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Report of the

FAO EXPERT WORKSHOP ON THE APPLICATION OF BIOSECURITY MEASURES TO CONTROL *SALMONELLA* CONTAMINATION IN SUSTAINABLE AQUACULTURE

Mangalore, India, 19–21 January 2010

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PREPARATION OF THIS DOCUMENT

This document contains the report of the FAO Expert Workshop on the Application of Biosecurity Measures to Control *Salmonella* Contamination in Sustainable Aquaculture" held from 19 to 21 January 2010 and hosted by the Microbial Resources Center (MIRCEN), College of Fisheries, Mangalore, India. This Workshop was supported by FAO Multidonor Partnership Programme (FMPP) under Objective D.1: Support to national biosecurity initiatives and policies. The Workshop consisted of presentation of background papers by the experts and drafting of the report in three Working Groups chaired by Ronold Lee, Balakrish Nair and Brett Koonse. The plenary sessions to discuss the draft were chaired by Alan Reilly. FAO technical support for this Workshop was provided by Iddya Karunasagar and Lahsen Ababouch from the Products, Trade and Marketing (FIPM) Branch of Fisheries and Aquaculture Department.

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ABSTRACT

This document contains the report of the FAO Expert Workshop on the "Application of Biosecurity Measures to Control *Salmonella* Contamination in Sustainable Aquaculture" held in Mangalore, India from 19 to 21 January 2010. The experts reviewed the current scientific evidence regarding the pathogen *Salmonella enterica*, its occurrence and survival in aquatic environment, possible pathways of contamination of aquaculture systems, serovars found in seafood and salmonellosis associated with fish and fishery products. The experts agreed that although *Salmonella* is a major foodborne pathogen, products of aquaculture are rarely involved in outbreaks of salmonellosis and the serovars which have been reported in products of aquaculture are rarely reported in cases of human salmonellosis in fish importing countries. The experts recognized that there are a variety of pathways reported as to how *Salmonella* can enter the aquaculture environment ranging from wild animals, domestic stock, poor sanitation and inappropriate disposal of human and animal wastes. Control of such pathways poses major challenges such as land runoff during rains, control of wild animals in the farm environment. There was agreement that very low level prevalence of *Salmonella* can be seen in products from aquaculture systems in developed countries but this has not led to any particular public health problem in these countries. The experts agreed that good hygienic practices during aquaculture production and biosecurity measures can minimize but not eliminate *Salmonella* in products of aquaculture. Biosecurity and control measures that would be useful in minimizing the risk of *Salmonella* contamination of aquaculture products were identified. The experts identified data gaps and made a series of recommendations to the national governments, national competent authorities, aquaculture industry and FAO.

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1. OVERVIEW OF THE EXPERT WORKSHOP

1.1 Introduction

International trade in aquaculture products has considerably increased in recent decades and the expansion of aquaculture production, particularly from Asia, has the potential to meet most of the growing global demand for fish and fishery products. The need to increase food production to feed an ever growing world population is more urgent now than ever as FAO has predicted that global food production will need to increase by 70 percent over current levels by 2050 (FAO, 2009a).

In addition to contributing to global food production, aquaculture is a major economic activity and an important source of foreign exchange for several developing countries. Currently aquaculture supplies about 50 percent of the global demand for fish and fishery products with about 90 percent of the aquaculture products coming from the Asian region (FAO, 2009b).

Ensuring food safety to protect public health and promote economic development remains a significant challenge for many countries worldwide. The application of risk-based approaches to food safety management is fundamental to international trade and underpins international food trade agreements. Harmonization of global trading standards relies on the application internationally agreed risk-based scientific principles which form the basis of the recommendations and standards of the Codex Alimentarius Commission. In the international market of fish and fishery products a major challenge faced by exporters is that different standards and regimes are applied by importing countries on producing countries to ensure that products meet the requirements of the target market.

Significant progress has been made in recent decades in improving the standards of post-harvest handling and processing of fish and fishery products with the introduction of food safety management systems based on the principles of the Hazards Analysis and Critical Control Point (HACCP) system. While food safety and hygiene standards have improved in the processing and manufacturing sectors, more still needs to be done to improve such food safety standards in the aquaculture production sector.

In recent years both FAO and WHO have advocated a risk based approach in food safety management and a current priority for both organizations is to promote risk based approaches to food safety management options in aquaculture production. Fish farmers are one of the first links in the food production chain and standards of final products depend on the application of good aquaculture practices being applied on the farm. A major challenge faced by many countries exporting aquaculture products is reducing the incidence of rejection of products because of microbiological contamination.

The presence of *Salmonella* remains a major cause of detention and rejection of shipments of raw aquaculture products in export markets. Though outbreaks of salmonellosis linked to products of aquaculture are rare, there is a concern in some importing countries that products coming from developing countries could be a source of *Salmonella* and this is exacerbated by detection of *Salmonella* in some raw fish and fishery products in import testing laboratories. Significant numbers of detections of this pathogen in fish and fishery products indicate that current strategies for *Salmonella* control in the aquaculture production and processing sectors are not adequate.

Our understanding of *Salmonella* ecology and reservoirs in various wild animals has been improving with number of studies looking at survival of *Salmonella* in rivers, soil and sources of contamination for aquatic environment. Application of microbial typing techniques such as serotyping and molecular typing has enabled better tracing of the source of pathogens. Outbreaks of salmonellosis associated with fresh produce have led to studies on presence and survival of *Salmonella* in irrigation water, which would be important consideration for aquaculture.

The objectives of this Expert Workshop were to:

i. review the current scientific knowledge regarding *Salmonella* ecology in aquatic environments, animal reservoirs and gain a better understanding of the pathways of contamination for aquaculture systems;
ii. consider epidemiological data on salmonellosis associated with fish and fishery products and facilitate application of risk based principles in the management of *Salmonella* problem in aquaculture;
iii. develop recommendations based on biosecurity and other measures for minimizing the contamination of products of aquaculture with *Salmonella*.

It is focused on the need for adopting novel biosecurity measures to minimize contamination and for using risk-based approach to develop management strategies for *Salmonella* in primary production systems to improve safety of fish for human consumption.

1.2 Summary of discussions and conclusions

The broad conclusions from the discussions of the workshop were:

(a) Public health risks due to *Salmonella* in products of aquaculture

- Although *Salmonella* is a major foodborne pathogen, products of aquaculture are rarely involved in outbreaks of salmonellosis.
- Serovars which have been reported in raw products of aquaculture are rarely reported in cases of human salmonellosis in fish importing countries.
- Very low level prevalence of *Salmonella* can be seen in raw products from aquaculture systems in developed countries but this has not led to any particular public health problem in these countries.
- This low level of prevalence may pose human health risk if aquaculture products are consumed raw, but even when low level of *Salmonella* is present, thorough cooking will eliminate the risk.
- While some marine fish caught offshore and handled hygienically and at low temperature according to the Codex Code of Practice for fish and fishery products (CAC/RCP/52-2003) may be suitable for raw consumption, it would be advisable to consume products of aquaculture only after cooking.

(b) Pathways of contamination of aquaculture environments

- There is a variety of pathways reported as to how *Salmonella* can enter the aquaculture environment ranging from wild animals, domestic stock, poor sanitation and inappropriate disposal of human and animal wastes. Control of such pathways pose major challenges such as land runoff during rains and, control of wild animals in the farm environment.
- *Salmonella* has been reported to survive for long periods of time in the aquatic tropical environment where aquaculture production takes place.
- There is currently disagreement in scientific literature as to whether or not *Salmonella* species are a part of the normal flora in the tropical aquatic environment. While long-term survival of some *Salmonella* serovars has been reported in these environments, it is unclear if these species are growing and multiplying and found an ecological niche. In some studies, the serotypes isolated from the environment are not those associated with humans and animals.

(c) Risk management

- Good hygienic practices during aquaculture production and biosecurity measures can minimize but not eliminate *Salmonella* in products of aquaculture.
- Current microbiological standards for fish and fishery products are based on fish from marine environment where pathogens like *Salmonella* are not expected to be present. Aquaculture is carried out in brackish water and inland freshwater environments, where opportunities for contamination with enteric organisms are higher.
- Good hygienic practices will minimize the opportunity for cross contamination in processing environments.
- Currently there is insufficient data to carry out quantitative risk assessment for *Salmonella* in aquaculture.

1.3 Data gaps and limitations

i. Product category-specific epidemiological data were only available for foodborne outbreaks in a proportion of developed countries. Further development of epidemiological surveillance systems should include the collection of such data on a wider basis.

ii. Comparison of the serovars isolated from seafood and the envrionment, and from seafood-associated human infections, infers that serovars may differ markedly in their potential to infect humans. The acquisition of further data on this aspect would inform potential future quantitative risk assessments and also inform hygiene controls and *Salmonella* standards for foods.

iii. The current dose response curves are determined using a variety of other foods. Studies on dose response using seafoods as the matrix using *Salmonella* isolates from seafoods and dose response information from outbreak data would be useful for quantitative risk assessments.

iv. There is very little quantitative data on *Salmonella* in various foods including seafoods. Exposure assessments would benefit from quantitative data at primary production and at the point of consumption.

1.4 Recommendations

To FAO

There are currently no internationally recognized guidelines for the safe production of aquaculture products. It is recommended that FAO work with key stakeholders to further develop these control measures into global guidelines for the safe production of aquaculture products.

FAO should work to harmonize the existing aquaculture certification systems.

To national governments

National governments should ensure that their food laws and regulations address the safe production of aquaculture.

National governments as a priority should implement an official food control programme that ensures the safe production of aquaculture products.

National governments should also ensure that adequate financial resources are available to implement the official food control programme for the safe production of aquaculture products.

To national competent authorities

National competent authorities should have a specific programme in place to minimize *Salmonella* contamination of aquaculture products.

National competent authorities should ensure that all staff working in the official food control programme working to minimize *Salmonella* contamination of aquaculture products should be adequately trained to allow them to perform their duties in a competent and consistent manner.

To the aquaculture industry

The aquaculture industry should assume responsibility for the production of safe aquaculture products and should implement a food safety management programme on the farm.

2. SALMONELLA: CHARACTERISTICS AND PUBLIC HEALTH OUTCOMES

2.1 General characteristics and association with human infections

Salmonella is a facultatively anaerobic, Gram-negative bacterium that can cause illness in humans and other animals. Most strains are motile by means of flagella. There are formally two species of *Salmonella*, *Salmonella enterica* and *Salmonella bongori*. Both species are divided into serovars. There are six subspecies of *S. enterica*: *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae* and *S. enterica* subsp. *indica*. The strains that cause human illness are primarily included in the subspecies *S. enteric*a subsp. *enterica*.

Salmonella are excreted in the faeces of animals (including birds) and humans that are infected with, or asymptomatically excreting, the organism. A biovar (previously known as S. Java) of *S.* Paratyphi B, strains of *S. enterica* other than subspecies *enterica*, and *S. bongori* are associated with cold-blooded animals such as amphibians, reptiles, although at least one serovar of *S. enterica* subsp. *diarizonae* has been found in sheep. Human infections have arisen from contact with both turtles and frogs kept in aquaria – the latter has included infection with *S.* Typhimurium (CDC, 2010). *Salmonella* has also been reported as being present in the guts of river fish (Gaertner *et al.*, 2008). Those workers suggested that cold blooded animals such as turtles provide habitats that allow the persistence of *Salmonella* in the environment but that they do not play a significant role in the dissemination of *Salmonellae* in the natural environment. It has also been put forward that *Salmonella* may be part of the normal flora in aquatic environments, at least in tropical regions (Reilly and Twiddy, 1992).

Transmission of *Salmonella* to humans is predominantly via water or food contaminated with faecal material, or cross-contaminated from other products containing the organism, or contaminated by infected food-handlers. Insects can also carry *Salmonella* in their gut and can contaminate food. In some types of birds, vertical transmission occurs from the female to the egg and humans can become infected by eating the latter. In developing tropical countries, the waterborne route predominates while in countries with better general public health the foodborne route is more important.

Salmonella strains contain a number of somatic ("O") and flagellar ("H") antigens. Many strains may, at any one time, express one of two different flagellar compositions, which give rise to two "H" phases. Assignment to serovar necessitates identification of the "O" and "H" antigens, including both phases if this is relevant. Other antigens may also be present, for example: Vi in some strains of *S.* Typhi, *S.* Paratyphi C and *S.* Dublin. Approximately 2 500 serovars have been described. Only a relatively small proportion predominates in reported human infections. Ninety nine percent of the human infections are due to *Salmonella enterica* which has about 1 500 serotypes. Based on an analysis of globally reported food borne outbreaks, the non-typhoid *Salmonella* serotypes most often encountered in human infections are Enteritidis followed by Typhimurium (Greig and Ravel, 2009). In a broad review of serovars reported from human infections, the following were the ten most commonly found: *S.* Enteritidis, *S.* Typhimurium, *S.* Hadar, S. Infantis, S. Newport, S. Typhi, *S.* Agona, *S.* Virchow, *S.* Heidelberg and *S.* Derby (Herikstad *et al.*, 2002). However, the predominant serovars found in human infections vary both geographically and with time. For example, *Salmonella* Weltrevreden was the second most common serovar in Asia during 2000-2001, but this serovar dropped to fourth place in 2002 surpassed by *S.* Rissen and *S.* Typhimurium (Galanis *et al.*, 2006).

S. Typhi is a common cause of human infection in Africa and South-East Asia, but less common elsewhere. Some of the common serovars, such as S. Enteritidis, S. Typhimurium and S. Virchow, as well as S. Typhi and S. Paratyphi, can be further subdivided using phage-typing.

The clinical outcomes of *Salmonella* can be considered as two separate groups:

Salmonella Typhi/Paratyphi – Strains of these cause enteric fever, a serious systemic illness. Incubation period ranges from 7 to 28 days. Symptoms include malaise, headache, fever, cough, nausea, vomiting, constipation, abdominal pain, chills, rose spots, bloody stools.

Non-typhoid *Salmonella* – Strains of these may cause gastroenteritis in humans. Incubation period ranges from 8 to 72 hours. Symptoms include abdominal pain, diarrhoea, chills, fever, nausea, vomiting and malaise. Systemic infection such as septicaemia may occur especially in susceptible patients such as the very young, very old and immunocompromised. The available data measuring illness as the endpoint suggests that no response is observed until a dose of 10^6 is reached (Coleman and Marks, 1998). Severe dehydration due to diarrhoea can on occasion require medical intervention through the administration of intravenous fluids and antibiotic treatment. However, occasionally some serovars of this pathogen may cause sepsis after entering the blood stream from the intestine and require intense medical intervention. Death is rare if patient is promptly hydrated and provided antibiotic treatment.

2.2 Pathogenicity and host factors

2.2.1 Host factors

Salmonella species are a leading cause of food borne illnesses worldwide and their incidence is dependent upon a variety of factors including host susceptibility. In general, the host factors that can affect outcome of exposure to *Salmonella* or any food borne pathogen by ingestion include age, nutritional status, socio-economic and environmental factors, immune status and underlying diseases. This susceptibility can often be associated with socio-economic status and demography.

Age and the general health of the exposed population are factors that should be considered when assessing the susceptibility of the host to infection. In addition to age, the immunological condition of the host apparently plays a significant role in disease. Children who have immature immune systems and people who are immuno-compromised show increased susceptibility to salmonellosis (FAO/WHO, 2002). It has been noted that children who have more neutral stomach pH are more susceptible due to the acid susceptibility of these pathogens. While stomach pH can affect host susceptibility, the matrix in which the pathogen is consumed may promote/protect the agent from low pH in the stomach. Following resolution of the acute phase, excretion of *Salmonella* ceases within several weeks, although a carrier state may evolve.

2.2.2 Pathogenicity factors

Pathogenicity of non-typhoid *Salmonella* strains is influenced by the presence of several pathogenicity islands in the genome – referred to as *Salmonella* pathogenicity islands (SPI). At present 12 different SPI have been described. Additional pathogenicity factors may be located on a plasmid. The islands and plasmid contain genes influencing attachment, invasiveness, production of toxins, and the survival and growth in the host.

A variety of fimbrial adhesins are involved in initiation of contact to host cells (Hensel, 2004). While the roles in pathogenesis of some SPI are well defined, the function in virulence of many genes within SPI are not understood (Hensel, 2004). The O side chains of the lipopolysaccharide molecules have also been shown to affect invasiveness and enterotoxin production (Murray, 1986). Other factors that affect the ability of the organism to cause disease include the presence of cytotoxins and diarrhoeagenic enterotoxins. The enterotoxin is released into the lumen of the intestine and results in the loss of intestinal fluids (D'Aoust, 1991). Antimicrobial resistant strains are somewhat more virulent than susceptible strains, in that, they cause more prolonged or more severe illness than do antimicrobial susceptible strains (Travers and Barza, 2002).

2.2.3 Effect of the food matrix on infectivity

Gastric acidity (pH 2.0) is considered an important defence against food borne pathogens. Though normally *Salmonella* grow at neutral pH, they have complex survival strategies that might facilitate their ability to tolerate pH fluctuations during pathogenesis. Most aquaculture products have neutral pH and *Salmonella* may be protected in this food matrix. The outcome may be affected by the amount of food ingested, the fat content and buffering capacity of the food, and the nature of contamination. In one outbreak linked to the consumption of scallop with egg yolk, 6.30 log cells resulted in a 56 percent attack rate (FAO/WHO, 2002). In fatty foods (e.g. chocolates, cheddar cheese) low infective dose may be observed and some aquacultured fish (e.g. salmon, catfish) may have high fat content, but there is no available data on outbreaks associated with these fish. Increased attack rates have been associated with ingestion of *Salmonella* between meals and it has been postulated that pyloric barrier may fail at this time and chocolates and ice creams may be consumed between meals (Mossel and Oei, 1975). Distribution of bacteria in food may also affect the outcome and due to the nature of bacteria to grow in colonies, agglomeration of cells may occur in foods and cells in inner layers of this might be protected (FAO/WHO, 2002).

2.3 Dose-response relationships

Nine studies have been published of experimentally induced salmonellosis, conducted between 1936 and 1970 using a variety of serotypes and strains (Table 1). However, some of these studies were deemed to be unsuitable to derive conclusions about the pathogenicity of *Salmonella* in general in humans. Severe illness resulting from salmonellosis can be exacerbated by antibiotic resistant strains of *Salmonella* and may be further complicated by the effects of other underlying illnesses.

There are number of human feeding trials performed using six different serotypes (Table 1). There were usually no illnesses at doses less than 10^6. However, outbreak investigations show that lower number of cells can cause infection depending upon the food matrix. There is no data with sea food matrix alone but in an outbreak of *S.* Enteritidis associated with scallop and egg yolk, a 56 percent attack rate was observed at a dose of 6.3 log Colony Forming Units (CFU). More information on the outbreaks, attack rate and doses involved is available in Table 3.14 of the FAO/WHO risk assessment of *Salmonella* in eggs and broiler chicken (FAO/WHO, 2002). Future risk assessments would benefit from data on levels of *Salmonella* in seafoods involved in any outbreaks.

Table 1. Human feeding trials using *Salmonella* (FAO/WHO, 2002)

No.	Serotype(s)	Strain(s)	References
1	S. Typhimurium		Hormaeche, Peluffo and Aleppo, 1936
2	S. Anatum		Varela and Olarte, 1942
3	S. Meleagridis S. Anatum	I, II & III I, II & III	McCullough and Eisele, 1951a McCullough and Eisele, 1951a
4	S. Newport S. Derby S. Bareilly		McCullough and Eisele, 1951c McCullough and Eisele, 1951c McCullough and Eisele, 1951c
5	S. Pullorum	I, II, III & IV	McCullough and Eisele, 1951d
6	S. Typhi		Sprinz *et al.*, 1966
7	S. Sofia S. Bovismorbificans		Mackenzie and Livingstone, 1968
8	S. Typhi	Quailes, Zermatt, Ty2V, 0-901	Hornick *et al.*, 1970
9	S. Typhi	Quailes	Woodward, 1980

3. SALMONELLA IN THE AQUATIC ENVIRONMENT

3.1 Occurrence in the aquatic environment

The presence of *Salmonella* in the external environment and food is considered a critical step to ensure the passage of the bacteria to new hosts. Existing studies on the presence of *Salmonella* in aquatic systems and seafood products have identified two different patterns according to the climate characteristics of the area. The results of a number of studies are summarized in Table 2. In different temperate and arid regions of Spain (Martinez-Urtaza *et al.*, 2004), Morocco (Setti *et al.*, 2009) and Mexico (Simental and Martinez-Urtaza, 2008) characterized by of presence of cold coastal seawaters, *Salmonella* have been detected in less than 10 percent of seawater and shellfish samples investigated. Similar low levels of *Salmonella* were obtained in marine samples from regions with similar oceanographic conditions to these areas and with temperate seawater temperatures, such as the United States and the United Kingdom, which have incidences of 7.4 percent, and 8 percent, respectively (Brands *et al.*, 2005; Martinez-Urtaza *et al.*, 2004; Wilson and Moore, 1996). These results contrast with the high prevalence in tropical areas where *Salmonella* incidence in seafood can reach up to 20 percent of the samples, as it has been reported for areas of Asia and Africa (Hatha and Lakshmanaperumalsamy, 1997; Heinitz *et al.*, 2000). In Vietnam, an incidence of 18 percent of positive samples for *Salmonella* has been reported for shellfish product (Van *et al.*, 2007), whereas in India, presence of *Salmonella* was found in 24.3 percent of different seafood products investigated (Rakesh Kumar *et al*., 2008a). A recent study of presence of *Salmonella* in rivers and coastal waters carried out in tropical areas of Mexico has shown an occurrence of this organism over 80 percent (Jimenez, Chaidez and Martinez-Urtaza, personal communication). A total of 75 percent prevalence has also been reported in Oconee river basin, Georgia, USA (Meinersmann *et al.*, 2008).

The dynamics of contamination of *Salmonella* in natural environments has been associated with specific seasonal patterns or climate characteristics. In temperate and tropical regions, presence of *Salmonella* in the environment has been detected linked to the periods of rains, and more specifically, after the days of the first heavy rains. Therefore, associations between storm-generated flows, torrential rains, and the monsoon season have been reported in studies in temperate and tropical regions of the world with frequent rainy periods (Baudart *et al.*, 2000; Brands *et al.*, 2005; Hatha and Lakshmanaperumalsamy, 1997; Martinez-Urtaza *et al.*, 2003; O´Shea and Field, 1991; Venkateswaran *et al.*, 1989), signalling the washing effect of torrential rains as one of the principal environmental drivers of *Salmonella* contamination in coastal areas (Martinez-Urtaza *et al.*, 2003). The arrival of *Salmonella* into marine environments is predominantly governed by the presence of persistent rains enough to transport the contamination from the original source points to the sea via aquifers, streams and rivers (Simental and Martinez-Urtaza, 2008). *Salmonella* has been found in several marine mammals like porpoises, sea lions, whales, dolphins, seals (Higgins, 2000). The permanence of *Salmonella* contamination in aquatic and marine systems appears to have been modulated by a combination of oceanographic characteristics and atmospheric conditions related primarily to the effects of sunlight. The presence of intense sunlight has been identified as a critical factor for the drastic reduction of *Salmonella* spp. in coastal areas of Spain, Morocco and Mexico (Martinez-Urtaza *et al.*, 2004; Setti *et al.*, 2009; Simental and Martinez-Urtaza, 2008). Water temperature has been proposed as playing an important role in the long-term survival of *Salmonella* in the environment. The presence of cold waters may reduce the survival of *Salmonella* spp. in the marine environment, while warm waters together with high levels of organic matter, typical conditions prevailing in tropical areas, may contribute to a more appropriate habitat for an increased survival of bacteria, as reflected in the disparate incidence of *Salmonella* described in diverse studies in temperate and tropical regions. *Salmonella* has been detected prevailing all year round in tropical areas in a recent study performed in Sinaloa, Mexico (Jimenez, Chaidez and Martinez-Urtaza, personal communication).

Table 2. *Salmonella* detection in the aquatic environment

Country (No of samples)	Type of sample	Positive sample (%)	Serotypes	Resistance (%)	Reference
Spain (5384)	Molluscs	3	Serotypes (N=20): Senftenberg 42.5% Typhimurium 15% Agona 9.4%	9	Martinez-Urtaza *et al.*, 2004
	Seawater	2.5			
Morocco (801)	Mussels	10	Serotypes (N=3): Blockley 43.8% Kentucky 29.8% Senftenberg 26.3%	49.1	Setti *et al.*, 2009
	Sediments	6.8			
	Seawater	4.1			
Mexico, Ensenada (1331)	Wastewater	16.2	Serotypes (N=20): Typhimurium 23.4% Vejle 6.2% Suberu 4.7%	–	Simental *et al.*, 2008
	Stream water	10.6			
	Molluscs	7.4			
	Seawater	2.3			
Mexico, Culiacan (138)	Water	80.4	Serotypes (N=29): Oranienburg 24,3% Saintpaul 9.0% Minnesota 6.3%	50.4	Jimenez, Chaidez and Martinez-Urtaza, personal communication
Asian countries (1234)	Shrimps	1.6	Weltevreden Paratyphi B Abaetetuba	–	Koonse *et al.*, 2005
	Holding pond water	2.5			
	Pond sediments	1.0			
	Pond grow-out water	3.5			
	Source water	5.0			
	Source sediment	24			
Viet Nam (50)	Shellfish	18.0	-	11.1	Van *et al.*, 2007
India, Cochin (443)	Fish	30.5	Serotypes (N=30): Weltevreden 8.2% Rissen 7.8% Typhimurium 6.7%	82%	Kumar *et al.*, 2008
	Shrimps	29			
	Clams	34.1			

The vast majority of studies looking at the presence of *Salmonella* in aquatic and marine environments have evidenced two main observations: only a small but constant number of serovars have been found in these environments and, in most cases, these do not coincide with the main zoonotic serovars identified in the surrounding areas (Catalao Dionisio *et al.*, 2000; Heinitz *et al.*, 2000; Martinez-Urtaza *et al.*, 2004; Polo *et al.*, 1999; Venkateswaran *et al.*, 1989; Wilson and Moore, 1996). In spite of the variability in sampling size (n= 37 to 251), in most of these studies the maximum number of serotypes identified has been around 20 (Catalao Dionisio *et al.*, 2000; Martinez-Urtaza *et al.*, 2004; Venkateswaran *et al.*, 1989; Wilson and Moore, 1996). Serovar Typhimurium has been shown to be the most common clinically significant serovar isolated from environmental samples in many parts of the world (Baudart *et al.*, 2000; Catalao Dionisio *et al.*, 2000; Martinez-Urtaza *et al.*, 2004; Polo *et al.*, 1999; Willson and Moore, 1996; Simental and Martinez-Urtaza, 2008), which attests to its capacity of adaptation and survival in external environments (Baudart *et al.*, 2000). *Salmonella* Senftenberg has been recognized one of the major serotypes identified in marine environments and raw seafood worldwide. It has been one of the predominant serovars detected in the coastal waters of Portugal (Catalao Dionisio *et al.*, 2000), in crustaceans from India (Hatha and Lakshmanaperumalsamy, 1997), in raw seafood imported into the United States especially from tropical countries (Heinitz *et al.*, 2000),

and in environmental samples in France and Brazil (Baudart *et al.*, 2000; Tavechio *et al.*, 2002). Serovar Senftenberg was the dominant serovar detected in coastal areas of Galicia over a 4–years study (Martinez-Urtaza *et al.*, 2004). *Salmonella* Weltevreden has been identified in recent years as one of the prevailing serovars in raw seafood products from Asian countries. Serovar Weltevreden has been detected as the dominant *Salmonella* serotype in fish and shrimp samples collected in India (Shabarinath *et al.*, 2007; Rakesh Kumar *et al.*, 2008), and in raw products of aquaculture in other Asian countries (Reilly and Twiddy, 1992; Koonse *et al.*, 2005).

Information about antimicrobial resistance among *Salmonella* strains isolated from environmental sources and food showed a differentiated incidence rate of resistant strains among isolates obtained from developed and developing countries. Antimicrobial resistance was detected in 9 percent of the total of strains isolated from environmental sources and shellfish over different studies in Spain (Martinez-Urtaza *et al.*, 2004b; Martinez-Urtaza *et al.*, 2005). Conversely, the presence of antimicrobial resistant strains among strains isolated from the marine environment in Morocco reached 49.1 percent of the strains (Setti *et al.*, 2009), whereas in Mexico, 50.4 percent of the strains recovered from water samples showed resistance to antimicrobials (Chaidez and Martinez-Urtaza, personal communication). In a study carried out in Cochin, India, 82 percent of the strains isolated from seafood products presented antimicrobial resistance (Rakesh Kumar *et al.*, 2008), whereas in Vietnam, antimicrobial resistance was observed in 11.1 percent of strains (Van *et al.*, 2007). However, these were observational studies and the methodology and spectrum of antibiotics used by different investigators vary and this may contribute to the high degree of variation observed.

3.2 Survival

Salmonella differs from *Escherichia coli* in that its enhanced survival in the external environment promotes transmission to a new host. Survival of *Salmonella* in soil, water, and on a variety of surfaces provides the bacterium with an increased probability of infecting a new host (Winfield and Groisman, 2003).

Salmonella can survive for 10 to 15 days in a septic system (Parker and Mee, 1982) and has high survival rates in aquatic environments (Chao *et al.*, 1987). *Salmonella* Senftenberg could be detected persisting in the marine environment for more than five years (Martinez-Urtaza and Liebana, 2005). *Salmonella* has a high survival rate following mixing of sewage effluent with brackish water (Mezrioui *et al.*, 1995). *Salmonella* can be widely disseminated in soil and sediment, even in the absence of active fertilization, as a result of water currents, underground springs, and rain runoff carrying contaminated material (Abdel-Monem and Dowidar, 1990; Chao *et al.*, 1987). *Salmonella* can survive and multiply for 26 months in soil (Davies and Wray, 1996; Thomason *et al.*, 1977).

3.3 Biofilm formation and persistence in the environment

A biofilm is defined as a microbially derived sessile community characterised by cells that are irreversibly attached to a substratum or interface or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription (Donlan and Costerton, 2002). Biofilms are almost universal in the natural environment and they can develop on innumerable surface types (Carpentier and Cerf, 1993; Costerton, 1999; Woodward *et al.*, 2000).

Recent studies have shown that *Salmonella enterica* also are capable of forming biofilms on a wide variety of contact surfaces, both inorganic and organic, and on the liquid-air interface (Hood and Zottola, 1997; Joseph *et al.*, 2001; Lapidot *et al.*, 2006; Solano *et al.*, 1998; Vestby *et al.*, 2009a; Vestby *et al.*, 2009b; Woodward *et al.*, 2000). In biofilms, bacteria are believed to be protected from various environmental stresses, and *Salmonella* in biofilms has been shown to be less sensitive to antibiotics and disinfectants than planktonic bacteria (Janssens *et al.*, 2008; Møretrø *et al.*, 2009; Scher *et al.*, 2005; Ueda and Kuwabara, 2007). Experiments have also shown that *Salmonella* can persist for several months in biofilm during long term desiccation and nutrient depletion (Vestby *et al.*, 2009b; White *et al.*, 2006). A correlation between biofilm forming abilities at room temperature and

persistence in feed factories has been found, thus indicating that biofilm forming abilities influence persistence in this kind of environment (Vestby *et al.*, 2009a). In this way, biofilms can serve as a long term reservoir for cross contamination of food products.

3.4 Persistence in live fish

Salmonella spp. have not been regarded as fish pathogens with the possible exception of *Salmonella arizona*. However, fish may be exposed to *Salmonella* spp. through consumption of contaminated feed or from the growing environment.

Salmonella have been reported to enter via the gastrointestinal tract into the internal organs and muscle tissue in several freshwater species, e.g. rainbow trout (*Salmo gairdneri*), Israeli mirror carp (*Cyprinus carpio*) and tilapia (*Tilapia aurea*), and in Atlantic salmon (*Salmo salar*) in saltwater (Baker and Smitherman, 1983; Buras *et al.*, 1985; Hagen, 1966; Heuschmann-Brunner, 1974; Nesse *et al.*, 2005). Although there are few comparable studies, results suggest that persistence and dissemination of high doses of *Salmonella* is analogous in saltwater and freshwater fish. When the administered dose is high, *Salmonella* may persist in the fish for several weeks. A few results may indicate longer persistence at higher temperatures than at lower temperatures.

Buras *et al.* (1985) studied the dissemination of *Salmonella* Montevideo in *Tilapia aurea (Saratherodon aureus)* and Israeli mirror carp *(Cyprinus carpio)* was studied. The results showed the existence of a threshold concentration of *Salmonella* causing their appearance in the muscles. The thresholds were 1.8 - 3.7 x 10^4CFU per fish when the bacteria were inoculated directly into the digestive tract, and 5.0 X 10^5CFU/ml when the bacteria were in the water. After 8 to 9 days the concentration of bacteria in the digestive tract was equal to or higher than the concentration in the water.

3.5 Possible pathways of contamination of aquaculture systems with *Salmonella*

Non-point water run-off.

> During rainfall events, increased run off of organic matter into ponds may occur and can contaminate the aquaculture system.

Animals (domestic animals, frogs, rodents, birds, insects, reptiles, etc.)

> A variety of animal waste has been shown to be potential sources of *Salmonella*. Animal waste can be introduced directly through bird droppings or frogs living in ponds or indirectly through runoff.

Fertilization of ponds.

> In some aquaculture systems animal manures are used in ponds to stimulate the production of algae. The use of non-composted manures can lead to production systems being contaminated with *Salmonella*.

Point source contamination.

> An example of this is having toilets discharging into ponds or integrated farming systems where animals are housed directly over an aquaculture pond.

Contaminated source water

> The water used in growout ponds, cages or tanks can be contaminated with *Salmonella* through wildlife runoff, untreated domestic sewage, discharge from animal farms, etc.

On farm primary processing

Aquaculture products can become contaminated with *Salmonella* through the use of unsanitary ice, water, containers, and poor hygienic handling practices.

Contaminated feed

Improperly stored feed or feed prepared on a farm under poor hygienic conditions can be a source of *Salmonella*.

4. *SALMONELLA* IN AQUACULTURE PRODUCTS AND ASSOCIATION WITH DISEASE

Aquaculture products are grown in both the marine and freshwater environments. Marine aquaculture products include finfish (various), crustaceans (prawns/shrimp) and bivalve molluscs (various). Freshwater aquaculture products include finfish (various) and crustaceans (prawns, crayfish). Marine aquaculture may take place close to shore and thus close to sources of contamination, or offshore in relatively clean environments. Freshwater aquaculture takes place in ponds of still or running water and in reservoirs and with a greater potential for exposure to sources of contamination. Aquaculture may also take place in brackish water, or in estuarine situations with varying salinity levels – these locations may be exposed to sources of contamination impacting on both the marine and freshwater environments. This report will not consider bivalve molluscs produced in aquaculture.

4.1 Detection of *Salmonella* in foods, including seafoods

Most laboratory testing of foods, for routine surveillance, outbreak investigations, and other purposes, is undertaken using presence/absence testing for the organism in a standard amount of food matrix, often 25 g, and utilizing conventional microbiological methods. Such methods, e.g. ISO 6579:2002, involve a primary non-selective enrichment in broth, a secondary selective enrichment in one or more liquid media, and subsequent subculture onto one or more selective agar media for the isolation of potential *Salmonella* colonies (ISO, 2002). Such colonies need to be subjected to further tests (conventional or molecular) to confirm that they are actually *Salmonella*. Once this has been done, the isolates may be subjected to serotyping, phage-typing or one or more molecular typing methods to discriminate between the many types that may be present. In foodborne outbreaks, such typing helps to confirm the potential involvement of one or more food vehicles.

More recently, molecular methods have been developed to detect *Salmonella* in foods without prior isolation of colonies (Levin, 2009). Such methods usually involve preliminary non-selective enrichment in order to achieve a sensitivity that is comparable to that obtained with conventional culture methods. The molecular methods generally do not require subsequent confirmation of results. However, they do not yield colonies on which further typing procedures can be undertaken.

Quantitative estimation of the concentration of *Salmonella* in foods has rarely been reported. The absence of quantitative data limits the potential application of quantitative risk assessment to product categories, including those from aquaculture. One study of imported seafood (353 samples of 29 types of seafood) in Japan found two samples of black tiger shrimp and the levels estimated by Most Probable Number (MPN) were <30 to 40/100g (Asai *et al.*, 2008).

4.2 Growth and survival in the seafood matrix

There is very little quantitative data on *Salmonella* in fish and fishery products. One study of imported fish in Japan showed a level (MPN)of about 30–40 cells/100g (Asai *et al.*, 2008). Considering that $>10^5$ cells are required to cause infection, it can be suggested that multiplication in fish would be necessary before the food is consumed. *Salmonella* is a mesophilic organism and the growth rate of this organism is markedly reduced at temperatures <15 ºC while the growth of most strains is prevented at <7 ºC (ICMSF, 1996). Most studies on minimum growth temperature have been done with beef, chicken or eggs using serovars like Typhimurium or Enteritidis common in these foods. However, these are not common serotypes in seafoods. In raw seafoods containing a variety of bacteria, *Salmonella*, if present has to compete with other flora for growth. *S*. Heidelberg had a generation time of 28 hours and 31 hours in the fish English sole and sterile crab respectively at 8 ºC (ICMSF, 1996). In cooked crab inoculated with *Salmonella* and stored at 8–11 ºC under modified atmospheres containing low levels of CO_2 (20–50 percent) proliferation of *Salmonella* has been reported (Ingham *et al.*, 1990). *Salmonella* has ability to proliferate at pH values ranging from 3.8 to 9.5 with optimum being 7.0–7.5 (ICMSF, 1996). Growth of *Salmonella* is generally inhibited at 3–4 percent NaCl, but salt tolerance increases with increasing temperature in the range 10–30 ºC (D'Aoust

and Maurer, 2007) and minimum water activity for growth is 0.94 (ICMSF, 1996). Though the resistance of *Salmonella* to drying varies, this organism may survive for months or even years in dried products and has been frequently isolated from fish meal, meat and bone meal, maize and soy products (Lunestad *et al.*, 2007). Decrease in *Salmonella* numbers occur during freezing and frozen storage, but this process does not guarantee elimination of *Salmonella*e in foods (ICMSF, 1996). *Salmonella* are heat sensitive and D-values are influenced by the water activity, nature of the solutes and pH of the suspending medium (ICMSF, 1996). Typical D-values reported for *Salmonella* are 0.176 min in chicken at 70 ºC, 0.36 min in ground beef at 63 ºC (FAO/WHO, 2002). Some strains of *Salmonella* like S. Senftenberg 775W may show higher heat resistance (ICMSF, 1996). Interestingly, S. Senftenberg is the serovar often isolated from fish feed (Lunestad *et al.*, 2007).

4.3 Serovars found in seafood

Salmonella serotypes are closely related genetically yet differ significantly in their pathogenic potentials. It is reported that *Salmonella* strains isolated from most of the clinical cases appear to be different from those found in shrimp and other aquaculture products resulting in the conclusion that these seafood constitutes a very low risk to public health (Feldhusen, 2000). However, this might not be true for all countries for example *S.* Weltevreden which is a common isolate from shrimp culture environments and shrimp products and is a common serotype involved in human infection in Thailand (Bangtrakulnonth *et al.*, 2004), Vietnam (Phan *et al.*, 2005) and Malaysia (Yasin *et al.*, 1995).

Table 3 compares the dominant serovars found in human infection with those found in seafood and the aquatic environment. Analysis of 11 312 imported seafood and domestic seafood over a nine year period (1992–1998), S. Weltevreden was the most common serotype followed by Senftenberg and Lexington. Among the serotypes involved in human infection, Enteritidis ranked fifth and Typhimurium ranked twelfth (Heinitz *et al.*, 2000). In India, data from the National Reference Centre for *Salmonella* indicate that during 2001–2005, sea foods account for 2.3 percent of the *Salmonella* isolated and poultry 19 percent and other animals 8.1 percent (Kumar *et al.*, 2009). In seafood the commonest serotype encountered was S. Worthington followed by Weltevreden. This pattern of dominance of S. Weltevreden seems to be reflected in reports from other countries in Southeast Asia (Reilly and Twiddy, 1992).

The table presented in Appendix 1 shows the distribution of serovars isolated from seafood in different regions of the world (Heinitz *et al.*, 2000; Iyer and Shrivastava, 1989; Rakesh Kumar *et al.*, 2008a, 2008b, 2009). *S.* Weltevreden, *S.* Enteritidis, *S.* Newport, *S.* Anatum, *S.* Senftenberg, *S.* Typhimurium and *S.* Saintpaul were the most commonly isolated serovars worldwide. The diversity of serovars associated with fish and fishery product was highest in Southeast Asia and next highest in South America. Though S. Typhi and S. Paratyphi are considered causative agents of typhoid fever, S. Paratyphi B appears to be heterogenous containing strains causing primarily systemic infection (typhoid fever) and strains associated with self-limiting gastroenteritis. The latter are also called biovar Java that is also associated with animal reservoirs. Seventy-eight percent of the *S.* Paratyphi B isolates in the study of Heinitz *et al*. (2000) belonged to biovar Java.

Table 3. Dominant *Salmonella* serotypes associated with human illness and seafood / aquaculture environment

Human illness associated global rank 2002[a]	**Seafood associated rank occurrence 1990–1998[b]**	**Aquaculture environment (not rank ordered) 2001–2003[c]**
Enteritidis (1)	Weltevreden (1)	Weltevreden
Typhimurium (2)	Senftenberg (2)	Paratyphi-B (predominantly biovar Java)
Newport (3)	Lexington (3)	Senftenberg
Heidelberg (4)	Paratyphi-B (4) (predominantly biovar Java)	Houten
Infantis (5)	Enteritidis (5)	Abaetetuba
Hadar (6)	Newport (6)	Derby
Virchow (7)	Thompson (7)	Aberdeen
Javiana (8)	Lanka (8)	Javiana
Saintpaul (9)	Virchow (9)	Hvittingfoos
Montevideo (10)	Hvittingfoss (10)	Give
Paratyphi B (16)	Typhimurium (12)	Newport
Weltevreden (20)	Derby(14)	

[a] Galanis *et al.*, 2006
[b] Heinitz *et al.*, 2000
[c] Data from: Hatha *et al.*, 2003; Koonse *et al.*, 2005; Kumar *et al.*, 2009; Norhana *et al.*, 2010

4.4 Salmonellosis and aquaculture products

There is very little specific data on the incidence of salmonellosis associated with aquaculture products. Epidemiological records of outbreaks associated with such food may be included under a general seafood category (even if they are freshwater products) or as fish or shellfish (possibly broken down into crustaceans and bivalve molluscs). Table 4 shows the incidence of salmonellosis associated with all food vehicles, and with seafood, for the European Union in 2007 (EFSA, 2009). Table 5 shows similar data for the United States (Lynch *et al.*, 1996). The general level of gastroenteritis and salmonellosis is much higher in developing countries. However, none of the outbreak data available for developing countries was subdivided by food category and thus cannot be presented in an analogous manner. Table 6 shows the overall 10 most common serovars isolated from humans, as well as from various foods, in Thailand between 1993 and 2002 (Bangtrakulnonth *et al.*, 2004).

The data for the European Union and the United States suggests that seafood is not a significant vehicle of salmonellosis in developed countries. It can therefore be inferred that aquaculture products, as a subset of this food category, is also not a significant vehicle. Other food products predominate with regard to the transmission of salmonellosis.

Salmonella has been isolated from aquaculture systems in both developing countries and developed countries. Studies done in Southeast Asia indicate that 16.1 percent of shrimp and 22.2 percent of water/mud samples were positive for *Salmonella* (Reilly and Twiddy, 1992). In US freshwater catfish ponds a prevalence of 5 percent (Wyatt *et al.*, 1979) was observed and from eel culture ponds in Japan, a prevalence of 21 percent (Saheki *et al.*, 1989) has been reported. *Salmonella* has also been isolated from pond water in a trout farm in Spain (Cesar-Javier *et al.*, 1999). A relatively high percentage of 33 percent in US catfish and 50 percent in Vietnamese catfish were reported to be positive for *Salmonella* by Pal and Marshall (2009) and this may be due to the methodology used for isolation. But these data show that low prevalence of *Salmonella* can be seen in aquaculture systems in all parts of the globe.

Table 4. Seafood associated outbreaks in the European Union, 2007

Food vehicle	Number of outbreaks	Number of *Salmonella* outbreaks	% of outbreaks associated with *Salmonella*
Fish and fish products	130	3*	2.3
Crustaceans, shellfish, molluscs, and products	75	2*	2.7
All food vehicles	2 025	590	29.1

Note:Verified outbreaks; *187 *Salmonella* outbreaks from Spain not included in product breakdown in original report as the Member State reported agglomerated data (therefore total broken down across all products = 403 rather than 590) – proportion due to seafood not known.

Table 5. Seafood-associated outbreaks in the United States, 1998-2002

Food vehicle	Number of outbreaks	Number of *Salmonella* outbreaks	% of outbreaks associated with *Salmonella*
Fish	337	4	1.1
Shellfish	151	2	1.3
All food vehicles	6647	585	8.8

Table 6. Distribution of the 10 most common serovars isolated from humans and foods in Thailand, 1993-2002 (modified from Bangtrakulnonth *et al.*, 2004)

			Reservoir and no. of isolates (%)			
Serovar	Humans	Frozen chicken	Frozen seafood	Frozen duck	Other food products	Water
Weltevreden	5,491 (12.5)	2,901 (19.9)	265 (26.3)	320 (12.0)	457 (6.6)	143 (14.5)
Enteritidis	5,010 (11.4)	423 (2.9)	14 (1.4)	-	309 (4.5)	22 (2.2)
Anatun	3,263 (7.4)	-	20 (2.0)	-	1,177 (17.0)	113 (11.5)
Derby	2,889 (6.6)	-	20 (2.0)	-	370 (5.3)	71 (7.2)
1,4,5, 12:i:-ssp.I	2,804 (6.4)	-	-	-	-	-
Typhimurium	2,322 (5.3)	-	12 (1.2)	-	198 (2.9)	-
Rissen	2,319 (5.3)	-	21 (2.1)	-	712 (10.3)	93 (9.5)
Stanley	1,688 (3.8)	-	20 (2.0)	279 (10.4)	-	-
Panama	1,474 (3.3)	-	-	41 (1.5)	254 (3.7)	47 (4.8)
Agona	1,096 (2.7)	452 (3.1)	-	80 (3.0)	273 (3.9)	39 (4.0)
Other	15,284 (35.9)	10,783 (74.1)	635 (63.1)	1,950 (73.1)	3,178 (45.8)	456 (46.3)
Total	44,087	14,559	1,007	2,670	6,928	984

4.5 Outbreaks associated with fish and fishery products

Among food borne outbreaks reported in international literature between 1988 and 2007, for which a source could be identified (n= 4093), 46.9 percent was due to *Salmonella* (Greig and Ravel, 2009) of which, seafood accounted for 1.7 percent compared to 14 percent associated with eggs (values calculated from Table 3 of Greig and Ravel, 2009). Non-typhoidal *Salmonella*e cause an estimated 1.4 million illnesses in the United States each year, resulting in an estimated 15 000 hospitalizations and 400 deaths (Voetsch *et al.*, 2004), but only about 5 percent of *Salmonella* infections in the United States are due to seafood (Bean *et al.*, 1997).

Outbreaks involving seafood has been reported from some countries. In Japan, *S.* Champaign was involved in 330 cases in children, who consumed cuttlefish that had been left to thaw at room temperature for 30 hours and then boiled for a short period (Ogawa *et al.*, 1991). Contaminated well water of a squid processing plant in Japan was found to be the source of *Salmonella* that affected more than 400 people in 1999 and during the same year, cuttlefish snack contaminated with *S.* Chester was

involved in an outbreak that affected more than 1500 people (D'Aoust and Maurer, 2007). *S.* Livingstone was the cause of an outbreak that occurred in Norway and Sweden in 2001 in which fish gratin manufactured in Sweden was implicated and the egg powder ingredient in fish gratin was suspected to be the source (D'Aoust and Maurer, 2007). One outbreak in which 16 people became ill after a reception in a hotel in the United Kingdom in 1981 was attributed to frozen prawns (PHLSC, 1983). Though the implicated food has not been tested, only those who ate prawns were affected and S. Bareilly and S. Hindmarsh were isolated from the patients. It is not clear whether the prawns were prepared with any other ingredients, which could be a source of *Salmonella*.

5. BIOSECURITY AND CONTROL MEASURES TO MINIMIZE THE RISK OF *SALMONELLA* CONTAMINATION OF AQUACULTURE PRODUCTS

In order to ensure that the interest of consumers is protected an integrated approach is required for the safe production of aquaculture products. The aquaculture farm is the first link in the food safety continuum and controls must be in place and implemented throughout the food safety chain.

Governments should set objectives to reduce the incidence of *Salmonella* in aquaculture products such as implementing pathogen reduction programmes and requiring Good Aquaculture Practices on farms.

The following are suggested control measures the National Competent Authority should consider when developing and implementing programmes to minimize the risk of *Salmonella* contamination of aquaculture products.

I. Aquaculture farm

1. Farm location

1.1 Aquaculture farms should be located in areas specifically zoned as suitable for aquaculture development.

1.2 Farms should be located in environmentally suitable areas where risks to food safety from chemical, biological and physical hazards from air, soil and water are minimized.

1.3 Farms should be secured from the entry of wild and domestic animals that may lead to the contamination of aquaculture products with *Salmonella*.

2. Farm layout, equipment and design

2.1 Farm is used for aquaculture purposes only (no livestock production).

2.2 Farm design and layout should be such that prevents cross contamination.

2.3 Septic tanks, toilet facilities and bathrooms/showers should be constructed and placed so drainage does not pose a risk of contamination of farm facilities.

2.4 Equipment such as cages, nets and containers should be designed and constructed to allow for adequate cleaning and disinfection.

2.5 Equipment such as containers and vehicles for feed, seed and harvested aquaculture products should be designed and constructed to allow for adequate cleaning and disinfection.

3. Source water

3.1 Farm source water should be free from sewage contamination and suitable for aquaculture production.

3.2 There should be sufficient quantity of water suitable for aquaculture throughout the year.

3.3 Farms should have settling ponds or waste water treatment in place to condition the output water prior to discharge.

4. Pest control

4.1 Building construction in combination with a pest control system should be designed to ensure that the risk of contamination of feed, equipment and the farming system is minimized. For example, feed sheds are properly constructed to prevent contamination by rodents or birds that may be a source of *Salmonella*.

5. Facilities

5.1 Separate buildings for feed, drugs and disinfection deposits are available and suitable.

5.2 Provision of facilities for keeping of dead/ill animals until safe disposal.

5.3 Provision of facilities for hygienic disposal of solid and liquid waste.

6. Staff facilities

6.1 Toilets are available and the number of toilets is adequate for the number of staff.

6.2 Toilets are located so that waste does not contaminate the farm.

6.3 Toilet facilities do not open directly into processing areas.

6.4 Adequate hand washing facilities are available and suitably located for staff to use.

7. Facility and equipment cleanliness

7.1 Farm and surroundings should be maintained clean and in a sanitary condition.

7.2 Measures should be taken to prevent animals and pests from causing contamination.

7.3 Containers, equipment and farm facilities are maintained in a good condition so they are easy to clean and disinfect.

7.4 Adequate procedures for cleaning and disinfection of containers, equipment and farm facilities are in place and implemented.

8. Pond preparation for stocking

8.1 Pond preparation practices should minimize the risk of cross contamination.

8.2 Fertilizers, probiotics, chemicals etc. are used according to manufacture instructions.

8.3 Animal manures used as fertilizers should be adequately composted before use to eliminate the risk of transferring pathogens to the pond water.

9. Feed management

9.1 Aquaculture feed, both domestic and imported, should come from a company registered with the national competent authority.

9.2 Additives, premixes and compound feeding material is from a company approved by the national competent authority.

10. Storage of feed

10.1 Feed should be properly stored according to manufacturing instructions and should not be allowed to be contaminated by vermin that could be a source of *Salmonella*.

11. Feed quality

11.1 The levels of additives and veterinary drugs should comply with national regulations.

11.2 Packages of feed should be properly labelled with a description of composition, proper storage conditions, expiry date, feeding rate and other necessary guidance.

12. Feeding

12.1 Feeding practices shall minimize the risk for biological, chemical and physical contamination of feed and animals.

12.2 Feeding practices should ensure the maintenance of water and sediment quality.

13. Feed production on farm

13.1 Ingredients, additives and veterinary drugs used in feed must be approved for aquaculture species.

13.2 Good hygiene practices are applied on the farm production of feed to minimize hazards with potential to compromise feed and food safety.

II. Management of veterinary drugs and chemicals

14. Only drugs and chemicals approved by the national competent authority are used in aquaculture.

14.1 Veterinary drugs, medicated feeds, chemical and biological substances are sourced only from registered or authorized manufactures and suppliers.

14.2 Veterinary drugs, medicated feeds, chemical and biological substances are permitted/registered by the national competent authority.

14.3 Substances requiring prescription are used under adequate supervision by qualified expert.

14.4 Veterinary drugs, medicated feeds, chemical and biological substances are labelled with clear information on the name, active substance, target species of animals, storage conditions, dosage, route of administration, expiry date and withdrawal period.

15. Storage and use of veterinary drugs and chemicals

15.1 Veterinary drugs, medicated feeds, chemicals and biological substances are adequately stored according to the label.

15.2 Veterinary drugs, medicated feeds, chemical and biological substances are used according to manufactures instruction and as specified on label.

15.3 Withdrawal periods and residues are verified by adequate testing.

III. Primary processing on farm

16. Ice and water supplies

16.1 Potable or clean water is available and used in sufficient amount for harvest, handling and cleaning operations.

16.2 Ice should be manufactured using potable water and produced under sanitary conditions.

16.3 Ice should be handled and stored under good sanitary conditions which precludes the risk for contamination.

17. Harvesting

17.1 Harvesting equipment and utensils easy to clean disinfected and kept in clean condition.

17.2 Harvesting is planned in advance to avoid time/temperature abuse.

17.3 Aquaculture products should be hygienically handled.

17.4 Records on harvesting is maintained for traceability.

18. On farm post-harvest handling

18.1 Utensils and equipment for handling and holding of aquaculture products is maintained in a clean condition.

18.2 Aquaculture products are cooled down quickly and maintained at temperatures approaching that of melting ice.

18.3 Operations such as sorting, weighing, washing, drainage, etc., is carried out quickly and hygienically.

18.4 All additives and chemicals (disinfectants, cleaning agents, etc) used in post-harvest aquaculture products should be approved by the national competent authority.

19. Transport of aquaculture products from farm

19.1 Transport is carried out in easy to clean and clean facilities (boxes, containers, etc.).

19.2 Conditions of transport should not allow contamination from surroundings (e.g. dust, soil, water, oil, chemicals, etc.).

19.3 Aquaculture products are transported in containers with ice or with ice + water, in sufficient amounts to ensure temperature around 0 ºC (approaching that of melting ice) in all products and during the whole period of transport.

IV. Record keeping

20. Adequate records shall be kept on:

- type, origin and use of feed and feed ingredients;
- veterinary drugs, chemicals, or other treatments administered;
- occurrences of diseases which may affect food safety;
- pond management activities (e.g. preparations and water quality controls);
- origin and type of seed used;
- harvest, transport and customer records are maintained to allow traceability.

21. Training of farm staff

21.1 Staff is trained and has knowledge on food safety issues related to handling of feed, veterinary medicines, chemicals, live animals and harvested products, adequate to the nature of duty.

22. Employee health

22.1 Staff should be medically fit to work and should be screened regularly to determine carriers of *Salmonella*.

23. National regulations on the production of safe aquaculture products

23.1 National governments should designate competent authorities with responsibilities for regulating the production of safe aquaculture products.

23.2 These responsibilities should include:

- the approval, sale, and use of veterinary medicines and chemicals used in aquaculture;
- the national competent authority should license or register aquaculture farms;
- the national competent authority should ensure the enforcement of aquaculture regulations; and
- the national competent authority should ensure an adequate funding to implement a national food safety programme for aquaculture production.

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APPENDIX 2

Salmonella serovars reported in fish and fishery products

Salmonella Serovar	India/ SE Asia	Africa	Central America	Central pacific	Eastern Caribbean	Europe and Russia	Mexico	Middle East	North America/ Multiple	South America
S. Abaetetuba			+							+
S. Aberdeen	+									
S. Agona	+						+			+
S. Ahepe		+								
S. Albany	+									
S. Anatum	+			+	+	+	+			+
S. Anfo	+									
S. Arizonae	+			+			+			+
S. Atakpame	+									
S. Augustenborg	+									
S. Baguida										+
S. Bareilly	+						+			
S. Biafra	+									
S. Blockley	+									
S. Bovis-mobificans	+			+						
S. Bradford	+									
S. Braenderup	+									
S. Brancaster	+									
S. Bredeney										+
S. Brunei	+									
S. Bullbay										+
S. Cannstatt							+			
S. Carrau			+							
S. Cerro	+									+
S. Derby	+			+						
S. Drypool	+									
S. Dublin	+									
S. Duesseldorf				+						
S. Emek	+									
S. Emek	+									
S. Enteritidis	+	+		+			+		+	+
S. Farmsen	+									
S. Gallinarum	+									
S. Georgia	+									
S. Gwaai					+					
S. Hadar	+			+			+	+		
S. Harmelen			+							
S. Havana	+									
S. Havana					+					
S. Heidelberg	+			+						+
S. Hilversum				+						
S. Houten			+	+						+
S. Houten	+									

Salmonella Serovar	India/ SE Asia	Africa	Central America	Central pacific	Eastern Caribbean	Europe and Russia	Mexico	Middle East	North America/ Multiple	South America
S. Hull		+								
S. Hvittingfoss	+									
S. Idikan	+									
S. Infantis	+		+		+					
S. Irumu	+									
S. Isangi	+									
S. Javiana	+			+						+
S. Kentucky	+			+			+			+
S. Kirkee	+									
S. Kottbus	+									
S. Krefeld	+									
S. Kumasi	+									
S. Lanka	+						+			
S. Lansing	+									
S. Lexington	+									
S. Liandoff							+			
S. Lindenburg	+									
S. Litchfield										+
S. Liverpool	+									
S. London	+			+						
S. London										
S. Manila				+						
S. Marina										+
S. Mbandaka	+		+							
S. Meleagridis	+						+			
S. Mendoza										+
S. Mgutani	+									
S. Miami		+								
S. Michigan							+			
S. Minnesota	+						+			
S. Montevideo	+									
S. Morehead	+									
S. Mosselbay										+
S. Muenchen	+						+			
S. Muenster	+				+					
S. Nairobi		+								+
S. Nchanga	+									
S. Newbrunswick							+			
S. Newington										+
S. Newport	+		+	+					+	+
S. Ohio	+						+			
S. Onireke		+								
S. Oranienburg	+				+		+			+
S. Orientalis				+						
S. Oslo	+		+							
S. Othmarschen	+									
S. Panama										+

Salmonella Serovar	India/ SE Asia	Africa	Central America	Central pacific	Eastern Caribbean	Europe and Russia	Mexico	Middle East	North America/ Multiple	South America
S. Paratyphi B	+			+						
S. Paratyphi B Java	+									
S. Parera		+								
S. Phoneix										+
S. Pomana			+				+			
S. Poona	+		+				+			
S. Potsdam	+									
S. Pullorum				+						
S. Reading	+									
S. Redba		+								
S. Reinikendorf	+									
S. Riggil	+									
S. Rissen	+									
S. Rissen	+									
S. Rubislaw		+	+				+			
S. Saintpaul	+			+			+	+	+	+
S. Saka		+								
S. Sandiego	+									+
S. Saphra							+			
S. Sarajane	+									
S. Schleisshein	+									
S. Schwarzengrund										
S. Senftenberg	+		+		+		+	+		
S. Singapore	+									
S. Srinagar	+									
S. Stanley	+			+						
S. Takoradi	+									
S. Tananarive	+									
S. Telelkebir		+								
S. Tennessee	+			+			+			
S. Thompson	+			+			+		+	
S. Tornow										+
S. Typhi	+									
S. Typhimurium	+		+		+		+		+	+
S. Uganda					+					
S. Urbana	+									
S. Virchow	+			+					+	
S. Wandsworth	+									
S. Washington	+									
S. Weltevreden	+		+	+		+	+			+
S. Weston	+									
S. Worthington	+									

This document contains the report of the FAO Expert Workshop on the Application of Biosecurity Measures to Control *Salmonella* Contamination in Sustainable Aquaculture" held in Mangalore, India from 19 to 21 January 2010. The experts reviewed the current scientific evidence regarding the pathogen *Salmonella enterica*, its occurrence and survival in aquatic environment, possible pathways of contamination of aquaculture systems, serovars found in seafood and salmonellosis associated with fish and fishery products. The experts agreed that although *Salmonella* is a major foodborne pathogen, products of aquaculture are rarely involved in outbreaks of salmonellosis and the serovars which have been reported in products of aquaculture are rarely reported in cases of human salmonellosis in fish importing countries. The experts recognized that there are a variety of pathways reported as to how *Salmonella* can enter the aquaculture environment ranging from wild animals, domestic stock, poor sanitation and inappropriate disposal of human and animal wastes. Control of such pathways poses major challenges such as land runoff during rains, control of wild animals in the farm environment. There was agreement that very low level prevalence of *Salmonella* can be seen in products from aquaculture systems in developed countries but this has not led to any particular public health problem in these countries. The experts agreed that good hygienic practices during aquaculture production and biosecurity measures can minimize but not eliminate *Salmonella* in products of aquaculture. Biosecurity and control measures that would be useful in minimizing the risk of *Salmonella* contamination of aquaculture products were identified. The experts identified data gaps and made a series of recommendations to the national governments, national competent authorities, aquaculture industry and FAO.

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