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Abalone herpes virus stability in sea water and susceptibility to chemical disinfectants

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ABSTRACT

Experimental infection models using immersion and injection challenges were developed to investigate the effects of various physicochemical treatments on the abalone herpes virus (AbHV), an emerging virus causing viral ganglioneuritis in abalone in Australia. To determine stability at different temperatures, the virus was held at 4, 15, or 25 °C for 1, 5, and 12 days prior to immersion challenge of naïve abalone. Mortality curves indicated that when held for 1 day in sea water at 4 °C and 15 °C the virus remained infectious and highly pathogenic. In addition, the virus retained partial infectivity after 5 days held at 4 °C. Histological examination of abalone tissues following viral exposure confirmed the presence of lesions typical of abalone viral ganglioneuritis in animals showing morbidity signs. An additional experiment was performed to determine the virucidal efficacy of three disinfectants (calcium hypochlorite, *Buffodine* and the non-ionic surfactant *Impress*). The disinfectants were used at various doses and durations to treat AbHV prior to injection and immersion challenges. Results showed that *Buffodine* and the non-ionic surfactant *Impress* were effective at inactivating the virus with no detectable adverse effects on the abalone's health. In addition, calcium hypochlorite showed a virucidal effect when used on lower titres of virus prior to immersion challenge.

1. Introduction

In December 2005/January 2006, a disease outbreak caused high mortality rates in abalone from two land-based farms in Victoria, Australia. Two other, marine-based farms also experienced disease but to a lesser extent. Histopathology performed on moribund abalone indicated a ganglioneuritis—infiltration of haemocytes in multiple ganglia and nerves (Hooper et al., 2007). Examination by electron microscopy revealed the presence of a herpes-like virus (AbHV) in the pleuropedal ganglion (unpublished data). Subsequently, the virus and disease, abalone viral ganglioneuritis (AVG), was observed in wild abalone in the vicinity of one of the affected farms and, currently, mortalities of wild populations of abalone continue to occur and spread along the Victorian coast approximately from Blue Nose to the west up to Cape Otway to the east. Affected reefs have suffered up to 95% mortality of their abalone populations (Victorian Abalone Diver Association, 2011). Most die-offs occurred in winter suggesting that colder water temperatures favour virus spread. To date, the abalone species *Haliotis rubra*, *Haliotis laevigata* and the hybrid of these species have shown susceptibility to AVG. In addition, juvenile and mature abalone are equally susceptible (unpublished results).

To limit the spread of the virus following outbreaks of AVG issues have been raised concerning biosecurity requirements and disinfection procedures to be implemented in farms, processing plants, as well as other coastal water facilities, equipment and premises where commercial and recreational fishing take place. Lack of knowledge on the biology of the virus and its ability to survive in sea water as well as its susceptibility to standard disinfection methods, makes it difficult to establish biosecurity measures aimed at virus inactivation to prevent the spread of infection. Furthermore, standard aquaculture disinfectants have not been specifically tested on AbHV to establish their virucidal efficacy. This study has investigated two aspects of AbHV biology. First the ability of the virus to remain infectious and pathogenic when held at three different temperatures 4, 15, and 25 °C for three periods of time, 1, 5, and 12 days prior to immersion challenge of naïve abalone. This mode of infection is aimed at simulating, under experimental conditions, virus spread and survival in the water column in the wild, in aquaculture facilities and in processing plants. Information from these experiments will contribute to our knowledge on the epidemiology of AbHV with regards to horizontal transmission and potential risks to infect abalone and cause disease. The second aspect of this study covers the virucidal efficacy of three chemical compounds (calcium hypochlorite, the iodophor *Buffodine*, and the non-ionic surfactant *Impress*) on AbHV and will inform industry and regulators on the conditions of use of these chemicals.

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2. Materials and methods

2.1. Experimental animals

Healthy abalone, blacklip (*H. rubra*) x greenlip (*H. laevigata*) hybrids (approximately 70 mm in diameter/2 years old), were obtained from a local abalone farm (Great Southern Waters Pty Ltd, Indented Head, Victoria) in an area of Victoria where there has been no history of abalone viral ganglioneuritis (AVG). In addition, abalone samples from this farm have consistently yielded negative results using the recently published AbHV specific real-time (Taqman ORF-49) PCR test (Corbeil et al., 2010). For all experiments the abalone were placed in aquaria containing aerated, filtered natural sea water maintained at 16 °C (representing water conditions in local farms) using central air-conditioning to maintain a constant room temperature, rather than cooling the water directly in individual aquarium. During each experiment, animals were fed commercial pellets (Halo from Skretting Inc., Tasmania, Australia) and each aquarium underwent 100% water changes daily. All animal experiments were approved by the Australian Animal Health Laboratory Animal Ethics Committee in accordance with the National Health and Medical Research Council's Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th Edition.

2.2. Production of AbHV infectious water

Virus stock was previously obtained from AbHV infected abalone tissues, homogenised, in Eagle's Minimal Essential Medium containing 20% foetal bovine serum, with a mortar and pestle on ice, filtered and stored in liquid nitrogen, until use. Infectious water used for immersion challenge experiments was produced by injecting five naïve abalone intramuscularly in the foot with 100 µL stock virus of the Victorian isolate of AbHV (designated Vic-1) (~1×10^5 viral gene copies (v.g.c.)/100 µL) (c.f. titration method in Corbeil et al., 2010). Inoculated animals were held in an aquarium containing 8 L aerated sea water with daily 100% water changes. Previous experiments had shown that water from day 4 post-inoculation contained high levels of infectious virus. After 4 days, the water was harvested, titrated using the AbHV TaqMan assay and used to generate challenge infectious water.

2.3. Stability in sea water

Infectious water prepared as described above was harvested and stored in 50 mL aliquots at 4 °C, 15 °C and 25 °C for 12 days before immersion challenge. The same procedure was performed twice more for producing infectious water to be held at 4 °C, 15 °C and 25 °C for 5 days and 1 day prior to immersion challenge. A total of 9 aliquots of infectious water were held. On the day of challenge (day 0), the nine aliquots of infectious water were placed in nine different aquaria containing 8 L of sea water (providing a final viral titre of ~12.5×10^4 v.g.c./mL which ensures that 100% mortality will result after immersion challenge with control untreated virus) Each 8 L of infectious water was then distributed into 8 small aquaria (1 L/tank). Eight naïve abalone were placed individually in these aerated aquaria and challenged for a period of 20 h (Fig. 1). A negative control group of abalone was immersed in sea water without virus. After the challenge, a daily water change was performed for the 10-day duration of the experiments.

2.4. AbHV susceptibility to chemical disinfectants: Injection challenge

Aliquots (1 mL) of Hank's buffered salt solution (HBSS) containing AbHV Vic-1 isolate (~200×10^6 v.g.c./mL) were placed into 6 tubes (2 mL volume). Calcium hypochlorite (Sigma-Aldrich), Buffodine (Malaguna PTY LTD) or the non-ionic surfactant *Impress* (Ultimate Cleaning Products NQ) was added to the tubes to obtain the final concentrations specified in Table 1. A positive control virus preparation did not receive any chemical treatment. After 10 min incubation at 16 °C the contents of the 6 tubes were transferred to 6 Vivaspin columns—30 kDa cut-off (Sartorius Stedim Australia PTY, LTD). The columns were then centrifuged for 10 min at 1200×*g* (IEC Centra 7R bench top centrifuge) at 4 °C. The filtrate was discarded and 2 mL HBSS was added to each column. The process was repeated twice more. After the third centrifugation the virus was resuspended in 1 mL HBSS and kept on ice. Groups of 6 abalone were injected intramuscularly with 100 µL of treated virus (20×10^6 v.g.c.) using a 1 mL syringe fitted with a 26 G gauge needle (Terumo Inc). This virus titre is approximately 100,000 times higher than that required to kill an abalone by injection. Abalone in the positive control group received 100 µL virus suspension that had not been exposed to any chemical. Six abalone forming a negative, uninfected control group received 100 µL of HBSS alone (no virus). Treatment control abalone received 100 µL of HBSS previously treated with calcium hypochlorite to evaluate its potential residual toxicity after filtration through the Vivaspin columns. Each abalone was placed in individual aquaria containing 1.5 L of sea water and monitored daily for the 10 days duration of the experiment. Animals that showed typical morbidity/clinical signs of AVG (e.g. lethargy and difficulty to attach to the

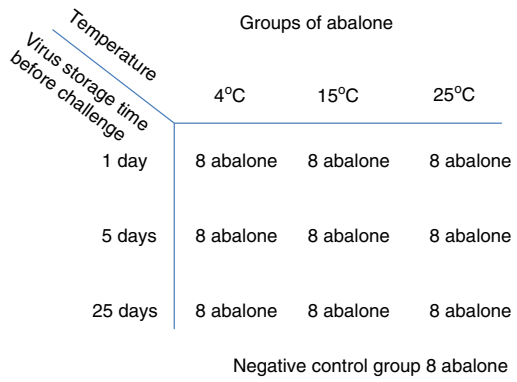


Fig. 1. Diagram showing various treatments for immersion challenge of abalone.

Table 1
Summary of the experimental groups to determine efficacy of chemical disinfectants.

Injection trial 1		
Chemical treatment	Low concentration	Medium concentration
AbHV + non-ionic surfactant *Impress*	1% (6 abalone)	5% (6 abalone)
AbHV + *Buffodine*	50 ppm (6 abalone)	nd
AbHV + calcium hypochlorite	5 ppm (6 abalone)	10 ppm (6 abalone)
Calcium hypochlorite alone	5 ppm (2 abalone)	10 ppm (2 abalone)
The positive control group (6 abalone) received AbHV in HBSS. The negative control group (6 abalone) received HBSS only. nd: Not done.		

Injection trial 2			
Chemical treatment	Low concentration	Medium concentration	High concentration
AbHV + calcium hypochlorite	5 ppm (6 abalone)	15 ppm (6 abalone)	20 ppm (6 abalone)
Calcium hypochlorite alone	5 ppm (2 abalone)	15 ppm (2 abalone)	20 ppm (2 abalone)

The positive control group (6 abalone) received AbHV in HBSS.
The negative control group (6 abalone) received HBSS only.

aquarium surface) were euthanased and tissue samples were fixed in 10% buffered formalin in sea water for histological examination.

2.5. Calcium hypochlorite treatment of AbHV infectious water: Immersion challenge

Three litres of infectious water containing 1.67×10^6 v.g.c./mL (a viral titre more than 100 times higher than is required to cause 100% mortality after immersion challenge) was distributed into three different aquaria (1 L per tank) and were either left as is (positive control water) or treated with calcium hypochlorite to a final concentration of 10 and 15 ppm. Two more aquaria containing normal sea water (virus-free) were also treated with calcium hypochlorite to a final concentration of 10 and 15 ppm to evaluate any toxicity effect on abalone during and after immersion challenge. Water was held at 16 °C for 15 min and then water samples from each treatment were taken to measure the level of residual free chlorine (using a ExStik CL200 Chlorine meter, Envco—Environmental Equipment) for each treatment. Following this treatment, 100 mL of each treatment was added to 900 mL sea water in five new aquaria. Healthy abalone were added to these new aquaria (6 abalone per aquarium containing the virus, and 2 abalone per aquarium containing control water now containing diluted calcium hypochlorite). Six abalone were also added to an aquarium containing sea water only and used as negative, uninfected control group. Abalone were challenged with the viral preparations for only 40 min to limit their contact with residual calcium hypochlorite, before transfer to individual aquaria containing 1.5 L of fresh sea water. Water was changed daily for the duration of the experiments.

2.6. Histopathology scoring

The formalin-fixed tissue sections containing the pleuropedal ganglion, nerve cords and peripheral nerves were prepared by routine histological procedures including dehydration through an alcohol series, paraffin embedding, sectioning (3–6 μm), and staining with haematoxylin and eosin. Typical AVG lesions have been described previously (Hooper et al., 2007), however, for this study lesions in tissues were evaluated according to the following scoring system:

Perineural sheath (*PNS*) 0: No viral lesions, 1: Scattered mild oedema and/or increased cellularity, 2: Extensive disruption of PNS by oedema and/or increased cellularity, 3: PNS almost completely disrupted by oedema and/or increased cellularity.
Grey matter (*GM*) *lesions* 0: No viral lesions, 1: Occasional neuronal necrosis; presence of small numbers of haemocytes and/or glia, 2: Moderate numbers of neurons remaining, but haemocytes/glia form the majority of cells in the GM, 3: No (or almost no) recognisable neurons in the GM.
White matter (*WM*) *lesions* 0: No viral lesions, 1: A slight increase in cellularity, 2: A moderate increase in cellularity, 3: A heavy increase in cellularity.
Transverse commissure lesions 0: No viral lesion, 1: A slight increase in cellularity of commissures, 2: A moderate increase in cellularity, 3: A heavy increase in cellularity.
Peripheral nerve lesions 0: No viral lesions, 1: A slight increase in cellularity in occasional, or many, nerves, 2: A moderate increase in cellularity in occasional, or many, nerves, 3: A severe increase in cellularity in occasional, or many, nerves.

2.7. Statistical analysis

Survival curves log-rank (Mantel–Cox) test was performed on the data (Graph Pad Prism version 5.02).

3. Results

3.1. AbHV stability in sea water

Groups of abalone undergoing immersion challenges with AbHV held at 4 °C for periods of 1 day and 5 days had 7 out of 8 (87.5% mortality) and 1 out of 8 (12.5% mortality) moribund abalone, respectively (Fig. 2a). Statistical analyses indicated that the 87.5% mortality curve was significantly different ($P = 0.0001$) from the negative control group but the 12.5% mortality was not different ($P = 0.2$) from the negative control group. No mortality was recorded in the group exposed to the virus held for 12 days at 4 °C prior to challenge (Fig. 2a). Virus held at 15 °C for 1 day induced mortality in 6 abalone out of 8 ($P = 0.001$ vs the negative control group). However, virus held for 5 and 12 days did not cause any mortality (Fig. 2b). Amongst the groups of abalone challenged with virus held at 25 °C only 1 out of 8 abalone died in the 1 day treatment group (Fig. 2c) but the difference in mortality curve was not statistically significant to the negative control group ($P = 0.2$).

A total of 72 abalone, including challenged and negative controls, were examined for histopathology in their pleuropedal ganglion, nerve cords, and peripheral nerves. AVG lesions were observed in tissues of abalone challenged with the virus held at 4 °C for 1 day (Table 2). The one animal that died after being challenged with the virus held at 4 °C for 5 days showed tissue damage too advanced for proper histological evaluation. Abalone challenged with the virus held at 15 °C for 1 day also showed AVG lesions in their tissues (Table 2) (some animals that succumbed to this challenge had degraded tissues and could not be analysed). The abalone that died after challenge with the virus held at 25 °C for 1 day did not show lesions in its tissues (results not shown). All other challenged abalone survived for the duration of the experiment and did not show signs of disease. Histological examination did not reveal any evidence of viral infection in these animals (results not shown). The negative control abalone survived for the duration of the experiment and no lesions typical of AVG were observed.

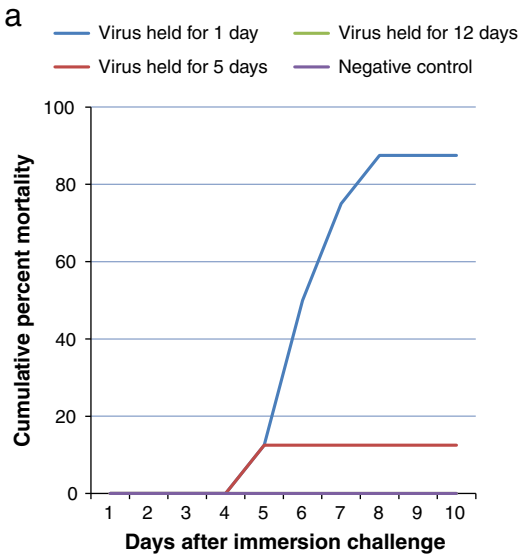
3.2. AbHV susceptibility to chemical disinfectants

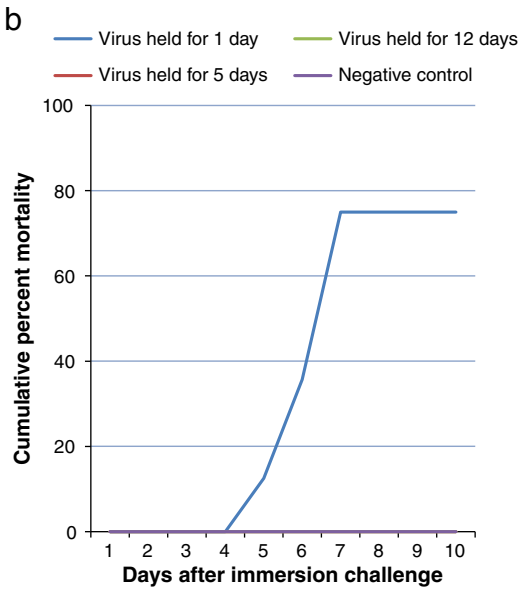
3.2.1. Injection trial 1

Abalone injected with AbHV alone showed a typical mortality curve reaching 100% (6 out of 6 abalone) within 7 days post-challenge (Fig. 3). The group of abalone injected with the virus treated with 5 and 10 ppm of calcium hypochlorite showed mortality rates of 100% (6 out of 6 abalone) and 66% (4 out of 6 abalone), respectively (Fig. 3). In addition, the 66% mortality rate (4 out of 6 abalone) of the 10 ppm group of abalone was delayed by 2 days in comparison to the 5 ppm group. Statistical analysis showed a significant difference between these mortality curves and the negative control group curve ($P = 0.05$). Histological examination carried out on 12 moribund animals (including 5 positive controls) revealed typical AVG lesions in their neural tissues (Table 3). The HBSS injected animals as well as all other groups of abalone challenged with treated virus (*Buffodine* and *Impress*) remained healthy for the duration of the experiment (Fig. 3). Histological examination of the target tissues for these animals revealed normal appearance with no AVG lesions (results not shown).

3.2.2. Injection trial 2

In this injection experiment, groups of abalone injected (intramuscularly) with the virus treated with 5 and 15 ppm of calcium hypochlorite showed mortality rates of 100% (6 out of 6 abalone) (Fig. 4) ($P = 0.03$). The virus treated with 20 ppm of calcium hypochlorite induced 66% cumulative mortality (4 out of 6 abalone) (Fig. 4), however, there was no difference with the accumulated 100% mortality (6 out of 6 abalone) of the positive control group





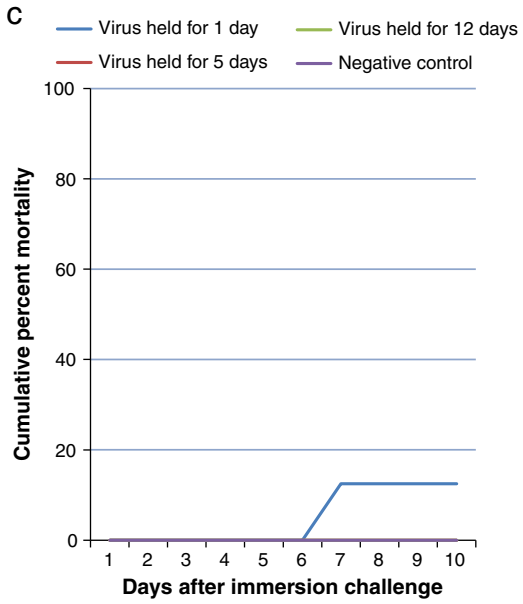


Table 2
Histopathological lesion scores[a] (virus stability in sea water experiment).

Animal number and treatment	Pleuropedal ganglion			Nerve cords				Peripheral nerves
	PNS	GM	WM	PNS	GM	WM	Trans comm	
1) 4 °C held 1 day	3	3	3	3	3	3	3	1
2) 4 °C held 1 day	2	3	3	2	3	3	2	1
3) 4 °C held 1 day	3	3	2	3	3	2	2	1
4) 4 °C held 1 day	1	1	1	1	1	1	1	0
5) 4 °C held 1 day	2	1	1	1	1	1	2	1
6) 15 °C held 1 day	3	3	3	3	3	3	2	0
7) 15 °C held 1 day	1	1	0	1	1	0	1	0
8) 15 °C held 1 day	3	2	2	3	2	2	1	1

[a] Lesions in tissues were evaluated according to the scoring system presented in Section 2.

(untreated virus) ($P = 0.44$). Histological examination carried out on 15 moribund animals (including 5 positive controls) revealed typical AVG lesions in their neural tissues (Table 3). Abalone injected with filtered HBSS solutions (without virus) containing calcium hypochlorite (to determine effect of chemical residue on abalone) all survived and did not show any lesions in their tissues (results not shown).

3.2.3. Immersion trial

In the immersion challenge experiment, the group of abalone exposed to untreated infectious water showed a 50% cumulative mortality (Fig. 5). In this group, 3 abalone out of 6 showed histopathology typical of AVG (Table 4). Groups of abalone exposed to infectious water treated with 10 and 15 ppm calcium hypochlorite (1.5 and 2 ppm of residual chlorine, respectively after 15 min exposure) all survived the challenge and did not show histopathological legions in their tissues (results not shown). Abalone exposed to the calcium hypochlorite treated water only (no virus) all survived (Fig. 5) and did not show any lesions in their tissues (results not shown).

4. Discussion

Knowledge of the ability of an aquatic virus to spread through water and infect its host is of prime importance for its control and/or eradication. The development of an experimental infection model for AbHV in abalone in this experiment allowed us to conclude that in “ideal conditions” (pristine sea water with no other biological or physical factors) AbHV can survive in the water column at 15 °C, remain infectious and cause disease 1 day after being shed in the water. It is likely that it could do so for up to 2 to 3 days although its infectivity/pathogenicity would be reduced in comparison to 1 day post-shedding (Fig. 2b; 85% mortality rate). Results also showed that increase in water temperature is not favourable for virus survival (Fig. 2c).

The results also confirm that direct contact between abalone is not required for horizontal transmission of this virus, as demonstrated in a previous study involving the Taiwanese isolate of an abalone herpes-like virus (Chang et al., 2005). Moreover, the virus appears to be stable for at least 1 day and perhaps even longer in sea water at 15 °C, under these experimental conditions. More natural conditions, i.e. the presence of other biological and physical factors, may modulate virus survival and water currents may influence the rate of spread. These results also show that virus survival is reduced at higher temperatures suggesting that transmission is more likely in the winter than in

Fig. 2. a. Mortality curves of abalone challenged with AbHV held at 4 °C for three different periods of time. b. Mortality curves of abalone challenged with AbHV held at 15 °C for three different periods of time. c. Mortality curves of abalone challenged with AbHV held at 25 °C for three different periods of time.

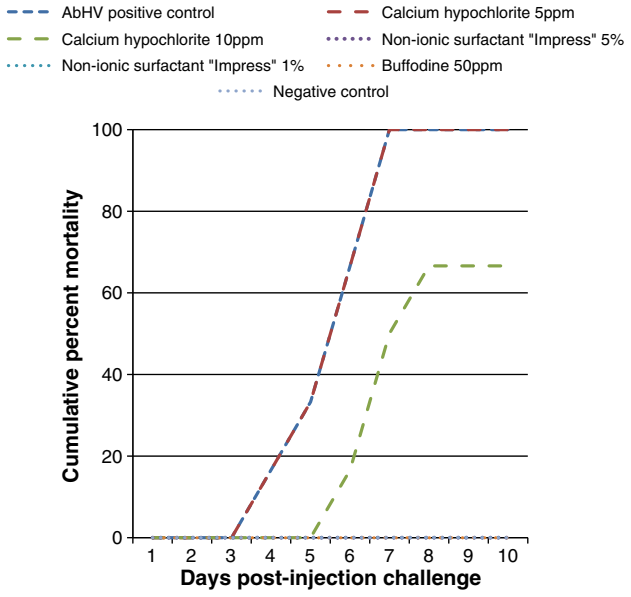


Fig. 3. Mortality curves of abalone injected with AbHV treated with chemical compounds.

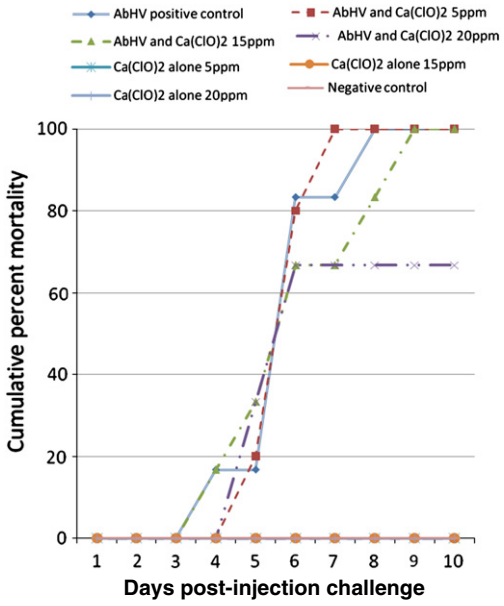


Fig. 4. Mortality curves of abalone injected with AbHV treated with calcium hypochlorite.

summer. This seemed to be the case in the wild as most abalone mortalities have been reported during winter months.

It is well recognised that for any infectious disease, prevention is the most effective control strategy and that use of chemical disinfectants plays an important part of this strategy (OIE, 2010). Chemical disinfectants are commonly used in aquaculture settings to reduce or prevent transmission of pathogens on eggs as well as for the treatment of hatchery influent or effluent waters, contaminated raceways, equipment and other fomites. Chlorine and iodophors have been found effective virucidal agents against fish pathogens such as infectious pancreatic necrosis virus (Elliott and Amend, 1978), infectious salmon anaemia (Smail et al., 2004), striped jack nervous necrosis virus (Arimoto et al., 1996), and white spot syndrome virus (Balasubramanian et al., 2006). In some cases the disinfectant efficacy depended on the concentration of the virus, the water pH and, mostly, the presence of organic matter in the water. Results presented here

Table 3
Histopathological lesion scores[a] (chemical treatments of virus: injection trials 1 and 2).

Animal number and treatment	Pleuropedal ganglion			Nerve cords				Peripheral nerves
	PNS	GM	WM	PNS	GM	WM	Trans comm	
1) Virus alone	1	0	0	1	0	0	0	0
2) Virus alone	1	0	0	1	0	0	0	0
3) Virus alone	2	3	2	N/A	N/A	N/A	N/A	0
4) Virus alone	1	0	0	N/A	N/A	N/A	N/A	0
5) Virus alone	1	1	0	1	1	0	0	0
6) Virus alone	1	0	0	1	1	1	0	0
7) Virus alone	2	2	2	2	2	2	2	2
8) Virus alone	1	2	1	1	2	1	1	0
9) Virus alone	2	2	1	2	2	2	1	0
10) Virus alone	3	3	2	3	3	2	1	0
11) $Ca(ClO)_2$ 5 ppm + virus	0	0	0	0	0	0	0	0
12) $Ca(ClO)_2$ 5 ppm + virus	2	3	2	2	2	3	2	0
13) $Ca(ClO)_2$ 5 ppm + virus	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0
14) $Ca(ClO)_2$ 5 ppm + virus	N/A	N/A	N/A	2	3	1	0	0
15) $Ca(ClO)_2$ 5 ppm + virus	3	2	2	3	2	2	0	0
16) $Ca(ClO)_2$ 10 ppm + virus	2	3	2	2	3	3	2	1
17) $Ca(ClO)_2$ 10 ppm + virus	3	2	2	N/A	N/A	N/A	N/A	1
18) $Ca(ClO)_2$ 10 ppm + virus	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
19) $Ca(ClO)_2$ 10 ppm + virus	1	0	0	N/A	N/A	N/A	N/A	0
20) $Ca(ClO)_2$ 10 ppm + virus	0	0	0	N/A	N/A	N/A	0	0
21) $Ca(ClO)_2$ 15 ppm + virus	1	0	0	0	0	0	0	0
22) $Ca(ClO)_2$ 15 ppm + virus	N/A	N/A	N/A	2	2	2	1	0
23) $Ca(ClO)_2$ 15 ppm + virus	1	1	0	1	1	0	0	0
24) $Ca(ClO)_2$ 15 ppm + virus	3	3	1	3	3	2	1	0
25) $Ca(ClO)_2$ 20 ppm + virus	2	2	1	3	3	2	1	0
26) $Ca(ClO)_2$ 20 ppm + virus	N/A	N/A	N/A	2	2	1	0	0
27) $Ca(ClO)_2$ 20 ppm + virus	0	0	0	0	0	0	0	0
28) $Ca(ClO)_2$ 20 ppm + virus	N/A	N/A	N/A	1	2	2	1	1

N/A: not available.
[a] Lesions in tissues were evaluated according to the scoring system presented in Section 2.

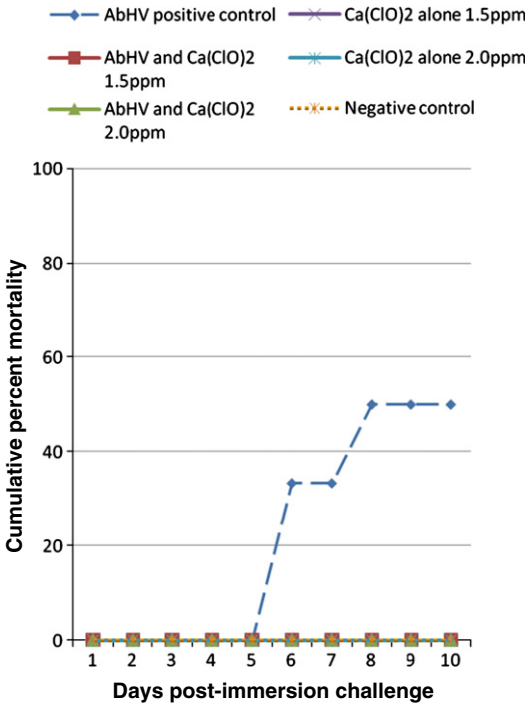


Fig. 5. Mortality curves of abalone immersed in infectious water treated with calcium hypochlorite.

on virucidal efficacy of disinfectants also showed that iodophors such as *Buffodine* and the non-ionic surfactant *Impress* are very effective at inactivating AbHV. Treatment of virus suspensions of high titres with *Buffodine* and *Impress* precluded any mortality in abalone challenged by intramuscular injection (Fig. 3).

It has been suggested that using high viral titres in virucidal tests has certain drawbacks including possible aggregation of viral particles making them more resistant to treatments (Papageorgiou et al., 2001; Thurman and Gerba, 1988). This may explain why calcium hypochlorite had only limited virucidal activity against AbHV (Figs. 3 and 4, injection experiments). Therefore, it is likely that extended exposure time to chlorine would be needed in facilities where high organic matter content is present in the water.

Further criticism has been raised regarding disinfection experiments that used high concentrations of virus; the use of such high concentrations of virus is unlikely to represent the real-world situation in hatcheries (Papageorgiou et al., 2001). It has been suggested that the virus would more likely be dispersed in the flow-through waters at low concentration (Smail et al., 2004). In order to reproduce more realistic aquaculture facility conditions, infectious water containing lower AbHV titres (1.67×10^6 v.g.c./mL) was treated with calcium hypochlorite followed by abalone challenge by immersion. Results indicated that 1.5 and 2.0 ppm of residual chlorine were sufficient to inactivate AbHV during a 15 minute exposure. Some disinfectants may be more efficacious at a temperature of 20 °C, however, the testing carried out at 16 °C reflected the lower ambient temperatures for abalone facilities.

Histological observations of tissues from challenged abalone showed that abalone injected with AbHV or with AbHV treated with calcium hypochlorite had increased cellular infiltration and/or lesions typical of AVG (Hooper et al., 2007) (Table 2). However, animals immersed in infectious water treated with calcium hypochlorite did not show signs of disease or histopathology, suggesting that in aquaculture facilities virus loads could be inactivated using calcium hypochlorite within a relatively short exposure time.

Although *Buffodine* and the non-ionic surfactant *Impress* have not been trialled in large amounts of water, it is likely that they will be also effective in aquaculture facilities if used at appropriate concentrations and exposure times.

Table 4
Histopathological lesion scores[a] (calcium hypochlorite treatments: immersion challenge experiment).

Animal number and treatment	Pleuropedal ganglion			Nerve cords				Peripheral nerves
	PNS	GM	WM	PNS	GM	WM	Trans comm	
1) Virus alone	1	2	0	2	2	1	1	1
2) Virus alone	2	2	1	1	2	1	0	1
3) Virus alone	3	3	3	2	3	3	2	0
4) Virus alone	0	0	0	0	0	0	0	0
5) Virus alone	0	0	0	N/A	N/A	N/A	0	0
6) Virus alone	0	0	0	0	0	0	0	0

N.B. The abalone from the negative control groups (not exposed to the virus and from the chemical exposure only) did not show any lesion in their tissues (results not shown).
N/A: not available.
[a] Lesions in tissues were evaluated according to the scoring system presented in Section 2.

5. Conclusion

To our knowledge this is the first report on the establishment of an experimental infection model for the testing of physical and chemical factors on a molluscan virus. Results indicated that AbHV stability in the water column is modulated by temperature and infectivity/pathogenicity is reduced by 100% within a few days under these experimental conditions. The non-ionic surfactant *Impress* and the iodophor *Buffodine* are very effective virucidal agents under these experimental conditions while calcium hypochlorite is also effective but on lower virus concentrations.

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