# SEAFOOD CRC RESEARCH TRAVEL GRANT: Research training at AAHL, Geelong

*Mr Vinh Dang*

AUSTRALIAN SEAFOOD COOPERATIVE RESEARCH CENTRE

## Project No. 2010/763

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# NON-TECHNICAL SUMMARY

**PROJECT NO 2010/763:** Seafood CRC research travel grant - Research training at AAHL, Geelong

**PRINCIPAL INVESTIGATOR**: Mr Vinh Dang

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**(PROJECT) OBJECTIVES OF RESEARCH TRAVEL GRANT/ INDUSTRY BURSARY:** To assist Mr Vinh Dang with research training at AAHL, Geelong

**NON TECHNICAL SUMMARY:**

I, Mr Vinh Dang, have carried two research trips in Australian Animal Health Laboratory (AAHL), Geelong, under the joint supervision of Dr Kirsten Benkendorff, Dr Peter Speck and Dr Mark Crane. Both trips lasted for three weeks, the first one in October 2010 and the second one in November 2011. It has been a great opportunity for me to work in that high safety standard PC-3 laboratory, where I have learned some new techniques (e.g. handling of abalone herpesvirus and other marine viruses, real-time PCR) as well as performed my own experiments (e.g. AbHV infection trial, abalone immune response during viral infection, and antiviral assay against marine fish viruses). In the end, I have achieved some satisfactory results, which are currently analysed and written up in manuscript format for publication. In addition, some interesting results will be presented at International Abalone Symposium in May 2012 in Hobart.

**OUTCOMES ACHIEVED TO DATE:** Satisfied with a summary of results provided below

**(PROJECT) OUTPUTS DEVELOPED AS RESULT OF TRAVEL GRANT/ INDUSTRY BURSARY:**

- The plaque assay has been optimised for the fish cell line (CHSE-214) and used to test the antiviral activity of abalone haemolymph samples against the fish viruses OMV, EHNV and IHNV. A preliminary result showed significant antiviral activity of abalone haemolymph (with different concentrations 5, 10, and 20%, v/v) against EHNV and IHNV but not OMV. This suggests antiviral activity in abalone is virus specific and further investigation with broader range of viruses may be required.

- Infection trials using emergence and injection methods were performed in collaboration with Dr Serge Corbeil and Dr Nette Williams at AAHL (Figure 1). Abalone immunity (e.g. total haemocyte count, superoxide anion level, and antiviral activity against human herpesvirus type 1) was measured at subclinical (days 1-3) and clinical stage (days 5-7) of viral infection. THC significantly increased across days after infection by emergence and injection methods while SO was found to increase by injection only (Figure 2 & 3). There was no significant effect of infection on antiviral activity.

• In the emergence infection trial, abalone started showing moribund signs (e.g. curly foot, protruded mouth and no firmed contact with tank) from day 5. In total, 18 moribund and 29 healthy abalone from the infection room and 27 negative control abalone from non-infection room were sampled for haemolymph and ganglion tissues at day 5-7 post infection. All moribund abalone were PCR+ve for AbHV (Ct < 35.8). For apparently healthy abalone, 23 abalone were PCR+ve and the other 6 abalone were PCR-ve. We found that THC and SO were lowest in moribund and PCR+ve group and highest in apparently healthy and PCR-ve group (Figure 4).

In conclusion, different abalone individuals of the same farmed family line showed different immune responses and susceptibility towards AbHV infection. Furthermore, superoxide anion production from hemocytes appears to be involved in resistance to ABHV infection in abalone.

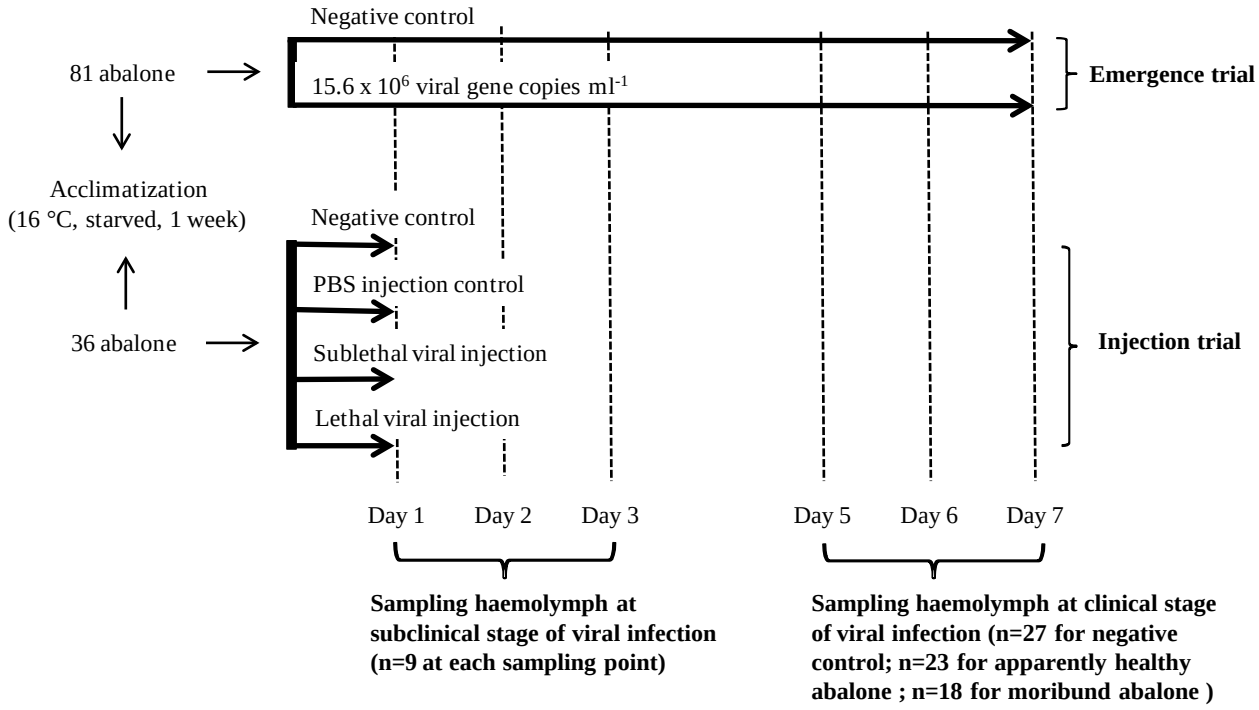


Figure 1. Summary of laboratory infection trials by emergence and injection. AbHV stock was prepared from previous infection trial for 6 abalone. For emergence infection trial, water containing AbHV viral particles (15.6 x $10^6$ viral gene copies $ml^{-1}$) was used to incubate with abalone (n=81) for 24h. For injection trial, two different ten-fold viral dilutions (104 g.c. $ml^{-1}$ and 105 g.c. $m^{l-1}$ in PBS) were injected into abalone foot muscle (100µl). Abalone were sampled for haemolymph at subclinical stage of viral infection (days 1, 2 and 3; n=9 for each day from triplicate tanks) and at clinical stage of viral infection (days 5, 6 and 7). Fresh haemolymph was measured for total haemocyte count and intracellular superoxide anion. The remaining haemolymph was centrifuged (3,000 rpm, 10 min, 4 °C) to obtain cell-free plasma, which were then stored at -80 °C for antiviral assay against HSV-1.

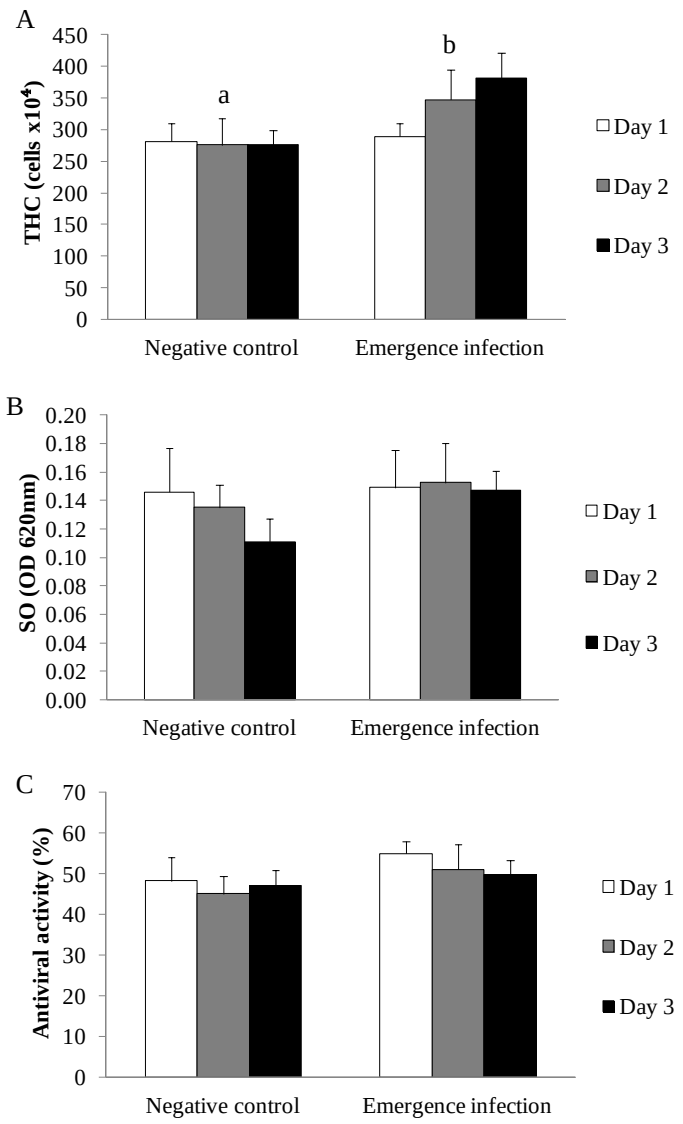


Figure 2. Effect of viral infection by emergence and at the subclinical stage on A) total haemocyte count (THC, cells x $10^4$ per ml), B) superoxide anion (SO, OD 620nm), C) antiviral activity (%) against HSV-1. Each immune parameter was measured from nine replicate abalone. Different small letters indicate significant differences ($p<0.05$) between infected and non-infection (negative control) groups across all days for THC.

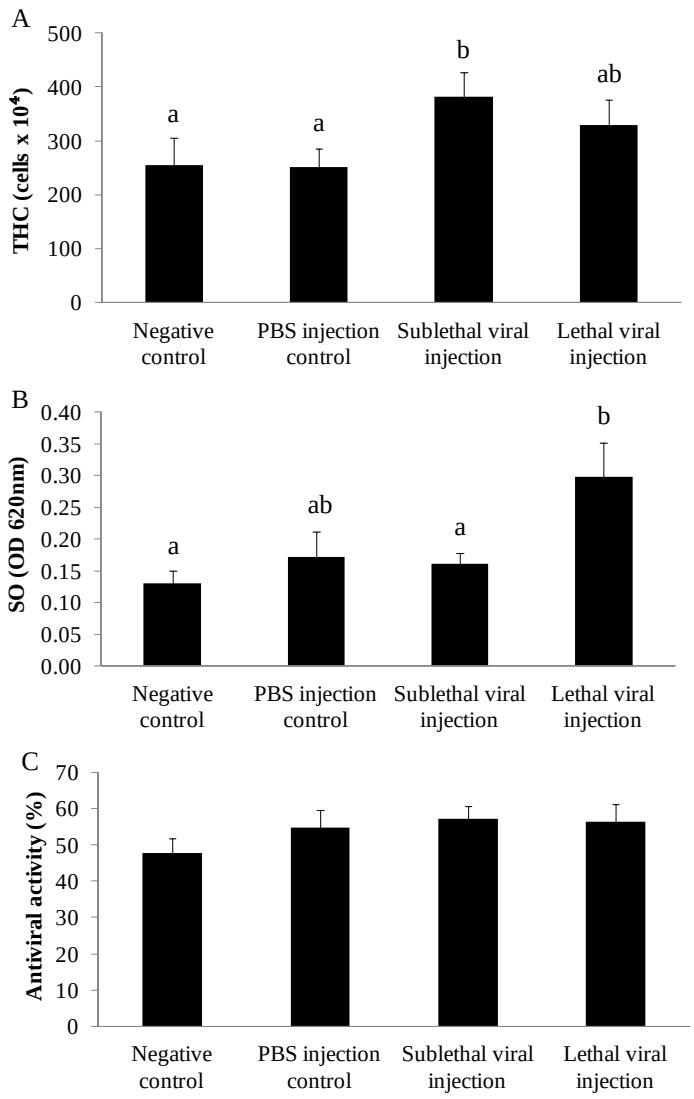


Figure 3. Effect of viral infection by injection with two different doses (104 g.c. $ml^{-1}$ and 105 g.c. $ml^{-1}$) on A) total haemocyte count (THC, cells x $10^4$ per ml), B) superoxide anion (SO, OD 620nm), C) antiviral activity (%) against HSV-1. Each immune parameter was measured from nine replicate abalone at day 1 after infection. Different small letters indicate significant differences ($p<0.05$) between negative control, PBS injection control, and two viral injection groups for THC and SO.

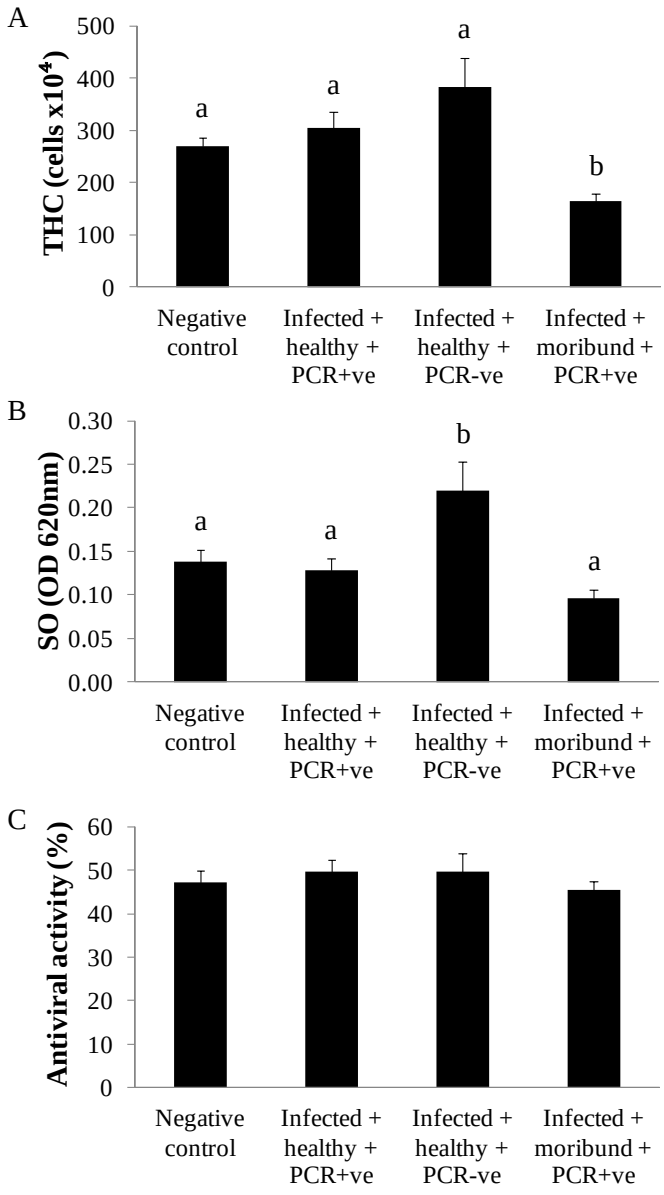


Figure 4. Different immune responses from emergence infection trial A) total haemocyte count (THC, cells x $10^4$ per ml), B) superoxide anion (SO, OD 620nm), C) antiviral activity (%) against HSV-1 between negative control (n=27), apparently healthy and PCR+ve (n=23), apparently healthy and PCR-ve (n=6), and moribund and PCR+ve (n=18). Different small letters indicate significant differences ($p<0.05$) between groups.

## ABOUT THE PROJECT/ACTIVITY

**BACKGROUND AND NEED:** Handling of abalone herpesvirus and other marine viruses needs to be conducted in high safety standard PC-3 laboratory, AAHL.

**RESULTS:** As provided above

## INDUSTRY IMPACT

### SUMMARY OF CHANGE IN INDUSTRY

Our results provide understanding about specific antiviral activity in abalone by testing a broad range of different marine viruses and understanding about immune responses in abalone when infected with its herpes virus. Importantly, we now can partly explain different resistance/susceptibility capability accross abalone individuals when challenged with abalone herpes virus.

### WHAT FUTURE AND ONGOING CHANGES ARE EXPECTED?

Antiviral compounds in abalone haemolymph will be isolated and identified. In addition, appropriate immune markers will be identified to select for abalone virus resistant lines.

### WHAT BARRIERS ARE THERE FOR CHANGES TO OCCUR?

N/A

### IF NOT ALREADY HAPPENING, WHEN WILL THE CHANGES OCCUR?

Further studies (e.g. post-doc research) are required to make such changes to abalone industry. If so, I expect an obvious change can be seen within next 24 months.

### WHAT IS THE LIKELIHOOD THAT THESE CHANGES WILL OCCUR?

>50% chance that abalone industry will adopt project findings.

### WHAT BARRIERS ARE THERE TO DOPTION OF THESE CHANGES AND WHAT ACTION COULD BE TAKEN TO OVERCOME THESE?

To adopt project findings, it will require group trainings to researchers and other people in abalone industry.

## COMMUNICATION OF PROJECT/EXTENSION ACTIVITIES

### WHAT IS THE OUTPUT THAT NEEDS TO BE COMMUNICATED?

Antiviral activity in abalone is non-specific and active against a number of different marine viruses (e.g. HSV-1, EHNV, IHNV).

Abalone have different virus-resistant capability so resistant line can be selected for breeding purpose.

**WHO IS/ARE THE TARGET AUDIENCE/S?**

All people within abalone industry as concerned about AVG disease outbreaks.

**WHAT ARE THE KEY MESSAGES?**

As provided in the output section.

**WHAT IS THE CALL TO ACTION?**

Until immune markers are found to select abalone virus resistant lines, it is important that abalone industry people work together to set up a selective breeding program for abalone family lines that are highly resistant to AVG virus.

**COMMUNICATION CHANNELS**

*(How can these messages be communicated and by who?):*

| ***Channel*** | ***Who by*** | ***When*** |
|---|---|---|
| Conference/symposium, publication, etc. | By all people including myself who have contributed to this project. | Within the next 12 months (e.g. 5-11$^{th}$ May at International Abalone Symposium) |

**LESSONS LEARNED AND RECOMMENDED IMPROVEMENTS**

**WHAT IS YOUR FEEDBACK?**

A delay between two research trips to AAHL has occurred. However, it did not affect the overall results of the project. We highly appreciate the extension agreement from CRC regarding this delay.

**FURTHER ACTION REQUIRED IN REGARDS TO COMMERCIALISATION?**
N/A

**ACKNOWLEDGEMENTS**

There has been a great contribution from my supervisors Dr Peter Speck and Dr Kirsten Benkendorff regarding project planning. I appreciate all people from AAHL, especially Dr Mark Crane, Dr Serge Corbeil and Dr Nette Williams for assisting me to complete the research training. Importantly, I would like to thank the Australian Seafood CRC for providing travel funds for this research training.