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DEGRADATION AND ITS CAUSES OF INFLUENZA A VIRUS IN EUTROPHIC FRESHWATER

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ABSTRACT

Avian Influenza (AI) can cause extremely high mortality in infected fowls, and the factors affecting its maintenance and infectivity in aquatic environment is still unclear. In this study, effects of ultraviolet (UV) irradiation, heat-sensitive materials and particle materials on influenza A virus infectivity in aquatic environment were analysed under laboratory condition. Influenza A virus was sensitive to both of the ultraviolet B (UVB) radiation and ultraviolet A (UVA) radiation. UVB may led to viral inactivation through both of the genome and protein damage, while UVA may inactive the influenza A virus through endogenous indirect inactivation. Heat-sensitive materials plays important role in influenza A virus decay process and contributed 8.7-25% to viral decay ratio. The particle material concentrations significantly affected the influenza A virus decay process. With the particle material concentrations increased 5 and 10 fold, the decay ratio increased from 22.56±2.35% to 55.67±2.08% and 70±2% respectively.

KEYWORDS:
Influenza A virus; Decay ratio; Ultraviolet radiation; Heat-sensitive substances; Particle materials

INTRODUCTION

As a subtype of influenza A virus, bird flu (Avian Influenza, AI) is recognized as a disease which caused extremely high mortality in infected fowls since its outbreak for the first time in 1878 [1] . All known subtypes of influenza A virus could be isolated from feral birds, especially ducks and geese [2]. Most avian influenza virus were excreted at high levels in feces, thus we could isolate influenza virus from lake water even without concentration. [3-5].

Without host, the influenza virus could not initiate the infection and replication, but at low-temperature, the virus could remain infectious in aquatic system even more than 207 days[6], thus the influenza virus could re-infect ducks in the next spring[7].

Many environmental factors can result in viral inactivity, such as protozoon grazing, attachment to labile colloids, degradation by heat-sensitive and high molecular weight dissolved material, and disinfection by the UV component of solar radiation[8]. In previous study, solar UV irradiation, especially UVB, is considered to be the dominant factor in controlling viral infectivity in the aquatic environments [9-12]. However, most of these studies were focused on aquatic original virus, such as bacteriophage and cyanophage. Only a few research investigated the effect of pH, temperature and salinity on persistence of avian influenza viruses in water [6, 13, 14]. Influence of most factors affecting virioplankton infectivity on the influenza A virus maintenance and infectivity in aquatic environment is still unclear. In this study, the decay of influenza A virus and its causes in eutrophic freshwater were analysed. The results may help us to understand the epidemic of influenza A virus in aquatic environment.

MATERIALS AND METHODS

Virus propagation. The influenza A virus (H1N1) was originally obtained from Hubei Provincial Center for Disease Control and Prevention. The virus was inoculated to 18-day-old embryonated chicken eggs through the allantoic cavity. After 48 h of incubation at 35°C, the allantoic fluid was collected and the virus was prepared as described by Tang et al.[15]. Viral titer was tested by the hemagglutination (HA) assay using freshly prepared 1% chick red blood cells. HA assay was performed according to the WHO guidelines[16].

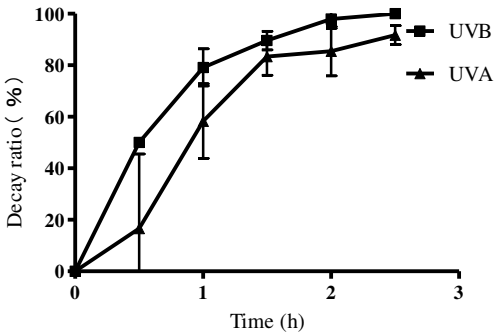
Viral decay caused by UV radiation. Influenza A virus suspension was mixed at ratio of 1:7 with natural water sampled from Honghu Lake (29°53'56.48" N, 113°15'44.60" E), a shallow eutrophic lake in Hubei Province of China，and the viral titer of the mixture was assayed as initial titer. Six Petri dishes without lid were infilled with 5 mL viral mixture, respectively. Three dishes containing

viral mixture were placed under an ultraviolet A (UVA) lamp (8.216-8.268 mw cm^2), and 3 dishes containing viral mixture were placed under an ultraviolet B (UVB) lamp (6.18-6.28 μw cm^2). The UV irradiation intensity was approximately equal to the solar UV intensity of sampling site. All dishes were placed under room temperature and 0.1 mL sample was collected from each mixture for HA test at 0 min, 30 min, 60 min, 90 min, 120 min, 150 min, 180 min and 240 min. Comparing to the initial titer, the percentage of viral titer decrease at each sampling time was denoted as the decay ratio caused by UV irradiation (%).

Viral decay caused by heat-sensitive substances. Influenza A virus suspension was mixed at ratio of 1:7 with natural water sampled from Honghu Lake， and the viral titer of the mixture was assayed as initial titer. Three Petri dishes without lid were infilled with 5 mL viral mixture, respectively. Dishes containing viral mixture as the test group were incubated at 22 ºC in water bath in the dark, and 0.1 mL sample was collected from each dish every 24 hours for titer assay. In the control group (for measuring the decay ratio caused by heat-stable substances), influenza A virus suspension was mixed at ratio of 1:7 with natural water restrained the heat-sensitive substances, and the other operations were the same as the test group. The heat-sensitive substances in water samples were restrained by heated to 70 ºC and maintained for 45 min, and then cooled to room temperature.

The viral decay ratios were calculated as the percentage of viral titer decrease at sampling time. The decay ratio of test group is the total decay ratio, and the decay ratio of control group is the decay ratio caused by heat-stable substances. The difference between the decay ratio of test and control groups was the decay ratio caused by heat-sensitive substances.

Viral decay caused by particle materials. Particle materials in the water samples were concentrated by 5 and 10 folds respectively through centrifugation at 8000 ×g for 30 minutes. The concentrated water samples were used to test the effect of particle materials on viral decay, and the un-concentrated water samples were applied as control. For each group, triplicate 7 mL water samples were mixed with 1 mL influenza A virus suspension, respectively. The mixtures were incubated at 22 ºC in water bath in the dark, and 0.1 mL sample was collected from each mixture every 2 hours for titer assay. The decay ratio was denoted as the percentage of viral titer decrease at the sampling time.



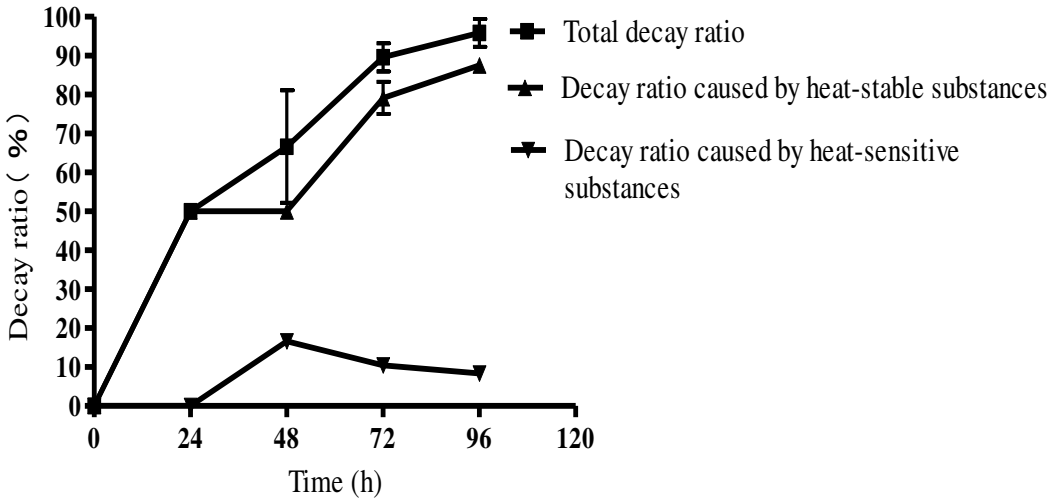
Decay ratio (mean ± SD) caused by UVA and UVB radiation

FIGURE 1
Influenza A virus suspension was mixed at ratio of 1:7 with natural water sample, and the mixture were divided into Petri dishes and exposed to ultraviolet A (UVA) radiation (8.216-8.268 mw cm^2) or ultraviolet B radiation (UVB) lamp (6.18-6.28 μw cm^2). The viral titer was monitored during the exposure, and the decay ratio (%) was calculated as the percentage decrease of viral titer.

RESULTS

Influence of UVA and UVB irradiation on viral decay. Results of UV radiation caused viral decay are shown in Fig. 1. UV radiation exposure led to rapid viral decay at the beginning time: viral decay ratio caused by UVB and UVA in 1.5 hour exposure was 89.6±3.6% and 83.3 ±7.2% respectively. The increases of viral decay ratio slowed down with the extended UV exposure, and at the end of exposure, the UVB and UVA caused viral decay ratio was 100% and 91.7±3.6%, respectively. Comparing the two treatment groups, the UVB caused viral decay ratio is significantly higher than that of UVA under the same exposure time ($P<0.05$, One-way ANOVA).

Influence of heat-sensitive substances on viral decay. Influence of heat-sensitive and heat-stable substances on viral decay is shown in Fig. 2. The viral decay process performed an average decay rate of 0.97%·h^{-1} and caused 95.87±3.58% of viral inactivation at the end. The viral decay ratio caused by heat-stable materials was significantly higher than that of heat-sensitive substances ($P<0.05$, One-way ANOVA). At the first 24 hours, heat-sensitive substances showed almost no influence on viral infectivity, and heat-stable materials contributed 100% to the total decay ratio. But after that, the contribution of heat- sensitive substances increased and maintained at a decay ratio of 8.4-16.6%, which was about 8.7-25% of total decay ratio.

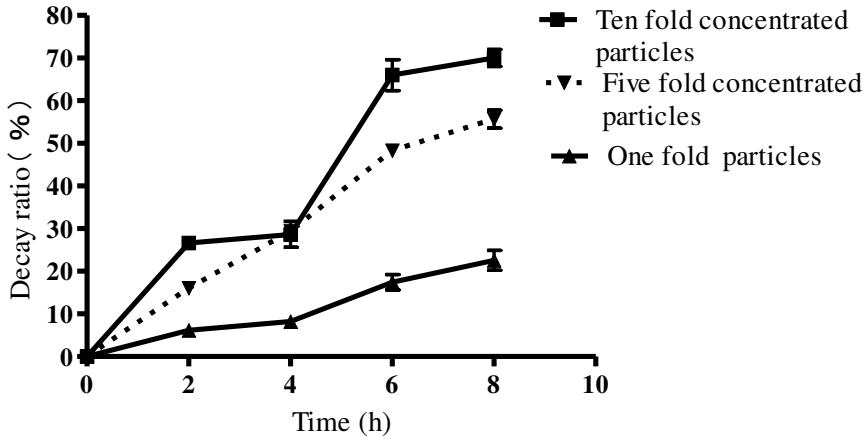


Decay ratio (mean ± SD) caused by UVA and UVB radiation

FIGURE 2

The heat-sensitive substances in water samples were restrained by heated to 70 ºC and maintained for 45 min. Influenza A virus suspension was mixed at ratio of 1:7 with both water samples with and without heat-sensitive substances. The mixtures were incubated in the dark, and the viral titer was monitored during the incubation, and the decay ratio (%) was calculated as the percentage decrease of viral titer. The total decay ratio was denoted as the viral decay ratio in water with heat-sensitive substances, the decay ratio caused by heat-stable substances was denoted as the viral decay ratio in water without heat-sensitive substances, and the difference between the two decay ratios was denoted as the viral decay ratio caused by heat-sensitive substances.

Influence of particle materials on viral decay. The viral decay ratio at different particle material concentrations varied significantly and increased with particle material concentrations ($P<0.05$, One-way ANOVA) (Fig. 3). The final viral decay ratios under three particle material concentrations were 22.56±2.35%, 55.67±2.08% and 70±2% respectively. Regression analysis indicated that the viral decay ratio was highly correlated to the particle material concentrations ($P<0.05$, $r^2=0.997$).



Decay ratio (mean ± SD) caused by UVA and UVB radiation

FIGURE 3

Particle materials in the water samples were concentrated by 5 and 10 folds respectively through centrifugation, and the un-concentrated water sample was applied as control. Influenza A virus suspension was mixed at ratio of 1:7 with above water samples and incubated in the dark. The viral titer was monitored during the incubation, and the decay ratio was denoted as the ratio (%) between the decreased viral titer at sampling time and the initial titer.

DISCUSSION AND CONCLUSIONS

Decay caused by UV irradiation. Our results indicated influenza A virus was sensitive to both of UVB and UVA. During a 2.5 hour period of irradiation, UVB and UVA equal to solar UV level led to a viral decay ratio of 100% and 91.7 ±3.6% respectively. The solar UV irradiation caused damage, especially the solar UVB caused dimes in the viral dsDNA was considered to be the main reason responsible for the loss of virioplankton infectivity [9, 17-19]. As to ssRNA virus like influenza A, the UV damage mechanism may be different. Research in human adenovirus type 2 (HAdV2) indicated UVB radiation was the major environmental factor challenging viral activation while UVA showed indirect photo-inactivation effect on HAdV2 [20]. Another research about HAdV2 demonstrated that genome damage induced by UVC light caused efficient inactivation, while the contribution of protein damage to the disinfection process was relevant for UVA combining exogenous sensitizer, and the full-spectrum sunlight may cause viral inactivation through both of genome and protein damage [21]. As to influenza A virus in this study, UVB may led to viral inactivation through both of genome and protein damage. However, UVA radiation alone also showed extensive inactivation effect, indicating that influenza A virus may contain endogenous sensitizers that could contribute to endogenous indirect inactivation.

Decay caused by non-light factors. In the absence of sunlight, heat-sensitive materials (especially heat-sensitive microparticles) [17] and heat-sensitive colloidal dissolved organic matter (DOM)[9] seemed to be an important factor regulating the viral infectivity. Our results indicated that heat-sensitive materials contributed 8.7-25% to influenza A virus decay. The proportion was close to that of viral decay observed in seawater (20% in average) [9]. The recent research in deep sea indicated that the extracellular enzymes controlled the virial decomposition through hydrolyzing the proteins of the viral capsids [22]. This suggests that the effect of heat-sensitive substances should be considered in assessing the maintenance of influenza A in eutrophic freshwaters.

Our results indicate that the viral decay ratio was highly correlated to the particle material concentrations. Similar phenomenon was observed in the Adriatic Sea. By studying the viral decay rates along a trophic gradient in the north Adriatic Sea, Bongiorni *et al* found out that particles appeared responsible for more than 56% of the total dark decay rate in eutrophic waters, while in oligotrophic waters the number was less than 6.6% [23]. The mechanism of this effect was contributed to the aggregate of virus and particles, since the viral recovery efficiency from the aggregate was very low [17]. These indicates that it will be more difficult for influenza A to maintain infectivity in eutrophic water than in oligotrophic water.

Influenza A virus was sensitive to both of UVB and UVA. UVB may led to viral inactivation through both of genome and protein damage, while UVA may inactive the influenza A virus through endogenous indirect inactivation. Heat-sensitive materials and particle material concentrations also play important role in influenza A virus decay process

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Influenza victims are also susceptible to potentially life-threatening secondary infections. Although the stomach or intestinal "flu" is commonly blamed for stomach upsets and diarrhea , the influenza virus rarely causes gastrointestinal symptoms. Such symptoms are most likely due to other organisms such as rotavirus, Salmonella , Shigella , or Escherichia coli . Description.Â Influenza outbreaks occur on a regular basis. The most serious outbreaks are pandemics, which affect millions of people worldwide and last for several months. The 1918â€“19 influenza outbreak serves as the primary example of an influenza pandemic. Pandemics also occurred in 1957 and 1968 with the Asian flu and Hong Kong flu, respectively. Influenza is a viral infection that attacks your respiratory system â€” your nose, throat and lungs. Influenza is commonly called the flu, but it's not the same as stomach "flu" viruses that cause diarrhea and vomiting. For most people, the flu resolves on its own. But sometimes, influenza and its complications can be deadly. People at higher risk of developing flu complications include: Young children under age 5, and especially those under 6 months. With respect to virus communities in eutrophic lakes, studies 93 by Green et al. [20], Skvortsov et al. [22], and Ge et al.Â Despite its designation over 30 years ago and ongoing remediation efforts, Hamilton 116 Harbour remains one of the most impaired sites in the Canadian Great Lakes [31]. 117 118 While Hamilton Harbour is in general an extensively studied system, the microbial community 119 has only been examined using microscopic techniques to investigate microbial diversity and 120 abundance.Â More research is required to better characterize freshwater virus 127 diversity, community structures, and patterns of abundance, and the factors that drive these. 6. bioRxiv preprint first posted online Jul. Epidemiology of Influenza A Virus. Replication in nucleus of Influenza A Virus. Pathogenesis of Influenza A Virus.Â Influenza A virus falls under the family Orthomyxoviridae. Influenza A virus particles are usually spherical and about 80- 120 nm in diameter. It is an enveloped virus and the envelope contains two glycoproteins, hemagglutinin (HA) and neuraminidase (NA), the membrane (M2) protein and is internally lined by the matrix (M1) protein. Source: Vincent Racaniello, http://www.virology.ws.Â It is estimated that annual epidemics of seasonal influenza cause 3â€“5 million cases of severe illness and 250,000â€“500,000 deaths worldwide. The economic impact of influenza A outbreaks is significant because of the morbidity associated with infections. The primary cause of environmental degradation is human disturbance. The degree of the environmental impact varies with the cause, the habitat, and the plants and animals that inhabit it. Humans and their activities are a major source of environmental degradation. (Wikipedia.com) Worldwide the greatest effects on the health of individuals and populations result from environmental degradation and social injustice. The two operate inconsort.