Curative Effect of 0.5% Salt Water Treatment on Carp, *Cyprinus carpio*, Infected with Carp Edema Virus (CEV) Results Mainly from Reviving the Physiological Condition of the Host

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**Abstract:** The mortality of carp infected with carp edema virus (CEV) was considerably reduced when infected fish were immersed in 0.5% saline, although CEV infectivity sufficient to kill the host remained. Increased hematocrit value and decreased plasma osmolality of CEV infected fish reverted rapidly to normal levels after the initiation of salt water treatment. Thus, the curative effect of salt water treatment in CEV infected fish results from an improvement in the physiological conditions of the host.

**Key words:** Carp; Carp edema virus; Curative effect; Salt water treatment

In Japan, outbreaks of viral edema of carp (VEC) have been occurring annually in early summer since the first reports in Hiroshima and Niigata prefectures in 19741). The pathology of VEC is characterized by degeneration of the epithelial cells of the gill lamellae and skin due to the infection by a pox-like virus named carp edema virus (CEV)2). In the case of this disease, treatment of infected fish with 0.5% salt water has proved to be effective in the prevention of mortality and treatment with crude salt is commonly conducted in the field of Koi carp culture3,4,5). However, the mechanism underlying the efficacy of this treatment has not yet been elucidated. In the present paper, the effects of salt water treatment on the infectivity titer of CEV and the blood parameters of the CEV infected fish treated with salt water will be described.

For preparation of the virus suspension, gills obtained from CEV infected fish were homogenized in 10 times the volume of Eagle's minimum essential medium supplemented with 2% FBS, centrifuged at 1500 × g for 10 min, filtered (450nm) and stored at -80°C until use.

To estimate the inactivating effect of salt on the infectivity titer of CEV, the virus suspension was mixed with an equal volume of crude salt (Naikai Engyo Co. Ltd. 97.8% NaCl) solution resulting in salinity of 0.1, 0.5, 1.0 and 3.0%. The mixtures were kept for 1 h at 20°C, and then serially diluted 3.16-fold with dechlorinated tap water. Five experimental fish (0.14g) were immersed in 300ml of diluted mixture (final dilution of 10-4.0-10-6.5) at 21°C for 1 h. Then, the fish were transferred to small aquaria containing 300ml of water and kept without feeding at 19.6-22.3°C for 15 days. The positive control fish group was inoculated with non-salt-treated virus. Since water-borne infection of CEV occurs easily, the infectivity titer cannot be expressed using the LD50 value. Thus, in this study, the infectivity titer are expressed in reciprocals of the end point dilution rate at which mortality was observed.

The effects of salt water treatment on the mortality of CEV infected fish were examined as follows. Fifty common carp fries (0.74g) were put into 500 ml of well water which contained 0.5 ml of virus suspension and allowed to stand for 1 h at 19.5°C. The fish were then transferred into a tank containing 9 l of well water and kept at 20°C without feeding. One liter of well water containing 5% pure NaCl (purity = 99.5% or higher: KOKUSAN Chemical Co. Ltd.) or crude salt was put into the aquarium when death of the fish due to the virus infection began to be observed. The positive control group did not receive the salt water treatment and the negative control group was treated the same as the other groups except for virus inoculation. The tanks were aerated and kept at 20°C without changing the water.

The effects of salt water treatment on the blood parameters of CEV infected fish were examined as follows. Common carp fries (2.33 ± 0.18g) were infected as described above. After infection, the fish were transferred into a tank containing 1.3 l of dechlorinated tap water and kept at 20°C without feeding. Salt water treatment by adding 200 ml of 2.6% salt water (0.5% final concentration) was started when changes occurred in the blood parameters. Blood samples were collected into hematocrit tubes by cutting off the caudal part of the fish. After measurement of hematocrit (Ht) value, plasma was recovered from the tube and used for measurement of the osmolality with an Osmometer (VOGEL Osmometer OM-801) by the freezing-point depression method.

Results of the infectivity titration following salt water treatment are shown in Table 1. The residual CEV titer for each saline concentration were 10^4.0 for 3%, 10^4.5 for 1%, 10^5.6 for 0.5%, 10^6.6 or 10^6.0 for 0.1% salinity and 10^6.0 for the positive control. Some reduction in CEV infectivity was observed for 0.5% salinity. However, even 3% salinity was inadequate to completely eliminate the virus infectivity.
Table 1. Infectivity titers of CEV after exposure to salinity at selected concentrations

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<tr>
<th>Dilution of homogenate, *1</th>
<th>Cumulative mortality of fish (%)</th>
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<tr>
<td></td>
<td>Salinity (%)</td>
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<td></td>
<td>0.1</td>
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<tr>
<td>10^4.0</td>
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<td>10^6.5</td>
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<td>Negative control *2</td>
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*1: Gill homogenates of CEV inoculated fish and equal volumes of salt water were mixed to give the indicated salinity, allowed to stand for 1 h at 30°C, serially diluted at 0.5log times and used to inoculate the experimental fish.

*2: Fish were inoculated with a homogenate of healthy fish gills (10^4 dilution).

NT: Not Tested.

Fig. 1. Suppression of the mortality of CEV infected fish by 0.5% salt water treatment. Each fish group was inoculated with CEV. Two groups received 0.5% salt water treatment using pure NaCl (●) or crude salt (●) at the time of the onset of death due to the virus infection. The positive control group (■) did not receive the salt treatment and the fish were not inoculated with CEV in the negative control group (●). ◀ indicates the initiation of salt water treatment.

On the other hand, 0.5% salt water treatment of fry with both crude salt and pure NaCl was extremely effective in reducing the mortality of CEV infected carp (Fig.1). The number of deaths did not increase following the treatment, while all of the fish died within 5 days in the positive control group. No death was observed in the negative control group.

CEV infected fish had higher Ht values and lower plasma osmolalities than the control fish on the 5th post inoculation day. These altered parameters reverted to normal levels on the day following the initiation of the salt water treatment (Fig. 2).

Salt water treatment was also effective in reducing the mortality of fish in the case of enteric red mouth disease⁶ and columnaris disease⁷. Although the growth of the pathogens Yersinia ruckeri and Flavobacterium columnare were not inhibited by the saline concentrations employed in in vitro tests, the adhesion abilities of the pathogens were inhibited. Therefore, the authors presumed that the curative effect of salt water treatment resulted from inhibition of the attachment of pathogens to the host. In the case of VEC, the inactivation of CEV was inadequate for 0.5% salinity commonly employed in the treatment of VEC. Thus, the curative effect of salt water treatment results from an improvement in the physiological condition of CEV infected fish. Studies on the hematology of CEV infected fish and fish undergoing salt water treatment are needed for elucidation of the mechanisms underlying the curative effect.

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Reference