

OCCURRENCE AND DISTRIBUTION OF *VIBRIO PARAHAEMOLYTICUS* AND RELATED ORGANISMS IN THE ARABIAN SEA

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ABSTRACT

The occurrence and distribution of *Vibrio parahaemolyticus* and related organisms were studied from offshore water samples (Lat. 10°N and Long. 65° to 69°E) collected at 5, 10, 50, 100, 200 and 500 m depths and in the surface water samples of Minicoy lagoon during January, 1982. *V. parahaemolyticus* like organisms contributed upto 94.4% of total viable counts in the offshore stations and 5.3% in lagoon stations. Although typical *V. parahaemolyticus* were not recorded from the open ocean, in the lagoon waters their density ranged from 0.002 to 0.66% of the total viable counts.

Key words: *Vibrio parahaemolyticus*, Arabian Sea.

INTRODUCTION

Vibrio parahaemolyticus is an etiological agent in the sea-food borne gastroenteritis. This halophilic bacterium is known to occur not only in the seas and estuaries but also in freshwater regions. While the widespread occurrence of these forms in near shore regions and in association with marine animals are well documented. (Colwell, Kaneko and Staley, 1972; Bockemuhl and Treimer, 1974; Vasconcelos, Stang and Laidlaw, 1975; Sutton, 1974 and Asakawa Akabane and Noguchi, 1974), their occurrence and distribution in the offshore waters is still a controversy. Although earlier reports by Aoki (1967a, b), Yasunaga (1964) and Gianelli, Cabassi, Allodi and Creschi (1970) indicated their presence in open ocean, later reports by Kaneko and Colwell (1973) showed that not only do *V. parahaemolyticus* like organisms progressively diminish with distance from shore but also that typical *V. parahaemolyticus* is totally absent. While the distribution pattern of *V. parahaemolyticus* in inshore Indian waters is well documented (Martin 1981; Pradeep and Perumalsamy, 1984), their distribution in offshore waters is not well understood (Loka Bharathi, Ramaiah and Chandramohan, 1986). This study describes their distribution pattern in the open ocean and in regions around the Minicoy coral reefs (Lakshadweep Islands).

MATERIALS AND METHODS

Samples were collected and analysed on board the research vessel, *Gaveshani* during its 104th cruise from 11th to 26th January 1982. Three

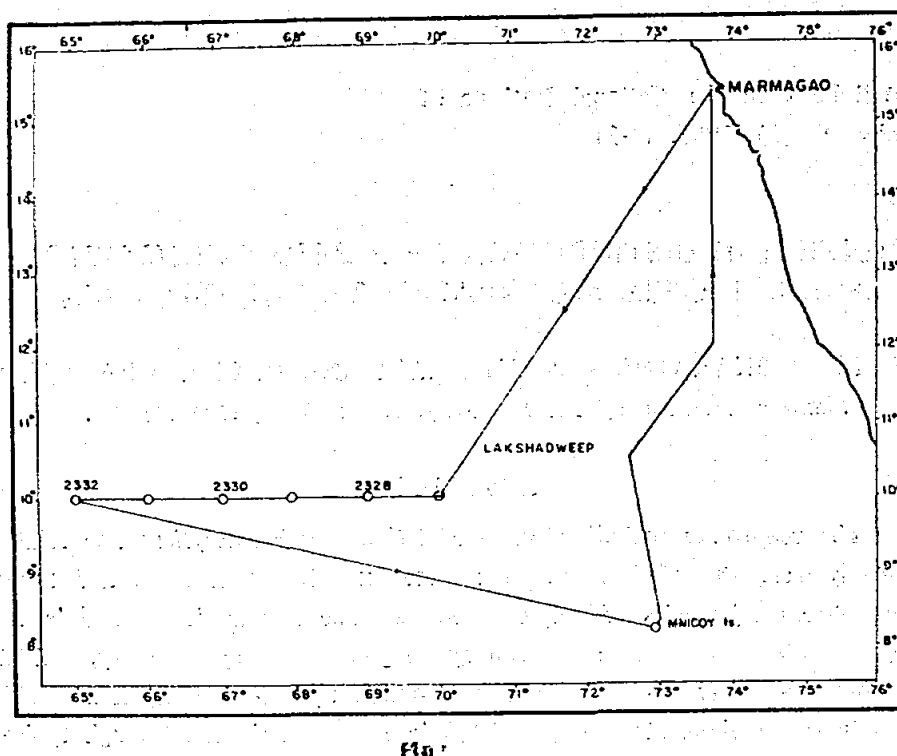


Fig. 1. Location of offshore sampling stations

offshore stations in the Arabian sea were selected for depthwise sampling (0, 10, 50, 100, 200, and 500 m) (Fig. 1). In the lagoon of Minicoy island surface water samples were collected from 10 stations (Fig. 2). While Zo-Bell's bacteriological samplers were used for the offshore stations, sterile glass stoppered bottles were used for lagoon samples.

Bacteriological analyses

Bacteriological analyses were carried out immediately after collection. Total viable counts (TVC) and vibrio counts were made following the membrane filtration technique (0.45 μ m pore size filters, Millipore Corporation, Bedford, Massachusetts, USA), Zo Bell's 2216 E medium was used to estimate total viable count (TVC), thiosulfate citrate bile salts sucrose agar (TCBS) was used to estimate vibrio like organisms (VLO) and *V. parahaemolyticus* like organisms (VPLO). Colonies that grow on TCBS agar were recorded as VLO and typical bluish green sucrose negative colonies on TCBS as VPLO (Kaneko and Colwell, 1973). Known volumes of water samples were filtered and the filters were placed on nutrient media or in TCBS petridishes. The colonies appearing on the filters were counted after 48 hours. The microbial populations were expressed as number per 100 ml.

Well isolated typical green colonies (VPLO) and atypical yellow and white colonies from various samples were removed from the membrane filters, streaked on fresh TCBS agar, checked for purity and maintained on nutrient

agar slants. A total of 134 isolates were obtained in pure cultures (54 from offshore marine and 80 from lagoon) and were subjected to 23 tests following the identification scheme of Sakazaki (1967), modified by Kaneko (1973) to confirm their identification as *Vibrio parahaemolyticus* (VP).



Fig. 2. Location of sampling stations in Minicoy lagoon

RESULTS

The physico-chemical parameters in the offshore stations did not deviate from the normal variations. Dissolved oxygen varied from 4 ml/l at the surface to 1 ml/l at 500 m. Phosphate and nitrate showed an increasing trend with depth. Phosphate ranged from 0.31 at the surface to 2.55 μg at P/1 at 500 m. Nitrate ranged from 0.4 at the surface to 26.5 μg at N/1 at the 500 m in these stations. Salinity varied from 36.7‰ at 0 m to 35.1‰ at 500m. The variation in temperature was from 29.2°C at 0 m to 11.3°C at 500 m.

Dissolved oxygen at the surface waters of the lagoons varied from 6.8 ml/l to 4.17 ml/l. Phosphate was < 1 μg at P/1 and nitrate < 1 μg at N/1. Salinity of lagoon waters was > 35.4‰ with an average of 35.6‰. Maximum temperature recorded was 29.6°C and the minimum was 28.8°C with an average of 29.3°C.

TVC estimated at different depths from the three offshore stations showed that it ranged from 42/100 ml at 500 m depth (stn 2332) to 448/100 ml at

Table I. Total viable counts, (TVC) Vibrio like organisms, (VLO) *Vibrio parahaemolyticus* like organisms (VPLO) and variations in their percentage composition in offshore waters and lagoon waters.

Date of collection	Stn. No.	Location	Station Depth (M)	Sampling Bacterial Population		Variation in Percentage composition						
				Depth (M)	*(TVC	VLO	VPLO	VPLO/VLO	VLO/TVC	VPLO/TVC	VP/VLO	VP/TVC
14.1.82	2328	10°00'N 68°45'E	4512	5	88	66	18	27.3	75.0	20.5		
				10	298	98	52	53.1	32.9	17.5		
				50	94	2	2	100.0	2.1	2.1		
				100	448	8	4	50.0	1.8	0.9		
				200	78	12	0	0.0	15.4	0.0		
				500	118	4	2	50.0	3.4	1.7		
15.1.82	2330	10°00'N 67°00'E	4446	5	156	94	76	80.9	60.3	48.7		
				10	226	130	130	100.0	57.5	57.5		
				50	194	80	72	90.0	41.2	37.1		
				100	144	48	40	83.3	33.3	27.8		
				200	164	48	48	100.0	29.3	29.3		
				5	252	250	20	100.0	35.7	35.7		
15.1.82	2332	10°00'N 65°00'E	4470	500	56	20	238	95.2	99.3	94.4		
				10	260	136	132	97.1	52.3	50.8		
				50	194	80	72	90.0	41.2	37.1		
				100	144	48	40	83.3	33.3	27.8		
				200	164	48	48	100.0	29.3	29.3		
				500	56	20	20	100.0	35.7	35.7		

15.1.82	2332 10°00'N 4470 65°00'E	5	252	250	238	95.2	99.3	94.4	
		10	260	136	132	97.1	52.3	50.4	
		50	254	146	132	90.4	57.5	52.0	
		100	296	178	166	93.3	60.1	56.1	
		200	246	134	90	67.2	54.5	36.6	
		500	42	26	22	84.6	61.9	52.4	
			(x 10 ⁴ /100 ml)						
20.1.82	2333-1 08°18'N 2 73°03'E	0	6000	256	140.8	55.0	4.3	2.3	
		0	7800	59.6	5.8	9.7	0.8	0.07	
		0	1350	6.4	0.4	6.3	0.5	0.03	16.7
		0	26000	2.3	0.4	17.4	0.01	0.002	0.005
		0	34700	604.8	3.2	0.5	1.7	0.03	0.002
		0	10600	1.8	1.3	72.2	0.02	0.01	
		0	3.8	0.7	0.2	28.6	18.42	5.3	12.5
		0	40.4	4.8	1.7	35.4	11.9	4.2	0.66
		0	61100	2.2	0.5	22.7	0.01	0	
		0	12400	0.5	0.3	60.0	0.01	0.002	

100 m depth (stn 2328). Percentage of VLO in TVC ranged from 2.1 at 50 m depth (stn 2328) to 99.3 at 5 m depth (stn 2332) (Table I). Though VPLO formed definite fractions of TVC in different depths in three stations contributing as much as 94.4% of TVC (5 m; stn 2332) no typical *V. parahaemolyticus* could be detected. In the lagoon stations, however, the number of TVC were 10^2 - 10^6 times greater than that observed in the open waters. VLO formed 0.01 to 18.42% of TVC and VPLO from 0.5 to 60% of VLO. *V. parahaemolyticus* were recorded only in 3 out of 10 stations and ranged from 0.002 to 0.66% of TVC (Table I).

Table II. Morphological cultural and Biochemical response of VLO isolated from Marine and lagoon samples

Tests	Percentage positive		Tests	Percentage positive	
	Marine*	Lagoon**		Marine	Lagoon
Gram	44.4	28.6	Urease	0.0	31.4
Motility	61.0	20.0	H/L	55.6	0.0
Growth at 43°C	100.0	100.0	Catalase	72.2	68.5
Growth in 0% NaCl		2.9	Oxidase	50.0	0.0
Growth in 3% NaCl		100.0	MR	72.2	57.1
Growth in 7% NaCl		94.3	VP	0.0	94.2
Growth in 10% NaCl		0.0	Lactose fermentation	66.6	48.6
Indole	0.0	62.9	Rhamnose fermentation	88.9	57.1
NO ₃ -reduction	55.6	88.6	Sucrose fermentation	72.2	14.3
Gelatinase	38.9	54.3	Manitol fermentation	33.3	5.7
Amylase	33.3	2.9			

* Total number of isolates = 18 (8-white, 1-yellow, 9-blue green)

** Total number of isolates = 35 (2-white, 12-yellow, 21-blue green)

The results of 23 test characteristics are summarized in Table II. Although 134 isolates were selected for series of tests to identify the VP strains, only 18 marine and 35 lagoon isolates were chosen for the tests based on their ability to grow at 43°C. This therefore includes not only typical blue green colonies but also a few of the 'atypical' white and yellow colonies or VLO.

While 44.4% of the offshore isolates grew at 0% NaCl, only 2.9% of the lagoon isolates tested showed growth in the absence of NaCl. Interestingly 44.4% of the tested offshore isolates grew in 10% NaCl while none of the lagoon isolates could grow at the same concentration. A greater percentage of the lagoon isolates were positive to indole, nitrate reduction, gelatinase, amylase and urease tests. Most of the lagoon isolates did not ferment the sugars tested while the marine isolates did so except in the case of mannitol where only 33.3% of isolates fermented it.

Consequently, none of the isolates from the offshore station was identified as VP. However, they were detected in 3 out of the 10 lagoon samples and were greater than 20% of VPLO and ranged from 0.002 to 0.66% of TVC. Of the total 80 lagoon isolates only 3 i.e. 3.75% were confirmed as VP. About 16.25% of the isolates differed only by lactose test and another 2.5% in lactose and mannitol and another 2.5% in mannitol test.

DISCUSSION

Irrespective of the number of TVC, maximum number of VLO and VPLO were always restricted to either 5 or 10 m depth in offshore stations. This could mainly be due to the higher temperature in surface waters, a factor that is known to influence their distribution. Martin (1981), Aoki (1967a, b) and Kaneko and Colwell (1973) have shown temperature to be a major limiting factor for the distribution of VP in tropical and subtropical waters. Likewise, minimum number of these forms were found at greater depths i.e. 500 m. The pattern of distribution of the percentage of VLO/TVC is also similar. The greater percentage of VPLO/VLO at deeper waters is not due to higher numbers of VPLO but to lower numbers of VLO. In the lagoon, however, high TVC did not always indicate high numbers of VLO or VPLO, though at Stn. 5 high numbers of both TVC and VLO were recorded. In offshore waters variation in salinity is not wide enough to explain disparity in distribution of VPLO or VLO with station or depth.

Recovery of VP from marine environment is complicated because *V. alginolyticus* which is often present in seawater may obscure colonies of VP (Kourany, 1983). In the present study, however, this drawback has been overcome by even isolating the yellow atypical sucrose positive strains for biochemical tests for VP screening. The presence of VP in stations 3, 5 and 7 in the lagoon samples showed that their distribution may in some way be related to the coral reef and its exudates. Studies carried out in the following year (1983) in the Lakshadweep sea by Loka Bharathi, Ramaiah and Chandramohan (1986) also showed their presence in these waters. The absence of VP in the open ocean partly confirm the findings of Kaneko and Colwell (1974) that these do not occur in regions away from the shore. However their presence in Minicoy lagoon waters show that in these tropical seas, their distribution may be influenced to a certain extent by the presence of coral reefs.

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