Viral depuration of *Mytilus galloprovincialis* in ozone treated water

Purification virale de Mytilus galloprovincialis *dans l'eau traitée à l'ozone*

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Abstract

The depuration effectiveness of *Poliovirus* 1 and HAV contaminated mussels, when subjected to flowing ozonized water, was evaluated.

Mussels were experimentally contaminated and then depurated up to 72 hours using a pilot depuration system with ozone treated and untreated water.

The results showed that viral infectivity titter decreased more rapidly in mussels subjected to ozonized water (from 10^4 TCID50 to $10^{1.5}$ TCID50 per ml of *Poliovirus* 1 but in the following period it remained unchanged.

Also HAV was detected in the mussels after 72 h of depuration.

Keywords: Mussel, viral depuration, ozone treated water.

Résumé

L'objectif est d'évaluer l'efficacité du traitement de décontamination de moules contaminées par le *Poliovirus* 1 et le virus de l'hépatite A (VHA), lorsqu'elles sont soumises à une eau courante ozonée.

Les moules ont été contaminées expérimentalement puis décontaminées pendant un temps variable pouvant atteindre 72 heures, en utilisant une installation pilote renfermant une eau traitée à l'ozone et une eau non traitée.

Les résultats montrent que le taux d'infestation décroît plus rapidement dans les moules ayant subi un traitement à l'eau ozonée (de 10^4 TCID50 à $10^{1.5}$ TCID/ml de poliovirus 1) : cependant, au cours de la phase suivante, ce taux demeure inchangé.

Le virus VHA a également été détecté dans les moules après 72 heures de décontamination.

Mots-clés : Moule, décontamination virale, eau traitée à l'ozone.

INTRODUCTION

Shellfish are important vehicles of food-borne disease by virtue of their ability to concentrate viruses and bacteria from the water. The risk of illness may be particularly high because these species are frequently eaten raw or lightly cooked (Gerba C.P., 1988).

Effective control of enteric bacterial disease spread by shellfish has resulted from the etablishment of bacteriological standards but these criteria are inade-

quate for the control of viral contamination. In fact no statistically significant relationship has been shown between concentrations of enteric viruses and fecal coliform bacteria in shellfish or shellfish growing waters (Goyal S.M. *et al*, 1979; Wait D.A. *et al*, 1983).

Several water desinfection processes for depuration of contaminated mussels are used (Richards G.P, 1988). However, while fecal indicator bacteria and enteric bacterial pathogens are eliminated at similar rate, this rate is not the same for coliform bacteria and viruses (Cook D.W. and Ellender T.D., 1986; Gerba C.P., 1988). The depuration of shellfish, if properly conducted, will reduce the risk, but the product can still contain viruses (Metcalf T.G. *et al*, 1980). Several epidemiological studies have suggested that shellfish may play a significant role particularity in the transmission of *Hepatitis* A virus (Gerba C.P., 1988; Mele A. *et al*, 1989; Sobsey M.D., 1987).

The present study was conducted to determine the effectiveness of a self-cleansing mechanism of mussels (*Mytilus galloprovincialis*) contamined with *Poliovirus* 1 and *Hepatitis* A virus (HAV), when subjected to flowing ozonized water.

Material and methods

Viruses and viral assays

HAV laboratory strain was grown and titrated in FRp/3 cell culture (Venuti *et al*, 1985).

Water and mussel samples were diluted 1:10 in cell culture medium and inoculated in cell monolayers. After 2 h absorption at 37°C, inoculum was removed, medium added and tissue cultures were kept at 34°C for 15 days, changing medium every 3-4 days. Cells were then freeze-tawed 3 times and tested by commercial Elisa (Enzyme linked immuno sorbent assay) test for HAV antigen (Hepanostika, Organon Teknika).

Poliovirus 1, *Mahoney* strain, was cultured and titrated in VERO cell line, using 96 wells tissue culture plates and 100 µl inoculum. Four wells were infected for each dilution and the cytophatic effect was assessed after 72 hours. The infectivity titter was calculated according the method of Reed and Muench (1938).

Virus extraction from mussels

Mussels (10) were rinsed with sterile distilled water; bodies and intervalve water were removed, diluted 1: 2 with sterile glycine buffer (0.05 M, pH 9.2), homogenised, freeze-thawed, gauze filtered and centrifuged at 10,000 rpm for 15 mn.

Appropriate dilutions of supernatant from mussels were made in Eagle Medium, modified Mac-Pherson-Stoker (BHK 0021 Eutroph), containing antibiotics (Kanamycin 20 mg; Streptomycin 120 mg; Penicillin 1000 U and Cotrimoxazole 30,000 U in 1 litre) and kept overnight at 4°C before testing in cell culture (Franco E. *et al*, 1990).

Mussels contamination

The contamination of mussels was performed in parallel with Poliovirus 1 and HAV.

Mussels (*Mytilus galloprovincialis*) were exposed to test viruses in tanks containing 1 litre per mussel of artificial marine water (d^{24} = 1020 -1022), prepared using synthetic marine salt (Istant Ocean-Aquarius System).

Viruses were added to a final concentration of about 10^4 TCID50/ml of water for *Poliovirus* 1 and 10^2 TCID50/ml for HAV. Sea water salinity was 0.30-0.3%, water temperature varied from 15°-18°C and oxygen values in the water were above 90% of air saturation, as measured using Dissolved Oxygen Meter YSI 58.

Mussels were exposed to viruses for up 2 hours, because in this time under our experimental conditions the maximal viruses concentration is reached (Franco E. *et al*, 1990).

Mussels depuration

Depuration was carried out using a pilot unit with a system for ozone treatment of water. Sea water was circulated between the aquarium and the ozone treatment tank by using a pump at a flow rate of approximately 6 liters/mn.

The instruments for the ozone production, yields about 360 mg/h of ozone for a sea water with d^{24} = 1020-1022.

The utilised ejector was constituted by a Venturi tube.

The mussels were elevated from the bottom of the aquarium on a perforated plastic tray to prevent recontamination with pseudofeces.

The depuration process was carried out with ozone treated and untreated water, under the physicochemical conditions above described.

Depuration was performed up to 72 hours. For each depuration time, i.e. 2, 24, 48 and 72 h ten mussels were examined.

Results

The results of depuration experiments, run using Poliovirus 1 contaminated mussels are show in table I.

Depuration carried out in untreated sea water showed that Poliovirus 1 infectivity titre decreased from 10^4 to 10^3 TCID50/ml within first 24 hours and only a limited further decrease was seen after 48 and 72 hours. In ozone treated water a bigger decrease of the viral titre during the first 24 h was seen, but in the following period it remained unchanged ($10^{1.5}$ TCID50/ml).

Similar results were obtained with HAV contaminated mussels in ozone treated or untreated water.

HAV antigen was detected in water, in mussels after contamination and in mussels depurated 2, 24, 48 and 72 hours.

Table I: Depuration of *Poliovirus* 1 contaminated mussels in ozone treated and untreated water

Time (h)	Poliovirus 1 (TCID50/ml) (*)	
	Untreated	Ozone treated water
0	$10^{4.0}$	$10^{4.0}$
2	$10^{3.5}$	$10^{3.0}$
24	$10^{3.0}$	$10^{1.5}$
48	$10^{2.7}$	$10^{1.5}$
72	$10^{2.5}$	$10^{1.5}$

(*) Mean of 5 experiments

DISCUSSION

The epidemiological evidence cleary showed that enteric viruses were present in depurated shellfish which met acceptable bacteriological criteria (Gill O.N. *et al*, 1983).

It was suggested that viruses, particularly *Hepatitis* A virus, may persist in shellfish for longer periods than bacteria, requiring depuration longer than 48 h (Sobsey M.D. *et al*, 1987).

Depuration system require a desinfection treatment of recirculating water to prevent microbial build-up and recontamination of the shellfish.

Several methods of water desinfection, such as hypochlorites, iodophor, ultraviolet and ozone, are used in the world. But they are often ineffective in reducing enteric viruses levels, without interfering with mussels edibility characteristics (Richards G.P. 1988).

In this study experiments were carried out to evaluate the effectiveness of the depuration when viral contaminated mussels were subjected to flowing ozonized water. Ozonization is effective in reducing virus level in the contaminated seawater, *Poliovirus* 1 inactivation is reached in a few minutes (Toti L. *et al*, 1990).

Ozone treated water must be vigorously aerated before addition to depuration tanks, because residual levels can be toxic to shellfish.

The results of our experiments showed that shellfish depuration performed in ozonized water caused a more rapid reduction of viral titre, perhaps because a very low amounts of residual ozone, so that not interfere with normal shellfish physiological processes, during regular pumping activities could contribute to viral inactivation *in situ*.

However depuration process, under our experimental conditions, carried out up to 72 h in untreated or ozone treated water do not give assurance about virus elimination by mussels.

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