Survival and growth of non-cholera vibrios in various foods

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SUMMARY

A study was made of the growth of three strains of non-cholera vibrio in a range of foodstuffs and of the effect of temperature and pH on their ability to grow.

Growth was tested at 4°, 10°, 22°, 30°, 37° and 43 °C in a range of foods likely to be incorporated into cold hors d'oeuvres, e.g. egg, cream, rice, cold meat, seafood, aspic and mayonnaise. Non-cholera vibrios grew well in all these foods except mayonnaise, the rate of growth increasing with increased temperature of storage.

At acid pH values the organisms died or grew very poorly but growth improved as the pH became more alkaline. None of the three strains showed any resistance to heat, an initial inoculum of $> 10^7$ organisms/g was reduced to < 100 organisms/g in 2–3 min at 55 °C.

INTRODUCTION

The non-cholera vibrios are organisms biochemically related to *Vibrio cholerae*, both the classical and el tor biotypes, but which do not agglutinate with O1 antiserum (McIntyre & Feeley, 1965). They have been increasingly reported as a cause of diarrhoeal disease in Asia and elsewhere (McIntyre *et al.* 1965; Chatterjee, Gorbach & Neogy, 1970) and are widely found in water and seafoods (Hobbs, 1975; De *et al.* 1977).

Vibrios can be divided into groups according to their pattern of fermentation of mannose, sucrose and arabinose (Heiberg, 1935; Smith & Goodner, 1965). True cholera vibrios fall into group I but other vibrio species, e.g. *V. alginolyticus*, *V. fischeri* and *V. proteus*, may also appear in this group. The non-cholera vibrios fall into groups I, II and V while the marine vibrios, *V. alginolyticus* and *V. parahaemolyticus*, mainly fit into groups III, IV, VII and VIII. A serological typing scheme has been developed for the cholera group of vibrios (i.e. true cholera and the non-cholera vibrios) based on the O antigens (Sakazaki *et al.* 1970). The cholera vibrio is type O1 and there are at least 52 other serogroups or serovars (Furniss, Lee & Donovan, 1978).

In recent years non-cholera vibrios have been incriminated in incidents of food poisoning. Table 1 gives details of four episodes of gastroenteritis, three occurred in Europe and one on a transcontinental flight. A variety of foods were involved although in two instances the contamination was probably introduced from water.

Table 1. *Incidents of gastroenteritis attributed to non-cholera vibrios*

Number of persons		Place	Food incriminated	Incubation period (h)	Symptoms	Organism incriminated	Reference
Ill	At risk						
56	180	Automobile Training Centre, Czechoslovakia	French potatoes	10	Vomiting, diarrhoea, headache and fever	Non-cholera vibrio 42/56 (Heiberg group II)	Aldová et al. (1968)
64	?	Transcontinental flight, London to Sydney	Grated egg on asparagus	5½–37½, average 11½	Nausea, vomiting, colic, watery diarrhoea, some fever	Non-cholera vibrio 10/12 (Heiberg group I 7/10, serovar 7*; Heiberg group II 9/10, serovar 39*)	Dakin et al. (1974)
1	1	Home, Sweden	Home pickled Baltic herring	48	Diarrhoea, dehydration	Non-cholera vibrio (serotype 166†)	Bäck, Ljunggren & Smith (1974)
1	1	Home, Sweden	?	?	Transient diarrhoea	Non-cholera vibrio (serotype 46†)	
13	?	Following consumption of products from a sausage factory, Bulgaria	Boiled pork sausage	½ to several	Vomiting, fever and diarrhoea	V. parahaemolyticus 9/15 Non-cholera vibrio 3/15 (Heiberg group I 1/15, Heiberg group II 2/15)	Zakhariev et al. (1976)

* Serotyping scheme of Sakazaki et al. (1970).   † American serotyping scheme.

Although there are many reports in the literature of studies on the survival of *V. cholerae* and the el tor vibrio in foods and beverages and on fomites (Felsenfeld, 1965; Pesigan, Plantilla & Rolda, 1967; Prescott & Bhattacharjee, 1969; Gerichter *et al.* 1975) and the growth of *V. parahaemolyticus* in various foods (Kodama, 1967; Beuchat, 1975; Nelson & Potter, 1976), we are unaware of similar reports on the behaviour of non-cholera vibrios in such situations apart from a recent study by Müller (1977) on the isolation and survival of these organisms in sewage and water.

The present study was therefore undertaken to determine the ability of non-cholera vibrios to grow in different foods and the effect of pH and temperature on their growth.

MATERIALS

Three strains of non-cholera vibrio were supplied by the Public Health Laboratory, Maidstone. Two were sucrose fermenters, serovar O24 (Heiberg group II) and serovar O37 (Heiberg group I) and the third a non-sucrose fermenter, serovar O32 (Heiberg group V). These strains represented serovars commonly isolated from cases of human diarrhoea.

Growth of the three strains was tested at 4°, 10°, 22°, 30° and 37 °C in most instances but in later experiments the temperature range was extended to include 43 °C. The range of foods included boiled rice, chopped hard-boiled egg, chicken meat, cooked peeled prawns, pasteurized cream, boiled potato, sliced cooked ham, canned tongue, aspic (vegetable stock and gelatin) and mayonnaise. Some of the foods, e.g. rice, egg, chicken, potato and aspic, were prepared in the laboratory; the prawns and tongue were taken from samples received for routine bacteriological testing; the ham was purchased, ready sliced, from a local butcher and the mayonnaise was a commercial bottled variety.

Sørensen's phosphate buffer at a range of pH from 4 to 8 was prepared as required. Plate counts were carried out on 5 % horse blood agar (BA) and thiosulphate citrate bile salt sucrose agar (TCBS) Oxoid CM 333.

METHODS

Growth at different temperatures

Each food was distributed in 10 g quantities in 1 lb screw-capped jars, one jar for each strain, temperature and sampling time. Overnight broth cultures of the three strains of non-cholera vibrio were diluted and 0·1 ml volumes distributed onto the surface of the food with a 50 drop/ml pipette to give an initial inoculum of *ca.* 10^3–10^4 cells/g of food. Sets of jars were stored at the range of temperatures for periods of time up to 3 days. Jars were removed at 6, 18, 24, 48 and 72 h after inoculation and 90 ml of quarter-strength Ringer's solution were added to each to give a 1/10 dilution. After thorough mixing further ten-fold dilutions were prepared and counts made using the modified surface drop technique (Thatcher & Clark, 1968) on BA and TCBS.

Growth at different pH

The effect of pH on growth of the three strains of non-cholera vibrio was determined in phosphate buffer (with and without the addition of 0·1 % peptone)

and in mayonnaise, prawn and luncheon meat slurries adjusted to the required pH. The range of pH values were: phosphate buffer at pH 4·9, 5·8, 6·7 and 7·7 and phosphate buffer with peptone at pH 5·3, 6·1, 7·0 and 8·0; mayonnaise (50% mayonnaise and 50% quarter-strength Ringer's solution, pH adjusted with N-NaOH) pH 4·1, 5·5, 7·6 and 8·6; prawns (50% prawns and 50% phosphate buffer) pH 6·4, 6·7, 7·4 and 7·9 (for the latter the buffer was replaced by quarter-strength Ringer's solution) and luncheon meat (50% luncheon meat and 50% phosphate buffer) pH 5·5, 5·8 and 6·4. All tests were carried out at ambient temperatures (21–24 °C). Ten-gram portions of the food slurries were inoculated with 0·1 ml volumes of suspensions of the organisms as previously described and counts made at 0, 6, 18, 24 and 48 h. For tests in the buffer solutions, 100 ml volumes were inoculated with the test strains and 1 ml volumes removed for counts after 0, 6, 24, 48 and 72 h.

Heat resistance

The heat resistance of the three strains was determined by heating suspensions of the organisms in phosphate buffer (pH 7) in sealed glass ampoules in a water bath at 50°, 52·5° and 55 °C. Counts of survivors were made initially by the conventional surface drop technique and later by a microdilution technique (Kramer, 1977).

<div align="center">RESULTS</div>

The three strains of non-cholera vibrio grew well in all foods tested except mayonnaise. As results were very similar, data are given for only a selection of the foods, namely rice, egg, prawns and aspic (Tables 2 and 3). In general at 4 °C the number of organisms inoculated remained about the same, or decreased slightly. At 10 °C there was usually a slight increase in numbers towards the end of the incubation period although in egg the numbers decreased and became undetectable. At 22°, 30°, 37° and 43 °C the organisms grew well, populations of 10^9 and 10^{10}/g of food being achieved in 24 h. Growth was most rapid in the cooked prawns (Table 3). The pH values of the foods tested were between 6 and 7 except for the mayonnaise (pH 4) and the prawns (pH 7·9). Growth did not occur in the mayonnaise, there was a steady decrease in numbers from an initial inoculum of 10^6–10^7/g to 10^2/g in 1 h. The sample of ham used in the growth experiments was found to have a high initial count (> 10^6 organisms/g) which suppressed the growth of the inoculated organisms. The remaining foods tested were cream, potato and canned tongue; the three strains of non-cholera vibrio grew well in these foods. There appeared to be little difference in the behaviour of the three strains in any of the foods tested.

Results obtained when the test strains were inoculated into phosphate buffer with and without the addition of 0·1% peptone at different pH were similar; results are given for plain phosphate buffer only (Table 4). At pH 4·9 all three strains died out, at pH 5·8 serovar O32 was undetectable after 18 h while the numbers of serovars O24 and O37 gradually declined over the 72 h period. At pH 6·7 the three strains showed an initial decline in numbers, then an increase

Table 2. *Growth of non-cholera vibrios in cooked rice and chopped hard boiled egg stored at 10°, 22°, 30° and 37 °C*

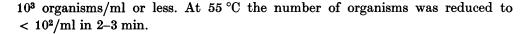| Food	Strain (serovar)	Storage temper- ature (°C)	Log count of non-cholera vibrio after storage (h)					
			0	6	18	24	48	72
Cooked rice	O24	10	2.44	NT	NT	3·04	3·64	4·30
		22	2·44	4·24	7·78	8·30	8·70	NT
		30	2·44	5·70	8·18	7·74	9·48	NT
		37	2·44	6·60	8·95	8·88	9·00	NT
	O32	10	2·71	NT	NT	2·30	2·30	3·20
		22	2·71	3·80	7·60	8·24	9·00	NT
		30	2·71	5·00	8·54	9·18	8·95	NT
		37	2·71	4·18	8·95	9·18	8·60	NT
	O37	10	3·49	NT	NT	3·11	3·04	3·57
		22	3·49	4·10	7·54	7·00	9·00	NT
		30	3·49	6·30	8·70	9·10	9·54	NT
		37	3·49	6·81	8·93	9·10	9·00	NT
Chopped hard boiled egg	O24	10	3·38	NT	NT	2·60	2·60	< 2·00
		22	3·38	2·60	8·48	8·40	10·24	NT
		30	3·38	5·48	9·88	10·18	9·78	NT
		37	3·38	7·00	10·10	10·54	9·30	NT
	O32	10	3·60	NT	NT	2·30	< 2·00	< 2·00
		22	3·60	2·95	8·65	8·81	10·18	NT
		30	3·60	5·18	9·81	9·10	9·10	NT
		37	3·60	6·40	10·00	10·48	8·18	NT
	O37	10	3·62	NT	NT	2·70	2·00	< 2·00
		22	3·62	3·20	8·92	9·74	10·00	NT
		30	3·62	5·90	10·10	10·44	9·88	NT
		37	3·62	6·60	10·10	10·48	9·60	NT

NT = not tested.

followed by a gradual decline. The three strains behaved in a similar manner at pH 7·7, showing the steepest increase in numbers and then maintaining their level before a gradual fall off. In mayonnaise at different pH (Table 5) serovar O37 grew least well, at pH 4·1 and 5·5 the organisms died in less than 6 h, at the higher pH values numbers did not begin to increase until 24 h after inoculation. Serovars O24 and O32 showed a rapid decrease in numbers at pH 4·1 and 5·5, while at 7·6 and 8·6 the numbers remained steady up to 18 h when they began to increase, serovar O32 showing a sharper increase than O24. In buffered prawn slurry the three strains grew well at all the pH values (6·4, 6·7, 7·4 and 7·9) and the growth curves were almost identical at each pH. In luncheon meat slurry at pH 5·5, 5·8 and 6·4 (Table 6) the organisms grew least well at pH 5·5 but as the pH became less acidic multiplication increased.

None of the three strains showed any resistance to heat, at 50 °C an initial inoculum of 10^9 cells/ml of each strain was reduced to $< 10^2$/ml in 12·5–15 min (serovars O24 and O32) and 15–20 min (serovar O37). At 52·5 °C the decrease in numbers was more rapid, after 7 min an inoculum of 10^6 or 10^7 was reduced to

Table 3. *Growth of non-cholera vibrios in cooked prawns and aspic stored at 10° or 14°, 22°, 30°, 37° and 43 °C**

Food	Strain (serovar)	Storage temperature (°C)	Log count of non-cholera vibrio after storage (h)					
			0	6	18	24	48	72
Cooked prawns	O24	10	3·51	NT	NT	4·18	2·78	4·65
		22	3·51	4·40	9·18	9·74	9·30	NT
		30	3·51	6·78	9·88	9·60	NC	NT
		37	3·51	7·74	9·85	8·70	NC	NT
	O32	10	3·18	NT	NT	2·00	2·30	4·00
		22	3·18	4·30	8·70	9·51	8·70	NT
		30	3·18	6·40	9·54	9·00	NC	NT
		37	3·18	7·30	9·30	8·65	NC	NT
	O37	10	3·70	NT	NT	3·04	2·78	3·88
		22	3·70	4·88	9·18	9·00	8·70	NT
		30	3·70	7·00	9·78	9·65	NC	NT
		37	3·70	7·30	10·00	9·18	NC	NT
Aspic	O24	14	3·70	NT	NT	4·18	5·48	9·16
		22	3·70	5·10	8·00	8·70	9·60	NT
		30	3·70	6·10	9·48	9·60	9·90	NT
		37	3·70	7·18	9·30	10·10	9·40	NT
		43	3·70	8·40	9·48	10·10	6·70	NT
	O32	14	4·48	NT	NT	5·30	7·10	8·24
		22	4·48	4·48	6·98	8·74	9·35	NT
		30	4·48	5·40	9·30	9·60	9·40	NT
		37	4·48	6·60	9·54	9·78	9·24	NT
		43	4·48	7·00	8·30	8·70	NC	NT
	O37	14	4·00	NT	NT	4·81	6·60	8·90
		22	4·00	4·78	7·30	9·00	9·54	NT
		30	4·00	5·93	8·81	9·78	9·74	NT
		37	4·00	6·78	8·88	9·90	8·78	NT
		43	4·00	7·40	9·35	9·65	NC	NT

* Prawns not stored at this temperature.
NT = not tested.   NC = not countable.

10^3 organisms/ml or less. At 55 °C the number of organisms was reduced to $< 10^2$/ml in 2–3 min.

DISCUSSION

The results of this study show that non-cholera vibrios grow well in a wide variety of foods if the ambient temperature is high and can reach populations *ca.* 10^8–10^9/g within 6–12 h which are probably sufficient to cause food poisoning. The foods selected covered the range of ingredients likely to be included in cold hors d'oeuvres, the type of dish implicated in at least one documented outbreak of gastroenteritis attributed to non-cholera vibrios (Dakin *et al.* 1974).

The fact that these organisms grew so well, particularly at high ambient temperatures, emphasizes the need for care in the preparation and storage of foods to be eaten cold, particularly in areas where vibrios, both cholera and non-cholera, are

Table 4. *Survival of non-cholera vibrios in phosphate buffer at different pH*

Strain (serovar)	pH	Log count of non-cholera vibrio after storage (h)				
		0	6	18	24	48
O24	4·9	6·55	3·08	< 2·00	< 2·00	< 2·00
	5·8	6·55	5·30	4·40	3·49	2·79
	6·7	6·55	6·18	7·30	7·40	6·78
	7·7	6·55	6·48	8·30	7·70	7·88
O32	4·9	6·16	< 2·00	< 2·00	< 2·00	< 2·00
	5·8	6·16	4·00	< 2·00	< 2·00	< 2·00
	6·7	6·16	5·78	7·18	6·30	6·11
	7·7	6·16	6·11	7·54	7·26	7·26
O37	4·9	6·01	< 2·00	< 2·00	< 2·00	< 2·00
	5·8	6·01	5·58	4·54	4·30	4·00
	6·7	6·01	5·85	7·30	7·18	6·88
	7·7	6·01	6·18	7·95	7·93	7·65

Table 5. *Growth of non-cholera vibrios in mayonnaise at different pH*

Strain (serovar)	pH	Log count of non-cholera vibrio after storage (h)				
		0	6	18	24	48
O24	4·1	3·80	< 2·00	< 2·00	< 2·00	< 2·00
	5·5	3·80	2·30	< 2·00	< 2·00	< 2·00
	7·6	3·80	3·73	3·40	4·30	NC
	8·6	3·80	3·40	3·48	4·00	7·18
O32	4·1	4·00	< 2·00	< 2·00	< 2·00	< 2·00
	5·5	4·00	2·00	< 2·00	< 2·00	< 2·00
	7·6	4·00	3·75	3·70	4·81	7·70
	8·6	4·00	4·10	4·00	6·00	8·18
O37	4·1	4·54	< 2·00	< 2·00	< 2·00	< 2·00
	5·5	4·54	< 2·00	< 2·00	< 2·00	< 2·00
	7·6	4·54	4·00	3·70	3·88	NC
	8·6	4·54	4·00	3·88	3·69	7·00

NC = not countable.

endemic in the population. Isolations of non-cholera vibrios from human stools in the United Kingdom are usually from persons who have recently returned from abroad but isolations have been made from environmental sources in this country and the organisms may be more common than was once appreciated (A. L. Furniss, personal communication).

The effect of pH on the growth of non-cholera vibrios was not surprising, the organisms not favouring an acid environment but growth improving at more alkaline pH values. This ability to grow at alkaline pH is used as a means of isolating both cholera and non-cholera vibrios from stools and other samples by using an alkaline peptone water as enrichment medium.

The organisms are also readily destroyed by heat, thus raw foods which are

Table 6. *Growth of non-cholera vibrios in luncheon meat at different pH*

Strain (serovar)	pH	Log count of non-cholera vibrio after storage (h)				
		0	6	18	24	48
O24	5·5	3·29	< 2·00	7·60	7·00	6·11
	5·8	3·29	3·53	7·88	7·65	9·30
	6·4	3·29	3·89	8·11	8·93	10·00
O32	5·5	2·80	< 2·00	4·88	6·11	6·40
	5·8	2·80	2·00	6·00	7·72	8·18
	6·4	2·80	< 2·00	7·36	8·11	9·18
O37	5·5	3·32	3·23	7·36	6·48	6·40
	5·8	3·32	3·84	8·00	8·00	8·36
	6·4	3·32	3·79	8·52	NT	10·00

NT = not tested.

contaminated with non-cholera vibrios should be safe to eat after normal cooking methods. The hazard lies in the recontamination of cooked food and subsequent poor storage at high ambient temperatures.

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