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Pathogenicity of a Birnavirus Isolated from Loach, *Misgunus anguillicaubulatus*

自泥鰍分離出 Birnavirus 之病原性研究

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Abstract

The infectivity and pathogenicity of virus isolated from loach (*Misgunus anguillicausbulatus*) were investigated. The virus is icosahedral with a diameter of approximately 70 nm. Neutralization test revealed a close relationship between this virus and AB strain of Infectious Pancreatic Necrosis virus (IPNV). LV-1 was able to initiate the cytopathic effect (CPE) against TO-2 cells at an incubation temperature between 18-34°C. The cumulative mortality of loach infected by an I. P. injection of LV-1 was significantly higher than those receving EVE and culture medium only.

Introduction

This study was initiated as part of a project investigating the viral diseases of fish cultured in Taiwan. Since 1980 there has been an increased interest in the establishment of fish cell line for detection of viral pathogens among cultured fish in Taiwan. Several fish cell lines have been established within the past few years (Chen and Kou, 1981, Chen *et al.*, 1982; 1983). However, very few papers have been published for the isolation of viruses in cultured fish of Taiwan. Ueno *et al.*, (1984) isolated Eel Virus European (EVE) from the cultured eel with nephroblastoma. The occurrence of viral infections in cultured fish, including eel (*Anguilla japonica*), tilapia and rainbow trout (*Salmo gairdneri*), was investigated between December 1981 and March 1982 (Chen *et al.*, 1984). Viruses serologically related to the AB serogroup of Infectious Pancreatic Necrosis virus (IPNV) were isolated from cultured eel reared in Northern, Central, Eastern Taiwan and Southern Taiwan. IPNV of VR 299 serogroup and infectious hematopoietic necrosis virus was found in rainbow trout.

The present report describes results of the experiment designed to demonstrate the infectivity and pathogenicity of a birnavirus, isolated from loach, *Misgunus anguillicaubulatus*, against cell cultures and fish.

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Materials and Methods

Cells, Medium and Viral Isolation

Monolayor cultures of EO-2 cell line were grown in 25 cm^2 Falcon plastic flasks in Leibovitz's L-15 medium (Flow Laboratories) supplemented with 10% foetal calf serum (FCS), 50 I. U/ml penicilline, 50 I. U/ml streptomycin and 2.5 μg/ml Fungizone. The cells were grown at 31°C. For virus isolation, kidney and spleen from suspected loach, *Misgunus anguillicaubulatus*, were ground in a homogenizer (Hihoneseiki, Tokyo, Japan). Filtered using 0.45 μm millipore and then inoculated into TO-2 cell cultures. The flasks were incubated at 18°C, 20°C, 25°C and 31°C respectively. When the cytopathic effect (CPE) of TO-2 cells was observed, the culture fluids were inoculated at 1:100 dilutions on fresh cell cultures. The subcultures were then repeated at least 3 times.

Virus identification

Virus identifications were performed by electron microscopy and serum neutralization using Anti-IPNV AB, VR 299 or SP hyperimmune serum described by Ueno (1984) and Medanial (1979) respectively.

Virus replication

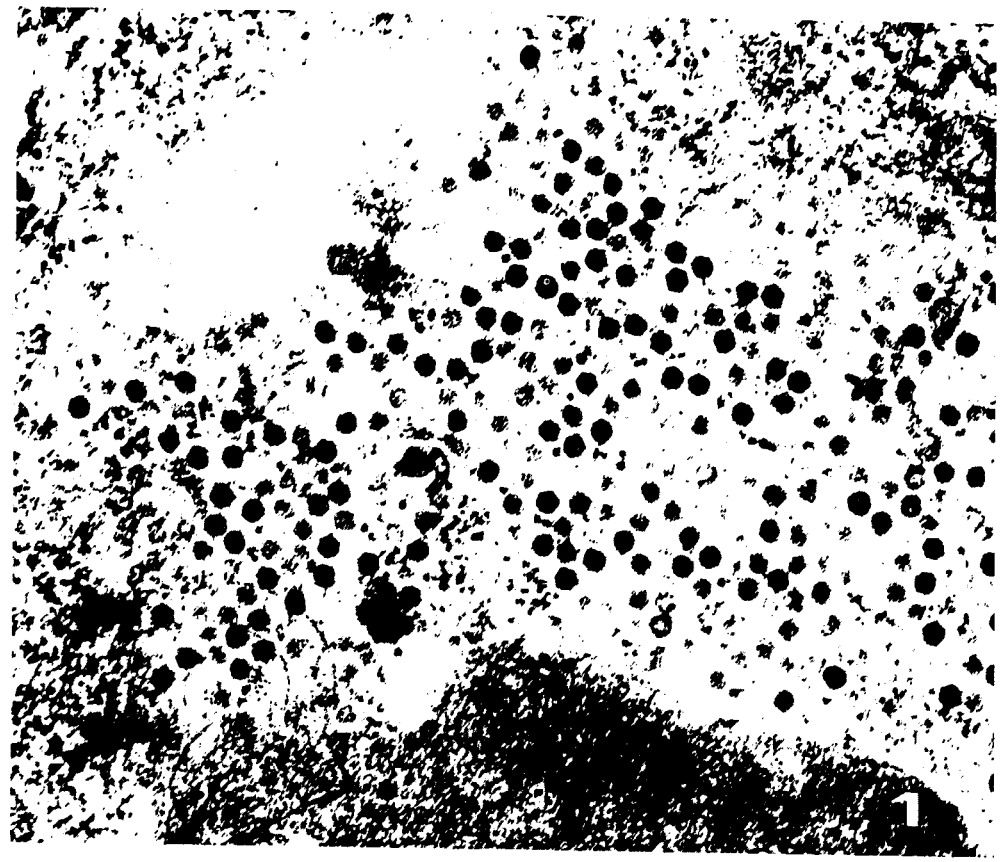
TO-2 cell line were used for the replication of isolated virus at different incubation temperature ranged from 10 to 37°C. The cells were then observed daily for the presence of CPE after inoculation of viral solutions. Eel virus European (EVE) was used for the control experiment. Each experiment was performed at least three times.

In vivo studies

The pathogenicity of virus was tested for healthy loach (*M. anguillicaubulatus*). Fish weighing 10-15 g each were injected intraperitoneally (i. p.) with $10^4 TCID_{50}$ of Virus in 0.1 ml L-15. Five groups of twenty fish each were injected with LV-1 and five control groups receiving only L-15 and $10^4 TCID_{50}$ EVE respectively were also performed for comparison. The fish were held in 100 ℓ static water aquaria at 25-28°C and observed daily for mortality. The dead fish were processed for viral isolation as described above. Each experiment was performed at least two times.

Results and Discussion

Virus isolated from loach is icosahedral morphology with a diameter of approximately 70 nm (Figs. 1 and 2) and disignated as LV-1. These observations showed that LV-1 is morphologically Similar to EVE (Sano, 1976) and IPNV (Wolf, 1966). However, when the LV-1 inoculated cells were incubated at temperatures between 10-37°C, CPE occurred at 18-34°C (Table 1). In comparison, CPE was only observed at an incubation temperature of 18 or 24°C when EVE was used (Table 1). It is interesting that LV-1 mutiply at an incubation temperatures above 30°C which was very rarely found in other fish viruses (Wolf, and Mann, 1980). Recently, several viral isolates obtained from cultured fish in Taiwan could also mutiply in cell lines with an incubation temperatures more than 30°C (unpublished data).



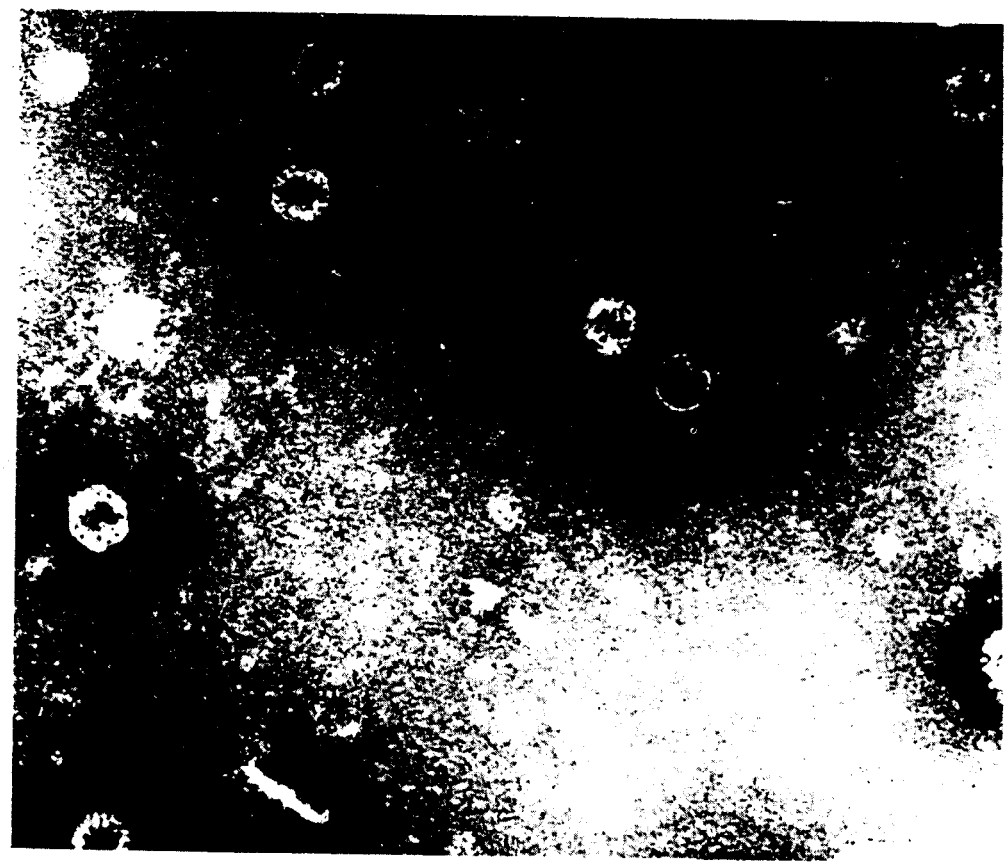


Fig. 1 and 2. LV-1 isolated from loach. 1. 50,000×, 2. 120,000×

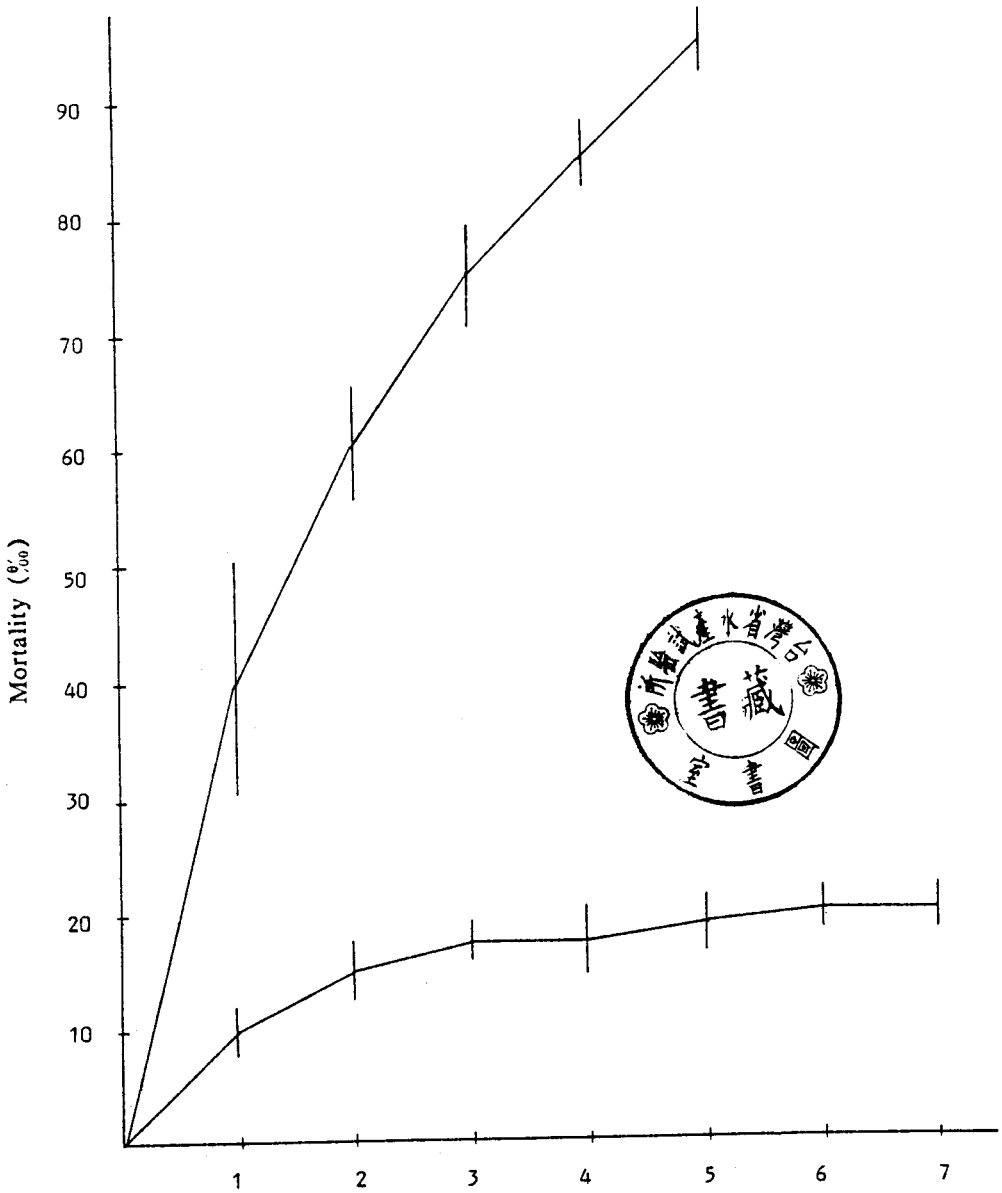


Fig. 3. Culmulative mortality of loach infected by an intraperitoneal injection of LV-1 at a concentration of 10^4 $TCID_{50}$ and maintained in a water temperature between 25 and 28°C.

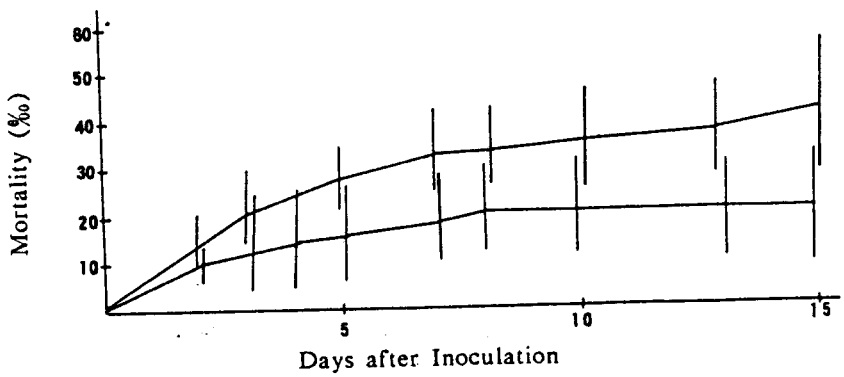


Fig. 4. Culmulative mortality of loach infected by an intraperitoneal injection of EVE at a concentration of 10^4 $TCID_{50}$ and maintained in a water temperature between 25 and 28°C.

Table 1. The Occurrence of Cytopathic Effect (CPE) in To-2 Cell Line Following Infection of LV-1 and EVE Viruses at a viral concentration of 3.5×10^3 $TCID_{50}$ Incubated at Various Temperatures

Temperature	hours after Infection									
	24		48		72		96		120	
	LV-1	EVE	LV-1	EVE	LV-1	EVE	LV-1	EVE	LV-1	EVE
10°C	−	−	−	−	−	−	−	−	−	−
18°C	−	−	−	−	+	−	+	+		
24°C	−	−	−	−	+	+				
28°C	−	−	+	−	+	−	+	−	+	-
32°C	+	−	+	−	+	−	+	−	+	−
33°C	+	−	+	−	+	−	+	−	+	−
34°C	+	−	+	−	+	−	+	−	+	−
35°C	−	−	−	−	−	−	−	−	−	−
37°C	−	−	−	−	−	−	−	−	−	−

+: CPE was observed −: No CPE was observed
Each experiment was repeated at least 5 times

Using cell neutralization technique, the activity of LV-1 was neutralized efficiently by hyperimmune serum prepared against EVE and IPNV AB serogroup. However, no neutralization ocurred when anti-VR 299 and SP IPNV hyperimmune sera were used. These results showed that LV-1 is very similar to reference strain AB IPNV or EVE. Studies are in progress for the biochemical analysis of LV-1 and the result will be published elsewhere.

The cumulative mortality of loach infected by an I. P. injection of LV-1 was higher then those receiving EVE and L-15 only (Figs. 3 and 4). Viruses including LV-1 and EVE were reisolated from dead fish in each experimental group. Our previous study revealed that Birnavirus related to AB strain of IPNV are widespread not only among eels but other cultured fish including tilapia and carp in Taiwan (Chen *et al.*, 1984). Although LV-1 is closely related to AB IPNV and EVE serologically, the results of infectivity and pathogenicity may suggest that each was unique. Further work is needed to detect the infectivity and pathogenicity of LV-1 against other cultured fish in Taiwan including tilapia, carp and eel.

中 文 摘 要

本實驗擬探討自泥鰍分離出的病毒 LV-1，對健康泥鰍之感染性與病原性。

LV-1 病毒形狀爲二十面體無被膜，直徑約爲 70 nm。中和實驗之結果顯示，其血清型和 IPN 病毒之 Ab 血清型非常相似。本病毒在 25°～34°C 均能使 TO-2 細胞株產生細胞病理變化 (Cytopathic effect)。腹腔注射 LV-1 於泥鰍中，其累積死亡率顯著的高於注射 EVE 或培養液的實驗對照魚。

Acknowledgement

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