# Iodine as a Disinfectant against Monodon Baculovirus (MBV)

**M.K. MANISSERI[1], K.M. SPANN and R.J.G. LESTER[2]**

*Department of Microbiology and Parasitology*
*The University of Queensland*
*Brisbane, 4072, Australia*

## Abstract

Virions of monodon baculovirus (MBV) were exposed to 0, 50, 200, 800, 3000 and 10000 ppm iodine at 4°C for 5 min and then used in a bioassay with *Penaeus monodon* postlarvae. Occlusion bodies were identified by histology in shrimp exposed to MBV treated with all iodine concentrations except 10000 ppm. Treatment with 3000 ppm reduced the prevalence and density of infection. However, only 10000 ppm iodine completely inactivated MBV.

# Introduction

Monodon baculovirus (MBV) was first described from *Penaeus monodon* shrimp cultured in Taiwan (Lightner and Redman 1981). MBV-like baculoviruses have been described for a number of penaeid species and occur in most parts of the Indo-Pacific region where penaeid shrimps are cultured (Brock and Lightner 1990). MBV is transmitted by ingestion of free virus and occlusion bodies (OBs) and by cannibalism (Paynter et al. 1992). Mortalities occur primarily among post-larvae in the hatchery, although disease may also occur among juvenile and adult shrimps (Johnson and Lightner 1988). MBV is controlled in the hatchery by avoiding contamination and by strict disinfection regimes. Infected animals should be eradicated and removed from the facility (Lightner 1988). All equipment and tanks should be disinfected routinely in between batches of larvae.

Iodine is widely used in aquaculture facilities to disinfect equipment and for the surface treatment of salmonid eggs (Liltvet and Landfald 1995; Goldes and Mead 1995). Iodophores are effective prophylactics against the bacteria *Aeromonas salmonicida, A. liquefaciens, Vibrio anguillum* and *V. ordalii* (Ross and Smith 1972; Sako et al. 1988).

*[1]Dr Mary K. Manisseri, Permanent address:*
*Central Marine Fisheries Research Institute, P. B. No 1603, Cochin-682014, India*
*[2]Corresponding author*

Iodophores have been proven effective against fish viruses such as: infectious hematopoietic necrosis virus (IHNV; Goldes and Mead 1995), infectious pancreatic necrosis virus (IPNV; Elliott 1978) and viral haemorrhagic septicemia viruses (VHSV; Amend and Pietsch 1972). In shrimp hatcheries, iodine is commonly used as a foot bath at the entrance of all buildings. It is also used as a rinsing solution for non-soakable hatchery and farm equipment, floors, and surfaces during facility clean-up following a disease outbreak (Wyban and Sweeney 1991). It is recommended that at least 200 ppm active iodine be used for these purposes and that foot baths be replaced regularly (Wyban and Sweeney 1991). Here we report the susceptibility of MBV to iodine disinfection.

## Materials and Methods

*Penaeus monodon* postlarvae were obtained from a hatchery in South-East Queensland and determined to be MBV-free by histological examination. The postlarvae were transported to laboratory aquaria in polythene bags containing oxygenated seawater. They were held in 5 l glass Ehrlenmeyer flasks which contained seawater maintained at 26°C and 30 ppt salinity. The postlarvae were allowed to acclimatise for three days prior to experimentation. Water was changed daily and the postlarvae were fed live 24 h old *Artemia salina* nauplii at a density of 10 *Artemia* nauplii · ml seawater$^{-1}$ during experimentation.

Homogenates of MBV-infected tissues were prepared from *Penaeus monodon* postlarvae (PL 8-9), known to be infected with MBV (Paynter et al. 1992), which had been stored at -70°C. Three grams of MBV-infected postlarvae were thawed and homogenised, on ice in 6 ml of 0.15 M phosphate buffered saline, pH 7.2, containing 1 mM disodium EDTA (Opdebeeck et al. 1988) using a glass Dounce homogeniser. One ml of homogenate was treated with five ml of either 50, 200 or 800 ppm of iodine produced by diluting the iodophore disinfectant Microshield PVP-5 (1% w/v iodine, Johnson and Johnson Medical Pty. Ltd., North Ryde, Australia) in distilled water. Homogenates were exposed to iodine for 5 min at 4°C. Homogenization of MBV-infected tissues may release cellular proteases which may damage any virions present (Vickers et al. 1992). To slow the activity of these proteases, homogenates of MBV-infected tissues were exposed to iodine solutions at 4°C.

Residual iodine was neutralised immediately after treatment by diluting the homogenates 1:1 with sodium thiosulphate at the rate of 0.0004 M thiosulphate solution for a test preparation containing 50 ppm of iodine (Amend and Pietsch 1972). The treated homogenates were then added to flasks containing 200 PL 22 postlarvae at a density of 80 PL · l seawater$^{-1}$. One group of postlarvae remained unexposed and was kept as a negative control while another group was exposed to 1 ml untreated viral homogenate (0 ppm iodine) and kept as a positive control. One group of postlarvae was exposed to homogenate which had been treated with sodium thiosulphate. The postlarvae were exposed to the homogenates for 24 h, after which time the water was changed and the density of postlarvae was reduced to 50 postlarvae · l seawater$^{-1}$.

Ten to fifteen postlarvae were sampled from each group on days 4, 8, 11 and 15, fixed whole in Davidson's fixative, sectioned and stained using haemotoxylin and eosin (Humason 1972).

To determine the relative density of viral infection, each histological section was first given an infection intensity rating according to the percentage of hepatopancreocytes which contained OBs; 0=0%, 1=0-10%, 2=10-40%, 3=40-70%, 4=70-100%. Each shrimp was then assigned an average intensity rating from the 10 or more sections examined. The relative density of infection for each sample of shrimp was calculated as the sum of average intensity ratings divided by the number of shrimps in the sample (Paynter et al. 1992; Spann et al. 1993). Prevalence of infection was calculated as the percentage of postlarvae in which OBs were detected.

A second experiment was carried out using younger *Penaeus monodon* postlarvae (PL7) held at a density of 160 PL · l seawater$^{-1}$. One ml of MBV-infected homogenate prepared as before, was treated with 5 ml of either 800, 3,000 or 10,000 ppm of iodine at 4°C. The iodine was neutralised after five minutes with sodium thiosulphate as with the first experiment. Treated homogenates were then added to flasks of postlarvae. Two groups of postlarvae were kept as positive and negative controls as with the first experiment. Postlarvae were exposed for 24 h, after which time the water was changed, the density of postlarvae decreased to 80 PLs · l seawater$^{-1}$ and the postlarvae were fed. Ten shrimps were sampled from each treatment group on days 3, 5, 7, 9 and 13, fixed in Davidson's fixative, sectioned, stained, and evaluated as before.

## Results

Roughly spherical, eosinophilic, baculovirus OBs were detected in the hepatopancreatic epithelial cells of *Penaeus monodon* postlarvae exposed to viral homogenates not treated with iodine and those treated with 50, 200, 800 and 3000 ppm of iodine (Table 1). The relative density of infection and prevalence of infection on the last day of sampling were similar among shrimps exposed to homogenates treated with 0, 50, 100 and 800 ppm of iodine. The prevalence and relative density of infection were much lower among shrimps exposed to homogenates treated with 3000 ppm of iodine than among the positive control shrimp (Figs. 1 and 2). The density and prevalence of infection among shrimps exposed to homogenates treated with sodium thiosulphate were comparable to that of the positive control shrimp, indicating that sodium thiosulphate itself is not effective against MBV. MBV OBs were not observed in the negative control shrimps or those exposed to viral homogenates treated with 10000 ppm iodine.

## Discussion

Chen et al. (1992) and Paynter et al. (1992) reported horizontal transmission of MBV by oral ingestion of occluded or free MBV virions. This suggests

Table 1. The prevalence (Prev.%) and relative density (R.Den) of MBV infection, and the efficiency of iodine in reducing the number of MBV occlusion bodies, on each sampling day for each iodine treatment in experiments 1 and 2.

Experiment 1

| Iodine Treatment (ppm) | Day 4 | | Day 8 | | Day 11 | | Day 15 | |
|---|---|---|---|---|---|---|---|---|
| | Prev.(%) | R.Den. | Prev.(%) | R.Den. | Prev.(%) | R.Den. | Prev.(%) | R.Den. |
| 0 (control) | 58 | 0.83 | 67 | 0.83 | 80 | 1.20 | 63 | 0.85 |
| 50 | 50 | 0.63 | 60 | 0.70 | 50 | 0.63 | 50 | 0.58 |
| 200 | 58 | 0.67 | 75 | 0.92 | 50 | 0.50 | 62 | 0.70 |
| 800 | 58 | 0.75 | 58 | 0.58 | 58 | 0.58 | 58 | 0.62 |
| Sodium Thiosulphate | 50 | 0.58 | 58 | 0.58 | 75 | 0.83 | 60 | 0.90 |

Experiment 2

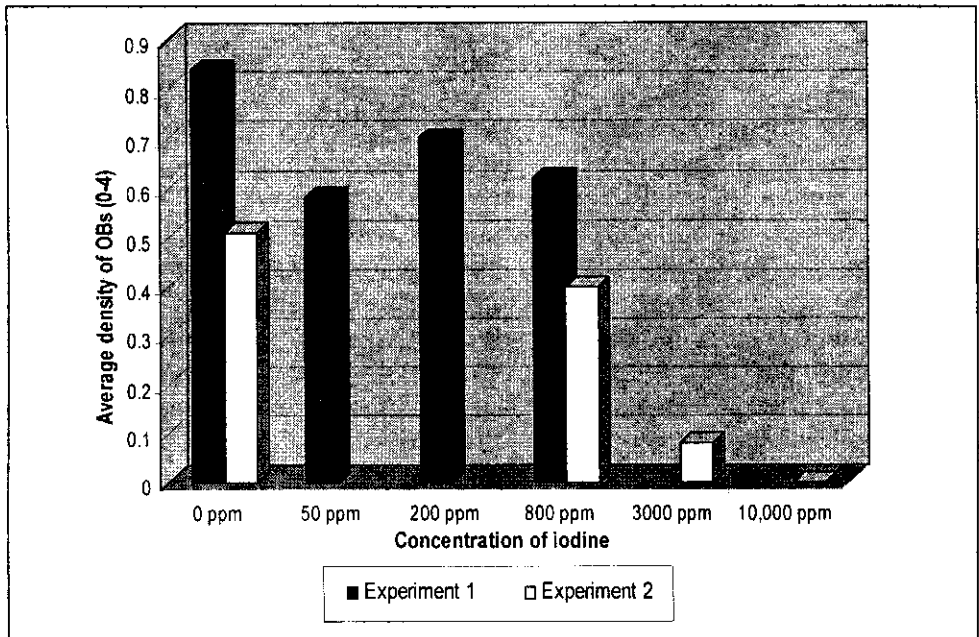| Iodine Treatment (ppm) | Day 3 | | Day5 | | Day7 | | Day9 | | Day13 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Prev. (%) | R.Den. | Prev. (%) | R.Den. | Prev. (%) | R.Den. | Prev. (%) | R.Den. | Prev. (%) | R.Den. |
| 0 | 30 | 0.70 | 33 | 0.33 | 33 | 0.70 | 38 | 0.75 | 52 | 0.51 |
| 800 | 12 | 0.40 | 33 | 0.33 | 25 | 0.50 | 25 | 0.63 | 25 | 0.40 |
| 3000 | 13 | 0.13 | 13 | 0.13 | 10 | 0.10 | 0 | 0 | 9 | 0.08 |
| 10000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |



Fig.1 The relative density of infection for shrimp from each treatment group sampled on the last day of experiments 1 and 2. The relative density of each sample was calculated as the average density of occlusion bodies (OBs) for a sample of shrimp. The shrimp in each sample were first assigned a density rating from 0 to 4 according to the percent of hepatopancreocytes containing OBs, where 0 = 0%, 1=0-10%, 2=10-40%, 3=40-70% and 4=70-100%.
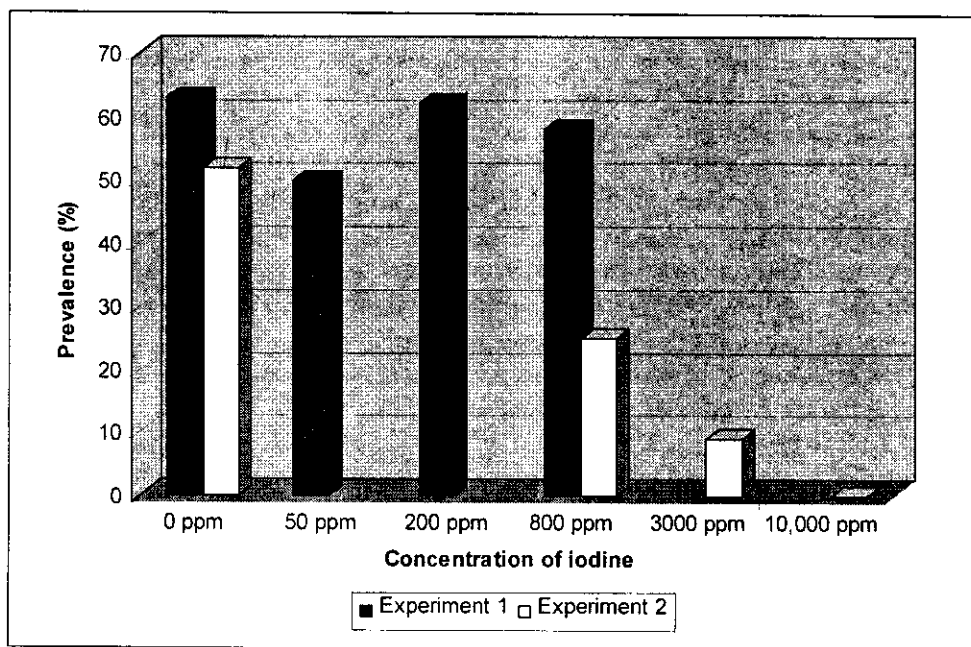
Fig 2. The prevalence of infection for shrimp from each treatment group sampled on the last day of experiments 1 and 2. The prevalence of infection was calculated as the percent of shrimp in each sample, which contained MBV occlusion bodies (OBs).

that MBV can only be controlled through the eradication of infected stock and by adherence to strict disinfection regimes within the hatchery. It is necessary that disinfection completely inactivates MBV, as infection can spread rapidly from one infected individual to another. Formalin, chlorine, and iodophores are the chemical agents most widely recommended for disinfecting hatcheries. The present study shows that treatment with a 10000 ppm solution of iodine for five minutes will effectively inactivate MBV OBs and virions.

Iodophores have low toxicity to fish eggs and are recommended as virucidal agents in fish hatcheries (McFadden 1969, Amend and Pietsch 1972). An iodine solution of 50 ppm is sufficient to inactivate striped jack nervous necrosis virus (SJNNV) if exposed for 10 min at 20°C (Arimoto et al. 1995). The salmonid viruses, IHNV, IPNV and VHSV are destroyed by 25 ppm iodine within 5 min in solutions near neutral pH (Amend and Pietsch 1972). Chen et al. (1992) tested various washing procedures in order to eradicate MBV infection in larval *P. monodon*. Total elimination of MBV was achieved by washing the nauplii or fertilised eggs thoroughly with 200-300 ppm formalin for 30 s followed by 20-50 ppm iodophore also for 30 s. The virucidal effect of iodine has also been reported for Baculoviral midgut gland necrosis (BMN) virus infecting *P. japonicus* (Momoyama 1989). BMN is inactivated by 25 ppm iodine for 10 min at 25°C. Wescodyne, a detergent containing at least 1.6% iodine, is commonly used in shrimp hatcheries in the USA. Overstreet (1994) reported that doses of 0.5-1% (v/v), as recommended on the product label, are sufficient to inactivate *Baculovirus penaei* (BP). MBV appears to be more resistant to iodine than other shrimp and fish viruses. MBV is also more resistant to chlorine as concentrations required to inactivate MBV are higher than for other shrimp viruses (Spann et al. 1993).

As part of a program to eradicate pathogens from Hawaiian shrimp culture, it has been recommended that iodophore footbath stations (50-100 ppm) be established at the entry of each area of a hatchery, and that tank exteriors, walls, and floors within a hatchery be washed with 50 to 100 ppm iodophore solution (Brock 1983). Wyban and Sweeney (1991) also suggest the use of footbaths containing 200 ppm of iodine for all staff entering specific pathogen free shrimp hatcheries. Our experiments suggest that these recommended concentrations of iodine are not sufficient to inactivate MBV and that iodine footbaths and disinfection solutions used in hatcheries in areas susceptible to MBV should contain at least 10000 ppm iodine.

The presence of organic matter appreciably reduces the level of iodine available for virus inactivation (Amend and Pietsch 1972). Frerichs (1990) observed that in the presence of organic matter there was no measurable reduction in snakehead rhabdovirus infectivity following treatment with 500 ppm iodine for 30 minutes. It is possible that resistance of MBV is further increased by the tough proteinaceous polyhedrin material of the baculoviral OBs which may act as a protective matrix (Federici 1986; Spann et al. 1993). Strong doses of iodine are required to disinfect hatcheries where free MBV virions are commonly enclosed within OBs and associated with organic matter, such as decomposing shrimp. Iodophores are effective disinfectants for use in shrimp hatcheries as they are non-corrosive and become visibly lighter in colour as the active iodine component weakens. Iodine is effective against MBV at high concentrations (10000 ppm) and for short exposure periods (5 min). However, it is important to regularly replace footbath soloutions to ensure that the concentration of active iodine does not fall below this dose.

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