Combating food poisoning from seafood

RIU

Validated RNRRS Output.

A rapid and highly sensitive DNA test is now available to screen seafood for bacteria. Infected seafood, particularly shellfish, can cause food poisoning. Outbreaks damage consumer confidence and producers suffer, especially the poorest. Previously, screening for bacteria was slow, taking up to 7 days, and was not always accurate. These diagnostic techniques have been extensively tested on coastal and estuarine seafood in India, Bangladesh, China, Malaysia and Japan. They've been used not only for detecting bacteria in seafood but also for food safety tests and for monitoring bacteria in coastal areas popular for water sports. Hundreds of laboratories have adopted these methods and they are widely accepted by international food safety authorities. They will probably become routine for ensuring that fish exports meet EU, US and Japanese import standards.

Project Ref: **PHF10:**
Topic: **5. Rural Development Boosters: Improved Marketing, Processing & Storage**
Lead Organisation: **Natural Resources Institute (NRI), UK**
Source: **Post Harvest Fisheries Programme**

Document Contents:

Description

PHF10

Research into Use
NR International
Park House
Bradbourne Lane
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UK

Geographical regions included:

Bangladesh, China, India, Malaysia,

Target Audiences for this content:

Fishers,

A. Description of the research output(s)

1. Working title of output or cluster of outputs.
In addition, you are free to suggest a shorter more imaginative working title/acronym of 20 words or less.

The development of a polymerase chain reaction (PCR) based method for the rapid and highly sensitive detection of aquatic *Vibrios*

Shorter title:

Sensitive polymerase chain reaction (PCR)-based detection of aquatic *Vibrios*

2. Name of relevant RNRRS Programme(s) commissioning supporting research and also indicate other funding sources, if applicable.

Post Harvest Fisheries Research Programme

3. Provide relevant R numbers (and/or programme development/dissemination reference numbers covering supporting research) along with the institutional partners (with individual contact persons (if appropriate)) involved in the project activities. As with the question above, this is primarily to allow for the legacy of the RNRRS to be acknowledged during the RIUP activities.

R5793

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4. Describe the RNRRS output or cluster of outputs being proposed and when was it produced? ***(max. 400 words)****. This requires a clear and concise description of the output(s) and the problem the output(s) aimed to address. Please incorporate and highlight (in bold) key words that would/could be used to select your output when held in a database.*

Pathogenic **Vibrio** bacteria are commonly found along inshore **coastal** and **estuarine** environments in **Asia** and can be isolated from water, sediment, plankton, fish and particularly **shellfish**. Shellfish concentrate a range of *Vibrio* spp. through their filter feeding and as a result **seafood** (e.g. **clams, cockles, oysters, lobsters, scallops, sardines, shrimp**) is commonly contaminated with vibrios. Human consumption of pathogenic vibrios usually gives symptoms ranging from mild **gastroenteritis** to severe debilitating **dysentery**, although for some vibrios such as *Vibrio* ***vulnificus****,* life-threatening septicaemia and eye, ear and **wound infections** can also occur especially in **immuno-compromised** individuals.

Gastro-intestinal infections are usually due to consumption of raw or semi-cooked shellfish, which is popular for several types of local Asian **food**s. Traditionally *Vibrio* spp. were detected using culture-based methods, employing enrichment media followed by isolation on selective culture media. Such culture-based methods suffer the disadvantages of being time-consuming (7 days for a positive identification) as well as cells of many *Vibrio* spp. entering a viable but non-culturable (VBNC) state. As a result in the mid 1990s, the **Post Harvest Fisheries Programme** funded research to develop much more rapid **polymerase chain reaction** (PCR)-based **diagnostic** techniques for pathogenic marine vibrios. PCR is a technique which relies on the amplification of a **nucleic-acid** fragment from short **DNA** sequences that have been identified as pathogen-specific. **PCR** techniques were developed for ***V. parahaemolyticus***, *V.* ***hollisae*** (reclassified as *Grimontia hollisae*) and *V.* ***cholera****e*.

These PCR-based techniques were shown in 1994-1996 under R5793 to have great potential for the quick and **sensitive detection** and diagnosis of pathogenic Vibrios in **seafood** in India. Since then, PCR-based techniques for detection and typing of *Vibrio* spp have been extensively validated not only within India, but also in many other countries (e.g. **Bangladesh, China, Malaysia, Japan**). PCR has been found to be useful for not only typing Vibrio infections, but also **real-time PCR** and alkaline-phosphatase **DNA probes** have been found to be rapid enumeration methods for evaluation of **postharvest treatments** to reduce *Vibrio* levels in seafood. PCR tools are also being used in selected laboratories worldwide for **food safety** testing, as well as monitoring the levels of pathogenic *Vibrio* spp. in coastal waters used for both recreational (e.g. water sports) and occupational purposes, such as **fishing**.

5. What is the type of output(s) being described here?
Please tick one or more of the following options.

Product	***Technology***	***Service***	***Process or Methodology***	***Policy***	***Other Please specify***
	x	*x*	*x*		

6. What is the main commodity (ies) upon which the output(s) focussed? Could this output be applied to other

commodities, if so, please comment

Seafood such as oysters, shrimp, eel, cockles, crab, clams, lobsters, scallops, sardines, and squid. The diagnostic tool outputs could be applied to other commodities that could have come into contact with contaminated seafood to assess these for the presence of pathogenic *Vibrio* spp.

7. What production system(s) does/could the output(s) focus upon?
Please tick one or more of the following options. Leave blank if not applicable

Semi-Arid	***High potential***	***Hillsides***	***Forest-Agriculture***	***Peri-urban***	***Land water***	***Tropical moist forest***	***Cross-cutting***
					x		*x*

8. What farming system(s) does the output(s) focus upon?
Please tick one or more of the following options (see Annex B for definitions).
Leave blank if not applicable

Smallholder rainfed humid	***Irrigated***	***Wetland rice based***	***Smallholder rainfed highland***	***Smallholder rainfed dry/cold***	***Dualistic***	***Coastal artisanal fishing***
						x

*9. How could value be added to the output or additional constraints faced by poor people addressed by clustering this output with research outputs from other sources (RNRRS and non RNRRS)? (**max. 300 words**).*

Please specify what other outputs your output(s) could be clustered. At this point you should make reference to the circulated list of RNRRS outputs for which proformas are currently being prepared.

The RNRRS output of project R5793 was to develop PCR techniques for *Vibrio parahaemolyticus*, *V. hollisae* and *V. cholerae*. Since the completion of these studies there have been 100s of reports from other labs using these and other PCR-based tools for detection of pathogenic *Vibrio* spp. Nucleic acid diagnostic techniques were in their infancy during R5793, and recently a range of highly sophisticated PCR-based techniques have been developed with which it would make sense to cluster outputs. These include:

- An Alkaline Phosphatase labelled probe for the detection and enumeration of trh+ *V. parahaemolyticus* in seafood by the same lab as carried out R5793 research (contact Dr Karunasagar).
- A multiplex Real-Time PCR Assay for detection of *Vibrio cholerae* (Dr Proll, Human Protection and Performance Division, Defence Science and Technology Organisation, Melbourne, Australia).
- A real-time PCR test for rapid and quantitative analysis of pathogenic *V. parahaemolyticus* (Kehe Huang lab, College of Veterinary Medicine, Nanjing Agricultural University, China).

With regard to RNRRS funded outputs, the *Vibrio* PCR tests developed would have potential value for a range of other outputs dealing with the food safety and market quality of seafood products, in particular

Aquaculture and Fish Genetics Research Programme
Promoting healthy peri-urban aquatic food supply (R8287; R8286; A11, A19; D03; D10) Prof. James Muir University of Stirling, UK)
Promoting networks for market quality(R8286, R8287, A11) Prof. James Muir University of Stirling, UK)

Crop Post Harvest Programme
Food safety - street foods (R7493, R8270, R8433, R8272, Mr K Tomlins and Dr A Graffham, Natural Resources Institute, UK)

Validation

B. Validation of the research output(s)

*10. **How** were the output(s) validated and **who** validated them?*

*Please provide brief description of method(s) used and consider application, replication, adaptation and/or adoption in the context of any partner organisation and user groups involved. In addressing the "who" component detail which group(s) did the validation e.g. end users, intermediary organisation, government department, aid organisation, private company etc... This section should also be used to detail, if applicable, to which social group, gender, income category the validation was applied and any increases in productivity observed during validation **(max. 500 words)**.*

The research outputs are sensitive nucleic-acid based diagnostic tests for the detection of a range of pathogenic *Vibrio* species. Scientific research under R5793 carried out by the Mangalore College of Fisheries (India) was used to validate these diagnostic tests and this was sufficiently rigorous to result in the following high impact peer reviewed publications.

Karunasagar, I; Sugumar, G; Karunasagar, I; Reilly, PJA (1993). Rapid detection of pathogenic marine vibrios by polymerase chain reaction (PCR). Int J Syst Evol Microbiol 53 (1993), 1615-1617

Karunasagar I, Sugumar G, Karunasagar I, Reilly A. (1995) Rapid detection of *Vibrio cholerae* contamination of seafood by polymerase chain reaction. Mol Mar Biol Biotechnol. 4:365-8

Karunasagar I, Sugumar G, Karunasagar I, Reilly PJ (1996) Rapid polymerase chain reaction method for detection of Kanagawa positive *Vibrio parahaemolyticus* in seafoods. Int J Food Microbiol. 31: 317-23

The scientific validation of the tests was done by adopting an approach of determining the sensitivity and specificity of the tests, together with ensuring they would work on seafood samples. Detection of a mere 10 cells of the highly pathogenic Kanagawa positive strains of *V. parahaemolyticus* was achieved using extracts prepared directly from fish homogenates and enrichment culture in alkaline peptone water. Similarly, *V. cholerae* contamination of seafood could be detected at 1000 *V. cholerae*/ml fish homogenate without enrichment and as few as 1-2 cells with prior enrichment in alkaline peptone water.

Since the completion of project R5793, there have been many research reports from the above, as well as other, laboratories using modifications of these and other PCR-based tools for detection of pathogenic vibrios.

Although validation was done on laboratory samples and did not result in any increases in productivity, the validation results would be most applicable to extreme vulnerable fisher communities as it is this income category that suffers most when consumer confidence in seafood safety is damaged.

*11. **Where and when** have the output(s) been validated?*
*Please indicate the places(s) and country(ies), any particular social group targeted and also indicate in which production system and farming system, using the options provided in questions 7 and 8 respectively, above **(max 300 words)**.*

The scientific research and validation under funding from the Post Harvest Fisheries Research Programme was carried out in 1993-1996 by Prof Iddya Karunasagar's research team at Mangalore College of Fisheries. However, nucleic acid diagnostic techniques were in their infancy during this project (R5793), and as a result the validations that were done were limited. In the past decade, DNA-based techniques have evolved and as a result a range of highly sophisticated similar PCR-based techniques have been developed for pathogenic vibrios not only in the above laboratory but also in institutes in the following RIUP target countries. The institutes (with active research contact names in brackets where available) include:

Bangladesh

- International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka-1212, Bangladesh (contact Drs N. Bhuiyan and G. Balakrish Nair)

China

- College of Veterinary Medicine, Nanjing Agricultural University (Dr Kehe Huang).
- Entry/Exit Inspection and Quarantine Bureau, Nanjing
- Key Laboratory of Subtropical Agro-ecology, Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Changsha, Hunan
- College of Biological Science and Technology, Shenyang Agricultural University, Shenyang (Dr Shuxia Lu)

India

- National Institute of Cholera and Enteric Diseases, Calcutta-700 010, India (Dr N. R. Chowdhury)
- Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore (Prof. I Karunasagar)

Malaysia

- Faculty of Food Science and Technology (Drs LM Bilung and S. Radu)
  Faculty of Veterinary Medicine (Dr A R Bahaman), and Faculty of Biotechnology and Biomolecular Sciences (Dr. R A Rahim and S Napis)
  University Putra Malaysia, 43400 UPM, Serdang, Selangor

- Department of Molecular and Cell Biology, Biotechnology Research Institute, Universiti Malaysia Sabah, Sabah, (Dr. CV Ling)

All validations target the coastal artisanal farming systems.

Current Situation

C. Current situation

12. ***How and by whom*** *are the outputs currently being used? Please give a brief description* ***(max. 250 words****).*

The diagnostic tests developed through project R 5793 have been superseded and no evidence has been found of their significant use. However, tests resulting from the improvement of R5793 tests, or of similar technologies, are being used by many research laboratories interested in food safety as well as clinical diagnostics.

International regulatory authorities, such as the United States Food and Drug Administration (USFDA) have constructed guidelines that state seafood should contain less than 10 000 cells of *V. parahaemolyticus* per gram. Sanitary and Phytosanitary Measures (SPS) established in 1994 under the Uruguay Round of GATT encourage member countries to use international standards, guidelines and recommendations where they exist.

Since the completion of R5793, laboratories in the Australia, Japan and the USA have been most active in assisting the RIUP target countries (see question 11) to improve their molecular diagnostic capability for pathogenic vibrio species.

PCR techniques for pathogenic vibrios are thus used to determine the presence of particular *Vibrio* genes in clinical samples, to monitor the spread of particular pandemics and to test the safety of retail and trade seafood samples. The diagnostic technologies also allow the effectiveness of appropriate hygiene measures to be measured and hence assist the development of measures to avoid infected seafood contaminating other foods through contact in retail outlets.
Recently the tests have also been proposed as valuable to assist the monitoring of the safety of particular estuarine waters for recreational as well as fishing purposes.

13. ***Where*** *are the outputs currently being used? As with Question 11 please indicate place(s) and countries where the outputs are being used* ***(max. 250 words).***

Modifications of the diagnostic tests developed are still being used actively by the Mangalore College of Fisheries (India). Detailed investigations on the prevalence and presence of particular genotypes of both *V. parahaemolyticus* and *V. vulnificus* in oysters from the southern Indian coast have been carried out using these tests (Environmental Microbiology (2005) 7, 995–1002). Recently, alkaline phosphatase probes have also been developed (research accepted for publication in Environmental Microbiology, 2006) to detect pathogenic vibrios in seafood. These probes have the advantage over PCR techniques in not being affected by the presence of PCR-

inhibitory compounds in seafood which can give rise to false-negative results.

The National Institute of Cholera and Enteric Diseases in Calcutta. India, is also using PCR tests to monitor the prevalence of the pandemic genotype of *V. parahaemolyticus*.

The International Centre for Diarrhoeal Disease Research in Dhaka, Bangladesh, has used PCR tests for *V. parahaemolyticus* to determine the presence of the particularly virulent pandemic genotype in hospitalised patients, as well as toxigenic *Vibrio cholerae* in the aquatic environment of Mathbaria (Alam *et al.,* (2006) Appl Environ Microbiol. 72: 2849–2855).

Various agricultural universities in China have been using PCR tests to demonstrate that retail seafood samples are commonly contaminated with *V. parahaemolyticus* (FEMS Immunol Med Microbiol 46 (2006) 180–186)

In Malaysia, universities (see 11) have used PCR-based tests to highlight estuarine waters that may be unsafe for recreational purposes. PCR tests have also shown cockle samples to contain high levels of infection (62%) highlighting the risk of consumption of such seafood in an undercooked state (Bilung *et al*., 2005. FEMS Microbiology Letters 252: 85–88).

14. What is the scale of current use? Indicating how quickly use was established and whether usage is still spreading ***(max 250 words).***

It has not been possible to obtain an accurate estimation of the current usage as the little data is available other than that published in scientific reports. The increased prevalence of scientific papers outlining the use of the techniques for clinical diagnoses and shellfish safety analysis in 2005-2006 suggests that PCR tests for pathogenic vibrios are becoming increasingly popular for detection, enumeration and typing of *Vibrio* spp. Of relevance here is that international regulatory authorities such as the USFDA now accept the application of nucleic acid tests such as PCR and probe hybridisation tests for food safety analysis.
Considering that these nucleic acid tests are rapid, highly sensitive and specific, it appears probable that they will become increasingly popular for routine analyses in food quality control laboratories particularly to ensure that fish products for exports meet EU, US and Japanese import regulations.

Regulations in the EU have tightened particularly in light of the first European incidence of gastroenteritis due to *V. parahaemolyticus*, derived from the consumption of live oysters in Spain, being reported in 2003. There will be an increasing requirement for the rapid and accurate detection of pathogenic vibrios against a background of numerically greater numbers of non-pathogenic vibrios and other heterotrophic bacteria to prevent the emergence of seafood-associated disease outbreaks. The non-radioactively labelled probes developed by the R5793 partner (post project funding) offer a very promising technique for such diagnostic purposes as they are less sensitive to the presence of a range of compounds present in shellfish than some of the PCR techniques.

15. In your experience what programmes, platforms, policy, institutional structures exist that have assisted with the promotion and/or adoption of the output(s) proposed here and in terms of capacity strengthening what do you see as the key facts of success? ***(max 350 words).***

Promotional activities for the diagnostic outputs outlined do not appear to be taking place. However, International

regulatory authorities such as the USFDA have indirectly assisted with the promotion of the outputs by recommending the use of non-radioactively labelled DNA probes for the enumeration of pathogenic *V. parahaemolyticus* to test whether shellfish to be certified meets food safety requirements.

The new EU General Food Law which became effective in 2006 is also resulting in EU importing countries tightening their food safety legislation and demanding the adoption by exporting countries of agreed inspection, examination and certification procedures.

Environmental Impact

H. Environmental impact

24. What are the direct and indirect environmental benefits related to the output(s) and their outcome(s)? ***(max 300 words)***

This could include direct benefits from the application of the technology or policy action with local governments or multinational agencies to create environmentally sound policies or programmes. Any supporting and appropriate evidence can be provided in the form of an annex.

The adoption of DNA-based diagnostic technologies assist the improvement of food quality control laboratories and the ability to meet international trade requirements regarding food safety.

25. Are there any adverse environmental impacts related to the output(s) and their outcome(s)? ***(max 100 words)***

The DNA based diagnostic tests involve the use of carcinogenic and mutagenic compounds that need to be disposed of correctly to ensure that there are no adverse environmental impacts. Certified waste disposal systems are sometimes lacking in developing countries.

26. Do the outputs increase the capacity of poor people to cope with the effects of climate change, reduce the risks of natural disasters and increase their resilience? ***(max 200 words)***

The outputs can be used as monitoring tools to determine the changed distribution of marine Vibrios due to climate change. For example, *V. parahaemolyticus* is widespread along marine coastal waters globally, with higher numbers usually encountered during warmer summer months. A slight increase in the temperature of waters due to global warming is likely to increase the incidence of Vibrio pandemics.

Annex

Annex 1. EU fish import bans: the case of Uganda

Between 1996 and 2000, the EU imposed three export bans of fish from Uganda for a number of reasons. In 1997, Spain and Italy rejected importation of fish originating from Uganda because they detected salmonella species in the imported products. In December 1997, when an EU Veterinary Inspection Mission was visiting Uganda, an outbreak of cholera was reported at some landing sites or beaches around Lake Victoria. A partial ban stopping the export of fresh-chilled fish products from Uganda was imposed. Early in 1998, suspected incidences of fish poisoning were reported in Lake Victoria and the Uganda government imposed a temporary ban on fish exports and the decision was communicated to the EU. Despite Ugandan efforts to put in place a monitoring system to ensure that no poisoned fish ended up in the market, the EU decided to impose a ban on imports of fish originating from Lake Victoria.

Fisheries are one of the main economic sectors in Uganda. Currently, fish exports (predominantly Nile Perch) is competing with coffee for the number one position in foreign exchange earnings. Ugandan export earnings from fisheries have increased significantly over the past decade from US$ 1.4 mn in 1990 to almost US$40 mn in 1998 and to over US$ 100 mn in 2002. According to several studies, the Uganda fish export bans resulted in losses of over US $30 mn. For example, UNIDO (2003) estimates that the ban of April to August 1999 alone resulted in a loss of US$36.9 million. It further estimated the loss to fishing communities in the form of reduced prices and less fishing activity at US$4.25 mn. In addition it is estimated that out of over 100,000 people who were directly employed in the fisheries sector, 32,000 people lost their jobs as a result of the ban while others earned less than one third of their average income. It is also estimated that over 300,000 people from families directly depending on fishing as a household activity were affected.

During the whole period of the ban (1997-2000), there were 11 operating fish factories in Uganda. The fish ban resulted into the closure of 3 of the 11 factories while the remaining ones had to operate at less than 20% capacity. This also resulted into factories laying off 60% to 70% of their labour force. Other auxiliary industries such as packing, the fishnet manufactures, the transport industry, the fuel industry and Uganda's economy in general were directly affected and all the people involved suffered the direct consequences of the EU fish export ban

Considerable efforts and a variety of measures were made by Uganda to comply with international fish trade requirements; it is estimated to have increased the operating costs of fish processing plants by 50%. In addition, costs were incurred as a result of efforts to streamline the fish inspection services and the capacity of the Department of Fisheries as the ‘Competent Authority’ (e.g. training of inspectors, provision of equipment, and introduction of a fish inspection manual).

Thus, the EU Nile perch export bans from Uganda represented major shocks for export the

sector. In the short-term this led to significant loss of foreign exchange earnings, bankruptcies and unemployment, However, in the medium- to long-term, the sector appears to have recovered well, with a smaller but better equipped processing sector, improved marketing strategy, and strengthened institutions. This case study clearly demonstrates the resilience of developing countries in the face of such measures. Nevertheless, despite the notably “post-ban” recovery, there is little doubt that there are also long-term losers, perhaps through increased polarization, and particularly related to the poor and vulnerable although little information exists on the extent of this problem.