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A study of the incidence of *Vibrio parahaemolyticus* in Malaysian shrimp undergoing processing for export

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SUMMARY

The incidence of *Vibro parahaemolyticus* in products of the Malaysian export shrimp processing industry was investigated through the stages from the catch to that of the cooked, peeled and frozen product. The organism was commonly found in freshly caught and landed shrimp, and could be detected by enrichment culture at all stages of processing. The numbers of *V. parahaemolyticus* in shrimp varied from nil to 4×10^4, and 19 of the 50 serotypes in the current antigenic scheme were found, O1-K38 and O1-K32 occurring most often. All the isolates were Kanagawa-negative; one strain was a sucrose-positive variant. The study indicated that specifications of $10^2 g^{-1}$ for *V. parahaemolyticus* in raw tropical shelfish are too stringent but that the Malaysian shrimp industry should be able to meet this requirement for cooked shrimp.

INTRODUCTION

Vibrio parahaemolyticus, a marine organism, was first isolated and identified as a cause of food-poisoning in Japan in 1951 by Fujino *et al.* (1953). Since that time it has received world-wide study and is now recognized as a common cause of food-poisoning resulting from the ingestion of fishery products, particularly in summer.

The earlier work on *V. parahaemolyticus* has been extensively reviewed (Sakazaki, 1969; Lee, 1973; Liston, 1973; Barrow, 1974; Barrow & Miller, 1976; Miwatani & Takeda, 1976; Richard & Lhuillier, 1977) and, in 1973, was the subject of an international conference held in Tokyo (Anon, 1974).

Despite its pathogenicity, the public health significance of *V. parahaemolyticus* when isolated from seafoods during routine surveillance is debatable. The pathogenicity of this organism is associated with a positive Kanagawa reaction – the ability to cause lysis of human blood – whereas most strains of *V. parahaemolyticus* isolated from fishery products are Kanagawa-negative, and it has been shown that very large numbers of cells of Kanagawa-negative strains (10^9–10^{10}) need to be ingested before symptoms of gastro-enteritis appear (Miwatani & Takeda, 1976).

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V. parahaemolyticus has a world-wide distribution and in the U.K. occurs in coastal waters, marine deposits and shellfish (Ayres & Barrow, 1978). We have also found it in trawled fish taken from deeper waters off the south coast of England. It is also found in about 1 % of imported, frozen, cooked, peeled, tropical shrimp (Gilbert, personal communication). Outbreaks of food-poisoning caused by *V. parahaemolyticus* are nevertheless uncommon in the U.K. and at present the public health authorities merely maintain a watching brief on the situation, using their discretion with regard to contaminated consignments of imported fishery products (Anon, 1975). Similarly, the International Commission on Microbiological Specifications for Foods (ICMSF, 1974) and the FAO/WHO Expert Consultation on Microbiological Specifications for Foods (Anon, 1977) have temporized in their assessment of the food-poisoning hazard from *V. parahaemolyticus* by recommending a limit of $10^2\,g^{-1}$, and postponing action respectively.

It is therefore of considerable importance that future international public health attitudes should be based on a realistic appraisal of what can be achieved by good commercial practice in those countries such as Malaysia where the incidence of *V. parahaemolyticus* is naturally high in the marine environment. Malaysia has, for many years, been a major exporter to the U.K. so, as part of an overall study of the bacteriological quality of their shrimp processing industry, a qualitative and quantitative survey of the incidence of *V. parahaemolyticus* in shrimp was carried out to obtain data for the formulation of realistic guidelines.

MATERIALS AND METHODS

The survey work was carried out in the Penang-Butterworth area of Malaysia where most of the processing of shrimp for export is done; in the U.K. it was carried out at the ports of London and Southampton. A mobile laboratory was used. Samples of shrimp were collected at various points in the production from catching, through handling, transportation and processing to the finished frozen product. Shrimp taken before factory peeling were examined with the shells on. Frozen samples were thawed at ambient temperature until sufficiently defrosted to allow separation for weighing and homogenization.

Bacteriological examination

Samples of shrimp of about 100 g were weighed accurately and homogenized in 400 ml of chilled, 0·1 % peptone, M15 phosphate buffer (pH 7·0) for 2 min, either with a homogenizer or the Colworth Stomacher.

In early work in 1974 enrichment cultures for *V. parahaemolyticus* were made with 100 ml of homogenate added to 100 ml of double strength TCBS agar but with omission of the solidifying agent. In the later work, 100 ml of homogenate was added to an equal volume of double strength salt colistin broth (Sakazaki, 1972). Enrichment cultures were incubated for 18–24 h at 37 °C and then sub-cultured onto TCBS agar (Oxoid) for incubation at 37 °C for 18 h.

Quantitative studies

Serial decimal dilutions of original homogenate were made in peptone-phosphate buffer, 0·1 ml volumes seeded onto TCBS agar plates using a modified Miles and Misra technique (Walker, Cann & Shewan, 1970) and incubated as before.

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Table 1. V. parahaemolyticus/*g in factory processed shrimp*, 1974

Sample	Factory A	Serology O	Serology K	Factory B	Serology O	Serology K	Factory C*	Serology O	Serology K
Raw	−	.	.	−	.	.	−	.	.
	+	8	22	.	.	.	−	.	.
Cooked	−	.	.	−	.	.	.	.	.
	−	.	.	+	NT		.	.	.
Peeled	−	.	.	−	.	.	−	.	.
	+	NT		+	1	25	−	.	.
	−	.	.	−	.	.	$1{\cdot}1 \times 10^3$	NT	
	−	.	.	.	.	.	$2{\cdot}9 \times 10^3$	NT	
	−	.	.	.	.	.	$2{\cdot}0 \times 10^1$	4	55
	+	7	19	.	.	.	.	.	.
Packaged	−	.	.	.	.	.	.	.	.
Frozen	−	.	.	+	1	38	−	.	.

* Product processed as raw peeled form.
\+ denotes organism present in 20 g using enrichment techniques.
− denotes organism absent in 20 g using enrichment techniques.
NT, not yet typeable.

Identification of V. parahaemolyticus

After incubation characteristic large (2–4 mm) deep green, dome-shaped colonies were counted and up to five colonies were picked from both direct and enrichment cultures, sub-cultured onto TCBS, checked for purity by further subculture on blood agar and subsequently identified as *V. parahaemolyticus* by the use of the tests cited by Barrow & Miller (1976). Presumptive isolates of *V. parahaemolyticus* were sent for confirmation and serotyping to the Public Health Laboratory, Truro, Cornwall, England.

The numbers of *V. parahaemolyticus* were calculated by multiplying the presumptive count by that proportion of the five colonies taken from the count plates which were confirmed as *V. parahaemolyticus*.

RESULTS

In 1974 *V. parahaemolyticus* was isolated from 7 of 10 samples of shrimp collected at sea from commercial vessels fishing off the island of Penang, from 8 of 12 samples taken from different landing places and from 9 of 27 samples examined during processing procedures. The stages of processing from which the organism, and its serotypes, was isolated from three processing plants are shown in Table 1. Additional serotypes isolated from the freshly caught and landed shrimp were O1-K1, O2-K28 (sucrose positive variant), O4-K34 and O4-K42; three isolates were untypeable. Subsequent examinations of 58 samples of frozen shrimp after shipment to the Port of London yielded two positive untypeable isolates.

During 1978–9 the organism was isolated from 5 of 9 freshly landed samples and from 49 of 86 sampled at production level in three processing plants (Table 2). It was not isolated from a total of 50 packages from two consignments of frozen shrimp either before or after shipment to the U.K.

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Table 2. *V. parahaemolyticus/g in factory processed shrimp*, 1978–9

Sample	Factory A	Factory C*		Factory D
Raw – shell on	$5{\cdot}0 \times 10^2$	$5{\cdot}0 \times 10^1$		+
	−	$4{\cdot}3 \times 10^3$		+
	+	$4{\cdot}0 \times 10^4$		+
	+	$5{\cdot}0 \times 10^2$		.
	−	.		.
Raw – peeled	.	.		$6{\cdot}0 \times 10^3$
	.	.		$3{\cdot}3 \times 10^3$
Cooked – shell on	−	−		+
	−	−		+
	−	+		−
	−	+		.
	+	.		.
Cooked – raw peeled	.	.		+
	.	.		+
		Machine peeled	Hand peeled	
Cooked shell on – peeled	$2{\cdot}5 \times 10^2$	−	+	+
	$5{\cdot}0 \times 10^2$	+	+	−
	+	.	.	−
	$3{\cdot}5 \times 10^2$	.	.	−
	+	.	.	+
	−	.	.	.
	−	.	.	.
	−	.	.	.
	−	.	.	.
Graded	−	−	−	.
	.	+	.	.
Packaged	−	−	−	+
	−	+	+	−
	+	.	.	+
	+	.	.	−
	+	.	.	+
Frozen	−	−	−	+
	−	−	−	$< 1{\cdot}5 \times 10^3$
	−	−	−	+
	−	+	+	+
	−	+	+	+
	.	+	+	.

\+ denotes organism present in 25 g using enrichment techniques.
− denotes organism absent in 25 g using enrichment techniques.
* Product processed as raw peeled form.

Of the 50 serotypes in the current antigenic scheme (Miwatani & Takeda, 1976) 17 were found in the samples tested (Table 3); of these O1-K38 and O1-K32 occurred most frequently. Forty-one of 126 isolates were, however, untypeable. Several different serotypes were often isolated from one primary culture. On one occasion four serotypes were identified amongst the five colonies taken from a TCBS plate culture.

Total counts of *V. parahaemolyticus* in shrimps varied from nil to 4×10^4 per gram. Samples containing countable numbers of the organism were usually from raw

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Table 3. *Incidence of serotypes among* 126 *strains of* V. parahaemolyticus *isolated from Malaysian shrimp in* 1978–9

Serotypes		No.
O	K	isolated
1	1	3
1	32	18
1	38	24
1	56	1
2	28	3
3	5	3
3	6	4
3	7	3
3	48	4
5	17	2
5	47	6
5	60	1
7	19	1
8	21	1
8	22	5
11	36	1
11	50	5
Not typeable		41
Total		126

shrimp but on one occasion a count as high as 5×10^2 per gram was obtained from a cooked, peeled sample (Tables 1 and 2). The counting of presumptive colonies of *V. parahaemolyticus* was complicated by the presence of the ubiquitious and often overwhelming concomitant growth of *V. alginolyticus*, which Barrow & Miller (1976) have suggested might well be used as an 'indicator' organism in assessing hygienic production.

All the isolates of *V. parahaemolyticus* obtained in both 1974 and 1978 were Kanagawa negative.

DISCUSSION

From the data obtained in both 1974 and during 1978–9, it is clear that *V. parahaemolyticus* is part of the usual flora of Malaysian shrimp as well as that of molluscs (Jegathesan *et al.* 1976), and there should always be strict attention to hygiene to prevent cross contamination of cooked from raw products. The organism is also present in sufficient numbers in raw shrimp to survive factory cooking procedures, which vary from only 30 s to 2 min, and they may thus persist throughout the production line, though usually in greatly reduced numbers when the product is frozen. However, the finding of a count of $1{\cdot}5 \times 10^3$ in one frozen sample supports the conclusions of Johnson & Liston (1973) and Cann (1976) that cold storage alone cannot be relied on to eliminate this organism from seafoods.

The counts of *V. parahaemolyticus* obtained in this work probably underestimate the true numbers. *V. alginolyticus*, for example, was almost invariably present in much greater numbers than *V. parahaemolyticus* itself. *V. alginolyticus* is closely related to *V. parahaemolyticus*, with which it grows competitively on TCBS agar.

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Until a medium is developed which will selectively inhibit *V. alginolyticus* in favour of *V. parahaemolyticus* direct counts will continue to give low results. The alternative method of a 9-tube most probable number count (ICMSF, 1978) is impracticable during field work of this type because of costs, laboratory time and manpower.

Comparison of the serotypes isolated in 1974 and 1978–9 showed one significant difference in that serotypes of O4 group were only isolated in 1974; this probably reflects selectivity of the different enrichment media used, as a similar phenomenon was observed during studies on trawled fish in the U.K. when both TCBS and SCB enrichment media were used (Cann *et al.*, unpublished results).

It is of interest to note that of the total of 21 different serotypes isolated from Malaysian shrimp, types O1-K38 and O1-K32 predominated, whereas in a survery in the U.K. (Ayres & Barrow, 1978) serotypes of O5-K30, O5-K17 and O2-K3 comprised more than 50% of the identifiable isolates. Since this major study of the incidence of *V. parahaemolyticus* in the coastal environment of the U.K. embraced 13 laboratories and a variety of enrichment techniques (Ayres & Barrow, 1978), including those used in the present work, differences in methodology can probably be discounted. It would appear, therefore, that the overall incidence of *V. parahaemolyticus* in the marine environment of the U.K. is unlikely to be the result of discharge of effluent from the processing of imported tropical crustacea, as reported from the Humber estuary (Ayres & Barrow, 1978) particularly as Malaysian processed shrimp has always comprised the bulk of imports of tropical crustacea into the U.K.

When considering the application of guidelines or standards for *V. parahaemolyticus* to fishery products, the results of this work suggest that more latitude should be given to tropical products than their cold-water equivalents. Fishbein *et al.* (1974) and Sutton (1974) found *V. parahaemolyticus* counts of $100\,g^{-1}$ or less in most instances and these findings are reinforced by experience during participation in the U.K. collaborative study when the senior author of this paper was never able to isolate *V. parahaemolyticus* other than by enrichment culture.

Raw fishery products should also be assessed differently from cooked products. The ICMSF blanket approach of applying limits of $10^2\,g^{-1}$ for all fishery products appears too severe in the light of *V. parahaemolyticus* numbers of up to $4 \times 10^4\,g^{-1}$ raw shrimp. To meet the ICMSF microbiological limits of $10^2\,g^{-1}$ the average outgoing quality of processed shrimp needs to be of the order of $12\,g^{-1}$ at a confidence level of 95% (Howgate, personal communication). Malaysian production of FCP shrimp should be able to meet this specification provided processors maintain good hygiene and standardize boiling times at 2 min so that a more effective reduction of *V. parahaemolyticus* can be achieved.

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