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ORIGINAL ARTICLE

Prevalence of *Salmonella* in fish and crustaceans from markets in Coimbatore, South India

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Seven hundred and thirty fishes and 276 crustaceans collected from various fish markets of Coimbatore, South India, over a period of 2 years (September 1990 to August 1992) were analysed for the prevalence of Salmonella*. Fishes (14·25%) and 17·39% of crustaceans were found to be contaminated with* Salmonella*. Of the different fishes analysed, the highest incidence of* Salmonella *was seen in Scopelidae (28%) followed by Trachnidae (26·9%). Among crustaceans* Portunus pelagicus *(33·33%) showed the highest incidence followed by* Scylla serrata *(28·57%). A well-marked seasonal variation in the incidence pattern was observed in both fishes and crustaceans with a higher incidence during monsoon season followed by post-monsoon and pre-monsoon. The region of the body that showed frequent isolation was the alimentary canal in fishes (41·33%) and gills (35·06%) in crustaceans. Serotyping of the isolates revealed prevalence of* Salmonella weltevreden, Salmonella typhi, Salmonella paratyphi B, Salmonella mgulani *and* Salmonella typhimurium *in both fishes and crustaceans.* Salmonella senftenberg *was isolated only from crustaceans.*

Introduction

Contamination of seafood by harmful Gram-negative bacteria is of great concern from a public health view point. Salmonellosis is one of the most prevalent zoonotic diseases. Despite continuous surveillance and concerted efforts, food-poisoning outbreaks due to salmonellosis are on the increase particularly in western countries. In those countries that maintain useful records of foodborne diseases, fishery products account for significant proportion of the outbreaks reported. The proportion varies from one country to another depending on climate, dietary customs and other social differences. In the USA, salmonellosis accounts for about 60% of all bacterial disease outbreaks (Bean et al. 1990); the actual number is close to 2 million (Beran et al. 1991). In Japan, where dishes based on raw seafood are extremely popular, about 70% of food-poisoning that occurs in summer months is caused by bacterial pathogens derived from fish products (Joseph et al. 1982).

In a developing country like India, there is no such constant monitoring system and the number of exact cases is not known. Fish, molluscs and marine crustaceans were implicated as vehicles of many cases of foodborne outbreaks. Therefore, in recent years,

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emphasis has been placed on the importance of marine fish and shellfish as vehicles of *Salmonella*-induced gastroenteritis outbreaks. In India, reports of *Salmonella* isolation from fishery products were limited to those by Iyer et al. (1986) in the samples from Bombay markets, Iyer and Shrivastava (1989) from shrimp, lobsters, cuttle fish, seer fish and cat fish, and Valsan et al. (1985) from the shrimps sold in Bombay markets. For the formulation and recommendation of quality standards, for fish in domestic trade, detailed background information and an in-depth study of the present status is necessary. However, despite being the focus of interest in medical bacteriology the world over, the ecology and seasonal incidence/ distribution of *Salmonella* in fish and crustaceans sold in domestic trade remains barely comprehended, and hence this study was undertaken.

Materials and Methods

Collection of samples

Fish and crustacean samples were collected from various fish markets around Coimbatore city, for a period of 2 years (September 1990–August 1992). The samples were collected individually in sterile polythene bags. Collections were made between 7 a.m. and 9 a.m. To study the seasonal variation in prevalence of *Salmonella*, the study period has been divided into pre-monsoon (February–May), monsoon (June–September) and post-monsoon (October–January). The samples collected were kept in portable ice-chests and transported to the laboratory. Processing and inoculation of the samples were completed within 2–4 h of collection. Aseptic procedures were strictly adopted during the analysis.

Bacteriological methods

The swab technique was used to sample the body surface, gill and alimentary canal of the fish and crustaceans. The swab technique was selected because it was inexpensive and practical. Although its sensitivity and reproducibility are not optimal, it is considered to be satisfactory (O'Brien 1988).

The swabs were pre-incubated in 10 ml lactose broth (Hi media laboratories, Bombay, India) at 37°C for 24 h. One millilitre of pre-enriched cultures were then transferred to 10 ml tetrathionate broth (Hi media) and selenite broth (Hi media) and incubated at 37°C for 24 h for selective enrichment. After selective enrichment a loopful of culture was streaked onto xylose–lysine–deoxycholate agar (Hi media), brilliant green agar (Hi media) and bismuth sulphite agar (Hi media) and incubated for 24–48 h at 37°C. Typical colonies were picked up, purified and subjected to preliminary biochemical screening, which involved hydrogen sulphide production in triple sugar iron agar (Hi media) and lysine iron agar (Hi media), indole production in tryptone broth (Hi media) and urea splitting ability on Christiansen's urea agar (hi media). Cultures that matched typical reactions of *Salmonella* in preliminary screening were further subjected to carbohydrate utilization involving lactose, sucrose, dulcitol and salicin, and further confirmed by slide agglutination test using polyvalent O sera (Wellcome Laboratories, Dartford, England). The confirmed cultures were then sent to National Salmonella and Escherichia Centre, Central Research Institute, Kasuali, for serotyping.

Statistical Analysis. Data obtained in this study were subjected to statistical analysis. Two-way analysis of variance (ANOVA) was used to study the significance. The F statistical value and level of significance are given in the footnotes of Tables 2, 3 and 4.

Results

The number of fishes analysed and the prevalence of *Salmonella* in different families of fishes are given in Table 1. The serotypes isolated from each group are also included in the Table. The fishes belonging to the family Scopelidae showed higher incidence of *Salmonella* (28%) followed by Trachnidae (26·9%) and Mugilidae (24·4%). While fishes belonging to Chirocentridae and Scarridae showed identical level of incidence (10%) of *Salmonella*, the fishes belonging to the families of Cyprinidae, Siluridae and Mullidae did not show prevalence of *Salmonella*. Five different *Salmonella* serotypes were isolated from fishes viz., *Salmonella weltevreden*, *Salmonella typhimurium*, *Salmonella paratyphi*, *B*, *Salmonella typhi* and *Salmonella mgul-*

Table 1. Incidence of *Salmonella* in fishes and the serotypes encountered in each group

Fishes analysed	Incidence (%)	Serotypes isolated
Scomberosocidae (18)	5·6	*Salmonella weltevreden*, *Salmonella paratyphi B*
Clupidae (235)	10·6	*S. weltevreden* *Salmonella typhimurium* *S. paratyphi B*
Scrombridae (93)	12·9	*S. typhimurium* *S. weltevreden*
Mugilidae (86)	24·4	*Salmonella typhi* *Salmonella mgulani*
Carangidae (85)	14·1	*S. paratyphi B* *S. typhimurium*
Scopelidae (25)	28·0	*S. weltevreden* *S. paratyphi B* *S. mgulani*
Percidae (59)	18·6	*S. typhimurium* *S. typhi*
Trachnidae (26)	26·9	*S. weltevreden* *S. paratyphi B*
Mullidae (19)	0·0	—
Stromatidae (15)	13·3	*S. typhimurium*
Carcharidae (5)	0·0	—
Cyprinidae (10)	0·0	—
Sphyraenidae (6)	0·0	—
Acanthuridae (7)	9·1	*S. weltevreden*
Pleuronectidae (10)	10·0	*S. paratyphi B*
Chirocentridae (10)	10·0	*S. paratyphi B*
Scarridae (10)	10·0	*S. mgulani*
Siluridae (11)	18·2	*S. typhi* *S. weltevreden*

Figures in parenthesis indicates the number of samples.

Table 2. Incidence of *Salmonella* in crustaceans and the various serotypes encountered

Crustacean	Number analysed	Number positive	Serotypes
Machrobrachium rosenbergii	30[a]	6 (20)	*Salmonella senftenberg* *S. typhimurium*
Parapenaeopsis stylifera	32	2 (6·3)	*S. weltevreden*
Portunus pelagicus	18	6 (33·3)	*S. weltevreden* *S. typhi* *S. paratyphi B*
Scylla serrata	21	6 (28·6)	*Salmonella mgulani* *S. senftenberg*
Penaeus indicus	85	18 (21·2)	*S. paratyphi B* *S. senftenberg* *S. weltevreden*
Penaeus monodon	90	10 (11·1)	*S. typhimurium* *S. typhi*

Figures in parenthesis indicates the percent of incidence.
[a]F value 12·79*, 2·03.
*Significant at 0·01 level.

ani. Three percent of the strains were rough and hence untypable.

The number of different crustaceans, incidence and *Salmonella* serotypes isolated from each are represented in Table 2. Though statistical analysis shows no significant variation in the incidence levels of *Salmonella* among various crustaceans, *Portunus pelagicus* (33·33%) and *Scylla serrata* (28·6%) showed a relatively higher level of significance. Six different serotypes were isolated from the crustaceans: *S. typhi*, *S. typhimurium*, *S. paratyphi B*, *Salmonella senftenberg*, *S. weltevreden* and *S. mgulani*. Six percent of the strains were rough. The F-values showed a significant variation between the number of samples analysed and the number of samples tested positive.

Table 3 represents the prevalence of *Salmonella* in fishes and crustaceans during various seasons. Samples collected during the monsoon yielded more positive samples in both fish and crustaceans, the percentage of incidence being 26·1% in fishes and 26·9% in crustaceans, when compared with samples collected in post-monsoon and pre-monsoon. Statistical analysis of the data showed significant variation in the incidence levels during various seasons. However, there was no significant variation in the incidence level between fishes and crustaceans analysed during various seasons.

Table 3. Seasonal variation in the incidence of *Salmonella* in fish and crustaceans

Season	Incidence in fish (%)	Incidence in crustacean (%)
Pre-monsoon	6·4[a]	6·4
Monsoon	26·1	26·9
Post-monsoon	7·1	10·9

[a]F value 1·1069, 67·272*.
*Significant at 0·01 level.

Prevalence of *Salmonella* in different body parts of fish and crustaceans is represented in Table 4. The results show a relatively higher level of incidence of *Salmonella* in the alimentary canal of fishes and gill in crustaceans. However there was no significant variation in the incidence levels at all three regions as per the statistical values.

Discussion

The present study highlights the considerably high prevalence of *Salmonella* in fish and crustaceans in retail markets of Coimbatore, South India. The prevalence was much higher than that reported by Iyer et al. (1986) in the retail markets of Bombay and the fishes sold in the retail markets of Cochin (Nambiar and Iyer 1990). However, Ramamurthy and Natarajan (1987) recorded a higher prevalence of *Salmonella* in fishes sold in the Parangipettai market, South India. Because the sample site was landlocked, most of the fish and crustaceans came from the neighbouring states of Kerala, Karnataka and from the coastal district of Rameswaram. During transportation, periodical dampening of seafood with contaminated water, as well as sprinkling with contaminated wet sand and packing it with cheap quality ice are customary to prevent overheating. The cumulative effect of such conventional practices, coupled with unhygienic handling during transportation explains the high level of *Salmonella* in marketed seafood. Secondary contamination by the use of contaminated water, contaminated beach soil, and excrement from aquatic birds were later reaffirmed by Iyer and Varma (1990). Lack of proper drainage facilities and heavy fly infestation in these markets also promotes secondary contamination to a great extent, especially during the mon-

Table 4. Incidence of *Salmonella* in different body parts of fish and crustaceans

Sample source	Total number of isolates	Number of isolates from each part		
		Body surface	Gill	Alimentary canal
Fish	150	48[a] (32)	40 (26·7)	62 (41·3)
Crustaceans	77	26 (33·8)	27 (35·1)	24 (31·2)

Figure in the parenthesis represents the % of incidence.
[a]F value 0·5759, 11·079 nonsignificant.

soon season. Shedding of *Salmonella* by human carriers while handling seafood is another major cause of contamination.

Results of the prevalence of *Salmonella* in different families of fishes showed a higher incidence in the fishes belonging to Scopelidae, Trachnidae and Mugilidae. Higher incidence in Mugilidae is reported earlier from Parangipettai and Cochin markets (Ramamurthy and Natarajan 1987, Nambiar and Iyer 1990). The high fat and lipid content in these fishes may offer a conducive environment for *Salmonella* to grow, and explains a higher frequency of *Salmonella* in these fishes. However, more detailed study is required to prove this. All the crustacean groups showed incidence of *Salmonella*. Though there was not any significant variation among the various crustaceans, penaeid prawn (*Penaeus indicus*) and crabs (*Scylla serrata* and *Portunus pelagicus*) showed a comparatively higher level of incidence than the other crustaceans. The level of incidence in these crustaceans were much similar to the findings of Venkateswaran et al. (1985).

During the study period, frequent isolation of *Salmonella* was encountered during the monsoon season. Earlier reports (Feachem 1974, Goyal et al. 1977) suggested that a reduced temperature is favoured by many pathogens, which is a well-marked feature during the monsoon months. High temperature results in the reduction of *Salmonella*, as seen in the reduced prevalence during premonsoon months. The seasonal variation in the incidence pattern shows a higher incidence of *Salmonella* during monsoon season, which may be due to the increased sewage and drainage inflow during monsoon season. Similar results were encountered in the samples collected from Mahe estuary by Gore et al. (1992). Considerable volume of land run-off into the coastal waters and prevailing unhygienic conditions during monsoon also results in higher contamination, and hence, increased prevalence of *Salmonella* during this season.

Though there was not any significant variation in the incidences of *Salmonella* among the various body parts analysed the present study revealed that the alimentary canal in fishes and gill in crustaceans was the most favoured habitat. This is contrary to the findings of Grunnet and Gundstrup (1969) and Yoshino and Cheng (1976) who indicated that the digestive tract did not offer a conducive environment for *Salmonella*. However, our results agree with the findings of Youssef et al. (1992) who reported extended survival of *Salmonella* in the intestine of catfishes. The higher incidence on the gill surface of crustaceans may be attributed to the filtering of polluted water and resultant accumulation of bacteria on the gill surface, when they are in the polluted coastal waters.

Among the various serotypes isolated during the study *S. weltevreden, S. typhimurium* and *S. paratyphi B* were frequently isolated from fishes and *S. paratyphi* B and *S. typhimurium* from crustaceans. Lyayman (1966) reported that *S. paratyphi* B was a common pathogen of fish. The existence of *S. typhimurium* in fishes and crustaceans appears to be due to its protracted capacity for survival in these two groups as suggested by Janssen (1974). Heuschmann-Brunner (1974) reported that in crabs persistence of *Salmonella* spp. was found, even after 10 days, in the intestine.

In 1973, the Food and Agricultural Organisation (FAO) published its code of practice for fresh fish which states 'fish as extremely perishable food and should be handled at all times with great care in such a way as to inhibit the growth of microorganisms. Fish quality deteriorates rapidly and the potential keeping time is shortened if they are not handled and stored properly. Much of all fish landed for human consumption is subjected to rough treatment. Fish should not be exposed to direct sunlight or to the drying effect of wind but should be carefully cleaned and cooled down to the temperature of melting ice, 0°C, as quickly as possible'. These conditions are seldom maintained, and it is reported that fish, molluscs and marine crustaceans were implicated as the vehicle in the majority of foodborne outbreaks of *Salmonella* (Bryan 1990). The high prevalence of *Salmonella* in fish and crustaceans is attributed to the poor and unhygienic handling practices and also during transportation from landing centres to fish markets. Environmental conditions prevailing during monsoon also favours a high degree of secondary contamination, as well as an extended survival of these organisms in the aquatic systems. Great care has to be taken to prevent such contamination and also to avoid cross contamination of other food stuffs, which cause serious health hazards. Though there was great awareness about this, as well as implementation of ISO and HACCP systems as far as the fishery products for export are concerned, much remains to be done in assuring the quality of seafood in the retail markets for domestic consumption. The high level of incidence of *Salmonella*, especially potentially hazardous strains like *S. typhi*, poses a serious health hazard to the public.

Programmes should be drawn by the central research institutes (Central Institute of Fisheries Technology and Central Marine Fisheries Research Institute) to educate the local fisherman about the importance of hygienic handling, as well as the use of clean impervious polythene boxes to carry the catch and use of crushed ice made out of potable water, which will help solve the problem to some extent. The Indian Standards Institution (ISI) has specified basic requirements for fish markets but very little attention is given to implement it. Immediate steps have to be taken to implement the recommendations, as well as to formulate and implement quality standards for all fishes and shellfishes sold in retail markets.

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