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Efficacy of Certain Chemicals Against Eel Virus European (EVE)

化學物質對歐洲鰻魚病毒之抑制作用

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ABSTRACT

The Virucidal efficacy of chlorex, extrant, salvant, 7x, alcohol and formalin was tested on Eel Virus European (EVE). Chlorex showed excellent virucidal activity producing inactivation at a concention of 1—5%. Formalin, caused viral inactivation at 250 ppm, but did not completely eliminate EVE after 3 days incubation at 20°C. Five percent 7x, 5% extrant, 5% salvant and 70% alcohol were not virucidal for EVE.

INTRODUCTION

An investigation of viral diseases among cultured fish in Taiwan showed that Eel Virus European (EVE), an agent similar to the AB strain of Infectious Parcreatic Necrosis Virus (IPNV), was widespread. (Chen *et al.*, 1985). This virus is considered to be the causitive agent for branchionephritis and has caused catastrophic losses among cultured eel (*Anguilla japonica*) in Japan (Egusa, 1970) and Taiwan (Chen *et al.*, 1985). The pathogenicity of EVE is closely related to water temperature and the physiological condition of the fish (Egusa *et al.*, 1971; Oka *et al.*, 1976; Chen *et al.*, 1985). Although the losses caused by EVE may be serious, there is no efficient treatment for diseased fish. The purpose of this work was the establish of sanitary measures for elimination of sources of infectious virus. Disinfectants tested *in vitro* on EVE for use in Taiwan included extrant, salvant, ethyl alcohol, 7x, formalin and chlorex on EVE *in vitro*.

MATERIALS AND METHODS

Cell lines

The cell line TO-2 (Chen *et al.*, 1983) was used for the proliferation and titration of virus. The procedures adopted for the experiment were similar to those described by Chen *et al.*, (1983) with slight modification. The cells were propagated at 28°C in Leibovitz's L-15 medium (L-15) (Flow Lab.) supplemented with 5% foetal calf serum (FCS) (Flow Lab.), 50 μg/ml streptomycin (GIBCO), 2.5 μg/ml fungizone (Flow Lab.) and 40 μg/ml gentamycin (Russel Lab.). For virual muliplication and titration,

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TO-2 cells were maintained in L-15 without FCS and incubated at 20°C.

Virual source and titration

The virus strain used in this study was originally isolated from an eel with nephroblastoma and identified as EVE (Ueno *et al.*, 1984). For testing a 0.1ml volume of viral strock solution containing $10^{8.5}$ TCID 50/ml was added into each flask. The ability of the virus to proliferate was measured by $TCID_{50}$ analysis using TO-2 cells in 96 well microplate and incubated at 20°C (Chen *et al.*, 1983).

Test chemicals

The six chemicals including 7x, extrant, salvant, alcohol, chlorex and formalin were tested for their ability to inactivate EVE. Viability of the treated virus was determined by seeding in TO-2 cells and observing for cytopathogenic effect (CPE). Comparison of these compounds indicates extrant and 7x are commercial detergents and chlorex contains 6% sodium hypochlorite. Salvant consists of 0.3% chlorhexidine gluconate (W/V) and 3% of certrimidine B. P. (W/V) and formalin contains 37% formaldehyde. Perior to these tests all compounds were filtrated using a 0.45 μm membrane pore size filter (Millipore) and the filtered solutions used as 100% stock solution for serial dilution using L-15 buffer.

Effect of Chemical Reagents on the Acitvity of EVE

The minimal toxic concentration of each chemical for TO-2 cells was determined by serial ten fold dilutions of each reagent using L-15 and added to monolayers of TO-2 cells seeded in 96-well microplats. Cells with L-15 alone were used as controls. After 7 days incubation at 20°C, the cells were observed by the light microscope for morphological changes. The appearance of control and exprimental cells were compered for any irregularity such as shrinkage, swelling, vaccuolization or dislogment. Altreations in cell appearance among treated cells were considered associated with the effect from tested reagents.

To test the effect of chemicals on the activity of EVE, 1 ml of 10-fold diluted extrant or salvant was made using L-15 and mixed with equral volume of EVE in a 10 ml test tube. Virus in L-15 only was used as control. Twenty four experimental tubes for each chemical reagent were prepared. Following incubation at 20°C for 0, 1, 2, 3, 4, 5, 6, 12, 24, 36, 72 and 96 hrs, viable virus was measured by titration using $TCID_{50}$ method (Chen *et al.*, 1983).

To detect the virucidal effect of alcohol, 70% alcohol solution was prepared by mixing 7 parts absolute alcohol with 3 parts virus solution intest tubes. The tubes were then incubated for 0, 1, 2, 3, 4, 5, 6, 12, 24, 48, 72 and 96 hrs at 20°C. Viable virus was again determined by $TCID_{50}$ method. One, 2 and 10% of chlorex and formalin at 50, 100 and 250 ppm were also tested for their ability to inactivate EVE using the same procedures as described above. All tests were performed at least in duplicate.

RESULTS

The toxicity of Chemical Reagents against TO-2

When extrant, salvant and chlorex 7x were added into cultures of TO-2 at a concentration leve 1 of 10^{-4}, no morphological change of the cells was observed. However, when the concentration of each was increased to 10^{-3} significant changes on cell morphology were observed.

It was also found that ethyl alcohol at a dilution level of 10^{-2} was not toxic to TO-2 cells. However, following incubation of TO-2 cells in culture medium with 10^{-1} ethyl alcohol for 24 hrs, the cells died immediately.

The toxicity of formalin for TO-2 cells was also tested. Results showed that formalin at a concentration of 10^{-4} or above caused abnormal cell morphology. Formalin at a concentration of 10^{-5} did not affect the morphology and cause alteration of cells.

The Effect of Chemicals on the Inactivation of EVE

The effect of incubating EVE in 5 and 1% chlorex solution at 20°C is presented in Fig. 1. When EVE was incubated in 5% chlorex solution for 10 min. virucidal no activity was detected in TO-2 cells. Following 2 hours incubation of EVE in 1% chlorex solution, the activity of EVE decreased to $10^{2.5}$ $TCID_{50}$.

The activity of formalin against EVE is shown in Fig. 4. EVE exposed to 50 or 100 ppm for 15 days remained active at $TCID_{50}$ of $10^{4.5}$ or above. When EVE was incubated in 250 ppm formalin for 3 days, the viral activity decrease to 10^{3} $TCID_{50}$.

Fig. 3 shows that when 70% alcohol and 5% 7x were incubated with EVE for 96 hours, no virucidal effect was observed. The activity of 5% extrant or salvant against EVE is presented in Fig. 4. The results showed that after 12 hours incubation of each solution with EVE, a slight decrease of virual was obtained after 96 hours' incubation viral concentrations of $10^{4.5}$ and $10^{6}TCID_{50}$ were obtained respectively.

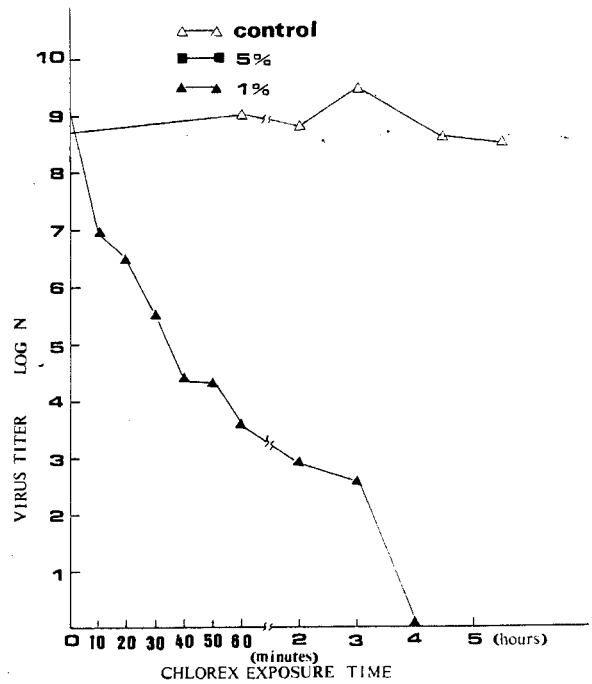

Fig. 1 Virucidal Effect of 1 and 5% of Chlorex at 20°C. LOG N=LOG_{10} $TCID_{50}$. Each point is the mean of two individual observations.

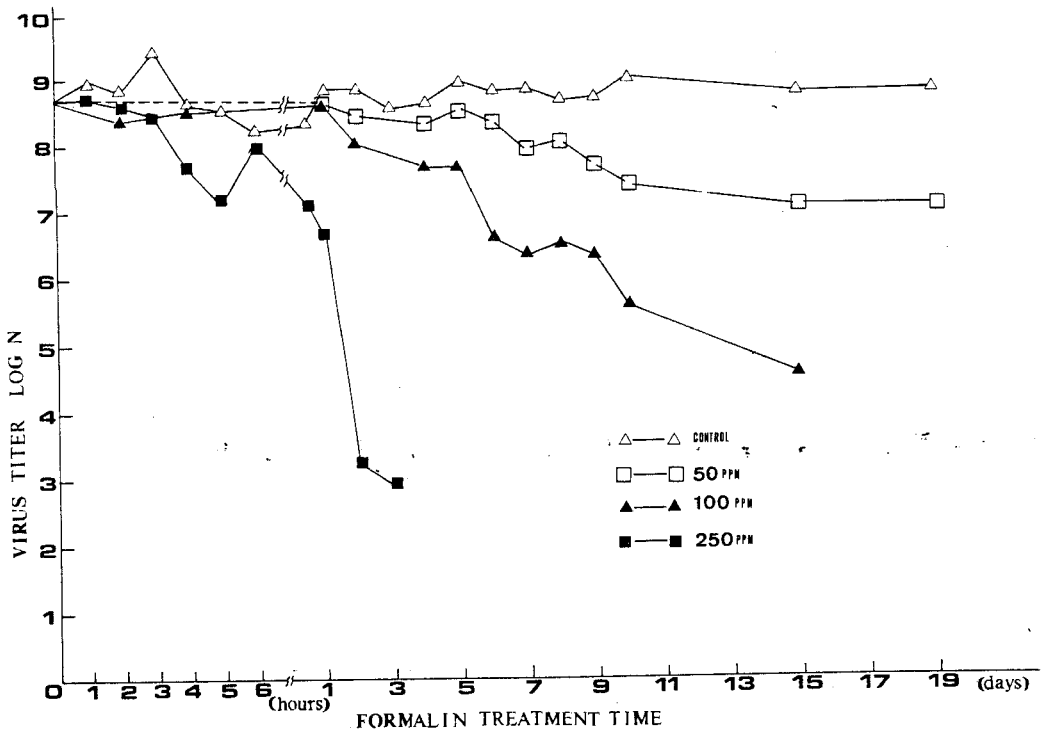

Fig. 2 Virucidal Effect of three concentrations of Formalin for Eel Virus European at 20°C. LOG N=LOG_{10} $TCID_{50}$. Each point is the mean for two individual observations.

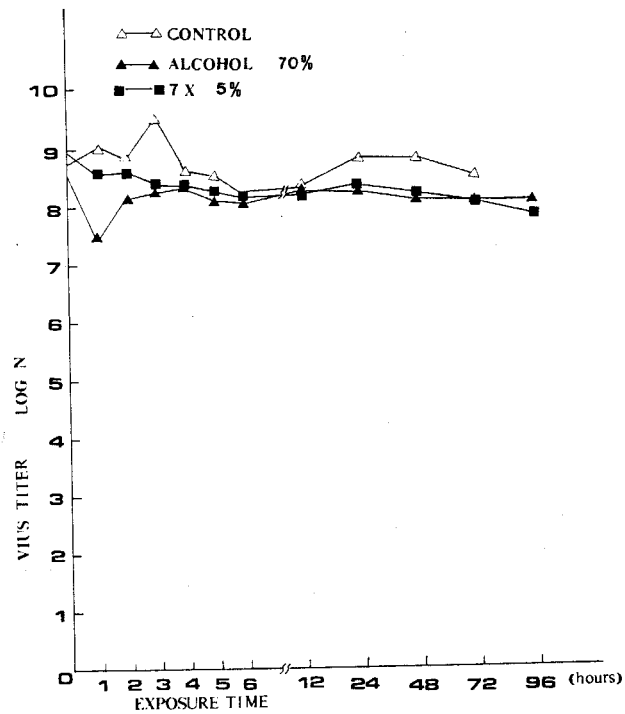

Fig. 3 Virucidal Effect of 70% Alcohol and 5% 7x for Eel virus European at 20°C. LOG N =LOG_{10} $TCID_{50}$. Each point is the mean of two individual observations.

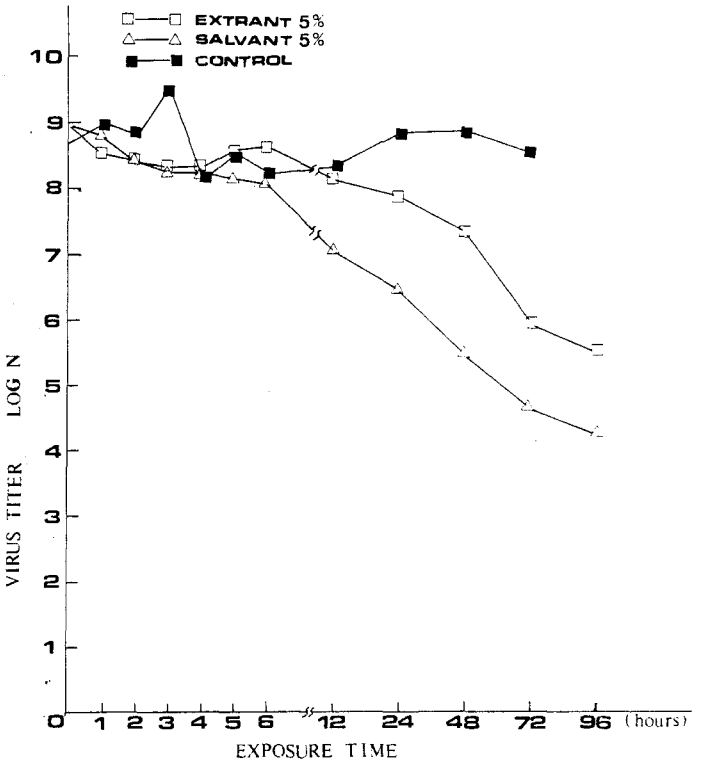

Fig. 4 Virucidal Effect of 5% of Extrant or salvant for Eel Virus European at 20°C. LOG N $=LOG_{10}$ $TCID_{50}$. Each point is the mean of two individual observations.

DISCUSSION

These results indicated that chlorex at concentrations of 1-5% were virucidal for EVE after 3 hours' incubation. The reagent was also demonstrated to be superior to the other five tesed disinfectants tested for the inactivation of EVE. Using chlorine, one of the main components for chlorex, Elliott and Amend (1978) demonstrated inhibition of Infectious Pancreatic Necrosis Virut (IPNV) isolated from rainbow trout (*Salmo gairderi*). Chlorex could be an excellent disinfectant for EVE in fish culture ponds. However, several factors including pH of water, and the concentration presence of organic matters in the water must be considered. Kabler *et al.*, (1961). Lawrence and Block (1968) and Elliott and Amend (1978) reported that antiviral activity of chlorine decreased as pH decreased. Kabler *et al.*, (1961) demonstrated that when proteinaceous organic mater is added to water containing chlorine, the formation of chloramines and N-chloro compounds not only reduces the amount of chlorine available but also revealed the virucidal activity. Elliott and Amed (1978) also concluded that the presence of calf serum decreased the efficacy of virucidal activity of chlorine. Therefore, too much organic materiala in ponds would be a seriouly hinder the virucidal efficacy of chlorex.

This study also showed that formalin at a concentration of 250 ppm exhibited some virucidal activity, but this chemical did not completely inactivate EVE after 3 days incubation at 20°C. Similarily, when 2% formalin was used only a portion of the IPNV exposed was inactived after 1 hour's incubation (Elliott and Amend, 1978). Morris and

Darlow (1971) reported that most viruses are inactivated by formalin, but the susceptibility varies greatly. Mackelvie and Desantels (1975) also demonstrated that formalin was not a good choice for disinfection. This may also be true with regard to of formalin in the inactivation of EVE.

Besides chlorex and formalin, several other disinfectants including iodine (Desantel and Maclcelvie, 1975) and acroflavine and acridines (Albert, 1966) have demonstrated virustatic or virucidal activity. Works are in progress to evaluate the virucidal efficacy of these chemicals against EVE.

摘　　要

本實驗擬測定 Chlorex, Salvant, 7x. 乙醇，福馬林，對歐洲鰻魚病毒之活性抑制作用。實驗結果顯示 Chlorex 在1—5％之濃度對歐洲鰻魚病毒有非常良好之活性抑制作用。福馬林於250ppm時對病毒雖有部份活性之抑制作用，但是在 20°C 下經3天之培育却不能完全抑制其活性。 5％ 的 7x 及 Extrant,Salvant 及70％之乙醇對歐洲鰻魚病毒則沒有殺滅作用。

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