REPORT ON THE EVALUATION OF LECTRA/SAN® MARINE SEWAGE TREATMENT SYSTEM FOR REMOVAL OF VIRUSES IN SEWAGE

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By

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SUMMARY

The survival of hepatitis A virus in faecal material was determined following its passage through the Lectra/San® marine sewage treatment system under normal boating conditions. The Lectra/San® marine sewage system was able to reduce the levels of infectious hepatitis A virus in human faeces by 4 logs following a single 2-minute electrolytic process with accompanying maceration. This indicates that, under optimal conditions, the Lectra/San® will inactivate hepatitis A virus and other less resistant enteric viral pathogens in marine sewage such that they no longer present a health risk. However, the potential variability in operation of the Lectra/San® system means that there could still be a risk of viral contamination from faecal material discharged from boats fitted with the Lectra/San® systems. The change in the Marine Pollution Regulations to no discharge within 500 metres of a marine farm or maitaitai reserve for Type B systems will ensure that marine farms and maitaitai reserves are better protected from sewage contamination.

The project has contributed scientific data on the inactivation of enteric viruses by the Lectra/San® system and provided new information for determining controls on treated sewage discharges from recreational boats. The information generated will assist government agencies, including regional and district councils in formulating policies for safe management of waters used for recreation, shellfish growing and boating.

INTRODUCTION

The Resource Management (Marine Pollution) Regulations control treated sewage discharges from boats in New Zealand. Increased recreational boating is impacting on water quality in many areas and there is growing concern about the health risk of sewage discharges from boats. Sewage contains pathogenic bacteria, viruses and, in some cases, parasites. When untreated sewage is discharged into the water, it creates a public health risk for those using the water. Boat sewage discharge is becoming a public health hazard, especially in areas where people are using the water for recreational activities, shellfish gathering and where commercial shellfish farms co-exist with boating activities. Bivalve shellfish such as mussels and oysters filter many litres of water per hour and can accumulate thousands of viral particles within a few hours. These viruses are retained in shellfish gut for many weeks or months, remain infectious and so present a risk to both shellfish farmers and consumers. Regional councils are able to restrict the discharges of untreated and treated sewage from recreational vessels but need advice when determining the safe separation distances for recreational boating, shellfish gathering and marine farming.

The Lectra/San® MC is a Type 1 Marine Sanitation Device (MSD) suitable for boats up to 65 feet or 19.8 m in length. This small marine sewage treatment system has been installed in many New Zealand boats. It operates by electrolysis of seawater with production of hypochlorous acid, a natural disinfectant. This hydrolytic system is quoted as removing or inactivating both bacteria and viruses from faecal material as it passes through the receiving tank. However, the evidence for this is not conclusive. An unpublished laboratory report on removal of hepatitis A virus (HAV) from seeded human and dog faeces stated that between 78 - 94% of viruses will be removed from faecal material by the Lectra/San® (Grohmann,1997). The report does not show the baseline recovery for HAV from the seeded faeces when the material is diluted with seawater during passage through the tanks without Lectra/San® treatment. Most virus recovery methods are less than 100% efficient, and depending on sample type and recovery method used, can be as low as 10-20% recovery of initial virus seed. Without the baseline data for comparison, there is no way to ensure that it is the Lectra/San® that has inactivated / removed the virus rather than dilition factors and deficiencies in the recovery methodology. The data presented also shows that, after treatment, between up to 21% of initial infectious hepatitis A virus particles are still present in the treated sewage. Given that the infective dose for HAV and most other enteric viral pathogens is very low (~10 particles) the presence of even a few (>10) infectious viral particles in the vicinity of filter-feeding shellfish presents a potential risk to human health.

The report also states that:
" the system will provide an effective viral barrier protecting any direct users of recreational water as well as shellfish farmers and shellfish consumers. After discharge of Lectra/San® EC treated water into the water body the resultant high dilution factor will further minimise any effect of viruses on the environment and human health."

The Lectra/San® device macerates waste into small particles. These small particles are more easily accumulated than large particles during filtration by bivalve shellfish. Viruses in particular bind to particulate matter, which then protects them from chemical and physical stressors. Enteric viruses are known to persist for long periods in the shellfish gut.

Apart from the above report, there is little supporting scientific data on the effectiveness of the system to eliminate or inactivate viruses. The data show that the unit does not provide a total virus kill and so sewage discharge from these units could still be a potential health risk when discharged in areas where there is a low dilution factor and flushing rate, high boating activity, proximity to bathing beaches, shellfish gathering and marine farming.

This project investigated the survival of hepatitis A virus in faecal material as it passed through the Lectra/San® marine sewage treatment system. Hepatitis A virus is a human enteric viral pathogen, which can be grown in cell culture and is resistant to environmental stressors and disinfectants. As such, it is an appropriate virus to use in inactivation experiments.

The project involved seeding faecal samples with hepatitis A virus (HAV) of known concentration and passing them, under optimal conditions, through a marine toilet system fitted with a Lectra/San® sewage treatment system. Treated effluent was collected and analysed to assess survival of the hepatitis A virus. The results were compared with those from control HAV-infected human faecal material after passage through the marine toilet system without Lectro/San® treatment**.** The efficiency of the treatment system for HAV inactivation under the given conditions was then determined.

This project contributes scientific data on the inactivation of enteric viruses by the Lectra/San® system and provides new information for determining controls on treated sewage discharges from recreational boats. The data generated also provide information for water quality and environmental management programmes and will assist government agencies, including regional and district councils in formulating policies for safe management of waters used for recreation, shellfish growing and boating

Experimental design

A cytopathogenic strain of Hepatitis A was used to determine the efficiency of the Lectra/San® sewage treatment system for small boats. Human faecal samples were seeded with known quantities of Hepatitis A virus (HAV) and passed through a marine toilet system with the Lectra/San® treatment system installed. Effluent was collected in plastic containers and subsequently analysed for infectious HAV following the treatment. A control sample was also included to assess the dilution factor and recovery of HAV from seeded faeces after passage through the marine toilet with no electrolytic treatment. This provided baseline viral levels for comparison. The infectivity titres of HAV were determined by the 50% tissue culture infectious dose/mL ($TCID_{50}$) on FRhK-4 cells. Three replicate samples were treated by the Lectra/San® system. Results were compared with those from the untreated control HAV-infected faecal material, and the efficiency of the treatment system for HAV inactivation under the given conditions was determined.

METHODS

Preparation of Hepatitis A Virus stocks

Hepatitis A virus (HAV) cytopathic strain HM175 and fetal Rhesus kidney-4 derived (FRhK-4) cells were kindly provided by Dr Mark Sobsey, (University of North Carolina, Chapel Hill, NC, USA). HAV was propagated in FRhK-4 cells cultured in Minimum Essential Media (MEM) (Gibco BRL Ltd) supplemented with 0.1mM non-essential amino acids (NEAA) (Gibco BRL Ltd), 100 units Penicillin G sulphate /100μg/mL streptomycin sulphate (Pen/Strep, Gibco BRL Ltd) and15% heat inactivated foetal calf serum (FCS).

Hepatitis A virus stocks were prepared from infected FRhK-4 monolayers by freeze-thaw lysis followed by sonication for 3 min in an Ultrasonic Cleaner (Model FX10, Unisonics Pty Ltd., Sydney, Australia). The HAV lysate was extracted by the addition of an equal volume of chloroform, then clarified by low speed centrifugation (400 x g, 5 min) and filtered through a 0.2µ membrane filter pretreated with FCS to remove viral aggregates. The preparation was aliquoted and stored at -70°C.

Titration of Hepatitis A virus.
FRhK-4 cells were added at a concentration of 10^4 cells per well in 2% FCS in MEM to wells of 96 well microtitre plate. This was followed immediately by the addition of 100uL of virus to each well (8 replicates per virus dilution). Plates were incubated for up to 21 days at 37°C in a humidified 5% CO_2 atmosphere. The infectivity titre of stock HAV was determined as the 50% tissue culture infectious dose / mL ($TCID_{50}$) (Block & Schwatzbrod, 1989) and expressed as $TCID_{50}$ per/mL cell culture medium. The limit of detection of the quantitation method was < $10^{1.6}$ $TCID_{50}$/mL.

Preparation of composite human faecal inoculum
Four hundred grams of a composite human faecal specimen were prepared by mixing faecal material from a number of faecal specimens. The material was combined by stirring at 4°C for ~1 hour with the addition of 50 mL sterile distilled water, then autoclaved to sterilise it. This quantity was sufficient for 4 samples to be passed through the marine toilet system.

Fifteen 1 mL vials of Hepatitis A virus stock (HAV) stored at -70°C (each containing approximately 10^{-7} virus particles / mL) were defrosted and added to 400g of autoclaved human faeces and the mixture stirred to incorporate the virus. Virus was allowed to adsorb to the faecal material at 4°C overnight. The initial viral titre of the seeded faecal material was determined as follows: 10 g were resuspended in 10 mL of phosphate buffered saline (PBS), centrifuged for 15 min at 3000 rpm and the resulting supernatant analysed by the microtitre plate $TCID_{50}$ described above. Dilutions to 10^{-8} were plated for determination of viral titre. The faecal sample was then divided into 4 equal portions of approximately 100 g each for the subsequent treatment trial.

Trial of Lectra/San® system.
The treatment trial was carried out on board a yacht fitted with a marine toilet system and a Lectra/San® treatment system. The yacht was moored in the entrance channel to Porirua Harbour during the experiment. The marine toilet containing the Lectra/San® boat sewage system was flushed thoroughly with seawater, then the effluent discharge pipe disconnected from the discharge pipe through the hull and bypassed into a collection vessel inside the boat. By pumping the toilet the effluent could be collected into large plastic containers. The total volume of the tanks is approximately 15 litres; this volume can be flushed through in 15-20 pumps of the marine toilet. Material moves from the toilet bowl into the first tank, then further pumping moves it to the second tank, displacing the contents of that tank out through the effluent pipe.

The experimental trial consisted of a faecal sample being placed in the toilet bowl, then via flushing with seawater being moved into the first tank. Twenty pumps was adequate to empty the toilet bowl and move the contents into the first tank. The Lectra/San® button was switched on; this macerates the material and sends an electric current through the tanks for 2 minutes, then automatically switches off. Further pumping then moved the treated material through the second tank to the discharge pipe. Once the faecal material in the effluent reached

the discharge pipe (as shown by the dark colour) it was collected in a 10 litre plastic container and stored at 4°C. This exercise was repeated twice more with 2 further samples. Before each run, the tanks were thoroughly flushed through with seawater to avoid cross contamination and build up of infected faecal material between samples. Under standard operation, material would be treated on passing through both tanks.

Following the 3 separate treatments, the remaining sample of seeded faeces was flushed through the system without electrolysis treatment. The entire effluent was pumped through and collected for assay. The tanks were sterilised by exposure to 5% formalin for 15 min, followed by flushing with 3% hypochlorite solution, to ensure that no residual infectious hepatitis A virus remained in the sewage system.

Virus recovery from effluent

Ten litres of boat sewage effluent were collected for each treated sample and 20 litres for the untreated control. After agitation, one litre of sewage was removed for analysis from the untreated sample and the three treated samples and concentrated by PEG precipitation (Green & Lewis, 1995). Following centrifugation at 3000 rpm for 15 min, the supernatant was discarded and 10 mL of PBS was added to the resulting pellet. The pellet was sonicated for 5 min to elute the virus, centrifuged at 3000 rpm for 15 min and the supernatant collected for analysis by microtitre plate $TCID_{50}$. Eight 100uL replicates of each dilution were plated into 96 well microtitre plates. Dilutions to 10^{-8} were plated for the untreated control sample and to 10^{-5} for the treated samples. Eight negative controls were included on each plate**.** Plates were incubated for up to 21 days at 37^{o}C in a humidified 5% CO_2 atmosphere. The infectivity titre of HAV was calculated as $TCID_{50}$ / mL sewage effluent.

RESULTS

The temperature of the seawater was 15°C during the course of the experiment. The final concentration of hepatitis A virus inoculum was titrated and found to give a $TCID_{50}$ of $10^{8.6}$ virus particles / g of faeces (Table 1). Both the treated samples and the untreated control sample were diluted 1/150 with seawater when passed through the marine toilet system and then concentrated 100-fold before assay. The HAV titre used to determine the log reduction in infectious virus following the Lectra/San® treatment was taken as $10^{6.4}$ /mL.

Table 1. Hepatitis A virus $TCID_{50}$ results for Lectra/San® trial

HAV seeded faecal sample	Initial titre	Treated 1	Treated 2	Treated 3
Initial inoculum	$10^{8.6}$/mL	$10^{8.6}$/mL	$10^{8.6}$/mL	$10^{8.6}$/mL
Untreated control*	$10^{6.4}$/mL	$10^{6.4}$/mL	$10^{6.4}$/mL	$10^{6.4}$/mL
Treated samples*	-	$10^{2.3}$/mL	$10^{2.1}$/mL	$10^{2.5}$/mL
Log reduction	2.2	4.1	4.3	3.9
Final titre w/out concentration step	$10^{4.4}$/mL	$10^{0.3}$/mL	$10^{0.1}$/mL	$10^{0.5}$/mL

*Concentrated 100-fold before assay

Table 1 shows that a consistent 4-log reduction in infectious hepatitis A virus was observed in each of the 3 samples subjected to electrolysis as they passed through the marine toilet system compared to the untreated control sample. The initial 150-fold dilution factor in seawater and the subsequent 100-fold concentration of effluent during the virus recovery procedure need to be taken into account when determining the final viral levels present in treated and untreated effluent.

DISCUSSION AND CONCLUSIONS

These experimental trials were carried out under conditions as close as possible to those in a normal boating situation, rather than in a laboratory situation. The amount of faecal material (~100g / treatment) used in the experiment was representative of that excreted by the average person on a western diet containing medium-low fibre content. Cases of hepatitis A excrete $>10^6$ viral particles / gram of faeces during the late incubation period and acute phase of illness, so the viral levels used in this project may be 1-2 logs higher than those that would be present in faeces of infected people.

The Lectra/San® marine sewage system was able to reduce the levels of infectious hepatitis A virus in human faeces by 4 logs following a single 2-minute electrolytic process with maceration. This indicates that, **under optimal conditions**, the Lectra/San® will inactivate hepatitis A virus and other less resistant enteric viral pathogens in marine sewage. There was 100-fold dilution of faeces and virus in seawater produced as the faeces passed through the sewage treatment system. Our recovery method from effluent included a 100-fold concentration step, and so in effect, the actual final titres of the treated effluent were <10 HAV $TCID_{50}$ / mL. Therefore, when in general use, provided the faecal material is fully pumped into the tanks and the electric current is at consistent maximum power, these results show that the Lectra/San® will inactivate hepatitis A virus in faecal material such that it no longer presents a health risk.

It is important to ensure that these units are operated under optimal conditions. There are a number of variables that could influence the efficiency of the operation. These include stability and voltage of electric current, salinity of water (units are not for use in freshwater), amount of pumping required to move material through tanks and out to discharge, the time faecal material is left in tanks between treatments and before discharge, and the quantity of excrement treated at one time. At least 15 –20 pumps are required to move material fully into the tanks for treatment. Less pump action would move material into the pipe only. The Lectrasan® electrolysis process is activated by pressing a button at the user's choice– it is not an automatic process. This means that faecal material may not be treated every time the toilet is used. Boat users may choose not to use the treatment system if the boat batteries were running low. Therefore, there is no way of knowing if the Lectra/San® treatment system is being used appropriately.

The revised Resource Management (Marine Pollution) Regulations, which come into effect on July 1 2002, state that Type B sewage treatment systems, such as the Lectra/San® system, can discharge treated sewage anywhere except within 500 metres of a marine farm or maitaitai reserve. Although this study has shown that, under optimal operating conditions, enteric viruses are inactivated by the Lectra/San® system, the potential variability in operating conditions described above means that there could still be a risk of viral contamination from faecal material discharged from boats fitted with the Lectra/San® systems. For this reason, we support the change in the Marine Pollution Regulations to no discharge within 500 metres of a marine farm or maitaitai reserve for Type B systems. This will ensure that marine farms and maitaitai reserves are better protected from sewage contamination.

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REFERENCES

Block J.C., Schwartzbrod L**.** (1989) Viruses in Water Systems: Detection and Identification. VCH Publishers Inc., New York, USA.

Green, D. H., Lewis, G.D. (1995) Enzymatic amplification of enteric viruses from wastewaters. *Water Science and Technology* **31,** No. 5-6, 329-336.

Grohmann G.S. (1997) Unpublished report to Sardik Engineering P/L., Australia.