	7/10 (70%)	<b>0/10 (0%)*</b>	<b>0/10 (0%)*</b>
14	4/10 (40%)	5/10 (50%)	3/10 (30%)

\* Statistically lower than untreated control ( $p < 0.05$ ,  $N = 10$ )

^ Statistically greater than untreated control ( $p < 0.05$ ,  $N = 10$ )

The time distribution of the incidence of animals with a drop in core temperature greater than or equal to 1% is shown graphically in Figure 5. The data in Table 2 show that the incidence of such animals is essentially the same for Groups A and B through Day 4 but is significantly lower for Group C through Day 4. From Day 5 through Day 7 both Groups B and C have a statistically lower incidence of animals with severe core temperature reduction compared to baseline. For Days 8 through 10 the incidence of animals with severe core temperature reduction is low in all groups and the number of animals in Groups B and C with core temperature higher than baseline by 1% or more is actually higher than for Group A on Day 9. Starting on Day 11 Group A again begins to show a high incidence of animals with core temperature drop of greater than or equal to 1% and this is significantly different from the incidence of such animals in Groups B and C. Three days later, on Day 14, there is no significant difference between Group B or C and Group A.

#### **Virus Shedding Phase –**

The viral particle counts for nasal and pulmonary tissues are provided in Tables 3 and 4 (respectively) below.

**Table 3: Nasal Viral Particle Count Data**

Nose

Group	Animal #	DPI	TCID50/g	GeoMean	SD	SE
A	33	1	6.00	6.1840639	0.23935678	0.11967839
	34	1	6.25			
	35	1	6.50			
	36	1	6.00			
	37	4	5.16	5.40480326	0.3923009	0.19615045
	38	4	5.25			
	39	4	5.25			
	40	4	6.00			
B	41	1	6.50	6.43419607	0.23935678	0.11967839
	42	1	6.25			
	43	1	6.25			
	44	1	6.75			
	45	4	3.75	5.11013545	0.96555252	0.48277626
	46	4	5.75			
	47	4	5.75			
	48	4	5.50			

**Table 4: Pulmonary Viral Particle Count Data**

Lung

Group	Animal #	DPI	TCID50/g	GeoMean	SD	SE
A	33	1	6.80	7.22888066	0.34161382	0.17080691
	34	1	7.13			
	35	1	7.46			
	36	1	7.55			
	37	4	<1.8	1.8	0	0
	38	4	<1.8			
	39	4	<1.8			
	40	4	<1.8			
B	41	1	7.55	7.35306376	0.42695628	0.21347814
	42	1	6.80			
	43	1	7.30			
	44	1	7.80			
	45	4	<1.8	1.8	0	0
	46	4	<1.8			
	47	4	<1.8			
	48	4	<1.8			

The viral inoculation procedure, using a 100 microL suspension of virus, allows viral suspension to be aspirated directly into the lung as well as exposing the nasal passages. This presents a very challenging test system for looking at the effect of SinoFresh on viral titers.

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Also, the treatment regimen for Group B is the same as for Group B in the infectivity phase. In the infectivity phase Group B appeared to have similar severity and infection rates as the untreated controls (Group A) but did show earlier recovery than Group A (see Tables 1 and 2 above).

Nasal viral titers were essentially the same at day 1 post-infection for Groups A and B in the virus shedding phase (see Table 3). At day 4 post-infection there is no difference in group mean nasal viral count between Group A and B. However, animal No. 45 is significantly lower than the other animals in Group B and in Group A. This is summarized in Table 5 below.

**Table 5: Comparison of Animal 45 to Other Animals in Virus Shedding Phase, Nasal Titer at 4-Days Post-Infection**

	<b>Titer</b>	Upper 95% CL	Lower 95% CL
<b>Group A</b>			
#37	5.16		
#38	5.25		
#39	5.25		
#40	6.00		
<b>Group Mean</b>	<b>5.42</b>	5.96	4.87
<b>Std. Dev.</b>	<b>0.392</b>		
<b>Group B</b>			
#46	5.75		
#47	5.75		
#48	5.50		
<b>Group Mean*</b>	<b>5.67</b>	5.93	5.41
<b>Std. Dev.</b>	<b>0.144</b>		
#45	3.75	n/a vs. Group A n/a vs. Group B	p < 0.05 vs. Group A p < 0.05 vs. Group B*

\* Excluding animal #45

These data suggest that in at least one animal in Group B SinoFresh treatment reduced nasal viral load at 4 days post-infection to a significant degree compared to the other animals.

As expected, due to direct lung infection in this model, group mean lung titers at day 1 post-infection are the same for Groups A and B.

**Histopathology Phase -**

Histopathology scores for nasal and pulmonary tissue are provided in Tables 6 and 7 below. Table 6 is for the olfactory epithelium in animals in the infectivity phase study and Table 7 is for the pulmonary epithelium in animals in the virus shedding phase.

**Table 6: Olfactory Epithelium Histopathology Scores for Animals from the Infectivity Phase**

Groups	Animal#	OE Damage	Path Score
A	79155	0	0
	79156	1	5
	79157	1	5
	79158	0	0
	79159	0	0
B	79265	0	0
	79266	0	0
	79267	0	0
	79268	1	5
	79269	1	5
C	79275	1	5
	79276	1	5
	79277	0	0
	79278	1	5
	79279	0	0

Raw Score to Path Score Conversion

- 0 = 0
- 1 = 5
- 2 = 25
- 3 = 75
- 4 = 100

The data in Table 6 indicate that there are no differences in the histopathology of the olfactory epithelium for animals in Groups A, B or C. This suggests that SinoFresh treatment three time daily per protocol (10 microL per administration) did not have any deleterious effect on the olfactory epithelium.

**Table 7: Pulmonary Pathology Scores for Pulmonary Tissue in Animals in Virus Shedding Phase**

Group	Animal #	Raw				Path Score			
		PB	PV	IP	AL	PB	PV	IP	AL
A	61837	2	2	3	3	25	25	75	75
	61838	2	2	3	3	25	25	75	75
	61839	2	2	3	3	25	25	75	75
	61840	2	2	3	3	25	25	75	75
B	61845	2	2	3	3	25	25	75	75
	61846	2	2	3	3	25	25	75	75
	61847	2	2	3	3	25	25	75	75
	61848	2	2	3	3	25	25	75	75

		Raw Score to Path Score conversion
PB	Peribronchiolitis	0 = 0
PV	Perivascularitis	1 = 5
IP	Interstitial Pneumonia	2 = 25
AL	Alveolitis	3 = 75
		4 = 100

The data in Table 7 indicate that there were no differences in lung histopathology between group A and Group B. The low level effects seen in both groups are attributable as sequelae to the influenza infection of the lungs.

## **Discussion and Conclusions**

The data reported here suggest that animals treated TID with SinoFresh recovered more rapidly from H1N1 infection than did untreated animals. These data also suggest that pre-application of SinoFresh compared to post-application decreased the severity and/or incidence of infection. This is reinforced by the observation that there is in the untreated Group A animals a second wave of core temperature depression occurring approximately 3 days after resolution of the first wave of core temperature depression but that this second wave of symptoms is not seen in the SinoFresh treated animals of Groups B and C.

The difference between the pre-treated and post-treated SinoFresh groups may possibly be due to the presence of a pre-existing amount of the actives in SinoFresh dissolved in the mucosa of the nasal passages at the time of viral instillation compared to administering the SinoFresh initial dose on top of a 10-fold greater volume of viral suspension. This difference could lead to a less effective local concentration of SinoFresh actives for Group B (post-treated) compared to Group C (pre-treated) at the critical time of presentation of the virus. In this context it is important to note that the volume of SinoFresh administered is only 0.01 mL whereas the volume of viral inoculant is 0.1 mL. This is a potential up to 10X dilution of the applied SinoFresh. Additional study will be needed to explore this difference more fully as well as to do dose ranging for the optimum dose of SinoFresh per application in this model and to control for treatment effects of dosing itself so as to be able to utilize body weight changes as an additional symptom index for infection. A study in which the animals were on a TID regimen of SinoFresh for at least a few days prior to viral inoculation could be useful in this regard.

The data for viral shedding study suggest that in the present model there was little effect on viral titer in the lung at day 1 post-infection (the typical peak time for viral expression in the lung in this model). For nasal viral titers there is little difference in either group comparing day 1 post-infection with day 4 post-infection. This suggests that the peak time for nasal expression of H1N1 in this model may be different than the peak of 4 days post-infection which has been reported for other influenza A strains in this model. At 4 days post-infection the group means were not significantly different comparing Group A and Group B. However, one animal in Group B was found to have a significantly lower viral titer than for animals in Group A or the other animals in Group B. This suggests that SinoFresh treatment may have had an effect on this animal's nasal viral titer. Also, the inoculation method used (100 microL of viral suspension delivered into the nose) assures that some viral suspension will get directly into the lung. This makes the present model very challenging for testing effects on killing or deactivating virus as it enters the nose. The timing (post-treatment) of initial SinoFresh treatment in the virus shedding phase was the same as used for Group B in the infectivity phase in which it was observed that a similar incidence of infection and degree of severity occurred as in the controls (Group A) but that pre-treatment resulted in lowered infection rates and severity of infection.

The present study, taken as a whole, suggests that regular use of SinoFresh may help reduce the risk of viral infection in the first place and/or the severity of symptoms of infection. Additional study is needed to determine an optimal use regimen in this regard. Also, additional study with smaller viral inoculant volumes is suggested so as to better investigate the effect on viral shedding and, also, provide for a study model which more closely mimics the human exposure condition.

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# Figures

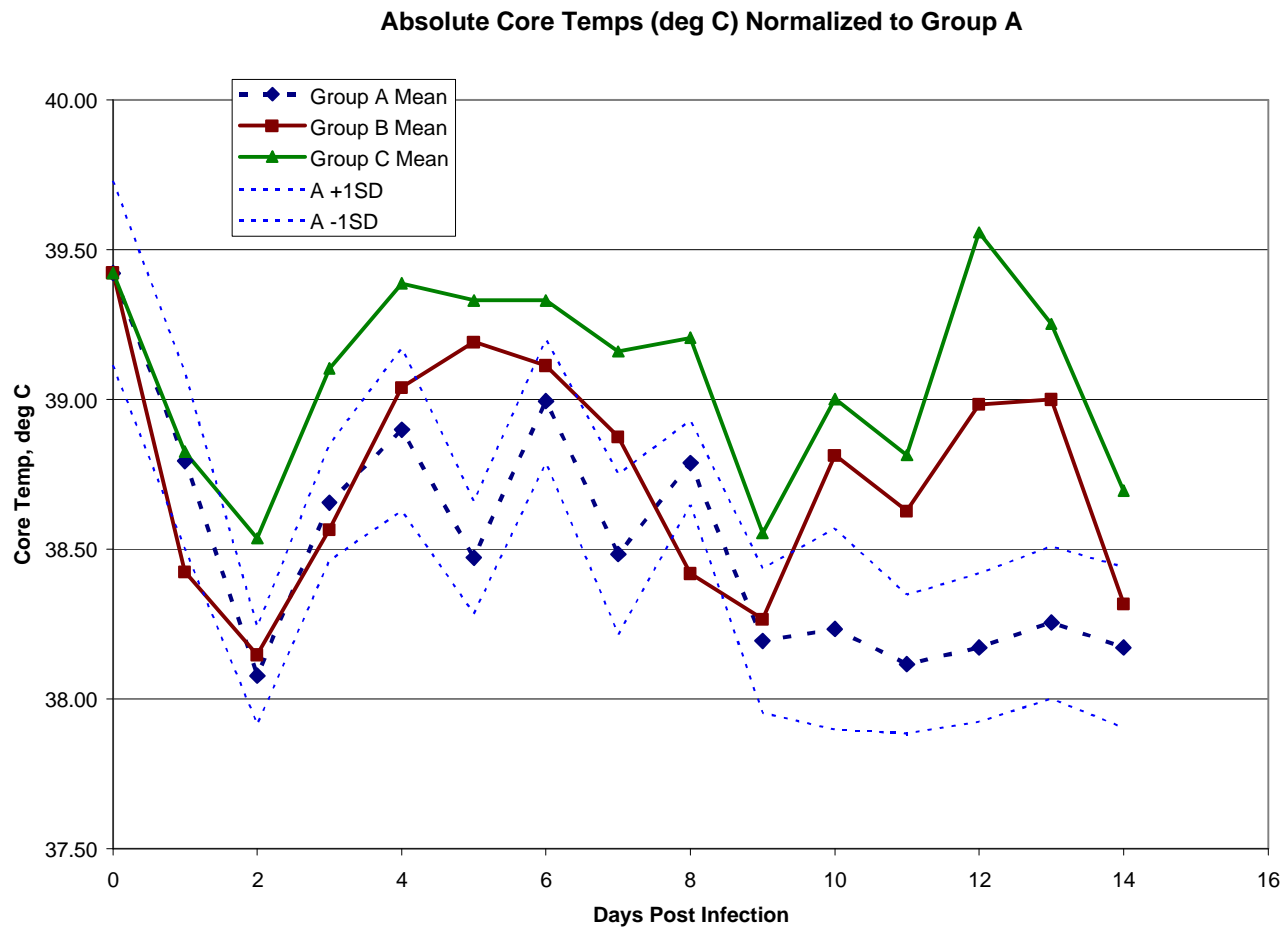


Figure 2

Percent Change in Core Temp (F)

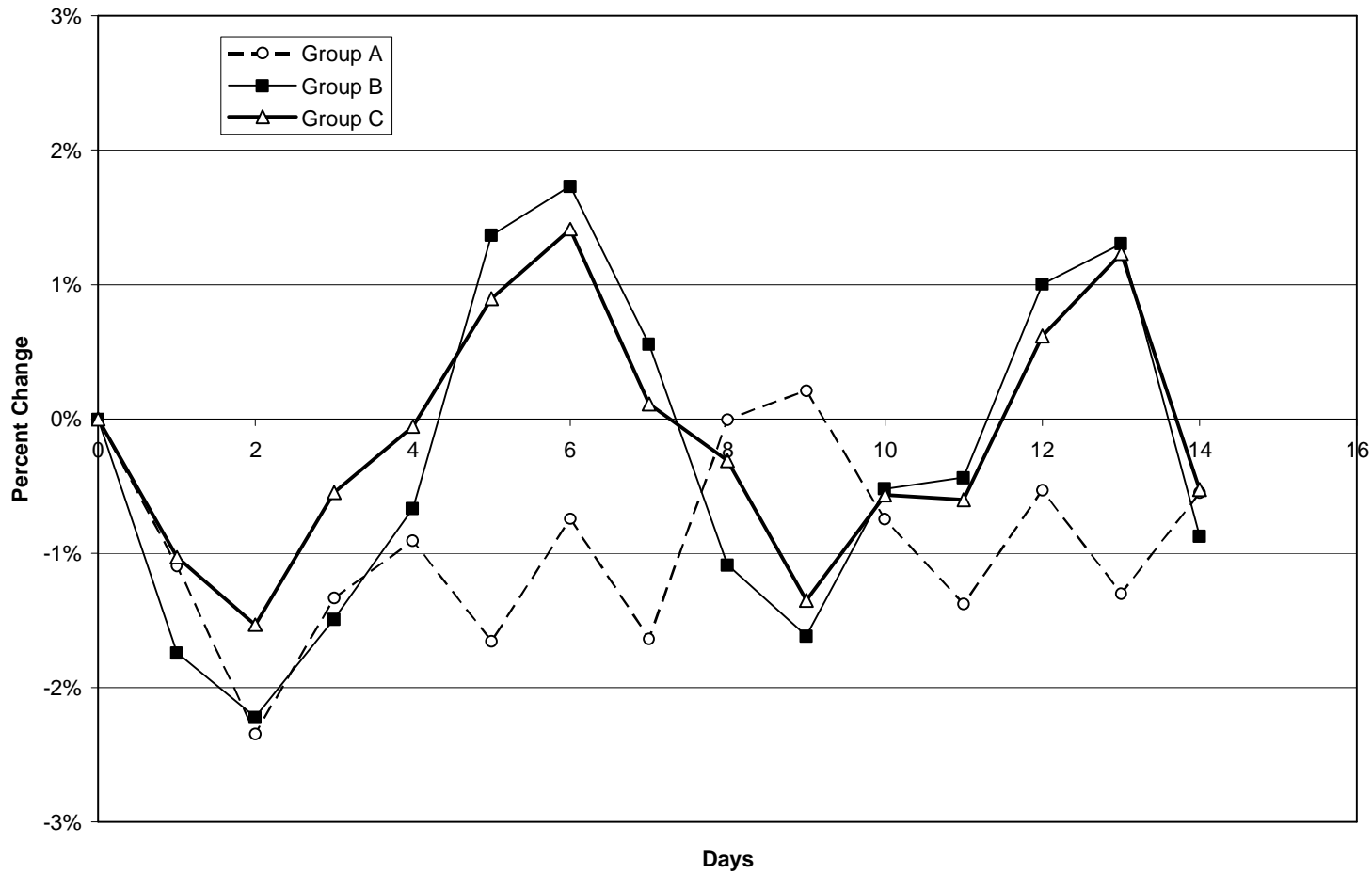


Figure 3

Percent Change in Core Temp by Periods

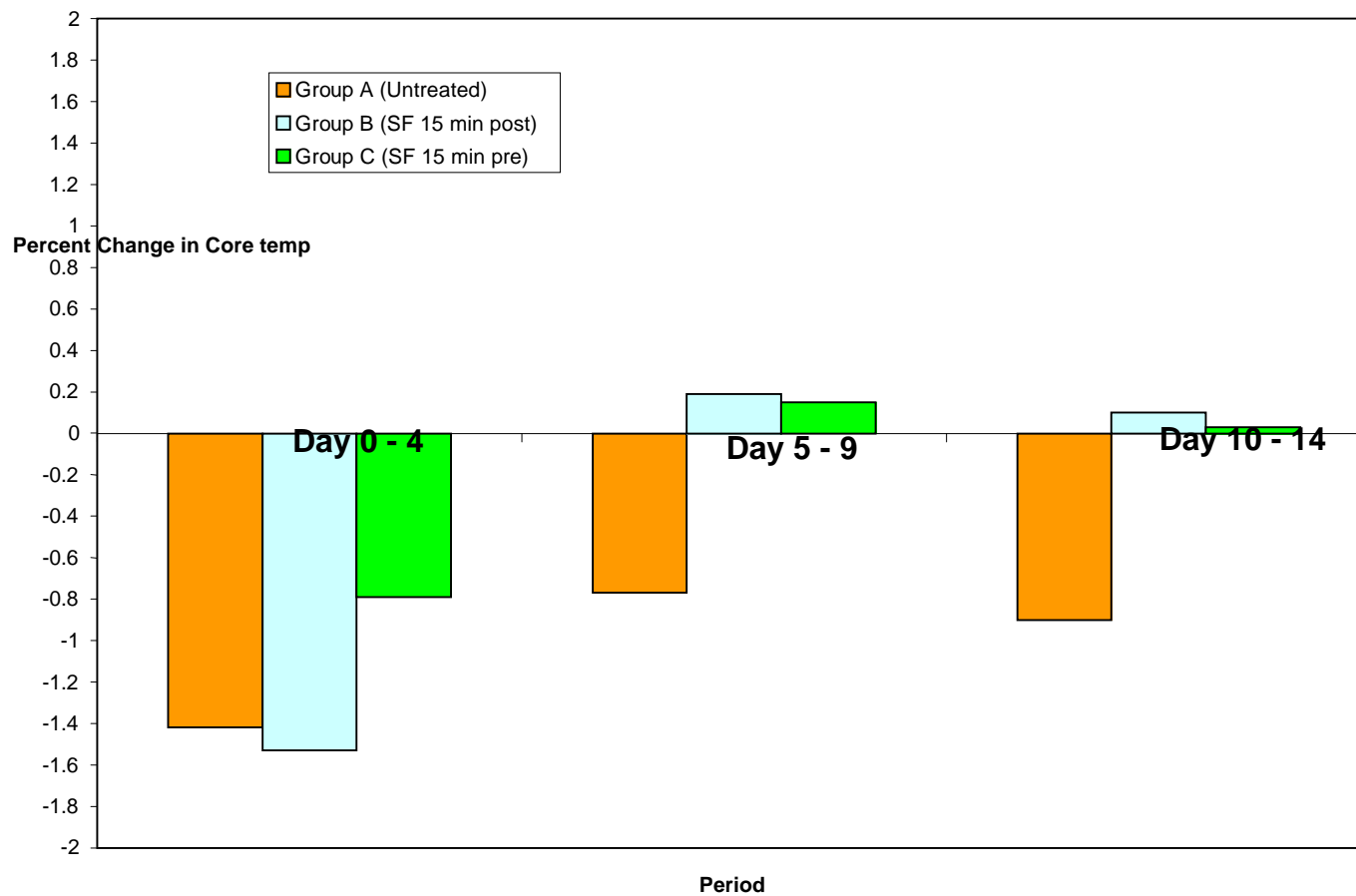


Figure 4

Percent Animals (N=10) w. Core Temp Drop  $\geq$  1%

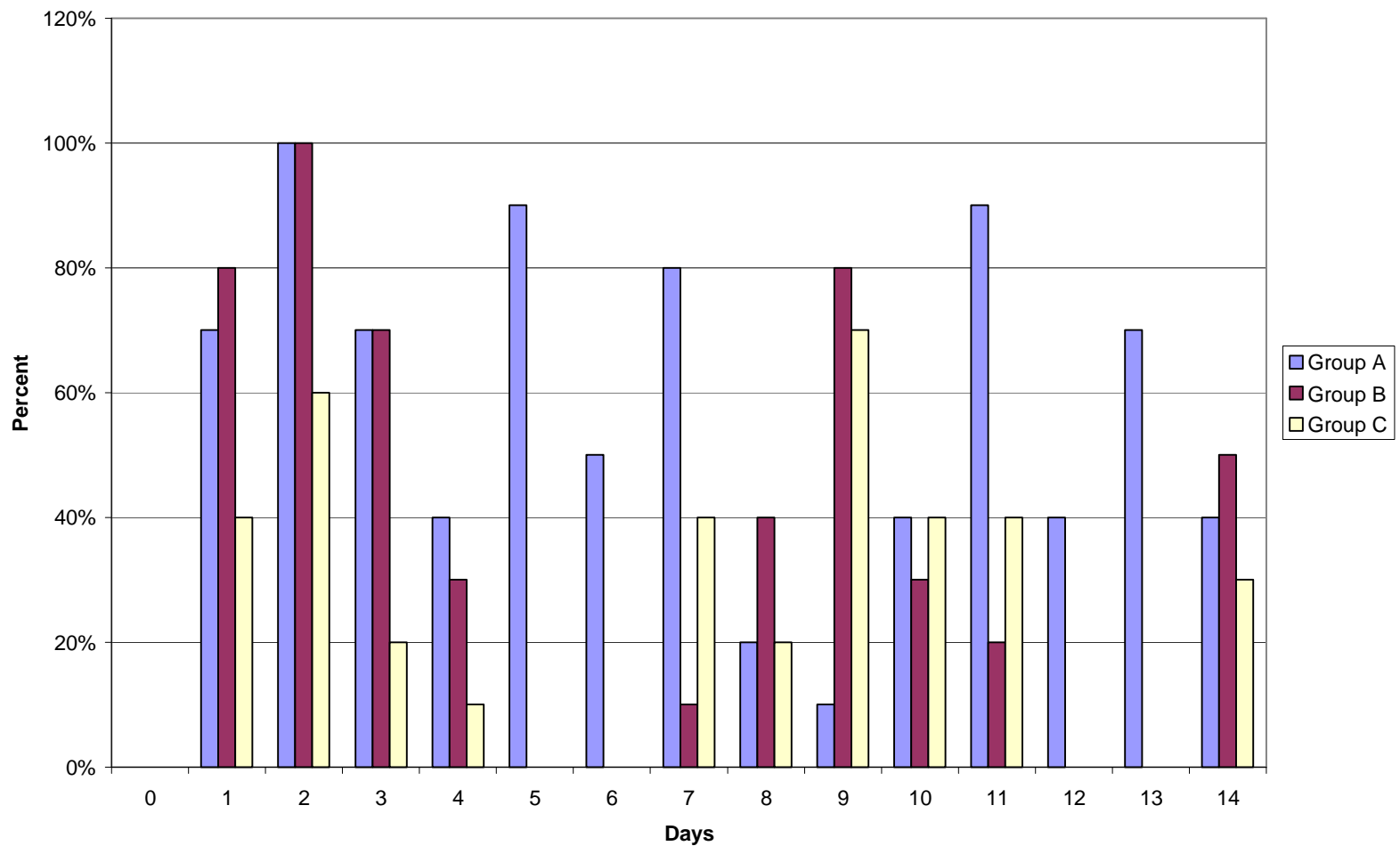


Figure 5