



Research Article

Ultra violet light in the Biosafety Cabinets fails to inactivate H5N1 Avian Influenza Virus in Poultry Feces

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ABSTRACT

Owing to their public health implications, handling of Highly Pathogenic Avian Influenza (HPAI) viruses should be carried out in BSL-3 laboratory inside a biosafety class II cabinet along with recommended personal protection procedures. Treatment with UV light is known to have virucidal activity and is commonly used in most of the laboratories to disinfect the working table. The present study was carried out to check the efficacy of UV light to inactivate HPAI virus (H5N1) inside biosafety cabinet. HPAI virus gets excreted in high concentration in feces of infected birds and these samples are most commonly processed in biosafety cabinets for virus isolation. In this study, fecal samples, wet as well as dry, having known EID₅₀ of virus were exposed to UV light to check its virucidal effect. The results indicated that UV light is unable to inactivate H5N1 virus even after 90 minutes of exposure in dry as well as wet feces. Thus there is a risk of improper disinfection of the biosafety cabinets if UV light alone is used as a source to disinfect the cabinet.

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Introduction

The H5N1 subtype of Highly Pathogenic Avian Influenza (HPAI) virus is included in risk group 3 infectious agents and the laboratory workers have high risk of acquiring occupational infection. Thus, handling of HPAI in the laboratory requires BSL-3 facility (OIE, 2009). Recommended personal protection procedures in the laboratory include mandatory handling of the suspected field samples that would include fecal samples from infected poultry inside a Class II Biosafety Cabinet (BSC). Ultraviolet (UV) light has been known to be effective in inactivating many viruses under some conditions and is routinely used for disinfecting the BSC in laboratories (Tseng and Li, 2007). Ability of UV light to disinfect biosafety cabinet surfaces again depends on a number of factors, viz., shelf life of the UV light, distance from the surface, duration of exposure, penetrability of the light waves in the medium, etc (Nicklin et al, 1999). However, its efficacy in BSCs has been questioned by many workers (NSF, 2004; Burgener, 2006). The present study evaluates the efficacy of UV light in inactivating the H5N1 Avian influenza virus in poultry feces under simulated laboratory conditions.

Materials and method

HPAI virus A/Ck/Sikkim/151466/2008 H5N1 accessed from repository of High Security Animal Disease Laboratory (HSADL), Bhopal was used in this study. The virus subtype was confirmed by virus isolation in embryonated chicken

eggs and identified by using tests HA, HI, RT PCR and real time PCR. The virus was amplified in 9–11 day-old embryonated chicken eggs and hemagglutination (HA) titre of the seed stock allantoic fluid was found to be 2⁹. The Embryo Infectious Dose 50 (EID₅₀) of the virus was 10^{10.33}/ml as estimated by Reed and Muench method (1938).

Fresh fecal samples were collected from Specific Pathogen Free (SPF) chickens, maintained in the SPF unit of the laboratory. The fecal samples were first divided into UV treated and control groups. The fecal samples from each group were further divided into two parts; one part was used as such as the wet feces and another part was dried aseptically in hot air oven at 50°C to bring the moisture level below 20% and used as the dry feces. In order to rule out the presence of any inherent toxicity or infective agent in the fecal sample, both the dry and wet fecal samples were processed and inoculated in eggs. The eggs were incubated at 37°C for five days and mortality, if any was observed in the eggs. All the inoculated embryos were found to survive up to five days and HA test of harvested allantoic fluids from these eggs were negative. The EID₅₀ of H5N1 A/Ck/Sikkim/151466/2008 virus-feces mixture (both dry and wet) was calculated and on its basis, both the dry and wet feces from UV treated group were spiked with the virus (diluted 1:100) in the ratio of 1:1 (100mg of feces: 100µL of diluted virus) to get final concentration of 100 EID₅₀ virus. The virus was added in feces and triturated in mortar pestle to ensure proper mixing. The spiked feces were kept at 37°C

for 30 min to ensure proper adsorption of the virus to the feces.

The UV radiation in the present experiment was supplied through freshly procured three-foot long 30 W tube fitted into the biosafety cabinet, which emitted UV-C radiation of approximately 12 W as per the manufacturer's literature. Proper recording of UV light usage in BSCs in form of logbooks is routinely done.

100 mg each of virus-spiked fecal samples (dry and wet) were taken in the petridishes and spread to form a thin layer. The petridish containing infected feces were kept open inside the running BSC (Class II B1 Biosafety cabinet, M/s Baker Co. USA). The airflow could not be switched off owing to the negative pressure requirements of the biocontainment laboratory environment. The spiked samples were subjected to UV light exposure for 15 min, 30 min, 45 min, 60 min, 75 min and 90 min time intervals. Similarly control group fecal samples were also kept open inside the running BSC, but were not subjected to UV light exposure.

One ml of phosphate buffered saline (PBS) (pH 7.2) was added to the spiked feces, mixed with the help of vortex mixer and centrifuged at 8000 rpm for 10 min. The supernatant was treated with antibiotic-antimycotic solution (HiMedia Laboratories, India) for one hour. The incubated samples were centrifuged at 8000 rpm for 5 min. The supernatant was inoculated into embryonated eggs for virus isolation as per the protocol by WHO (2006). Isolation of the virus was confirmed by carrying out HA test. To determine the complete inactivation of the virus and considering the allantoic fluids to be negative for virus isolation, the HA negative allantoic fluids were re-passaged

upto third passage and tested for HA. The entire experiment was replicated thrice to calculate the percent infectivity and to minimize the error.

Twenty five µL of PBS was dispensed into each well of a plastic V bottomed microtitre plate (M/s. Greiner, Germany). In the first well 25 µL of harvested allantoic fluid was dispensed and two fold serial dilutions of virus were made from 1:2 to 1:2¹². Further, 25 µL PBS was dispensed in each well and finally, 25 µL of 1% (v/v) chicken RBCs were dispensed in all the wells. The last row of the microtitre plate was kept as RBC control, which was prepared by dispensing 50 µL of PBS and 25 µL of 1% chicken RBCs. The plate was gently tapped for mixing and incubated at the room temperature (20–25°C) for 30 min in a BSC. The HA titer was determined by tilting the plate and observing the presence or absence of tear shaped streaming of the RBCs against the RBC control. The reciprocal of the highest dilution giving complete HA (no streaming) was taken as HA titer (OIE, 2005).

Efficacy of UV disinfection procedure and that of control was analyzed by observing the time required to completely inactivate the virus. The percent infectivity to estimate the effectiveness of the treated as well as control group was calculated as:

$$\% \text{ Infectivity} = \frac{\text{Infected embryos (No. of HA positive)}}{\text{Total embryos inoculated}} \times 100$$

The percent infectivity of treated samples was compared with the control group. The data was analyzed for significance using t-test ($p \leq 0.05$)

Table 1: Percent infectivity of H5N1 AIV in feces on exposure with UV light

Time (Minutes)	Percent infectivity		Wet feces	
	Dry Feces UV Treated	Control	UV Treated	Control
15	80	86.7	80	100
30	73.4	80	66.67	93.4
45	66.67	73.4	66.67	86.7
60	60	73.4	60	66.67
75	53.4	66.7	60	53.4
90	53.4	60	40	53.4

Results and Discussion

HSADL is an OIE referral laboratory for Avian Influenza diagnosis and surveillance and has been involved in routine surveillance and diagnosis of avian influenza from India and the neighboring countries. Since H5N1 HPAI is included in Risk Group 3 infectious agents and the laboratory worker has high risk of getting occupational infection, handling of the virus requires a minimum of BSL-3 facility (OIE, 2009). As a part of this diagnosis, a large number of fecal samples and cloacal swabs from the suspected poultry in the field are processed inside the BSCs before their inoculation in the embryonated eggs for isolation of the virus. Spillage of such samples during processing is common and alongwith the use of disinfectants, ultraviolet light fitted inside the biosafety cabinet is commonly used in many laboratories as a part of BSC disinfection protocol. In view of the high throughput of the samples being processed in the biosafety

cabinets, there is always a risk of the biosafety cabinets not being disinfected thoroughly with disinfectants and the UV inside the biosafety cabinet is the only source relied upon to disinfect the cabinet. In our study, poultry feces were spiked with H5N1 avian Influenza virus and were exposed to UV light in biosafety cabinet and were compared to control group fecal samples. The results indicated that as compared to control groups, UV light had no significant deleterious effect on the virus replicating ability even after 90 min of exposure (t- test, $p = 0.08$ for dry feces and $p = 0.10$ for wet feces), although, percent infectivity was reduced in fecal samples (Table 1; Figure 1 and 2). This indicated that the fecal matter was probably protecting the virus as the UV light was not able to reach every virus particle because the solid fecal particles shielded the virus. The results are compatible with the report of Chumpolbanchorn et al, (2006) who indicated that the infectivity of the H5N1

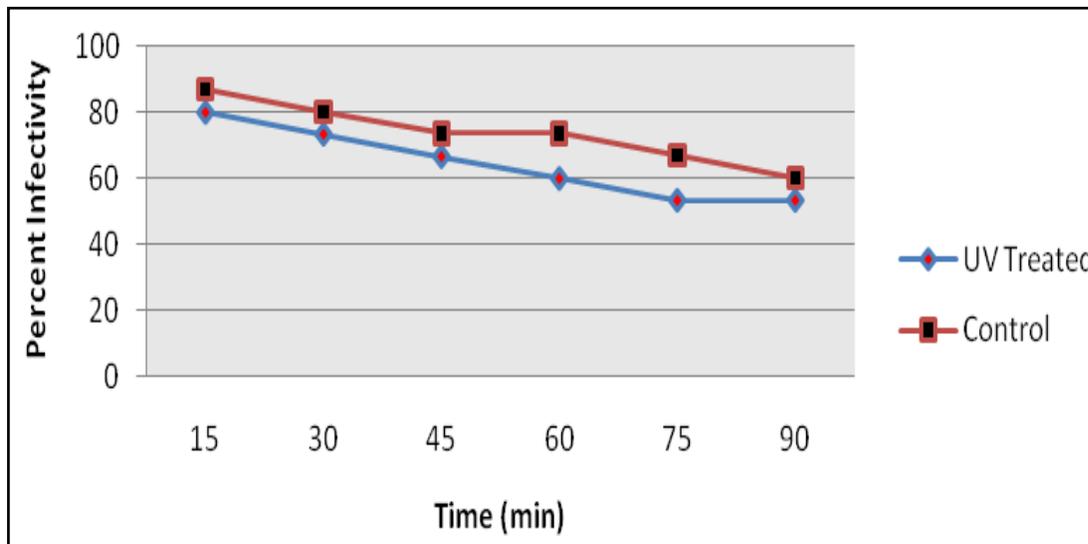


Figure 1: Effect of UV light on H5N1 HPAI in dry feces

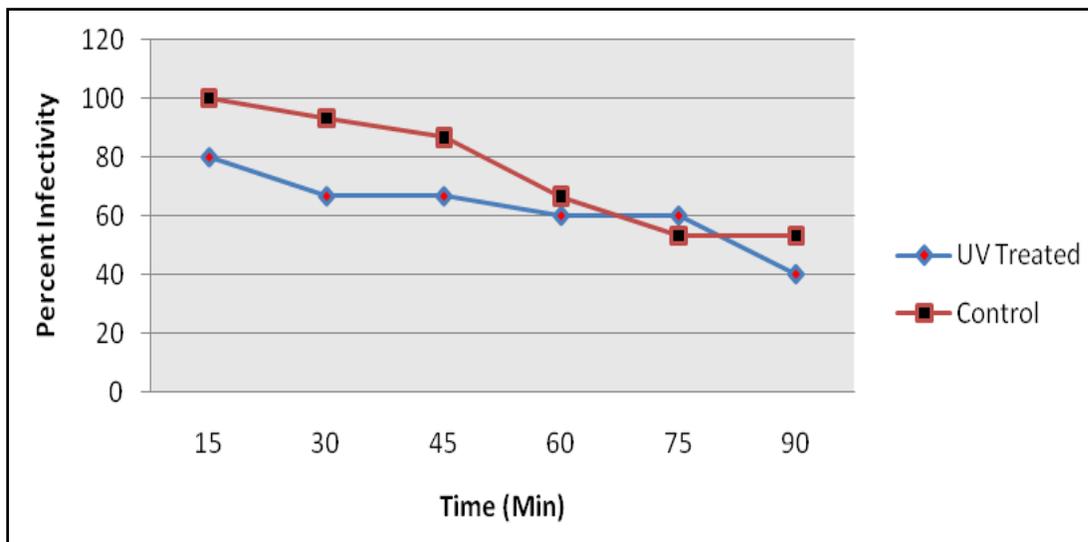


Figure 2: Effect of UV light on H5N1 HPAI in wet feces

(Thai field strain) in chicken fecal manure with initial concentration of $2.38 \times 10^{5.25}$ ELD₅₀ could not be destroyed on exposure to ultraviolet light at room temperature.

Similar results were obtained by Shahid et al., (2009) who found no deleterious effect on the virus replicating ability after 60 minutes of UV light exposure. In another study by Fasina et al., (2010), the exposure to UV light up to 180 minutes was not found to have any effect on the HPAI H5N1 virus ability to haemagglutinate chicken RBC, pathogenicity in eggs and haemagglutination titre. Effectiveness of UV light depends on various factors such as presence of organic matter, humidity, cleanliness of UV light bulbs, age of UV lamps, and the pattern and duration of use of the UV light for disinfection (Burgener, 2006; Tseng and Li, 2007). UV radiation cannot be considered an appropriate method for disinfection of premises such as a BSC as it is efficacious only on surfaces free from organic matter and well cleaned and when light source is positioned very close to the surfaces to be disinfected. The biosafety cabinets inside our laboratory have to be kept operational continuously since

the air circulation and maintenance of negative pressure inside the containment laboratory has been calibrated taking into account the continuous operation of the biosafety cabinets. Since the biosafety cabinet in which the UV treated was given, was operational during exposure studies, the air currents inside the biosafety cabinets could probably be distorting the UV rays falling on the fecal samples reducing its microbicidal effect. The theory that the UV lamps should not be used as the primary means of decontamination and disinfection of biosafety cabinets has been purported by many workers and biosafety organizations. (NSF, 2004; Burgener, 2006) as there is always a risk of giving the worker a false sense of security as far as sanitization of the workbench is concerned. Proper cleaning of BSCs before and after handling of infected feces should be done. Disinfectants like 70% ethanol can effectively be used in laboratory (Kurmi et al., 2014).

CONCLUSIONS

The results of present study indicated that the UV light is ineffective on the virus replicating ability and unable to inactivate virus completely even after 90 minutes of exposure, which indicated that UV Light alone cannot be considered as an appropriate method for disinfecting H5N1 AIV from premises such as a BSCs. The result from the study necessitates the use of proper disinfectants inside the BSCs and ensuring that there are adequate numbers of air exchanges inside the BSC before and after the handling of infectious agents inside a BSC.

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