THE EFFECT OF TEMPERATURE AND UV LIGHT ON INFECTIVITY OF AVIAN INFLUENZA VIRUS (H_5N_1, THAI FIELD STRAIN) IN CHICKEN FECAL MANURE

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Abstract. Normal chicken fecal manure (pH 8.23 and 13.7% moisture) was investigated for infectivity of the avian influenza virus (AIV; H_5N_1). The manure was divided into three groups; each group was inoculated with AIV at 2.38 x $10^{5.25}$ ELD_{50}. After viral inoculation, the first group was incubated at 25°C. The second group was kept at 40°C, and the last group was exposed to ultraviolet light at 4-5 μw/cm^2 at room temperature. After incubation, a 20% suspension of manure was filtered and the filtrates were inoculated into 9-11 day-old embryonated chicken eggs per WHO protocol (2002). The results showed that at 25°C the virus lost its infectivity within 24 hours, and at 40°C within 15 minutes. UV light, however, could not destroy the infectivity of the virus even after exposure for 4 hours.

INTRODUCTION

Avian influenza (AI) is an emerging zoonotic disease in Southeast Asia and throughout the world. In Southeast Asia, more than 60 people have died from the disease since the first case in Hong Kong was reported in 1997 (Yuen ***et al***, 1998; WHO, 2005). In Thailand, there were 13 deaths in 21 cases reported from December 2003 to November 2005 (WHO, 2005). There has been a great economic loss in the poultry industry; the export of frozen chicken meat to EU and Japan amounts to over 45,000 million bahts (Department of Livestock Development, 2005).

Avian influenza virus can survive outside the host for certain periods of time depending on the environmental conditions. AIV is susceptible to heat and dryness and is easily destroyed by strong acid or alkaline conditions and by disinfectant. Webster ***et al*** (1978) found that AIV could live for 4 days in water at 22°C and up to 30 days at 0°C. In droppings, Beard ***et al*** (1984) reported that AIV (H_5N_2) in fecal droppings kept at 4°C could be recovered until the 30th-35th day, but for periods of 7 days at 20°C. Lu ***et al*** (2003) studied AIV (H_7N_2) in chicken feces. They found the virus died within one week at 15-20°C. At high temperatures the virus is destroyed in minutes, the higher the temperature the shorter the survival time of the virus. At 70°, 75° and 80°C, AIV was destroyed in 30, 5 and 1 minutes, respectively (Department of Primary Industries and Energy, 1996).

Two studies demonstrated the pH stability of AIV (H_5 and H_7) was best between pH 5.5 - 8.0. At a pH of 2 at 56 °C the virus survived only 30 minutes (Scholtissek, 1985; Lu ***et al***, 2003).

In Thailand, Songserm ***et al*** (2005) studied the survival of AIV (H_5N_1) under various conditions. In allantoic fluid or fresh feces at 33-35°C, the virus can live for only 30 minutes. It has a longer survival time if kept at room temperature, up to 10 days. Dryness is a factor that affects survival time. In the same study, the virus was mixed with chicken feces and spread on an egg-shell and an egg-tray and allowed to dry; the following day, the virus could not be recovered from either object.

Ultraviolet (UV) light has also been used to destroy microbes. UV light consists of types A, B and C. Only type UV-C, which has a wavelength of 250-270 nm, can cleave the hydrogen-bond of microbial DNA resulting in destruction. The UV light bulb in the general laboratory has

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plenty of UV-C. UV light can not pass through even a thin glass. The consistency of feces is not solid. UV light may be used to destroy AIV in infected fecal material.

Onion exports from Thailand to Japan were cancelled due to concern over the use of chicken manure for cultivation. A study on the effect of temperature and UV light on the infectivity of AIV (H_5N_1) was warranted. The findings of this study should be useful to control the spread of this virus and the safe use of chicken manure.

MATERIALS AND METHODS

Avian influenza virus (H_5N_1, Thai field strain)

Virus titration was performed using embryonated chicken eggs. The virus titer was calculated by the method of Reed and Muench (1938). The seed virus used had a titer of $5x10^{5.25}$ ELD_{50}/ml.

Sample preparation and study groups

Chicken manure was tested using an Avian influenza test kit (Smart vet®) to ensure no contamination with AIV occurred before the study. The manure samples were measured for humidity (AOAC) and pH (Bartlett, 1975), and found to have a 13.67% moisture content and a pH of 8.23.

The manure was dried in an incubator at 60°C for 72 hours. One hour later, the manure was then divided into 26 aliquots of 3 grams each and placed in a small plastic Petri dish (Nunclon™, Nunc; diameter 3.5 cm) which made the height of the manure approximately 0.8 cm. Four hundred seventy-five microliters of seed virus was inoculated into each sample. This gave about 13.67% moisture content (w/w); similar to the original. All 26 samples were divided into 3 groups as follows:

The first group (7 samples) was tested at 25°C for 7 days. After each 24 hours of incubation, one sample was taken out and added to sterile PBS to make a 20% suspension. The suspension was filtered with a 0.45 µm plastic filter (Minisart®, Sartorius). The filtrate was kept at -20°C until egg inoculation took place.

The second group was composed of 8 samples and was tested at 40°C for 120 minutes. The incubation interval for each sample was 15 minutes. The viral suspension was prepared the same as in the first group.

The last group (11 samples) was subjected to UV light exposure at a density of 4-5 µw/cm^2 (25°C), which was the average density during the day at the study site. The cover of the Petri dish was opened when the samples were exposed to the light. Sample numbers 1-6 were collected every 10 minutes, sample numbers 7 and 8 at 30 minutes intervals, sample numbers 9 and 10 at 1 hour intervals and sample no. 11 was collected $1^1/_2$ hour after sample no.10.

The recovery of the virus

The filtrate from each of the 3 groups was inoculated into three 9-11 day-old chicken embryonated eggs per WHO protocol (2002). After 48 hours of incubation, the allantoic fluid was harvested and hemagglutination (HA) and hemagglutination inhibition (HI) tests were used to identify the recovered virus.

RESULTS

In the first group (25°C), 3 embryoes from 2 samples (2/1, 6/2 and 6/3) died during the incubation time. All the fluids, except the fluid from sample 6/2, gave HA-negative results. Although the fluid from sample 6/2 gave an HA-positive result, tested as AIV negative, based on the HI test (negative) as seen in Table 1.

In the second group (40°C), 4 embryoes from 3 samples (75/3, 90/2, 90/3 and 120/3) also died, but all the samples gave HA-negative results as shown in Table 2.

In the last group (UV light exposure), 10 embryoes from 7 samples (10/1, 10/2, 10/3, 40/3, 60/1, 60/3, 90/3, 120/3, 180/3 and 240/1) died during incubation. The HA test showed that 7 out of 10 fluid samples had positive results. Fluid from samples 60/3, 90/3 and 120/3 gave HA-negative results as shown in Table 3. The longest exposure time after which the virus was recovered was 240 minutes.

DISCUSSION

When the sample was kept at 25°C, the virus could not be recovered after 24 hours of incubation. As stated before, the virus is susceptible to heat and dryness. The manure used in

Table 1
The recovery of AIV (H_5N_1) at 25°C.

Time (days)	Sample	Embryo	HA test	HI test
1	1/1	alive	- ve	
	1/2	alive	- ve	
	1/3	alive	- ve	
2	2/1	death	- ve	
	2/2	alive	- ve	
	2/3	alive	- ve	
3	3/1	alive	- ve	
	3/2	alive	- ve	
	3/3	alive	- ve	
4	4/1	alive	- ve	
	4/2	alive	- ve	
	4/3	alive	- ve	
5	5/1	alive	- ve	
	5/2	alive	- ve	
	5/3	alive	- ve	
6	6/1	alive	- ve	
	6/2	death	+ ve	- ve
	6/3	death	- ve	
7	7/1	alive	- ve	
	7/2	alive	- ve	
	7/3	alive	- ve	

Table 2
The recovery of AIV (H_5N_1) at 40°C.

Time (minutes)	Sample	Embryo	HA test
15	15/1	alive	- ve
	15/2	alive	- ve
	15/3	alive	- ve
30	30/1	alive	- ve
	30/2	alive	- ve
	30/3	alive	- ve
45	45/1	alive	- ve
	45/2	alive	- ve
	45/3	alive	- ve
60	60/1	alive	- ve
	60/2	alive	- ve
	60/3	alive	- ve
75	75/1	alive	- ve
	75/2	alive	- ve
	75/3	death	- ve
90	90/1	alive	- ve
	90/2	death	- ve
	90/3	death	- ve
105	105/1	alive	- ve
	105/2	alive	- ve
	105/3	alive	- ve
120	120/1	alive	- ve
	120/2	alive	- ve
	120/3	death	- ve

Table 3
The recovery of AIV (H_5N_1) after UV light exposure (25°C).

Time (minutes)	Sample	Embryo	HA test	HI test
10	10/1	death	+ ve	+ ve
	10/2	death	+ ve	+ ve
	10/3	death	+ ve	+ ve
20	20/1	alive	- ve	
	20/2	alive	- ve	
	20/3	alive	- ve	
30	30/1	alive	- ve	
	30/2	alive	- ve	
	30/3	alive	- ve	
40	40/1	alive	- ve	
	40/2	alive	- ve	
	40/3	death	+ ve	+ ve
50	50/1	alive	- ve	
	50/2	alive	- ve	
	50/3	alive	- ve	
60	60/1	death	+ ve	+ ve
	60/2	alive	- ve	
	60/3	death	- ve	
90	90/1	alive	- ve	
	90/2	alive	- ve	
	90/3	death	- ve	
120	120/1	alive	- ve	
	120/2	alive	- ve	
	120/3	death	- ve	
180	180/1	alive	- ve	
	180/2	alive	- ve	
	180/3	death	+ ve	- ve
240	240/1	death	+ ve	+ ve
	240/2	alive	- ve	
	240/3	alive	- ve	
330	330/1	alive	- ve	
	330/2	alive	- ve	
	330/3	alive	- ve	

this study was dry with about 13.67% moisture content (w/w) compared with fresh fecal droppings which have at least 60% moisture. Songserm *et al* (2005) found that the virus can survive for 4 days in fresh fecal droppings. The moisture content of the environment surrounding the virus is an important factor for the survival period of the virus. When farmers buy manure from an unknown area, which may or may not be contaminated with the virus, they should choose one that is dry.

One fluid from sample 6/2 gave positive result on the HA test, but negative on the following test. The hemagglutination may have been caused by other microbes, such as Paramyxovirus, Adenovirus or *Mycoplasma* spp (Wongwatcharadumrong, 1990).

At 40°C, the virus in the manure was killed in a short time. After only 15 minutes of incubation, the virus could not be recovered. This finding is similar to a study by Songserm *et al* in 2005. They found the virus was destroyed within 30 minutes when they mixed the virus with allantoic fluid or fresh fecal droppings and put the containers in sunlight with an ambient temperature of approximately 33-35°C. In a chicken house, the temperature can be successfully raised to 40°C by an ordinary heater used for chick brooding. Rising the temperature in an empty house can help to disinfect it, especially in an endemic area. This is useful for the prevention and control of the spread of the virus.

UV light does not seem to be useful for viral destruction in fecal material. Although fecal material is not solid, it can still shield the virus from direct light. Therefore, only microbes on the surface of material and in the air are killed by UV light (Nicklin *et al*, 1999).

This study gives data about the effects of temperature and humidity on the infectivity of AIV (H_5N_1) in manure. These findings should be given to farmers who use chicken manure. It is important for the consumer to know that plants fertilized by contaminated manure, such as the case of the onions, can not spread the AIV (H_5N_1), since the virus does not remain viable for long, even at room temperature.

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