Final FAO/IAEA Research Co-ordination Meeting (RCM) of the Co-ordinated Research Project on Determination of Profiles of Human Bacterial Pathogens in Foods for Export by Introduction of Quality-Assured Microbiological Assays

Mexico, 22-26 July 2002

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INTRODUCTION

International trade in food and agricultural commodities is governed by Agreements of the World Trade Organization (WTO). With respect to food safety matters, relevant provisions of the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) apply. The overall objective of the SPS Agreement is to permit countries to take legitimate measures to protect the life and health of their consumers (in relation to food safety matters), while prohibiting them from using those measures in a way that unjustifiably restricts trade.

The SPS Agreement calls on members to harmonize sanitary and phytosanitary measures on as wide a basis as possible and on the basis of international standards, guidelines and recommendation when they exist. With regard to human health and food safety, sanitary measures which conform to standards, guidelines and recommendations of the Codex Alimentarius Commission are presumed to be consistent with the provision of the SPS Agreement. In spite of the existence of this SPS Agreement a proportion of food exports are still rejected by importing countries on the grounds of unacceptable contamination, such measures sometimes are neither scientifically based nor statistically sound and may be an impediment to fair trade.

The SPS Agreement recognizes the right of members of WTO to protect their consumers at a level they consider necessary by applying measures which result in a higher level of protection if there is a scientific justification.

To attain the SPS objectives, it is necessary to carry out several important activities such as:

- The collection of data on the major microbiological contaminations in food implicated in rejection of food shipments in international trade and their economic impact.

- Use of analytical methods in food microbiology (conventional and rapid methods) certified by national or international bodies and analyze the possibility for harmonization of such methods.

- To improve the quality of the reference laboratories, especially in developing countries.

- Training of the personnel especially from the developing countries in the reference laboratories.

Some of these activities can be supported by the FAO/IAEA Joint Division of Nuclear Techniques in Food and Agriculture and its Training and Reference Center for Food and Pesticide Control under its mandate “to assist Member States and their institutions to
fulfill requirements to support the implementation of international standards/agreement relevant to food safety and control”.

In 1997, a FAO/IAEA consultants meeting on microbiological contamination of food was held in the IAEA Headquarter in Vienna, Austria (18-22 August). As a result of the discussions a plan of action was recommended. This Action Plan included four types of activities i) Training of regulators and processors in HACCP-based food production ii) Enhancement of laboratory activities for specific purposes iii) Compilation of microbiological methods and iv) Research elements. Among the research activities 2 research projects were identified, one of them being the Coordinated Research Project (CRP) on Determination of profiles of human bacterial pathogens in foods for export by the introduction of quality assured microbiological assays. This CRP started in 1997 with 15 participants countries.

OBJECTIVES

The Overall Objectives of this CRP was to assist national food control authorities and institutions to improve food safety and stimulate international trade in foods by determining profiles of (selected) human bacterial pathogens of concern to importers on (selected) raw materials and/or products, thereby increasing assurance in their food control measures and facilitating international trade. Food that are microbiologically safe would be identified.

THE MEETING

The Final Research Co-ordination Meeting on the determination of profiles of human bacterial pathogens in foods for export by the introduction of quality assured microbiological assays was held at the Facultad de Medicina Veterinaria y Zootecnia, de la Universidad Nacional Autonoma de Mexico (UNAM) from 22-26 July 2002.

It was attended by 11 participants who were research agreement and contract holders of this CRP and 2 observers (Annex 1). Dr. Luis Alberto Zarco, Director of the Facultad de Medicina Veterinaria and Dr. Fernando Nunez, the national counterpart and participant in this CRP, welcomed the participants and indicated the importance of this Research Project. On behalf of the Directors General of FAO and IAEA, Dr. Tatiana Rubio, Scientific Secretary to the CRP and representative of FAO/IAEA, expressed her appreciation for the organization of the meeting to UNAM through the Facultad de Medicina Veterinaria y Zootecnia for accepting to host the Research Co-ordination Meeting.

Dr. Tatiana Rubio was the chairperson of the meeting and Dr. Ken Newton was invited to serve as the rapporteur. The programme of the meeting is attached as Annex 2.
During the opening activity of the programme, Dr. Newton provided information about the laboratory complying with ISO 17025 and the importance of laboratory quality control system. Subsequently, the participants gave a complete report on their research findings, particularly for the period after the second RCM in Bogor, Indonesia, in November 2000.
Compilation of Microbiological Testing Results for Human Pathogens and Indicator Bacteria in Foods Imported by Australia during Recent Years.

Dr. Ken Newton
Australian Government Analytical Laboratories

Analytical data for certain foods imported into Australia during the calendar years 1999 to 2001 has been collated and is summarized below. A total of 302 seafood samples was tested for *Vibrio cholerae* and 955 samples were tested for staphylococcal enterotoxin. No positive results were obtained.

<table>
<thead>
<tr>
<th>prawns/lobsters</th>
<th>10-100</th>
<th>E2-3</th>
<th>E3-4</th>
<th>E4-5</th>
<th>E5-6</th>
<th>&gt;E6</th>
<th>Total</th>
<th>E. coli</th>
<th>Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan99-Dec99</td>
<td>29</td>
<td>23.5</td>
<td>37.3</td>
<td>30.6</td>
<td>4.5</td>
<td>1.2</td>
<td>510</td>
<td>475 1+ve</td>
<td>470 8+ve</td>
</tr>
<tr>
<td>Jan00-Dec00</td>
<td>6.3</td>
<td>23.4</td>
<td>39.2</td>
<td>23.2</td>
<td>6.1</td>
<td>1.8</td>
<td>380</td>
<td>60 2+ve</td>
<td>60 0+ve</td>
</tr>
<tr>
<td>Jan01-Dec01</td>
<td>8.1</td>
<td>26.5</td>
<td>44.9</td>
<td>17.7</td>
<td>1.6</td>
<td>1.3</td>
<td>385</td>
<td>385 0+ve</td>
<td>375 1+ve</td>
</tr>
</tbody>
</table>

SPC results >E6 exceed the relevant standard.

PEPPER
Jan99-Dec99 123 samples 7+ve
Jan00-Dec01 139 samples 2+ve in each year

PAPRIKA
Jan99-Dec99 48 samples 1+ve
Jan00-Dec01 43 samples 0+ve each year

COCONUT
Jan99-Dec99 26 samples 2+ve
Jan00-Dec00 24 samples 0+ve
Jan01-Dec01 39 samples 1+ve

The number of prawn and lobster samples failing to comply with the relevant standard on plate count over the three years reported here remained at the low level achieved in 1998. Eight prawn and lobster samples were *Salmonella* positive in 1999 (1.7% of samples tested) <1% were positive in 2000 and 2001. The figures suggest an improvement after the 1998/99 rise in detections. In pepper, paprika and coconut, *Salmonella* continues to be an intermittent low level problem as was observed for 95-98.
Completion of the project seems an appropriate time to prepare the results of the collation for publication.

**STUDIES ON METHODS FOR DETECTION OF SALMONELLA SP. IN MEAT WITH REGARD TO EQUIVALENCY AND COMPATIBILITY 10466**

P. Paulsen, F. J. M. Smulders
Institute for Meat Hygiene, Meat Technology and Food Science, University for Veterinary Medicine Vienna,

*Salmonella* is still the most important infective agent of foodborne disease with known epidemiology in Austria. However, in some provinces the number of reported cases of campylobacteriosis is going to exceed that of salmonellosis, which is in accordance with a European trend. Antibiotic resistance has been at a constant level the last years. There is still a need for improved surveillance of (food borne) salmonellosis in Austria. The foundation of effective surveillance include regularly updated information on the epidemiology of a given pathogen in different countries, and the quality of the diagnostic methods employed to generate data. So, this research project aimed at compiling currently used procedures for detection of *Salmonella* in meat and, where feasible to generate data on (promising) procedures.

In 2001, studies on the use and mode of action of DIASALM medium were completed. The major findings were: (a) In contrast to its addition to MSRV medium, the addition of Novobiocine to DIASALM (factory recommendation: 10 mg/l) was found useful. (b) The mode of action of DIASALM medium was tabulated.

The second subtask was to compile methods for detection of *Salmonella* in foods (protocols were taken from scientific or technical publications and from national or international standards).

The series of studies undertaken demonstrate the effectiveness of motility media of detection of *Salmonella* in poultry meat. However, these media have to be used by skilled personnel. Automated EIA system offer both a high level of quality assurance and good agreement of results with motility media and ISO 6579 method. Contamination on poultry is common both in domestic and imported poultry, with a prevalence of above 30 %. Notably, no difference between imported and domestic poultry could be detected. The *Salmonella* load per positive sample is usually small, with 10 – 10000 cfu / carcass. However, it is probable
that cross contamination or improper handling of raw poultry meat in kitchens may account for food borne diseases.

The results obtained allow the recommendation, that motility media offer a rapid and cheap alternative to solid selective media for *Salmonella* detection. Improved *Salmonella* testing of raw poultry meat may contribute to reduction of foodborne salmonellosis, by allowing the design of improved preventive measures at the pre-harvest stage and education of the consumer is also necessary for more effective safe handling procedures in the kitchen.

**PRESENCE OF SALMONELLA SPP AND E. COLI O 157:H7 IN RAW MEAT IN S. PAULO CITY - BRAZIL, AND EVALUATION OF LOW TEMPERATURE (REFRIGERATION AND FREEZING) RESISTANCE OF THESE BACTERIA**

Instituto Adolfo Lutz - Public Health Laboratory -

The incidence of *Salmonella* spp and *E. coli* O 157:H7 was determined 256 samples of raw meat (bovine, swine and poultry) in S. Paulo city.

The survival pattern of *Salmonella enteritidis* (the predominant serotype) and *E. coli* O 157:H7 at different times after refrigeration and freezing in ground beef was also evaluated.

From 256 samples, 9% (23 samples) were positive for *Salmonella*. The serotype most frequently isolated was *S. enteritidis*. (8 samples). One strain of *S. typhimurium* and one of strain of *S. emek* were lysine decarboxilase negative - they may be misdiagnosed as *Citrobacter* sp.by inexperience analyst during biochemical typing. All samples were free of *E. coli* O 157:H7. *S. enteritidis* in ground beef was not influenced by refrigeration, but it was reduced in numbers by freezing temperature. *E. coli* O 157:H7 was sensitive to refrigeration and freezing temperatures.
DETECTION OF PATHOGEN BACTERIA IN FOODS FOR EXPORT AND THEIR RAW MATERIAL

Instituto de Salud Publica

84 frozen vegetable samples were analyzed; 48 of final product and 36 raw material. The samples were obtained from 2 companies that produce frozen vegetables for exportation.

The following microbiological analysis were carried out: Enumeration of mesophilic aerobes bacteria, Bacillus cereus, Clostridium perfringens and Escherichia .coli (MPN) and the isolation of Salmonella and Listeria monocytogenes.

One company use raw material with a high number of mesophilic aerobes bacteria. Nevertheless the final product was of good quality because the company applied a special treatment to reduce the bacterial load in the raw material.

In the second company, the raw material was of a good sanitary quality because this company had a very good environmental management of production including a good bacteriological quality of irrigable water and good protection against contamination by animals and people. The harvest was mechanical with good hygienic control. The process included a blanching (heating 95 – 99°C for 3 – 5 minutes), to inactivate the enzymes, which also reduced microbial load 1 or 2 fold. Bacterial growth in products before freezing was rare because the short time elapsed between blanching and freezing. The good bacteriological quality of the final product resulted from the good technological process which controlled and reduced the bacterial contamination.

MICROBIOLOGICAL QUALITY OF SOME MAJOR FISHERY PRODUCTS EXPORTED FROM INDIA

Food Technology Division, Bhabha Atomic Research Centre

The export quality marine and aquaculture fish and fishery products were collected from European Union Approved (EUA) and EU-non-approved (EUN) plants located at east and west coast of India and were analyzed for the presence of human bacterial pathogens
using standard bacteriological techniques. A total of 60 samples comprising of 18 freshwater prawn, scampi (*Macrobrachium rosenbergii*), 6 cuttle fish (*Sepia* sp.), 30 rohu (*Lobia rohita*) and 6 long fin herring (*Citrocentrus* sp.) were analyzed. The samples were screened for aerobic plate count (APC) and pathogens including *Salmonella* sp., *Vibrio cholerae*, *V. parahaemolyticus*, *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, and *Yersinia enterocolitica*. It was observed that the marine products from EUN were of poorer microbiological quality as compared with products from EUA plants. *Salmonella* contamination was observed in 16.7% of the cuttle fish samples from EUN plants. Whole herring samples were of acceptable microbiological quality. Of the freshwater items analyzed, whole rohu samples had higher microbial load as compared to processed rohu samples. All the rohu samples were free from the pathogens, however, 25% of the rohu steak samples had *E. coli* exceeding the limit of 20 cfu/g. Both whole as well as headless scampi harboured higher microbial load; 50% of the whole and 41% of the headless scampi samples were contaminated with *Salmonella* sp. The results suggested need for implementation of better hygienic practices for the improvement of microbial quality of products from EUN plants. In addition, it is necessary to adopt stringent hygienic practices for production of good quality aquacultured fishery items.


The fishery samples from EUA plants were of better microbiological quality as compared to those from EUN plants. The whole scampi (50%), headless scampi(41%) and cuttle fish (16.7%) samples from EUN plants were contaminated with *Salmonella*. There is a urgent need to improve the hygienic conditions in the processing plants as well as to follow good aquaculture practices.

**DETERMINATION OF CONTAMINATION PROFILES OF HUMAN BACTERIAL PATHOGENS IN SHRIMPS OBTAINED FROM JAVA, INDONESIA**

R. Dewanti-Hariyadi¹,², Suliantari², and L. Nuraida²

¹ Center For Assessment Of Traditional Foods, Bogor Agricultural University,
² Department of Food Technology and Human Nutrition, Faculty of Agricultural Technology, Bogor Agricultural University
Shrimp continues to be an important export commodity for Indonesia and contributed significantly to the country’s revenue. However, shrimp export has been frequently rejected due to filth, *Salmonella* and insanitary condition. This study was conducted to evaluate the profiles of bacterial contaminant in the shrimp during the production of frozen shrimp. Aqua cultured black tiger shrimp samples were obtained at six sampling points during frozen shrimp production i.e. (1) during receiving, (2) after head removal, (3) after sizing and grading, (4) after final rinsing in water containing 30 ppm chlorine (5) after arrangement and water filling and (6) after freezing. Samples were analyzed according to the Bacteriological Analytical Method (AOAC, 1995) for total plate counts, Staphylococcal counts, *Escherichia coli* counts, *Salmonella*, *Vibrio cholerae* and *V. parahaemolyticus*. The microbiological analysis on the samples revealed that processing reduced the number of total bacterial, *E. coli*, and staphylococcal counts to log CFU/g of 5.4, 0-<2, and 1.3, respectively thus meeting the standard for frozen shrimp. However, processing did not effectively reduce the number of samples positive for *Salmonella* or *V. parahaemolyticus*. The number of samples positive for *Listeria* increased during processing, while *V. cholerae* was not isolated in any of the samples.

Contamination by bacteria including pathogen in shrimp has occurred in the raw material which is in agreement with our previous study. Sizing and grading as well as arranging of shrimp before freezing were considered as the critical points where bacteria should be controlled to inhibit growth and cross contamination. Inadequacy of the process to significantly reduce *Salmonella* and *V. parahaemolyticus* may have been caused by inadequate control of the use of chlorine.

Implementation of Good Agricultural Practices in production of shrimp as well as Hazard Analysis Critical Control Point at the line processing is expected to improve the quality of fresh and frozen shrimps.

**DETERMINATION OF PROFILES OF FOOD PATHOGENS IN IMPORTED SEAFOOD IN KOREA**

ChangMin Kim, GunJo Woo, DongHa Lee, YunSook Kang and JongSuk Park
Korea Food and Drug Administration

In this study, major foodborne pathogenic bacteria such as *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Escherichia coli*, and *E. coli* O157:H7 were monitored against various seafood products. A total of 347 samples
including smoked salmon, tuna, frozen shrimp, small octopus, frozen cod and pollack flesh, and jelly fish were obtained from retail markets. Both qualitative and quantitative tests were conducted at first then positive isolates were characterized through biochemical or molecular methods. The overall of target bacteria was 16.1% (56 out of 347), which was considered fairly high. Among the tested microorganisms, *S. aureus* was the most frequently detected. It was isolated from 42 samples (12.1%) in which 29 were from frozen shrimp, 7 from tuna, 4 from smoked salmon and 2 from small octopus. In the enterotoxin test on *S. aureus* isolates, 57.1% was type A, whereas no type B, C or D was detected. Seven smoked salmon were contaminated with *L. monocytogenes* and it could be a threat to public health. Four smoked salmon and 3 frozen pollack were contaminated with *E. coli*. The quantification test for all positive samples showed the average contamination was less than 100cfu/g. In the comparison of prevalence rate by importing countries, products from China were the most contaminated with a positive rate of 39.1%. Products from Thailand were the least contaminated. *V. parahaemolyticus* was not detected at all. The reason might be due to the temperature tolerance characteristics of *V. parahaemolyticus*, which is able to grow between 10 and 40°C. Since all products tested in this study were frozen, there might be no or slight chance of survival or growth of contaminating *V. parahaemolyticus*.

From the results of this study, it can be concluded that the sanitation quality of imported seafood products, especially in raw state is quite poor. The regulatory agencies such as KFDA (Korea Food and Drug Administration) or Ministry of Maritime Affairs and Fisheries should put more attention to the sanitation quality of imported seafood products and try to find out solutions to improve it such as surveillance programme reinforcement and/or requirement to exporting countries for maintaining better sanitation quality for their products. Also, consumer education programme for the prevention of food poisoning occurrence by seafood especially in summer season is important and should be implemented.

**DETECTION OF Salmonella sp, Shigella flexneri and sonnei and Vibrio cholerae O1 BY POLIMERASE CHAIN REACTION (PCR) IN EXPORTED SHRIMP FROM THE MEXICAN NORTHEAST COAST.**

Fernando Núñez Espinosa
Universidad Nacional Autónoma de México, Facultad de Medicina Veterinaria y Zootecnia

The objective of the present work was to use the PCR technique for the simultaneous detection of *Salmonella sp, Shigella* and *Vibrio cholerae* O1 in frozen shrimp for export. The DNA segments located in the gene A [284 pares of bases (pb)] from *Salmonella sp; locus
ial (217 and 320 pb) from Shigella flexneri and sonnei and the gene ctxA and ctxB (777 pb) from V. cholerae O1 were amplified. The different primers that amplify these segments were assayed in a PCR reaction for the simultaneous detection of DNA from the microorganisms. However, it was not possible to amplify the gene of S. sonnei and S. flexneri under the assay’s conditions employed. Those from Salmonella sp and Vibrio cholerae O1 were successfully amplified.

The amplification conditions for the PCR were: 94°C, 58°C and 72°C during 30 cycles, PCR allowed a reduction from 15 days with the official microbiological methods to 28 hours (24 for the pre-enrichment and 4 for the PCR).

Samples of raw-frozen-headless shrimps were taken from production plants located in the State of Sinaloa, Mexico. A random sampling procedure was used, according to the guidelines described by the International Commission of Microbiological Specifications for Foods (ICMSF, 1999). Five packages per lot per production plant were obtained. From each individual package (5 pounds 80 oz ~ 2.27 kg) three samples were taken for the bacteriological and PCR assays to search for Salmonella sp and Vibrio cholerae O1, respectively. Results showed that none of the samples was positive by PCR or bacteriological testing to any of the studied bacteria. However, bacteriological testing identified other Vibrio species Proteus and Acromobacter. These results confirmed PCR’s rapidity, sensitivity and specificity.

Unfortunately, during the year 2000, harvest shrimp plants stop collaborating with this project. Therefore we were not able to sample plants in Mérida, Yucatán, México.

DETERMINATION OF PROFILES OF HUMAN BACTERIA PATHOGENS IN NIGERIAN FISH AND SEAFOOD FOR EXPORT

Falana A.A; Mainasara O. N.; Ubiaru M. E; Udegbunaam C.N.N; Adesanlu A; Babatunde A; Uwamadi C. U; Uzegbu G; Adewusi E; Ibrahim H; Ezejimofoor M. C; and Ochogu C.

National Agency for Food and Drug Administration and Control

Fish and seafood export to the European Union market has increased to about tenfold with reference to revenue from 1994 to 2001. The need to control and guarantee the safety of the exported seafood products with reference to bacterial contamination and its potential for poisoning can not be over-emphasized.
The prevalence of *Vibrio cholerae*, *V. parahaemolyticus*, *Listeria monocytogenes* and *Staphylococcus aureus* was determined in 838 frozen and packaged seafood samples destined for the European market. The sample comprised of 49 Cuttle Fish, 55 Crabs, 31 Fillets and 703 Shrimps.

The methods employed for the study was as stated in the Bacteriological Analytical Manual (BAM).

Majority of the samples were received from the months of April to December which are generally referred to as the rainy or wet seasons. None of the samples was found unsatisfactory for *S. aureus*. The range of counts obtained was: Cuttle Fish 11-75 cfu/g; Crab 9-43 cfu/g; Fillet 3-21 cfu/g and Shrimps <3-21 cfu/g.

*L. monocytogenes* and *V. cholerae* were not detected in any of the samples. *V. parahaemolyticus* was isolated from some of the samples and the range of colonies obtained was as follows; Cuttle Fish <3-15 cfu/g, Crab <3-23 cfu/g, Fillet <3-9 cfu/g and Shrimps <3-43 cfu/g.

From the above results, it can be inferred that the absence of *L. monocytogenes* and *Vibrio cholerae* in all the samples was due to the implementation and strict adherence to safety measures such as Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Procedures (HACCP) being enforced on the exporters by the European Union designated Competent Authority. The low incidence of *V. parahaemolyticus* and *S. aureus* can be attributed to these same controlling factors.

**HUMAN BACTERIAL PATHOGENS IN EXPORTED FOODS AND EVALUATION OF METHODS OF ANALYSIS**

Alicia Lustre, Juanita Ramos, Rachel Elano, Clarine Co and Zoraida Manalastas
National Food Authority, Food Development Centre

Phase I consisted of comparison of pond prawns, frozen prawns, and imported frozen fish fillet for detection of *Salmonella* using six available rapid methods vs conventional cultural method. The best rapid method is S2 which was based on lateral immunoprecipitation.

In Phase II *Salmonella* was monitored in 120 samples of prawns, catfish, milkfish, and tilapia from ponds. Tecra Visual Immuno Assay (VIA) for *Salmonella* had 99% agreement with the BAM/AOAC method.
During Phase III *Salmonella* was monitored further in 83 samples of aquaculture prawns and milkfish. Tecra VIA for *Salmonella* remained number 1 in accuracy with 99% agreement with the BAM/AOAC method. Further, conventional selective plating media and three innovative agars were compared for sensitivity in growing characteristic *Salmonella* colonies based on morphology. Hektoen Enteric Agar, Xylose Lysine Decarboxylase Agar and Chrom Agar Salmonella SA ref 130 were the most sensitive agars amongst the six agar media tested.

**DETERMINATION OF PROFILES OF SALMONELLA AND PATHOGENIC VIBRIO SPP. IN BLACK TIGER SHRIMP FOR EXPORT BY INTRODUCTION OF QUALITY ASSURED MICROBIOLOGICAL ASSAYS**

Fishery Technological Development Institute, Department of Fisheries,

An survey of *Salmonella* and pathogenic *Vibrio* spp. in samples of aquaculture black tiger shrimp, canal water supplying the ponds (before treatment), pond water, shrimp feed, and fresh and frozen shrimp was undertaken.

*Salmonella* was found in 3/35 samples of canal water (8.57%), and 1/57 samples of pond water (1.75%). *Salmonella* was not detected in 35 feed samples collected at farms (0%). *Salmonella* was found in 3/57 samples of fresh shrimp collected at farms (5.26%), and was found in 31/99 samples collected at wholesale shrimp markets (31.31%). In frozen shrimp destined for export sample from processors, *Salmonella* was not found in 118 samples.

*Vibrio parahaemolyticus* was found in samples of canal water, pond water, fresh black tiger shrimp collected at farms, fresh black tiger shrimp collected at wholesale shrimp markets and frozen black tiger shrimp destined for export at levels 2.3%(2/86), 5.3%(7/131), 14.3%(18/126), 48 %(60/125) and 0.2% (1/468) respectively. *V. cholerae* non 01 was only found in 1 sample of water from culture pond of 131 tested (0.8%).
MPN of *V. parahaemolyticus* in various samples is shown as follow:

<table>
<thead>
<tr>
<th>Samples for MPN of <em>V. parahaemolyticus</em></th>
<th>No. of sample</th>
<th>&lt;0.3 (No.)</th>
<th>0.3 - &lt;3 (No.)</th>
<th>&lt;3 (No.)</th>
<th>3-10 (No.)</th>
<th>10-100 (No.)</th>
<th>100-10000 (No.)</th>
<th>&gt;10000 (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canal water which supplied culture pond</td>
<td>81</td>
<td>79 (97.5)</td>
<td>1 (1.2)</td>
<td>-</td>
<td>1 (1.2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Water from culture pond</td>
<td>119</td>
<td>114 (95.8)</td>
<td>4 (3.4)</td>
<td>-</td>
<td>-</td>
<td>1 (0.8)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fresh black tiger shrimp from culture pond</td>
<td>115</td>
<td>-</td>
<td>-</td>
<td>99 (86.1)</td>
<td>4 (3.5)</td>
<td>8 (6.9)</td>
<td>4 (3.5)</td>
<td>-</td>
</tr>
<tr>
<td>Fresh black tiger shrimp from wholesale shrimp market</td>
<td>122</td>
<td>-</td>
<td>-</td>
<td>76 (62.3)</td>
<td>21 (17.2)</td>
<td>14 (11.5)</td>
<td>10 (8.2)</td>
<td>1 (0.8)</td>
</tr>
</tbody>
</table>

It was found that 2.67% (7/262) isolates of *V. parahaemolyticus* were TDH (KP+) and 1.15 % (3/262) were TRH (urease reaction) positive.

A very high percentage of fresh black tiger shrimp collected from wholesale shrimp markets was found to be contaminated with *Salmonella* and *V. parahaemolyticus*.

Good handling practices and HACCP system for shrimp distributors/producers should be applied e.g. appropriate temperature control during handling and transportation. Shrimp should be maintained at not higher than 5 ºC. Improper refrigeration of shrimp will allow proliferation of the microorganisms which increases the possibility of infection of consumers.
ACHIEVEMENTS OF CRP

The participants were grouped into a seafood group and a miscellaneous group in order to discuss the results obtained during the development of this CRP.

Group 1: miscellaneous

Australia, Austria, Brazil, Chile

Seasonal variations:

No seasonal variations in the profiles of pathogenic bacteria in meat were reported in this working group. For vegetables, a higher aerobic total plate count was observed in summer than in winter (Chile).

Methodology

Most participants used standardized methods for the detection of *Salmonella* (APHA, BAM). The participant from Austria employed MSRV medium, an in-house method which was periodically validated against ISO 6579 modifications (1990, 1993). For *E. coli* O157, an APHA procedure was applied.

Customers specifications

The specifications for a given type of food vary with the importing country. So, for *Listeria monocytogenes* in smoked fish, U.S., Australia and Korea require the absence in 25 g, and the E.U. requires < 100 cfu/g.

Accreditation

Brazil is progressing and expects accreditation in 1 –2 years. This is a public health laboratory. They currently participate in Food and Environmental Proficiency Assessment Scheme (FEPAS). Chile has obtained US Food and Drug Administration (FDA) certification for some tests this year.

Australia has national accreditation (ISO 17025) for their microbiology laboratories.

Impact

Some of the results may be politically sensitive.

Results from studies conducted in South American countries reveal a very low incidence for *E. coli O157* in meat.
Training activities were carried out by the Australian and Austrian partners for colleagues from Nigeria, Ethiopia and Egypt.

**Results collation**

This groups is composed of various, non-seafood commodity-oriented projects. Therefore, the collation of results is hardly possible. So, in a general view, *Salmonella* remains the main pathogen of concern, while the prevalence of *L. monocytogenes*, *E. coli* O157 and *Staphylococcus aureus* is of less international importance. It was observed, that the serotypes of *Salmonella* isolated in frozen shrimp imported from Thailand to Australia matched with the ones found in aquaculture waters and shrimp in Thailand. Further transnational cooperation is suggested to identify the *Salmonella* reservoirs and mode of transmission to food supplies.

**Problems encountered**

Variation in specifications of customer countries are counteracting the intentions to harmonize international trade. The same holds true for the harmonization of testing methodology.

**Working Group II : Seafood**

India, Indonesia, Nigeria, Korea, Mexico, Philippines and Thailand

**Seasonal Variation**

During the last period of work, no study was carried out to determine the effect of seasonal variation of sampling on the microbiological quality and the prevalence rate of the various bacterial pathogens. In Nigeria, samples were mostly collected between April-December which is rainy season. In India samples were collected between January-April and September-December (dry season) During monsoon months (June - September) no fishing is allowed by law, thus no samples can be obtained. Participants observed that in the past two years, climate has been unpredictable thus it was difficult to correlate the results of the study with seasonal variation.

**Methodology**

Most of the microbiological assays conducted for the studies were according to the BAM FDA methods. In addition, ISO, Canada PHLS were also used for some studies. Korean, Philippines, and Mexico laboratories also employed rapid detection based on ELISA and PCR. In order to speed the screening process, rapid detection methods such as ELISA tests of
proven performance should be considered. Confirmation testing by molecular methods may be useful for rapid results.

**Customer Requirements**

All countries require *Salmonella* and *Vibrio cholerae* to be absent in all seafood products. The required standards for the total count, coliform, *Staphylococcus aureus* (coagulase positive), *Listeria monocytogenes* and *Escherichia coli* counts vary from country to country.

**Accreditation Process**

Participants were either from research laboratories working on various aspects of foodborne bacterial pathogens or government laboratories inspecting exported and imported foods. None has been yet accredited by any international organization. Several laboratories are working towards obtaining accreditation.

**Impact**

The results of the studies showed the level of contamination of various pathogens in exported or imported food in each participating country. For exporting countries the results have been and continues to be used to improve the safety of exported product. For importing countries the results can be used for regulating sanitation quality of imported seafood. Another important impact of the research was that the government of Mexico has called for National Competitive Grants for research in the area of detecting foodborne pathogens using PCR methods.

**Results-Correlation**

Results showed that it is possible to produce safe and wholesome seafood for export which can meet international requirements by following Good Agricultural Practices, Good Hygiene Practices and HACCP System.

**Problems encountered**

In some countries, availability of reagents sometimes is a constraint. Collecting samples for analysis from processing plant and exporting companies was difficult for some participants.
CONCLUSIONS AND RECOMMENDATIONS

The participants discussed the results from the work carried out during the CRP (5 years) and drew the following conclusions and made the recommendations.

Conclusions

1) Profiles of selected human bacterial pathogens in some foods, raw materials and products were identified. *Salmonella* remains a major concern in export quality food and is responsible for rejection of some of the consignments. *E. coli* O157:H7 is of low incidence in raw meat.

2) *E. coli* O157:H7 is of low incidence in raw meat in Brazil and Chile.

3) In general, freshly caught seafood and aquaculture fishery products were found to have some level of contamination of bacterial pathogens.

4) Environmental contamination of the coastal area as well as the aquaculture ponds affected the microbiological quality of the seafood products originating from those sources.

5) Samples obtained from processing plants which followed GMP and HACCP were of good microbiological quality and able to comply with the standards of importing countries. The results demonstrated that Good Practices in agriculture, processing and distribution can produce improvement in the quality of food in trade. This is reflected in the improving quality of the foods observed by importing countries. It is recognized that in a certain food producing areas environmental problem are difficult to address.

6) The use of internationally recognized methodology and combined results from participating countries give an overall picture of the prevalence of pathogens in foods.

7) Good progress has been made in the development of laboratory quality systems by participants and evaluation of rapid methodology by some participants. These developments mean improved confidence and timeliness of results are possible.

8) The findings of the CRP has contributed to the improved national food safety programmes in some countries. The results of the CRP will be useful to assist national food control authorities and institutions to improve food safety and to facilitate international trade.
**Recommendations**

1) In recognition that laboratory analysis is only one part of the total food chain, it is recommended that attention should be directed by national authorities to the improvement of the microbiological safety of the environment, agricultural practices, processing, transport and distribution.

2) Implementation of Good Manufacturing Practices and HACCP based quality systems should be accelerated to assure the products of good quality are produced that meet international specification/standards.

3) International organizations should continue to support the national governments to implement the HACCP based food safety systems in order to facilitate international trade in food commodities.

4) It is recommended that the incidence of *Campylobacter* and other emerging foodborne pathogens to be examined in the near future

5) It is recommended that training activities in methodology and quality systems based on ISO 17025 be organized and supported by national and international organizations. Three possible schemes are suggested:
   - A centralized facility such as Seibersdorf to which trainees attended training courses.
   - The provision of training fellowships for trainees to attend an existing training laboratory
   - Provision of funding for an expert to visit laboratories for training purposes

6) Recent advancement in microbiological analysis using molecular and immunological techniques increases the need for training for the personnel involved in food analysis

7) An advisory/information center be established to collect, update, and disseminate information on national and international standards. Efforts to harmonize international standards and testing methodology as undertaken by Codex and ISO should be encouraged and supported. Central development of protocols or guides for laboratory documentation required for Technical and Quality Systems accreditations, which could be made available to National laboratories for accreditation, would greatly facilitate the process.
LIST OF PARTICIPANTS

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Dr. Eliana Marambio
Instituto de Salud Publica de Chile
Av. Marathon No. 1000-Nunoa
Santiago, Chile
Tel. +56.2.350.7375
Fax. +56.2.350.7589
emarambio@entelchile.net

Dr. Ratih Dewanti-Hariyadi
Bogor Agricultural University
Centre for Assessment of Traditional Foods, IPB Campus Darmaga
Box 220, Bogor 16002, Indonesia
Tel/Fax : - 62251-626 564
pkmt-ipb@indo.net.id;
hariyadi@bogor.wasantara.net.id

Dr. Juanita Ramos
National Food Authority
Food Development Centre
FTI Complex, Taguig, Metro Manila
Philippines
Tel. 632 818 7110
Fax. 632 838 4692
infofdc@pacific.net.ph

Dr. Niracha Wongchinda
Fisheries Technological Development Institute,
Department of Fisheries
Kaset-Klang, Chatuchak
Bangkok 10900
Thailand
Tel. 622.940613045/4302
Tel.6229406200
nirachaa@fisheries.go.th
Dr. Peter Paulsen  
Veterinary Medical University  
Microbiology Laboratory  
Institute for Meat Hygiene, Meat Technology and Food Science  
Veterinarplatz 1  
A-1210 Vienna, Austria  
Tel. 250-77-3318  Fax. 250-77-3390  
Peter.Paulsen@vu-wien.ac.at

Dr. Miyoko Jakabi  
Instituto Adolfo Lutz  
Laboratorio Central de Saúde Publica  
Av. Dr. Arnaldo 355  
Cerqueira Cezar, CEP 01246-902  
Sao Paulo SP, Brazil  
Tel. 55.11.306 829 32  
Fax. 55-11-853.35.05  
mijakabi@ial.sp.gov.br

Dr. Fernando Nuñez  
Facultad de Medicina Vet. y Zootecnia  
Universidad Nacional Autónoma de México  
Av. Universidad, 3000  
Ciudad Universitaria  
Circuito Exterior, Delegación Coyoacan  
C.P. 04510 México D.F.  
Tel. 0052-55-5622 58 58  
fer@cuauhtli.veterin.unam.mx  
Fax. 0052-55-5622 59 31

Dr. Abiodun Falana  
National Agency for Food and  
Drug Administration and Control,  
NAFDAC Microbiology Laboratory Oshodi,  
P.M.B. 12525, Ikoiyi, Lagos, Nigeria  
Tel. 234.1.452.1213, 452.4259, 452.4280  
Fax. 234.1.269.3104  
falanaabiodun@yahoo.com  
nafdacos@beta.linkserve.com
OBSERVERS

Dr. Maria de la Salud Rubio Lozano
Facultad de Medicina Vet. y Zootecnia
Universidad Nacional Autónoma de México
Av. Universidad, 3000
Ciudad Universitaria
Circuito Exterior, Delegación Coyoacan
C.P. 04510 México D.F. México
Tel: 0052-55-58480515
Fax: 0052-55-58480514
msalud@seridor.unam.mx

Dr. Ahmad Maznah
Makmal Kesihatan Awam Veterinar
Jabatan Perkhidmatan Haiwan
Persiaran Barat
46630 Petaling Jaya
Selangor D.E, Malaysia
Tel. 603-795 70 960
Fax. 606 787 47 970
maznah@po.jaring.my

FAO/IAEA

Tatiana Rubio Cabello
International Atomic Energy Agency Food and Environmental Protection Section, Wagramestrasse 5
P.O. Box 100
A-1400 Vienna. Austria.
Tel. 43-1-2600 21639 Fax. 26007
T.Rubio@iaea.org
PROGRAMME


Monday 22 July

09:00 - 09:30  Inaugural Session, Dr. Luis Alberto Zarco, Director, Faculty of Veterinary Medicine and Zootech.; Dr. Fernando Nuñez, (UNAM)  Dr. Tatiana Rubio (FAO/IAEA)
09:30 - 10:00  CRP. Final report and publication of results (Dr. Tatiana Rubio)
10:30 – 10:45  Break
10:45 - 11:00  Complying with ISO 17025 and the importance of laboratory quality systems (Dr. Ken Newton)
11:00 - 14:00  Lunch
14:00 - 15:00  Australia report
15:00 - 16:00  Austria report
16:00 - 16:30  Break
16:30 - 17:30  Brazil report

Tuesday 23 July

09:00 - 10:00  Chile report
10:00 - 11:00  India report
11:00 - 11:15  Break
11:15 - 12:15  Indonesia report
12:15 - 13:15  Mexico report
13:15 - 14:00  Lunch
14:00 - 15:00  Nigeria report
15:00 - 16:00  Philippines report
16:00 - 16:30  Break
16:30 - 17:30  Korea report
Wednesday 24 July
09:00 - 10:00    Thailand report
10:00 - 11:00    Malaysia report
11:00 - 11:30    Break
11:30 - 12:30    Working groups
12:30 - 13:00    Lunch
13:00 – 15:00    Working groups
15:00 - 17:30    Working report

Thursday 25 July
09:00 - 10:30    Writing report
10:30 - 11:00    Break
11:00 - 12:30    Writing Report
12:30 - 14:00    Lunch
14:00 - 17:00    Visit to Veterinary Medicine Faculty. UNAM

Friday 26 July
09:00 - 10:00    Revision of the written report
10:00 - 10:30    Break
10:30 - 11:00    Approval of the report
11:00 - 12:00    Closing ceremony