Inspector-General of Biosecurity's review of the circumstances leading to the 2017 suspension of uncooked prawn imports into Australia and the biosecurity considerations relevant to future trade in uncooked prawns

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Scope of review

The scope of this review covers operational policy and activities relevant to biosecurity risks associated with importation of uncooked prawns and prawn meat into Australia. The review will consider the following areas:

- the effectiveness of biosecurity controls and their implementation for managing the biosecurity risks of importation of uncooked prawns and prawn meat into Australia;
- the effectiveness of post-entry surveillance measures and 'end use' import conditions for uncooked prawns and prawn meat into Australia; and
- areas for improvement in the biosecurity risk management framework and its implementation for future trade in prawns and related seafood.

Background

White spot syndrome virus is the lone virus (and type species) of the genus *Whispovirus* (**whi**te **spo**t), which is the only genus in the family *Nimaviridae*. It is responsible for causing white spot syndrome in a wide range of crustacean hosts.[1] **White spot syndrome** (WSS) is a viral infection of penaeid shrimp(prawns). The disease is highly lethal and contagious, killing shrimps quickly. Outbreaks of this disease have wiped out within a few days the entire **populations** of many shrimp farms throughout the world. (Wikapaedia).

The virus itself has no affect on humans and other than in countries like Australia where it I screened for, so that a large percentage of the world's population has actually no idea that the shrimp they eat may be affected by or contain WSSV itself.

WSSV is present in the prawns in most of the oceans/seas especially in Asia where it may be carried by all types of crustaceans including and other marine species such as polychaete worms and small

crustaceans such as crabs and copepods. These may be infected at low levels and act as carriers of WSSV without manifesting any symptoms.

Disease problems are normally manifest when WSSV gets into a prawn farm but may remain at low infectious levels for long periods and only manifest when the prawn population in a pond becomes overcrowded or has a number of other environmental factors change. Typically changes to the situation of the pond itself (water, salinity, temperature etc). Generally WSSV affects highly inbred prawns and may not cause disease in the wild.

The 2009 IRA

As part of the 2009 IRA (Import Risk assessment for imported prawns) devised over two or more years and available from the DAWR website. This over three hundred page document outlines in much greater detail the scientific reasoning and implementation of a protocol for the importation of uncooked green prawns or prawn derivatives or products to minimise the disease risk for three viruses. These included WSSV, YHV and IHHNV. This is by no means an exhaustive list of potential nasty pathogens that could be imported into Australia in prawns. The requirement for IHHNV testing was subsequently dropped from the requirements when found to be not exotic , leaving only WSSV and YHV for screening of every and any container arriving into Australia containing wild caught and/ or farmed raw green prawns prepared in Asia or elsewhere. This leaves out a very substantial list of other potential pathogens which have emerged and are currently affecting and causing disease and major economic losses in aquaculture in Asia in particular. What is the reason for excluding testing for these diseases as being present in imported prawns potentially? As diseases emerge and change this list should be revisited and the risk decided.

Any IRA is required by the OIE under an ALOP (Appropriate Level of Protection) and to fulfill the protection requirements of the OIE to devise a protocol for the sampling and testing of imported prawns in order to reduce the risk of WSSV (or any other disease) transferring infection to Australian prawns to be a low risk. It specifically under the OIE guidelines and protocol for the protocols to ensure LOW RISK not NO RISK. The scientific basis for the 2009 IRA was carefully and extensively debated by an expert panel with significant contributions from expert scientists, both in Australia and overseas as well as local importer and farmer and public input.

There was an expert scientific review panel to review the submissions from all stakeholders including the importers as well as the local prawn farmers and anyone or any body who had to do with the importation and testing. While not a perfect document or seemingly accepted by some after two years work it seemed to have most if not all the requirements to fulfill the requirements under the OIE and the acceptance of ALOP especially to have a **low level of risk** for any imported prawns to cause a transfer of infection.

Two parts of the IRA

The IRA implementation consisted of two major elements fulfilled by two parties. One was the sampling of prawns from the containers and second to test them for the presence of viruses. The basis of the sampling was on the concept of a container representing a " batch" of prawns from a "pond" in Asia (or other country) which had been harvested and sent for processing in the same or another country. When the container was opened here in Australia a statistical sample of 13X5=65 prawns was to be taken AT RANDOM from the inside of the container and then the 13 bags of prawns sent to an accredited testing lab of choice of the importer for testing at a choice of laboratory using a NATA (National Australian Testing Authorities) accredited method according to ISO/IEC 17025:2005. The results were then sent to the department of Agriculture, currently called DAWR,

and the importer for the release of those prawns to the market. The importer paid the cost of sampling attendance by DAWR officers, for the testing and transport of the prawns. The costs are in the thousands of dollars all together for each container.

A "Pond" or " batch" ?

The concept of a "pond " or "batch". Whether the concept of the "pond" was adequately thought out is debatable. The original concept was that a container would have the contents of one pond in it. This is not what happens at the processing and filling of a container overseas. Different types of prawns, peeled, head on, head off, breaded, crumbed, wild caught, or farmed all end up in one container if the import volume is small for a small importer. Large importers may spread into several containers. They maybe all from one source or one pond but given the nature and size of different operations in the world this may be from several sources or a mixture of ponds.

The paperwork associated with each import is then supposed to contain one "batch number". In recent times it has been realised that inadequate details are on the consignment or import papers usually handled by importers brokers to indicate either the different types of products or sources of the prawns. Mostly that information may be only available to the exporter or not known at all. This has lead to the somewhat ludicrous arrangement of front line Biosecurity officers inspecting containers to try and second guess the source or types of prawns in the container costing hours of work (fully charged to importer) labeling one batch as two or more batches just because one had tail on and the other tail off and yet could be from same farm or even wild caught. The costs therefore potentially to be imposed on the importer then also reached ludicrous levels. In one case one container was declared to have seven or more batches based on labels on boxes or types thought to be in the container.

This situation cannot be left to front line biosecurity officers who have no means of deciding or sufficient . From an IRA risk point of view unless it is clearly known or identified eg wild caught or farmed in the same container some sort of measure associated with risk would seem to be that simply persisting with taking thirteen boxes at random and then selecting 5 prawns from each box should suffice to potentially capture the likelihood of finding at least one positive prawn which would be sufficient to condemn the entire container. To otherwise would increase the costs to small importers of sampling and transport and testing to levels making the importation not affordable or feasible.

The two elements of the IRA

If either of the two elements ie the sampling or the testing was to not be carried out correctly then the entire process would fail. The laboratories could only test on an "as received" basis, so if the prawns were not a random sample of the contents of the container and a statistical sample representing the "pond" being taken then the results would be biased and incorrect not in value but in misleading anyone into the actual percentage of potentially positive prawns per container and the level of risk to meet the ALOP requirement.

The sampling required that the officers representing DAWR at the border would be fully understanding of their role in the process and that they understood sufficiently the need for statistical independent correct sampling of the container to ensure a properly representative result. This statistical sampling regime was unique and quite unlike other food sampling processes for the testing of eg chemicals or other biological pathogens in a container.

This in turn required a ”sampling protocol” a work instruction document (not publicly available) which appears to be an internal document of the department and only referred to in the subsequent investigation by the Inspector General of Biosecurity in a review conducted when a container of prawns was accidently released and found subsequently to contain WSSV infected product. Without an adequately informed and trained front line force of sampling officers understanding their role in the whole process then the whole regime was likely to fail. The accuracy and completeness and thorough understanding by any Biosecurity officer inspecting a container is vital.

Training

Initial training sessions in 2009 called a “road show “ was done by two departmental members Dr Mike Nunn and Dr Geoff Grossel of the department shortly after the process outlined in the IRA was instigated. This was rolled out to the departmental officers in each port of entry in each State at the time but over the intervening years as staff left or were replaced at the front line it would appear that none of these officers were trained or had any proper instruction or had any use of the “sampling protocol” which could explain their role in the failure of overall sampling and testing of containers.

Eventually some years ago also both Dr Mike Nunn and Dr Geoff Grossel also left the Department to take other roles elsewhere and as a consequence of that the whole basis and scientific reasoning and understanding of the IRA and its two major elements sampling and testing were also lost and not replaced by senior staff understanding the whole picture with regard to the WSSV story, the IRA and how to get the right representative results from the sampling and testing , the two elements required. Where was the adequate training of new staff?

It was obvious at the testing laboratories that the sampling process was being poorly handled. Prawns would arrive unrefrigerated, sent on the wrong day, not properly bagged in 13 bags containing 5 prawns and many many other problems related to inadequate or improper sampling or transportation to the labs in Sydney. At Advanced Analytical (I was then CEO) and one of the major testing labs, there was an officer almost full time on the phone trying to educate the various State officers supposedly sampling prawns according to a “ sampling protocol” clearly not doing so and despite trying to do this over many years there appeared no one at the department who would either listen or understand the importance of proper sampling or the testing would subsequently fail. There often even appeared to be no understanding of the 2009 IRA and its scientific content and meaning and intent.

Anecdotal information was however supplied to the laboratories especially from brokers and importers of inadequate and improper sampling of containers not being accessible for inspection at the time, stacked atop each other and so on or of officers just simply requesting that someone else from the importer or their agent asked by the officer to bring them 65 prawns of such and such a batch number. The officers didn’t enter the containers. This in turn clearly lead to the potential of exploitation by unscrupulous agents of brokers or importers of inadequate or potentially substituted “ negative” prawns . But the lack of adequate understanding or training of the front line officers themselves and their important role in the process to get the a representative result was also significantly the cause of subsequent inadequate or false non statistical test results .

Likewise despite the significant paperwork and the requirements of that paperwork, often and mostly handled by brokers of importers there was wrong information, lack of complete information so that no one least of all anyone in the department knowing what results meant. It appeared also that no one at the department was monitoring what the results were and what they meant

scientifically. The reality is that the testing laboratories had the most information. It was they who interacted with importing clients, their brokers as it was the importer who paid the labs' bills. But there was no forum or mechanism to share results between labs or with the department in fact any communications were actively discouraged. There was no contact between the two main labs testing at that time Advanced Analytical (testing actually subcontracted internally to Agrigen scientists) and EMAI or CSIRO AAHL either. This has lead to significant breakdown on a scientific basis of the understanding of the testing methodology which has evolved over the years as well as highlighting the problems with the sampling and these issues also. The labs simply went on testing prawns sending data to the department for years with no feedback or review or discussion of the results being obtained or what they meant. Despite my repeated calls to the department from 2009 onwards nothing was ever organised no meeting amongst those who knew the most of what was happening in the sampling and testing of prawns for these viruses.

The need for a virus test for commercially imported prawns

Australia has a unique problem. No one else in the world is interested in sampling every container at the border and testing it for these viruses and other pathogens. There is no major incentive to sample and test except to try and prevent major economic losses of production in other countries. The countries are varied in their responses to control the diseases which are mostly ubiquitously present in the oceans/ seas around them as well. Some countries are more successful than others in having proper biosecure facilities in their countries and rigidly enforcing testing. But is often inadequate or incomplete.

For importers in Australia it is enormously difficult to find exporters or processing facilities or even farmers who can reliably provide prawns negative for these diseases including WSSV. There is no real incentive for exporters who can sell much greater volumes of product to other destinations such as Japan or Europe or America who have no testing requirement or need for it in their countries. So importers try to secure suppliers of negative prawns but this is a constantly changing scene with problems of inadequate testing, cross border movement of broodstock, lack of biosecurity and so on meaning that a prawn pond may be negative this season and positive the next. Finding a reliable source of totally negative prawns for the importer is an almost impossible task. Such sources when found are guarded severely from the any other importer finding out.

And on testing for WSSV or any other viruses there is no overseas protocols to pick up for routine testing of commercially imported prawns as there might be for many other pathogens, ie to have a uniform accepted testing protocol which would be specifically applicable to routine commercial testing of imported material. This is not a problem for chemical testing where international bodies such as CODEX have international agreements on testing levels etc. So Australia does use this mechanism adopting testing for other agents especially in other human or other veterinary pathogens. For WSSV and YHV instead the laboratories simply used the international literature and emerging information on DNA/PCR testing to devise their own protocols and methods and have them hopefully accredited by NATA as OIE equivalent methods.

The OIE protocols are designed to help counties who have an outbreak of disease. They are NOT designed for routine diagnostic border testing. The only way to have done this was to set up mechanisms and scientific discussion to bring about standardised protocols and inter-laboratory proficiency testing overseen by independent providers of proficiency testing services. Yes, under accreditation requirements of ISO/IEC 17025:2005 the international requirements accredited by NATA there is some proficiency testing conducted. The labs in Australia have participated if and when this has been possible, but no subsequent discussion or evaluation of this process and the

suitability and robustness of the method being used by a particular or any lab was is done as there was no mechanism to do it.

With other viruses of international interest such as say Norovirus in food including particularly oysters and recently found in raspberries etc, there are international labs across the world and especially in Europe to bring about standardised international protocols/ methods, many laboratories contribute and participate in that process and then an international committee oversees the implementation of the testing to all labs. In addition particularly where qPCR methods are used for virus detection, reports are also standardised to an extent to allow easy international interpretation of any result by an importer exporter or regulator.

The dilemma of report interpretation- infectivity

On an international basis for viruses measurement in particular the qPCR DNA results have shifted specifically and preferentially to equate them with viral genome copy number. The purpose is to try and decide whether a scientific qPCR DNA (Ct, Cq) value is actually representative of a number of virus particles which may be infective. qPCR DNA techniques do not measure live infective particles. They measure fragments of specific pieces of DNA. They are very accurate and sensitive at doing that, but without infectivity studies in parallel cannot be used to tell anything about a prawn containing viruses and whether infective. The virus maybe alive or dead. You are only measuring DNA.

But studies have been done with many viruses eg Norovirus to interpret how many viral particles are needed for that virus to be considered infective in that oyster, raspberry etc if it were eaten. This for Norovirus has been considered to be in the vicinity of 600-800 virus particles from the extracted material. And Norovirus is a very infectious agent. Studies at low infective numbers for WSSV have not been done or remain unpublished if they have been done.

In WSSV in recent times WSSV OIE reference laboratories such as the University of Arizona reference lab has done studies and now provides reports in viral genome equivalents. The theoretical limit and Level of Detection (LOD) using qPCR is single viral genome equivalent for the qPCR test but that is theoretical only and the research and methodology of this reference lab has indicated that it is only possible to report to 10 viral genome equivalents as the LOD with values below 10 as being unreliable and not reproducible or repeatable. So they give out reports on that viral genome equivalent basis and are accepted world wide on that basis. This report still does not tell you specifically if a value of 10 viral genome equivalents would produce an infection were that prawn be eaten by another prawn. This could be done in practical experiments but only by using well characterised infective material to test in pathogen free prawns to see if that is infective. You need facilities not present in Australia to do that. Where this has been done it appears that thousands of viral genome equivalents are needed to obtain infection.

It is therefore very important not to rely entirely on a qPCR result especially when the number of viral genome equivalents is very low or at the LOD. At high numbers this is not an issue but over interpretation of very low numbers to imply that such WSSV prawns may contain infectious virus is not reasonable. The risk presented would be extremely low of providing an infectious source and well outside the ALOP.

Monitoring WSSV

WSSV is a virus that cannot be cultured in any cell culture system. The presence of WSSV in carriers or prawns at sub clinical manifestation cannot be recognised easily. When WSSV is at sufficient

clinical pathological level to cause morbidity and mortality it may then be noticed as actual white spots present on the carapace and be at very high infectious levels.

To effectively monitor the presence of WSSV at low levels and in carriers and hatchery or larval stock to stock ponds the only useful test is based on DNA technology to measure the actual WSSV genome (DNA sequence) rather than the virus itself. This is currently done on a world wide basis using what are called DNA/PCR techniques. RNA viruses such as yellow head virus (YHV) is also tested for in a similar way. PCR techniques rely on the known DNA sequence of the WSSV and a short segment on the DNA is very specifically amplified and multiplied by the use of the PCR (polymerase chain reaction).

Detection of WSSV using DNA methods and PCR techniques is described in scientific literature dating well back into the nineties to current day. PCR/DNA techniques have evolved over the years since the early nineties as the whole PCR methodology has developed. The entire reaction mechanisms are well understood and it is not a "black box" technology. Methods and instrumentation to get reliable, reproducible, robust and quantitative methods have been developed for WSSV and are published in the scientific literature where they can be peer reviewed and subsequently relied upon for usage in diagnosis. The current best PCR techniques are (quantifiable) PCR methods. qPCR methods are used today for all types of medical, animal, plant, bacterial, and viral monitoring of the agents causing diseases of all types.

When an outbreak occurs in a country in the world the local authorities will try and deal with the disease. The diagnosis of an outbreak requires the use of some sort of veterinary or aquatic scientifically based facility able to diagnose disease.

On a world wide basis this diagnosis is covered specifically in the aquatic diseases manual of the OIE (World organisation for Animal Health) with WSSV diagnosis covered in the chapter 9.7 of the OIE AQUATIC ANIMAL HEALTH CODE
http://www.oie.int/index.php?id=171&L=0&htmfile=chapitre_wsd.htm
This chapter outlines specifically the OIE code to deal with infections by WSSV the code of any country dealing with outbreaks of WSSV, notifications to the OIE and conditions for considering a country WSSV free or otherwise. It outlines the OIE regulations regarding this disease.

In addition the OIE AQUATIC Manual of Diagnostic Tests for Aquatic Animals has a chapter specifically on WSSV Chapter 2.2.7 see
http://www.oie.int/index.php?id=2439&L=0&htmfile=chapitre_wsd.htm
Which outlines the disease its diagnosis the methods of doing so and also includes as part of this diagnosis are details of the PCR methods that could be used for detecting WSSV DNA sequences of the WSSV genome. The section 4.3.1.2.4. Molecular techniques and 4.3.1.2.4.1. Polymerase chain reaction (PCR) are included.

The PCR method for WSSV detection is according to the latest manual described as "The PCR protocol described here is from Lo et al., 1996a and Lo et al., 1996b, and uses sampling methods from Lo et al., 1997. It is recommended for all situations where WSSV diagnosis is required. A positive result in the first step of this standard protocol implies a serious WSSV infection, whereas, when a positive result is obtained in the second amplification step only, a latent or carrier-state infection is indicated. Alternative PCR assays have also been developed (e.g. Nunan & Lightner, 2011), but before use they should first be compared with the protocol described here"

So that the method described in the chapter in the OIE manual is in fact based on original methods for 1996 and 1997 and are in molecular science terms considered very old by modern standards. The

method of Nunan and Lightner, 2011 comes from the WSSV laboratory which is considered the most up to date advisory and testing facility at the University Of Arizona USA. (Prof Lightner was an original contributor to the setting up of the 2009 IRA by the Department of Agriculture). Currently the laboratory is headed by Dr Kathy Tang Nelson and considered and used by most laboratories as the best international back up reference laboratory for the testing of WSSV. She is a reference adviser for WSSV for the OIE. (it is not CSIRO AAHL which is OIE reference lab for Yellow Head Virus, YHV).

Diagnosis of an out break of WSSV has been dealt with by CSIRO AAHL Aquatic diseases unit and the QLD dept of Agriculture in the current outbreak. The methods have included veterinary diagnostic methods as they have facilities for pathological examination of specimens and are best placed for these techniques. This does not however mean necessarily that that these laboratories have the staff equipment or best diagnostic methodology or routine and ongoing expertise or experience to diagnose and quantify WSSV in prawns by the use of qPCR methodology for routinely imported prawns. From their reports, they appear to use three qPCR methods and in WSSV detection one based on the OIE although not following the published OIE method (Taqman OIE) , and one based a so called CSIRO Taqman method. The details of this second method is unpublished and also not accepted by the OIE as a method of choice.

Three other diagnostic laboratories Agrigen Pty Ltd, Advanced Analytical Australia Pty Ltd and The Elizabeth Macarthur Agricultural Institute a(EMAI) as part of NSW DPI have been specifically approved by DAWR to perform routine testing of imported prawns using PCR diagnostic methods according to OIE or equivalent methods. Since 2009 these three laboratories have tested hundreds of thousands of prawns for the presence of these viruses and would have to be considered the experts in the routine testing and reporting of commercially imported prawns and prawn meat for the viruses WSSV and YHV. CSIRO AAHL did not and has not since 2009 conducted routine commercial testing of WSSV or YHV in imported prawns.

qPCR testing in Australia

In Australia the qPCR testing has been expressed on reports as Ct or Cq values which pertains to the sigmoidal curve graphic plot obtained in the amplification of the DNA extracted from the start material. Its value which is used internationally by laboratories using qPCR for all types of diagnostic methods to indicate 1 viral genome equivalent in the extract. The acceptable level of this Ct Cq value is around a maximum value of 35 cycles. Anything greater than 35 cycles represents on a logarithmic scale hundreds or thousands of less likelihood of a single viral DNA equivalent being found and has been specifically found by the international reference laboratories doing qPCR to not reproducible or reliable. In reality, this also represents an extremely low level of potential infectivity of such material. In effect it represents absolute zero tolerance or no virus. In effect for any importer such a low level is impossible to achieve ion practice.

The actual level of reliable value to place on a report for LOD to be equivalent to 10 viral genome copies and would be a value of 32-34 cycles or Ct Cq cycle values if that was being used on the report. Such reports should also contain the values of reference positive and negative material to ensure confidence in the quality of the report. Normally positive control material and would be at Ct Cq values of 20 or greater and represent millions of viral genome equivalents.

Even specific pathogen free prawns have shown unreliable results of cycle value of 36-37 cycles (personal communication) which is why these are discarded by international reference laboratories. Such values above 35 cycles means less than 1 viral genome equivalent. Any value of > 40 cycles is entirely meaningless. In fact the OIE reference manual refers to a maximum of 40 cycles being the

used in PCR methods , and that manual as already discussed is for diagnosis in an outbreak not for routine testing of imports to satisfy the ALOP and OIE.

The laboratories testing currently routinely (not CSIRO AAHL) have by their existing extensive experience of testing commercially imported prawns in hundreds of thousands simply devised the LODs and limitations of the testing itself. Where a value is questionable such as at the 35 Ct value the whole assay is repeated to determine whether the same result can be obtained and if not discarded as a false positive. Both pathogen free prawns and even wild caught or farmed Australian prawns often show false positive results >35 cycles or < 1 viral genome copy.

The whole issue of the limitations of qPCR testing is also well covered from the technical point of view. A large number of websites on the internet cover the whole issue of the limitations of qPCR and the issue of false positives beyond 35 cycles. These very educative websites for teaching the use of qPCR techniques to technicians and scientists cover the limitations in terms of the chemistry of the qPCR technique. In general terms the qPCR technique breaks down beyond 35 cycles when it plateaus and reagents become exhausted and the fluorescently labeled probe also breaks down liberating non specific fluorescing chemicals creating false results. The international published literature does not recommend qPCR use beyond 35 cycles should be relied upon for any pathological agent and certainly not beyond 40 cycles which is also the OIE recommended absolute cutoff point. (see report below)

Results from existing reports from all labs

DAWR has collected a huge amount of Ct Cq data and gets every report from the testing of the laboratories. All laboratories have been obliged to collect and pass on all the Ct Cq data in reports to DAWR for more than a year now. What precisely has been done with this data? What collation or collection of this data? What interpretation of this data and scientific discussion has taken place on this with scientists or experts in qPCR? None to my knowledge outside DAWR. Its all still held in secret. Where is the forum or advice or expert scientific committee properly familiar with qPCR to deal with these findings?

The original IRA and subsequent methods required that the laboratories testing do so independently and that an importer had the choice of provider as required under general competition laws for purchasers of services. If an importer then was unhappy with the results of a test the importer could request a second test by CSIRO AAHL lab if the result was positive. In reality this presented no problems because the result over all came back as “positive” and was backed up. But then DAWR suddenly and without consultation decided to retest “negative” material. This presented huge problems for everyone. The laboratories themselves already accredited by NATA to OIE and approved by DAWR and testing according to OIE or equivalent methods and having set their own LODs were confronted with an “ enhanced “ test which then from the reports of CSIRO AAHL were declared “positive” and importers containers condemned for destruction or re-export at significant expense. In this case these importers in recent times had gone to considerable effort and expense to ensure by prior testing at source that the materials were negative. It include also wild caught prawns form southern Argentina never known to contain WSSV being declared positive even though twenty other containers had been passed and tested negative previously. This left no credibility in the eyes of importers certainly not in the CSIRO AAHL qPCR tests and a general distrust in qPCR testing in general even though it is a well established well regarded test if done properly.
Again there seemed to be no way for anyone to get a review of this CSIRO AAHL testing. They also absolutely refuse to allow any examination of the raw data, curves or results. They refuse to allow testing of the prawns by any other lab? This practice is certainly unique as all testing labs would normally at least show the data to the aggrieved party on request. While CSIRO AAHL may be

regarded as an expert veterinary testing laboratory for diagnosing diseases in all manner of animals it is in diagnosis that their expertise lies. Since 2009 CSIRO has not participated in routine qPCR testing of commercially imported prawns. That expertise lies with the three accredited commercial and government labs testing since 2009.

The huge number of reports currently held by DAWR have not been subject to any independent analysis by any qPCR expert. One lab Agrigen Pty Ltd and its owner and qPCR scientist of twenty years expert qPCR experience in all types of animal and plant qPCR, Dr David Croan has examined a limited number of the reports given him by the importers and also compared with the rounds of proficiency testing in which the labs participated. (These are organised by ANQAP but are done blind). There are also others conducted internationally by the OIE WSSV reference lab of University of Arizona, USA.

An analysis of these reports is given in a separate document attached to this submission . This document clearly shows the incorrectness and false positives that are generated by the use of the CSIRO AAHL methods. The conclusion is that the CSIRO AAHL reports are inaccurate and incorrect and cannot be relied upon for negatively tested prawns from the other laboratories. CSIRO scientists do not appear to understand the limitations and scientific method of qPCR detection techniques. This report should also be considered as a failure of the testing according to the IRA principles. (see attachment report to NATA by Dr David Croan).

Monitoring the success of sampling and testing to achieve low risk

To separately monitor the success or otherwise of the sampling and testing regime also required that independent surveys be conducted to measure the effectiveness at the retail level in Australia. This is done currently by DAWR for the purposes of their IFIS program to monitor the level of chemicals in products as well as microbial contamination. The process is well established and is done every few years in what is called a market basket survey to ensure compliance. Clearly no equivalent process was put in place to monitor the sampling and testing of WSSV and YHV until last year when it has emerged from the Senate enquiry so far, that an operation was conducted in secret to test retail outlets, storage facilities and other sources such as fishing bait for WSSV in such materials. Given the lack of proper separate sampling for years it is not surprising that the results of this testing did not reflect the testing being conducted by the appointed three labs. As stated above the test becomes meaningless when the samples provided are not reflective of a statistical sample of the “pond”. Why wasn’t independent end sampling done and tested previously to 2016/2017?

As a consequence we now have some emerging data from DAWR indicating that the level of “positivity” of containers is not what it was thought to be. If the results of testing from 2009 were examined it would also be seen that there was a high percentage of positives in the early stages of testing when the sampling was being conducted properly. Then at some point the percentage of positives fell to low levels (<10%) . Was this assumed that everyone was simply suddenly successful in finding negative prawns to ship or was this the start of results obtained from poor sampling or substitution or other practices . As a consequence of the survey in 2016 and changes then being made to the sampling regime and the actions of DAWR some importers then redoubled their efforts to find “ negative prawns to ship. This is not easy as previously stated as some exporters may simply not know the status of the prawns and the availability of reliable testing services in different countries is very variable. In addition the statistical sampling of prawn ponds is not conducted for cost reasons. Many exporters in Asia are simply not interested in exporting for the costs of insurance against potential rejection of the shipment, placing even more pressure on finding the illusive negative prawn.

There has been some attempts by DAWR in recent years to potentially certify certain processing facilities in certain countries. This is a difficult task and the one DAWR certified facility currently in Thailand would appear to have a limited capacity to process prawns and seems to only handle the prawns caught by Australian fishers or prawn farmers for subsequent re-export to Australia. Exporting and processing facilities in Asian countries are excellent but have no interest in obtaining certification for one country Australia when it is considered such a small and very demanding market when they simply can export to the rest of the world not needing WSSV negative prawns.

But one company Agrigen Pty Ltd has been helping both prawn importers and exporters in Asia in finding negative prawns by simply taking the latest qPCR technique and equipment to Asia to the processing factory and to the pond. This process has been very successful in improving the testing and availability of prawns for import. There are many Australian importers that have taken the opportunity offered. According to the reports of the labs testing these containers in the last six months they have been negative when they arrived. ALL this has now ceased since the introduction of the CSIRO AAHL testing of this negative material already tested in the three labs to be negative but then found positive by the CSIRO "enhanced" testing. And in practical terms not only has imported testing ceased, but so have shipments of any prawns and others are sitting in limbo with "false positive" results according to the fact they have two or even three tests showing different results. (one form the accredited lab and two different from CSIRO AAHL Lab?). This is an impossible situation for importers. It is suspected likewise that there are large numbers of imported existing prawns released on the original labs testing but condemned in warehouses etc for potential destruction. Are the results from CSIRO AAHL correct? or are these prawns a real risk under the ALOP? No results of the surveys and testing are available to anyone outside DAWR currently.

The reputation of CSIRO AAHL and the faith of qPCR testing in general is now severely damaged in the eyes of the importers. How can that be restored? What is the future for qPCR testing for any important pathogen for Australias biosecurity situation? It could start by significantly improved communication from DAWR to stakeholders.

It is also important to note again that this disease WSSV in prawns is not just one pathogen but one of many and there are others affecting all forms of aquatic and marine life as well as potential terrestrial animals. Yes we need specialised diagnostic and research facilities like AAHL to do that research and diagnosis when outbreaks or breaches of Biosecurity occur. But there is also a role of other commercially focused testing labs to also do routine testing and even sampling and surveying for these diseases. Facilities such as CSIRO AAHL and State diagnostic research facilities should use those resources for research and diagnosis not as commercially testing services where private labs can do this work efficiently and quickly. Trust and communication are however required.

There should be lessons to learn from the IRA on viral pathogens. The next one is just around the corner. Can Australia's current biosecurity resources, testing and staffing cope with two or more outbreaks at the same time? And the ongoing monitoring required? What would take priority?