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# Virucidal Effects of Ultraviolet, Heat Treatment and Disinfectants against Koi Herpesvirus (KHV)

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**ABSTRACT**—The virucidal effects of ultraviolet (UV) irradiation, heat treatment and disinfectants against koi herpesvirus (KHV) were evaluated using KF-1 cells. KHV (KHV-I strain) was completely inactivated by UV irradiation at a dose of $4.0 \times 10^3$ $\mu$ Ws/cm$^2$ or heating to temperatures above 50°C for 1 min. KHV was completely inactivated by 200 mg/L iodophor, 60 mg/L benzalkonium chloride solution or 30% ethyl alcohol for 20 min. When chlorine concentration was measured after mixing with virus supernatant containing medium and fetal bovine serum, 97.5% and 98.5% reduction in infectivity was observed at 0.30 mg/L of chlorine for 30 s and 20 min, respectively. In practice, a tenfold concentration of chlorine (i.e. 3 mg/L) will be recommended to inactivate KHV completely.

**Key words:** koi herpesvirus, KHV, virucidal effect, disinfectant, ultraviolet

Since 1998, high mortalities of *Cyprinus carpio* (koi and common carp) have been reported in North America[1], Europe[2–4] and Asia[5–7]. Also in Japan, similar outbreaks have occurred since 2003[8], causing big losses at numerous farms. A herpes-like virus, designated koi herpesvirus (KHV)[1] or *Cyprinid herpesvirus* 3 (CyHV-3)[9], was isolated from diseased fish and identified to be the cause of these mortalities.

Although development of vaccines against the virus is needed to prevent disease outbreaks, it's very important to prevent spreading of the disease by quarantine, and/or, killing of infected or possibly infected carp. Also disinfection of equipments at affected farms is important. The virucidal effects of ultraviolet (UV) irradiation and disinfectants against other fish pathogenic viruses have been studied and utilized to control diseases[10]. However, there is little information on control of KHV using these methods.

In this study, the virucidal effects of UV irradiation, heat treatment and disinfectants against KHV were evaluated.

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## Materials and Methods

The koi fin cell line (KF-1) was used for a propagation of KHV thankfully provided by Dr. R. P. Hedrick (University of California Davis). KF-1 cells were cultured with the Leibovit's L-15 medium containing 10% (v/v) fetal bovine serum (FBS) (L-15$_{10}$) at 20°C. The virus isolate (KHV-I) provided by Dr. R. P. Hedrick was inoculated to sub-confluent KF-1 cells, the supernatant was then collected after 14 d and stored at –80°C until use. Virus infectivity was measured by the plaque method using KF-1 cells with a modification of the Kamei's method[11].

In the case of UV irradiation, virus supernatant in L-15$_{10}$ ($1.0 \times 10^5$ PFU/mL) was spread to a thickness of approximately 0.2 mm in a petri dish and exposed to UV irradiation at doses of 0.5, 1.0, 2.0, 3.0, 4.0 and $5.0 \times 10^3$ $\mu$ Ws/cm$^2$. The UV dosage was measured using a UV photometer (Iuchiseieido). For heat treatment, the virus supernatant ($1.6 \times 10^4$ PFU/mL) was heated in 40, 50, 60 and 70°C for 0.5, 1, 3 and 5 min, and the infectivity was measured. Disinfectants used in this study were iodophor (Meiji Seika), sodium hypochlorite solution (Wako), benzalkonium chloride solution (Takeda) and ethyl alcohol (Japan Alcohol Trading Corporation). Chlorine concentration of sodium hypochlorite solution was measured by the N,N-Diethyl-*p*-phenylenediamine (DPD) method. Levels of iodine and benzalkonium chloride were calculated from dilutions of a stock mixture.

A virucidal effect of disinfectants was measured by a method reported by Hatori *et al.*[11]. Briefly, the virus supernatant, adjusted to $1.0\sim1.5 \times 10^4$ PFU/mL by L-15$_{10}$, was mixed with equivalent volume of disinfectants and treated for 30 s or 20 min. Samples were then diluted with nine times volume of L-15$_{10}$, and 200 $\mu$L of the diluted solutions were inoculated to pre-seeded cells. Sodium thiosulfate was used to neutralize chlorine.

In a separate experiment, water collected from three goldfish ponds and two rivers at or near our university campus was mixed with sodium hypochlorite. The amount of chlorine required to achieve a final concentration of 3 mg/L in pond or river water was determined.

## Results and Discussion

UV irradiation at a dose of $4.0 \times 10^3$ $\mu$ Ws/cm$^2$ was effective (100%-plaque reduction) against KHV. A previous study[12] showed that *Oncorhynchus masou* virus (OMV), infectious hematopoietic necrosis virus (IHNV), hirame rhabdovirus (HIRRV), channel catfish virus (CCV) and chum salmon virus (CSV) were inactivated at the same dose. In the case of heat treatment, although 26.8~71.4% of KHV remained active at 40°C for 0.5~5 min, KHV was inactivated completely in excess of 50°C

**Table 1.** Minimum concentration of disinfectants showing 100% plaque reduction

| Disinfectant | Treatment time | Temperature | | |
|---|---|---|---|---|
| | | 0°C | 15°C | 25°C |
| Iodophor (mg/L) | 30 s | 130 | 200 | 200 |
| | 20 min | 130 | 200 | 200 |
| Sodium hypochlorite solution (mg/L) | 30 s | > 400 | > 400 | > 400 |
| | 20 min | 200 | 200 | 250 |
| Benzalkonium chloride solution (mg/L) | 30 s | 60 | 60 | 30 |
| | 20 min | 60 | 60 | 30 |
| Ethyl alcohol (%) | 30 s | 40 | 40 | 30 |
| | 20 min | 40 | 30 | 25 |

for one min. These results indicate that infectious KHV can be easily and effectively removed from water for culture using standard UV irradiation or heat treatment.

Minimum concentrations of disinfectants showing 100%-plaque reduction of KHV at different temperatures and reaction times are shown in Table 1. Minimum concentrations showing 100%-plaque reduction at 15°C for 20 min was 200 mg/L of iodophor, 200 mg/L of sodium hypochlorite solution, 60 mg/L of benzalkonium chloride solution and 30% of ethyl alcohol. There was no difference in virucidal effects between 30 s and 20 min using iodophor, benzalkonium chloride solution and ethyl alcohol. Compared with virucidal effects against other fish viruses, examined using the same method[13], KHV was most unstable in benzalkonium chloride solution. However, KHV was stable rather than OMV in iodophor and hypochrolite solution.

It's generally known that the disinfectant effect of halogens is decreased dramatically in the presence of organic matter. Therefore, as high survival rate of KHV in sodium hypochlorite solution was observed, chlorine concentration was measured after mixing with the virus supernatant. Infectivity reductions of 97.5% and 98.5% were found at 0.30 mg/L of chlorine for 30 s and 20 min, respectively (Fig. 1). This result indicates that chlorine concentration dropped from 200 mg/L to 0.3 mg/L just after mixing due to organic matter contained in the virus-culture supernatant. Similar results can be expected for iodophor. As 0.3 mg/L of chlorine inactivate KHV at nearly 100%, a tenfold concentration (i.e. 3 mg/L) will be recommended to inactivate KHV completely at fish farms for the purpose of safety. In a separate experiment, the amount of chlorine required for reaching 3 mg/L after mixing with pond or river water was found to be 11.2 mg/L at most (Table 2). Therefore to effectively inactivate KHV by sodium hypochlorite, more than 11.2 mg/L must be added so as to achieve final concentrations of at least 3 mg/L.

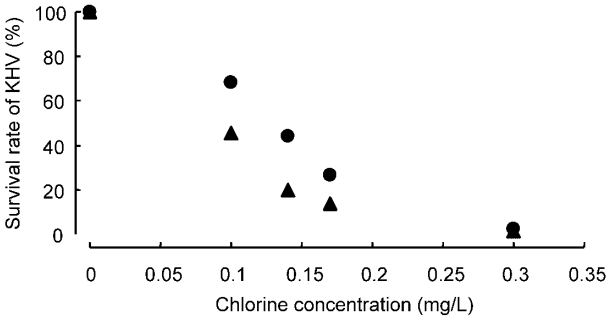


**Fig. 1.** Survival rates of KHV in sodium hypochlorite solution for 30 s (●) and 20 min (▲).

**Table 2.** Initial amount of chlorine (mg/L) required to achieve a final concentration of 3 mg/L in pond or river water

| Chlorine concentration | Pond water | | | River water | |
|---|---|---|---|---|---|
| | No. 1 | No. 2 | No. 3 | No. 1 | No. 2 |
| Initial (Distilled water) | 5.2 | 11.2 | 4.8 | 7.2 | 6.6 |
| Final | 3.4 | 2.9 | 2.8 | 3.0 | 2.7 |

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