Table 17-7. *Survival of* V. cholerae *in sewage*

Source	*Biotype and initial concentration per milliliter*	*Type of sample*	*Temperature*	*Survival*[a]
Altukhov and others (1975)[b]	Classical (Ogawa) 10^3	Sewage of a bath house (USSR; BOD = 320 milligrams per liter)	37°C	> 10 days
	El Tor (Ogawa) 10^5			> 10 days
Daniel and Lloyd (1980*b*)	El Tor 2×10^6	Strong sewage at refugee camp (Bangladesh)	22–25°C	Concentration fell by 1 log in 6 hours and remained steady for further 42 hours
	Non-O1 (sewage isolate) 2×10^5			Concentration rose to 4×10^6 in 6 hours and remained steady for a further 42 hours
Flu (1921)	Classical	Sewage in septic tanks	Ambient temperature (Batavia)	2 days
Gerichter and others (1975)	El Tor	Sewage (Jerusalem)	20–28°C	Two phase decline: t_{90} = 1.8 days for first 5 logs and t_{90} = 8 days subsequently. *V. cholerae* not detected after 24 days[c]
Howard and Lloyd (1979)	El Tor 10^6	Raw sludge 1 percent solids	25°C	t_{90} = 2 days max survival = 14 days
		5 percent solids		t_{90} = 3 days max survival = > 14 days
Kott and Betzer (1972)	El Tor 10	Diluted sewage (Haifa; BOD = 200 milligrams per liter)	Room temp. (Israel)	1 day
Mukerjee, Rudra and Roy (1961)	Classical 2×10^6	Sewage (Calcutta) Raw	Room temp. (Calcutta)	1–5 days
		Autoclaved		4–24 days
		Filtered		2–7 days
	El Tor (clinical isolate) 2×10^6	Raw		2 days
		Autoclaved		9 days

Table 17-7 (*continued*)

Source	*Biotype and initial concentration per milliliter*	*Type of sample*	*Temperature*	*Survival*[a]
	El Tor (water isolate) 2×10^6	Raw		2 days
		Autoclaved		10 days
	Non-O1 (clinical isolate) 2×10^6	Raw		2 days
		Autoclaved		8 days
	Non-O1 (water isolate) 2×10^6	Raw		2 days
		Autoclaved		8 days
Ohwada (1924); cited by Pollitzer (1959)	Classical	Sewage	4°C	12 days
			Room temp. (Japan)	4 days
			37°C	1 day
Zaidenov and others (1976)	El tor (Ogawa) 10^4	Locomotive depot wastewater	18–24°C	> 39 days
		Domestic sewage		3 days
		Dairy effluent		14 days
	10	Oil and water		> 14 months
		Diesel fuel and water		> 14 months

Note: Older literature is reviewed by Pollitzer (1959).

a. Times given, for instance, as 6 days are durations at which viable organisms could not be detected. Times given as > 10 days indicate that organisms were still viable at that time but that sampling was discontinued.

b. These experiments were discontinued after 10 days, at which time the concentration of classical *V. cholerae* was 5×10^2 while that of El Tor had risen to over 10^8 per milliliter. Data from the bath house suggested that *V. cholerae* (El Tor, Ogawa) survived for at least 13 months in the sewerage system (temperature 20–25°C) despite repeated disinfection and no known external recontamination.

c. t_{90}: time for 90 percent reduction.

and *V. cholerae*, 7 hours. Pandit and others (1967) found that *V. cholerae* (El Tor) survived 2 to 5 times longer than *E. coli*, *Pseudomonas* spp., and *Aerobacter* spp. when they were added to artificial well water and stored at 25°C.

Prolonged survival in water and wastewater

Pollitzer (1959) cited several early studies that reported prolonged survival of *V. cholerae* in various waters. Examples are up to a year in sterilized spring or well water, up to a year in sterilized river water, and over 9 months in sterilized seawater.

Sayamov and Zaidenov (1978) studied the survival of classical and El Tor *V. cholerae* in mineral waters from a spa at Matsesta (USSR). In raw mineral water, survival did not exceed 22 days for either biotype. In boiled mineral water at 20–24°C, initial concentrations of 9×10^5 per milliliter remained steady for 4 years for both biotypes. Other results from these experiments are given in table 17-4.

More remarkable are reports of prolonged survival in raw sewage. Altukhov and others (1975) studied a bath house in the USSR. *V. cholerae* (El Tor, Ogawa) was isolated from 49 percent of samples of wastewater from the bath house over a 13-month period. Repeated attempts to disinfect the wastewater system had no effect on *V. cholerae* isolation. There was no known cholera infection in the community. *V. cholerae* was not isolated from the incoming water supply, nor from large numbers of samples of human feces, water, fish, and frogs that were examined. Serological surveillance also failed to detect evidence of *V. cholerae* infection. *V. cholerae* was isolated from river water contaminated by the discharge from the bath house. In laboratory experiments, wastewater from the bath house

(BOD_5 = 320 milligrams per liter, pH = 7.6) was inoculated with an El Tor (Ogawa) strain previously isolated from the bath house and with a reference strain of classical *V. cholerae* (Ogawa), and stored at 37°C. The concentration of El Tor organisms was 10^5 per milliliter at the start, rose to over 10^8 per milliliter after 3 days, and maintained this concentration up until 10 days when sampling was discontinued. The concentration of classical organisms was 10^3 per milliliter at the start, rose to 10^5 after 3 days, and fell back to 5×10^2 after 10 days. The investigation failed to discover how the bath house sewerage system became infected, but it was clear that, once infection had taken place, *V. cholerae* (El Tor, Ogawa) maintained itself in the warm sewage (20–25°C) and was remarkably resistant to disinfection.

A very similar experience was reported by Zaidenov and others (1976). A sewerage system serving a locomotive depot and a housing estate was investigated. Wastewater from the locomotive depot (450 cubic meters per day) was rich in oil products and passed through oil traps and a flotation chamber before being mixed with domestic sewage (150–250 cubic meters per day). The mixed sewage was then pumped to treatment fields. Because hot water was used in the locomotive depot, the sewage was warm, even in winter, and temperatures of 19–24°C were recorded throughout the year. The pH of the sewage was 7.1 to 9.3. Over a 17-month period 1,454 samples of sewage from various points in the system were examined, and 17 percent were positive for *V. cholerae* (El Tor, Ogawa). The wastewater from the locomotive depot was far more frequently infected (18–42 percent) than the domestic sewage (5 percent). The oil traps and flotation chamber were most frequently infected. The *V. cholerae* strain isolated was always the same and was nontoxigenic. Fecal examination of 2,708 people in the depot and the housing estate revealed only three infections with non-O1 *V. cholerae*. When one oil trap was isolated from the system, *V. cholerae* were shown to survive in it for 36 days (the temperature in this oil trap fell to 10°C after isolation from the sewerage system). In laboratory experiments, the El Tor strain isolated from the locomotive depot was inoculated into various wastewaters and stored at 18–24°C. In mixtures of oil plus water and diesel fuel plus water, survival was for over 14 months, with an initial concentration of 10 per milliliter. In domestic sewage, survival was less than 3 days; in locomotive depot wastewater, survival was over 39 days; and in dairy effluent (included for comparison), survival was less than 14 days. All experiments were performed with initial inocula of 10^4 *V. cholerae* per milliliter. The source of infection of the sewerage system was not discovered. Repeated disinfection failed to clear *V. cholerae* from the network until massive doses of chlorine (to achieve 10

Table 17-8. t_{90} *values in hours for various types of* V. cholerae *in various waters and wastewaters*

Type of water environment	*Classical O1*			*El Tor O1*			*Non-O1*		
	No.	*Arith. mean*	*Range*	*No.*	*Arith. mean*	*Range*	*No.*	*Arith. mean*	*Range*
Dechlorinated tap water	8	22	3–48	8	49	2–163	ND	ND	ND
Well water	1	36	NA	13	116	5–264	ND	ND	ND
Surface water	8	18	0.16–36	10	53	1–230	4	8	8–8
Seawater	3	95	0.36–161	7	56	3–235	ND	ND	ND
Sewage	1	12	NA	9	66	8–240	2	8	8–8
Sterilized well water, surface water or sewage	7	34	3–65	9	59	32–168	6	39	31–50

No. Number of results.
Arith. mean Arithmetic mean.
ND No data.
NA Not applicable.

milligrams per liter throughout the system) and sulphuric acid (to lower sewage pH to 3–4) were added. Following this, no *V. cholerae* were isolated for the next 12 months.

Further evidence of multiplication and prolonged survival in some wastewater is provided by reports of the multiplication of *V. cholerae* (El Tor, Inaba) in a clinic septic tank in Japan (MMWR 1979) and the multiplication of *V. cholerae* (non-O1) in a trickling filter in Bangladesh (Daniel and Lloyd 1980*b*). These occurrences, and their relationship to environmental reservoirs of some atypical and non-O1 *V. cholerae*, await clarification.

A possible aquatic reservoir for V. cholerae

Perhaps the greatest upset to traditional concepts of cholera epidemiology and bacteriology has come from the recent discoveries of *V. cholerae* and related organisms occurring in surface waters not known to be fecally contaminated or in areas where no human infection has been recorded. *V. cholerae*, El Tor and non-O1, were frequently isolated from wells, tanks, and rivers in India in the 1930s and 1940s, but their close relationship with classical *V. cholerae* O1, and their potential pathogenicity, were not recognized at that time (Read and Pandit 1941; Taylor and Ahuja 1938; Venkatraman, Krishnaswami and Ramakrishnan 1941).

Colwell, Kaper and Joseph (1977) reported the isolation of non-O1 *V. cholerae* from various parts of Chesapeake Bay (USA). Subsequently, Kaper and others (1979) described the ecology of non-O1 *V. cholerae* in Chesaspeake Bay in some detail. Concentrations were up to 7 per liter, and isolations were only made at sites with salinities between 0.4 and 1.7 percent. There was no correlation between *V. cholerae* counts and counts of total bacteria, coliforms, fecal coliforms, or salmonellae. *V. cholerae* were not especially associated with bottom sediment or oysters.

In a recent publication (Colwell and others 1980), data on *V. cholerae* isolations from various brackish and estuarine environments are summarized. *V. cholerae* isolations in Chesapeake Bay were dependent on salinity and temperature, with the highest recoveries (up to 46 per liter) being reported at salinities of 0.3 to 1.7 percent and during the summer when water temperatures were 28°C. *V. cholerae* isolations were not correlated with known fecal contamination, nor with fecal coliform counts, thus suggesting that *V. cholerae* "is an autochthonous species in the estuarine ecosystem". Both non-O1 *V. cholerae* serotypes and *V. cholerae* O1 (Inaba) have been isolated from Chesapeake Bay. *V. cholerae* O1 (Inaba) has also been isolated from Louisiana salt marshes. Some of the *V. cholerae* O1 and *V. cholerae* non-O1 strains isolated from the Chesapeake Bay and the Louisiana coast showed evidence of toxin production. A marked association of *V. cholerae* non-O1 with zooplankton was found both in the Chesapeake Bay and in surface water samples collected in Bangladesh.

Bashford and others (1979) and West, Knowles and Lee (1980) reported the isolation of up to several hundred *V. cholerae* per milliliter from streams and drainage ditches in Kent (England), including sites where there was no known sewage contamination. Isolations were more common during the summer. Except for one occasion, all isolations have been of non-O1 serotypes, and all have been nontoxigenic (J. Lee, personal communication). Müller (1978, 1979) isolated non-O1 *V. cholerae* from 33 percent of river water samples in the Federal Republic of Germany, but not from sewage treatment plant effluents. Isolations were more numerous in summer.

V. cholerae O1, atypical *V. cholerae* O1 and non-O1 *V. cholerae* have been isolated variously from freshwater, saline water, and wastewater in Australia, Bangladesh, Brazil, England, Germany, Guam, Japan, the USA, and the USSR (WHO Scientific Working Group 1980). Most of these isolates have been found to be nontoxigenic and nonpathogenic. They have been found in areas where cholera cases or infections are not known to occur (for example, Brazil, England, and the USA) and in waters that are not thought to have received any human fecal contamination (for example, England and the USA). It is very probable that some of these *V. cholerae* isolates are free-living aquatic organisms. Whether they are in any way related to human disease or to the epidemiology of cholera remains to be determined.

The speculation concerning a possible environmental reservoir for atypical and non-O1 *V. cholerae*, and possibly also for *V. cholerae* O1, has been increased by findings on the affinity of these organisms for chitin. Nalin and others (1979) found that about 70 percent of *V. cholerae* O1 organisms, which were shaken for 6 hours with powdered crabshell in a 4.2 percent salt solution at pH 6.2 and 20°C, adsorbed to the chitin particles. These adsorbed *V. cholerae* were then somewhat resistant to an acid environment simulating the stomach (pH) 1.6–1.8 for 13 minutes). *V. cholerae* also multiplied (>4 log increase) when incubated for 2 days at 37°C in 4.2 percent salt solution containing chitin. Other studies have shown that *V. cholerae* O1 (classical and El Tor) and non-O1 can

produce chitinase (Dastidar and Narayanaswami 1968) and that non-O1 *V. cholerae*, like *V. parahaemolyticus*, can adsorb to, and multiply on, chitinous fauna such as crab, shrimp, and zooplankton (Colwell, Kaper and Joseph 1977; Kaneko and Colwell 1973, 1975, 1978; Kaper and others 1979; Nalin 1976; Sochard and others 1979).

In sweat

Dodin and Félix (1972) found that *V. cholerae*, El Tor, was still viable after seven weeks at 28°C in human sweat and on gauze pads soaked in sweat and stored in humid conditions. From one quantitative experiment a t_{90} of 215 hours at 28°C in sweat can be computed. This is much longer than typical t_{90} values at that temperature (table 17-8). Dodin and Félix considered that these findings had considerable relevance to the epidemiology of cholera in arid areas of West Africa. Isaacson and Smit (1979) showed that *V. cholerae* (El Tor, Inaba) multiplied, and could survive for at least 120 hours, in pooled human sweat. Multiplication of *V. cholerae* in sweat was believed to have promoted the transmission of cholera among South African gold miners undergoing heat acclimatization (Isaacson and others 1974). It is not known whether *V. cholera* survives well in sweat on the skin.

On surfaces

V. cholerae survival on surfaces is usually limited because of the sensitivity of the organism to desiccation. Four studies on *V. cholerae* on various household items are summarized in table 17-9.

Table 17-9. *Survival of* V. cholerae *on surfaces*

Source	*Biotype*	*Type of surface*	*Temperature*	*Survival*[a]
Felsenfeld (1965)	Classical and El Tor	Absorbent materials		
		Cotton	28–30°C	5–7 days
		Chopsticks		2–3 days
		Paper		2–3 days
		Shoes		2–3 days
		Silk		3–5 days
		Non-absorbent materials		
		Aluminium foil	28–30°C	1 day
		Coins		1 day
		Tin cups		1 day
		Plastic envelopes		1–2 days
		China plates		1–2 days
		Metal utensils		1–2 days
Gohar and Makkawi (1948)	Classical	Linen	Room temp. (Egypt)	6 days
		Wool		5 days
		Leather		3 days
		Paper and rubber		10 hours
		Coins		6 hours
Pesigan, Plantilla and Rolda (1967)	El Tor	Frying pan	30–32°C	4 hours
		China plates		4 hours
		Pestle and mortar		4 hours
		Drinking glass		4 hours
		Metal utensils		24 hours
		Kitchen knife		48 hours
		Wooden chopping block		24 hours
Shousha (1948)	Classical	Cotton and cloth	Room temp. (Egypt)	4 days
		Bank note		3 days
		Postage stamp		2 days
		Coin		1 day

Note: Older literature is reviewed by Pollitzer (1959).

a. Times given are those at which viable organisms could no longer be detected.

The longer persistence on absorbent materials, especially cotton, is interesting and suggests that clothing (especially clothing soaked in sweat) may act as a temporary habitat for *V. cholerae*. It is also noteworthy that survival times are markedly shorter than those reported for other enteric bacteria—for instance, *Shigella* (chapter 16)—on similar surfaces.

In soil

Experiments in Israel (Gerichter and others 1975) found that *V. cholerae* (El Tor) in soil survived for up to 4 days when the soil was allowed to dry slowly, but for up to 10 days when the soil was regularly remoistened with uncontaminated sewage (initial concentrations were 10^7 per gram of soil, and the storage temperature was 20–28°C). Nalin and others (1980) reported survival for over 6 days when *V. cholerae* (El Tor) was inoculated into sterile potting soil and stored at 26°C. In the same experiments it was found that common earthworms (*Lumbricus terrestris*) ingested *V. cholerae* in soil and subsequently died. *V. cholerae* multiplied in the earth worms and were isolated at concentrations up to 10^7 per milliliter of worm homogenate.

On food and crops

In looking at the potential of food for transmitting cholera, it is important to make the distinction between food that acts as a primary vehicle for cholera, becoming infected through direct contact with the stools of a case or carrier, and food that acts as a secondary vehicle of spread, becoming contaminated by polluted water. Most documented occurrences of foodborne cholera are of the second kind, and the most numerous of these incidents are those involving fish and shellfish. Alternatively, food can act as a secondary vehicle of cholera through the use of polluted water to irrigate or freshen vegetables.

The evidence for foods acting as the primary vehicles for cholera is very limited. This is to be expected because few studies have examined the domestic environment in a cholera area during an outbreak and carried out a systematic investigation of food for *V. cholerae*. Table 17-10 summarizes some literature on the survival of *V. cholerae* on food. It is clear that survival times of several days are commonly achieved, even at around 30°C. Survival is longest in moist, nonacidic, and sterile (that is, cooked) foods. Only two studies (Felsenfeld 1965; Neogy 1965) directly compared the survival of the classical and El Tor biotypes, and both found that El Tor survived for longer. It seems likely that some foods can and do act as a primary vehicle for spreading cholera, especially within the household or at feasts and markets.

Inactivation by Sewage Treatment Processes

There is very little information on the fate of *V. cholerae* in sewage treatment plants partly because, as mentioned above, most people with cholera produce no sewage; therefore *V. cholerae* is only very rarely found in sewage, and even then in low concentrations.

Flu (1921) studied seeded *V. cholerae* in septic tanks in Batavia (now Jakarta; Indonesia). A total of five septic tanks were studied, and in only one was *V. cholerae* detected in the effluent. Early studies reviewed by Kabler (1959) reported a 98 percent reduction of *V. cholerae* in an activated sludge plant.

Kott and Betzer (1972) studied a 70-liter model waste stabilization pond with a retention time of 5 days. The pond was fed with diluted sewage ($BOD_5 = 200$ milligrams per liter) spiked with *V. cholerae* (El Tor). Influent coliform and *V. cholerae* concentrations were 3×10^6–8×10^8 and 1×10^3–8×10^3 per 100 milliliters, respectively. Effluent coliform and *V. cholerae* concentrations were 8×10^4–4×10^7 and 0–2 per 100 milliliters respectively. The addition of 8 milligrams per liter of chlorine to the waste stabilization pond effluent eliminated all remaining *V. cholerae*.

Daniel and Lloyd (1980*a*) studied two Oxfam Sanitation Units in refugee camps near Dacca (Bangladesh). These units treated very strong sewage (17,000 and 7,400 milligrams of suspended solids per liter) in two unbaffled, flexible tanks connected in series. Each tank had a volume of 18 cubic meters, and the flow of sewage was 2.5 to 3 cubic meters per day. Thus, the total mean retention times were 12–15 days. The geometric mean inflowing concentrations of non-O1 *V. cholerae* at the two camps were 2.6×10^3 and 1.6×10^2 per 100 milliliters, respectively. The geometric mean effluent concentrations were 6.5 and 5.3 per 100 milliliters. Thus, overall removal rates at the two camps were 99.8 and 96.4 percent, respectively. These removal rates give t_{90} values of 106 and 257 hours, respectively, which are longer than those reported in table 17-7, especially if the warm ambient temperature is taken into account. This suggests either short-circuiting in the tanks, which is quite probable, or non-O1 *V. cholerae* multiplication in the warm sewage in the tanks.

Table 17-10. *Survival of* V. cholerae *on food and crops*

Source	*Biotype*	*Type of food*	*Temperature*	*Survival*[a]
		A. Meat		
Cheng (1963)	El Tor	Beef	Day 1: 22°C Thereafter: 3–4°C	5 days
Felsenfeld (1965)	El Tor and classical	Raw beef	2–4°C 28–30°C	5–7 days 1–2 days
		Cooked beef	2–4°C 28–30°C	1–2 weeks 3–7 days
		Sausages (surface and inside)	2–4°C 28–30°C	1 day 1 day
Pesigan, Plantilla and Rolda (1967)	El Tor	Raw meat	5–10°C 30–32°C	4–9 days 2–4 days
		Cooked meat	5–10°C 30–32°C	3–5 days 2–5 days
		B. Fish		
Cheng (1963)	El Tor	Lice-eye fish Sliced sword-fish	Day 1: 21.5°C Thereafter: 4°C	16 days 10 days
Felsenfeld (1965)	El Tor and classical	Shrimp	2–4°C 28–30°C	1–3 days 1–2 days
		Catfish Raw	2–4°C 28–30°C	1–2 weeks 2–4 days
		Dried	2–4°C 28–30°C	3–5 days 1–2 days
		Salted	2–4°C 28–30°C	1–2 days 1 day
		Cooked	2–4°C 28–30°C	2–7 days 1–6 days
Pesigan, Plantilla and Rolda (1967)	El Tor	Various fish and shellfish	5–10°C 30–32°C	4–9 days 2–4 days
		C. Vegetables and fruit		
Cheng (1963)	El Tor	Horseradish Cucumber Tomato Orange	Day 1: 22°C Thereafter: 3–4°C	21 days 23 days 16 days 14 days
El Shawi and Thawaini (1967)	El Tor	Date Melon	Room temp. (Iraq)	3 days 2 days
Felsenfeld (1965)	El Tor and classical	A comprehensive survey of a wide range of cooked and uncooked fruits and vegetables	2–4°C 28–30°C	Up to 4 weeks Up to 7 days (except inside melon, which was 2 weeks); survival was especially long on cabbage, cucumber, eggplant, melon, okra, peas, and potatoes.

Table 17-10 (*continued*)

Source	*Biotype*	*Type of food*	*Temperature*	*Survival*[a]
Gerichter and others (1975)	El Tor	Parsley	20–26°C	1 day
		Tomato and carrot		1.5 days
		Cucumber, pepper, and okra		1–2 days
		Lettuce		2–3 days
				Mean death rates for all the above were 4–6 log units per day
		Parsley		
		Wet	20–28°C	2 days
		Dry		1 day
		Lettuce		
		Group of leaves	18–26°C	68 hours
		Single leaf		44 hours
		Tomato		
		in sunlight	22–30°C	4 hours
		Parsley	4°C	2 days
		Lettuce		4 days
Gohar and Makkawi (1948)	Classical	Date	Room temp. (Egypt)	4 days
		Vegetables		6 days
Neogy (1965)	El Tor and classical	Papaya	Room temp. (India)	1 day
		Cucumber		>1 day
		Pineapple		15 minutes
		Boiled rice soaked overnight		1 hour
Pesigan, Plantilla and Rolda (1967)	El Tor	Cooked fruit and vegetables	5–10°C	3–5 days
			30–32°C	2–5 days
			5–10°C	2–3 days
		Fresh fruit	30–32°C	1 day
		Fresh vegetables	5–10°C	6–9 days
			30–32°C	2–5 days
Prescott and Bhattacharjee (1969)	El Tor	Lime, lemon, and date	20–25°C	1 hour
		Orange, grape, fig, raisin, and tomato		1 day
		Banana, guava, papaya, onion, eggplant, pea, celery, green bean, bean sprout, and rice.		2–5 days
		Okra, lima bean, pumpkin, and potato		6–8 days
Shousha (1948)	Classical	Onion and date	Room temp. (Egypt)	4 days
		Garlic, rice, lentil, and grape		3 days
		Orange and lemon		7 hours

Table 17-10 (*continued*)

Source	*Biotype*	*Type of food*	*Temperature*	*Survival*[a]
		D. Milk and Milk products		
Felsenfeld (1965)	El Tor and classical	Butter, unsalted	2–4°C 28–30°C	1–2 weeks 1 week
		Cheese	2–4°C 28–30°C	2–3 weeks 1 week
		Custard	2–4 °C 28–30°C	3–4 weeks 1–2 weeks
		Ice cream	2–4°C 28–30°C	3–4 weeks 5–7 days
		Milk	2–4°C 28–30°C	3–4 weeks 1–3 weeks
Lema, Ogawa and Mhalu (1979)	El Tor	Milk	4°C 30°C	3 weeks 4 days
Neogy (1965)	El Tor and classical	Milk desserts	Room temp. (India)	1 day
Pesigan, Pantilla and Rolda (1967)	El Tor	Milk, ice cream, and butter	5–10°C 30–32°C	1 week- > 2 weeks 5–14 days
Prescott and Bhattacharjee (1969)	El Tor	Milk desserts	20–25°C	1–2 days
Shousha (1948)	Classical	Milk Sour milk Butter Cheese	4°C Room temp. [illegible]	> 2 days 2 hours > 2 days 7 hours
		E. Other foods		
El Shawi and Thewaini (1967)	El Tor	Barley and wheat	Room temp. (Iraq)	2 days
Felsenfeld (1965)	El Tor and classical	A comprehensive survey of a wide range of cooked and uncooked foods	2–4°C 28–30°C	Up to 4 weeks Not more than 7 days, except for coconut cream (10 days), coconut dishes (3 weeks), and noodles (2 weeks)
Gohar and Makkawi (1948)	Classical	Honey and treacle	Room temp. [illegible]	3 hours
Neogy (1965)	El Tor and classical	Sweet and sour curd [illegible] and *sandesh*	Room temp. (India)	5 minutes 1 day
Pesigan, Plantilla and Rolda (1967)	El Tor	Cooked noodles, rice cake, and jam	5–10°C 30–32°C	3–5 days 2–5 days

Table 17-10 (*continued*)

Source	*Biotype*	*Type of food*	*Temperature*	*Survival*[a]
Prescott and Bhattacharjee (1969)	El Tor	Wheat and nuts	20–25°C	3 days
		Spices	20–25°C	1–5 days
Shousha (1948)	Classical	Sugar	Room temp. (Egypt)	4 days
		Bread		3 days
		Honey		2 days
		F Beverages		
El Shawi and Thewaini (1967)	El Tor	Soft drinks	Room temp. (Iraq)	1 day
Felsenfeld (1965)	El Tor and classical	Beer, carbonated water, carbonated soft drinks, lime and whisky	2–4°C	1 day
			28–30°C	1 day
		Cocoa	2–4°C	1–2 weeks
			28–30°C	3–5 days
		Coffee	2–4°C	1–2 days
			28–30°C	1 day
		Ice cubes	2–4°C	4–5 weeks
		Lemonade	2–4°C	2–3 weeks
			28–30°C	5–7 days
		Tea	2–4°C	1 week
			28–30°C	2–3 days
Lema, Ogwa and Mhalu (1979)	El Tor	Coconut fluid	4°C	4 days
			30°C	2 days
		Beer, gin, and traditional alcoholic beverages *chibuku* (maize and beans) and *mbege* (bananas and millet)	4°C	1 hour (except for *mbege*, in which survival was 2 days)
			30°C	1 hour
Pesigan, Pantilla and Rolda (1967)	El Tor	Coca cola	5–10°C	2 days
			30–32°C	4 hours
Prescott and Bhattacharjee (1969)	El Tor	Coca cola	20–25°C	1 day
		Rosewater		2 days
		Ground coffee		1 hour
		Tea leaves		1 day

Note: Older literature is reviewed by Pollitzer (1959).

a. Times given, for instance, as 2 days are durations at which viable organisms could no longer be detected. Times given as >2 days indicate that organisms were still viable at that time but that sampling was discontinued.

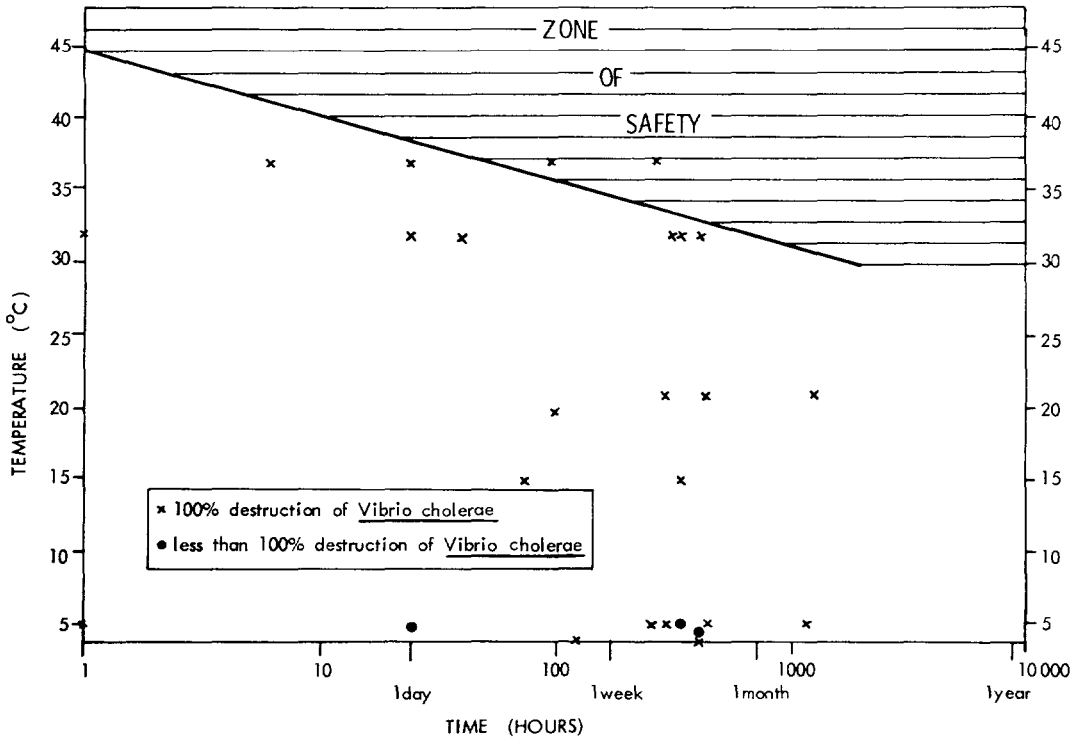

Figure 17-3. *The influence of time and temperature on* V. cholerae. The points plotted are the results of experiments done under widely differing conditions. The line drawn represents a conservative upper boundary for death

Daniel and Lloyd (1980*b*) reported that small trickling filters were installed to treat further the effluents from these Oxfam Sanitation Units. Influent concentrations (effluents from the second tank of the main unit (non-O1 *V. cholerae* were 3–9 per 100 milliliters, while effluents from the trickling filters contained 3–2,400 per 100 milliliters. The authors concluded that non-O1 *V. cholerae* was multiplying in ponded sewage in the trickling filters.

Inactivation by Night Soil and Sludge Treatment Processes

No reports of *V. cholerae* reduction during night soil or sludge treatment were located. The data given in table 17-6 suggest that *V. cholerae* will be eliminated by any process having a retention time of appreciably more than 5 days in a warm climate. Time-temperature combinations lethal to *V. cholerae* are given in figure 17-3. It appears that *V. cholerae* will be eliminated by almost any sludge digestion, composting, or storage process and will certainly be removed far more readily than *E. coli* and other fecal indicator bacteria (chapter 13).

Literature Cited

Abel, R. and Claussen, R. (1895). Untersuchungen über die lebensdauer der cholera-vibrionen in fäkalien. *Zentralblatt für Bakteriologie, und Parasitenkunde*, I, **17**, 77–81 and 118–130.

Altukhov, A. A., Ivanov, S. I., Semiotrochev, V. L., Bulegenov, E. I., Lebedev, K. K., Filimonova, L. V., Zakharov, N. I. and Rybak, E. I. (1975). Prolonged detection of El Tor cholera vibrio in sewage of a bath-house. *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii*, **2**, 41–44.

Azurin, J. C. and Alvero, M. (1974). Field evaluation of environmental sanitation measures against cholera. *Bulletin of the World Health Organization*, **51**, 19–26.

Azurin, J. C., Kobari, K., Barua, D., Alvero, M., Gomez, C. Z., Dizon, J. J., Nakano, E-I., Suplido, R. and Ledesma, L. (1967). A long-term carrier of cholera: Cholera Dolores. *Bulletin of the World Health Organization*, **37**, 745–749.

Bart, K. J., Khan, M. and Mosley, W. H. (1970). Isolation of *Vibrio cholerae* from nightsoil during epidemics of classical and El Tor cholera in East Pakistan. *Bulletin of the World Health Organization*, **43**, 421–429.

Bashford, D. J., Donovan, T. J., Furniss, A. L. and Lee, J. V. (1979). *Vibrio cholerae* in Kent. *Lancet*, **1**, 436–437.

Blake, P. A., Rosenberg, M. L., Florencia, J., Costa, J. B., Quintino, L. D. P. and Gangarosa, E. J. (1977). Cholera in Portugal, 1974. II. Transmission by bottled mineral water.

American Journal of Epidemiology, **105,** 344–348.

Briscoe, J. (1978). The role of water supply in improving health in poor countries (with special reference to Bangla Desh). *American Journal of Clinical Nutrition*, **31,** 2100–2113.

Cash, R. A., Music, S. I., Libonati, J. P., Snyder, M. J., Wenzel, R. P. and Hornick, R. B. (1974). Response of men to infection with *Vibrio cholerae*. I. Clinical, serologic and bacteriologic responses to a known inoculum. *Journal of Infectious Diseases*, **129,** 45–52.

Cheng, C. T. (1963). Survival of El Tor vibrio outside the human body. *Nagasaki Igakkai Zassi*, **38,** 870–876.

Colwell, R. R., Kaper, J. and Joseph, S. W. (1977). *Vibrio cholerae*, *Vibrio parahaemolyticus*, and other vibrios: occurrence and distribution in Chesapeake Bay. *Science*, **198,** 394–396.

Colwell, R. R., Kaper, J., Seidler, R., Voll, M. J., McNicol, L. A. Garges, S., Lockman, H., Maneval, D. and Remmers, E., Joseph, S. W., Bradford, H., Roberts, N., Huq, I. and Huq, A. (1980). Isolation of O1 and non-O1 *Vibrio cholerae* from estuaries and brackish water environments. In *Proceedings of the 15th Joint Conference on Cholera, US-Japan Cooperative Medical Science Program*, pp. 44–51. Bethesda, Md.: National Institutes of health.

Cvjetanović, B. (1979). Sanitation versus immunization in control of enteric and diarrhoeal diseases. *Progress in Water Technology*, **11,** 81–87.

Cvjetanović, B., Grab, B. and Uemura, K. (1978). *Dynamics of Acute Bacterial Diseases, Epidemiological Models and their Application in Public Health*. Geneva: World Health Organization.

Daniel, R. R. and Lloyd, B. J. (1980*a*). Microbiological studies on two Oxfam Sanitation Units operating in Bengali refugee camps. *Water Research*, **14,** 1567–1571.

—— (1980*b*). A note on the fate of El Tor cholera and other vibrios in percolating filters. *Journal of Applied Bacteriology*, **48,** 207–210.

Dastidar, S. G. and Narayanaswami, A. (1968). The occurrence of chitinase in vibrios. *Indian Journal of Medical Research*, **56,** 654–658.

Dizon, J., Fukumi, H., Barua, D., Valera, J., Jayme, F., Gomez, F., Yamamoto, S-I., Wake, A., Gomez, C. Z., Takahira, Y., Paraan, A., Rolda, L., Alvero, M., Abou-Gareeb, A. H., Kobari, K. and Azurin, J. C. (1967). Studies on cholera carriers. *Bulletin of the World Health Organization*, **37,** 737–743.

Dodin, A. and Félix, H. (1972). Du role de la sueur dans l'épidémiologie du cholera en pays sec. *Bulletin de l'Académie Nationale de Médicine*, **156,** 845–852.

El-Shawi, N. N. and Thewaini, A. J. (1967). El Tor vibrios isolated in Iraq and their survival on some foods and beverages. WHO/Cholera Information/67.9 Geneva: World Health Organization, Unpublished document.

Feachem, R. G. A. (1976). Is cholera primarily water-borne? *Lancet*, **2,** 957.

———(1981). Environmental aspects of cholera epidemiology. I. A review of selected topics reports of endemic and epidemic situations during 1961–1980. *Tropical Diseases Bulletin*. **78**, 675–698.

———(1982). Environmental aspects of cholera epidemiology. III. Transmission and control. *Tropical Diseases Bulletin*, **79,** 1–47.

Felsenfeld, O. (1965). Notes on food, beverages and fomites contaminated with *Vibrio cholerae*. *Bulletin of the World Health Organization*, **33,** 725–734.

——— (1974). The survival of cholera vibrios. In *Cholera*, eds. Barua, D. and Burrows, W., pp. 359–366. Philadelphia: W. B. Saunders.

Flu, P. C. (1921). Investigations of the duration of life of cholera vibriones and typhoid bacteria in septic tanks at Batavia. *Mededeelingen van der Burgerlijken Geneeskundigen Dienst in Nederlandschindie*, **3,** 289–297.

Forbes, G. I. Lockhart, J. D. F. and Bowman, R. K. (1967). Cholera and nightsoil infection in Hong Kong, 1966. *Bulletin of the World Health Organization*, **36,** 367–373.

Gangarosa, E. J. and Mosley, W. H. (1974). Epidemiology and surveillance of cholera. In *Cholera*, eds. Barua, D. and Burrows, W., pp. 381–403. Philadelphia: W. B. Saunders.

Gerichter, C. B., Sechter, I. Gavish, A. and Cahan, D. (1975). Viability of *Vibrio cholerae* biotype El Tor and of cholera phage on vegetables. *Israel Journal of Medical Science*, **11,** 889–895.

Gildemeister, E. and Baerthlein, K. (1915). Beitrag zur cholerafrage. *Münchener Medizinische Wochenschrift*, **62,** 705–708.

Gohar, M. A. and Makkawi, M. (1948). Cholera in Egypt: Laboratory diagnosis and protective inoculation. *Journal of Tropical Medicine and Hygiene*, **51,** 95–99.

Greenough, W. B. (1979). *Vibrio cholerae*. In *Principles and Practice of Infectious Diseases*, eds. Mandell, G. L., Douglas, R. G. and Bennett, J. E., pp. 1672–1687. New York: John Wiley.

Grieg, E. D. W. (1914). On the vitality of the cholera vibrio outside the human body. *Indian Journal of Medical Research*, **1,** 481–504.

Gunn, R. A., Kimball, A. M., Pollard, R. A., Feeley, J. C. and Feldman, R. A. (1979). Bottle feeding as a risk factor for cholera in infants. *Lancet*, **2,** 730–732.

Hornick, R. B., Music, S. I., Wenzel, R., Cash, R., Libonati, J. P., Snyder, M. J. and Woodward, T. E. (1971). The Broad Street pump revisited: response of volunteers to ingested cholera vibrios. *Bulletin of the New York Academy of Medicine*, **47,** 1181–1191.

Howard, J. and Lloyd, B. (1979). Sanitation and disease in Bangladesh urban slums and refugee camps. *Progress in Water Technology*, **11,** 191–200.

Isaacson, M., Clarke, K. R., Ellacombe, G. H., Smit, W. A., Smit, P., Koornhof, H. J., Smith, L. S. and Kriel, L. J. (1974). The recent cholera outbreak in the South African gold mining industry. *South African Medical Journal*, **49,** 2557–2560.

Issacson, M. and Smit, P. (1979). The survival and transmission of *V. cholerae* in an artificial tropical environment. *Progress in Water Technology*, **11,** 89–96.

Jamieson, W., Madri, P. and Claus, G. (1976). Survival of certain pathogenic microorganisms in sea water. *Hydrobiologia*, **50,** 117–121.

Kabler, P. (1959). Removal of pathogenic micro-organisms by sewage treatment processes. *Sewage and Industrial Wastes*, **31,** 1373–1382.

Kaneko, T. and Colwell, R. R. (1973). Ecology of *Vibrio parahaemolyticus* in Chesapeake Bay. *Journal of Bacteriology*, **113,** 24–32.

——— (1975). Adsorption of *Vibrio parahaemolyticus* onto chitin and copepods. *Applied Microbiology*, **29,** 269–274.

——— (1978). The annual cycle of *Vibrio parahaemolyticus* in Chesapeake Bay. *Microbial Ecology*, **4,** 135–155.

Kaper, J., Lockman, H., Colwell, R. R. and Joseph, S. W. (1979). Ecology, serology, and enterotoxin production of *Vibrio cholerae* in Chesapeake Bay. *Applied and Environmental Microbiology*, **37,** 91–103.

Khan, M. and Shahidullah, M. (1980). Cholera due to the El Tor biotype equals the classical biotype in severity and attack rates. *Journal of Tropical Medicine and Hygiene*. **83,** 35–39.

Khan, S. and Agarwal, M. N. (1929). On the duration of the life of vibrios in the Ganges and Jumna river water. *Indian Journal of Medical Research*, **16,** 993–1008.

Koch, R. (1884). An address on cholera and its bacillus. *British Medical Journal*, August 30, 403–407 and 453–459.

Konchady, D. Prescott, L. M., Datta, A. and De, S. P. (1969). Survival of *Vibrio cholerae* in some of the waters of Calcutta. *Indian Journal of Medical Research*, **57,** 60–65.

Kott, Y. and Betzer, N. (1972). The fate of *Vibrio cholerae* (El Tor) in oxidation pond effluents. *Israel Journal of Medical Science*. **8,** 1912–1916

Lahiri, M. N., Das, P. C. and Malik, K. S. (1939). The viability of *Vibrio cholerae* in natural waters. *Indian Medical Gazette*. **74,** 742–744.

Lema, O., Ogwa, M. and Mhalu, F. S. (1979). Survival of El Tor cholera vibrio in local water sources and beverages in Tanzania. *East African Medical Journal*, **56,** 504–508.

Levine, R. J. and Nalin, D. R. (1976). Cholera is primarily waterborne in Bangladesh. *Lancet*. **2,** 1305.

McFeters, G. A., Bissonnette, G. K., Jezeski, J. J., Thomson, C. A. and Stuart, D. G. (1974). Comparative survival of indicator bacteria and enteric pathogens in well water. *Applied Microbiology*, **27,** 823–829.

Mhalu, F. S., Mmari, P. W. and Ijumba, J. (1979) Rapid emergence of El Tor *Vibrio cholerae* resistant to antimicrobial agents during first six months of fourth cholera epidemic in Tanzania. *Lancet*, **1,** 345–347.

MMWR (1979). Cholera surveillance—Japan. *Morbidity and Mortality Weekly Report*. **29,** 98–99.

Mukerjee, S., Rudra, B. C. and Roy, U. K. G. (1961). Observations on cholera endemicity in Calcutta and survival of *Vibrio cholerae* in the water sources. *Annals of Biochemistry and Experimental Medicine*, **11,** 31–40.

Müller, H. E. (1978). Occurrence and ecology of NAG vibrios in surface waters. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektions krankheiten und Hygiene, I. B.* **167,** 272–284.

——— (1979). NAG-vibrionen, choleraähnliche erreger in unseren gewässern. *Forum Städte-Hygiene*, **30,** 211–214.

Nalin, D. R. (1976). Cholera, copepods and chitinase, *Lancet*, **2,** 958.

Nalin, D. R., Daya, V., Reid, A., Levine, M. M. and Cisneros, L. (1979). Adsorption and growth of *Vibrio cholerae* on chitin. *Infection and Immunity*, **25,** 768–770.

Nalin, D. R., Robbins-Browne, R., Levine, M. M., Daya, V. and Noble, P. (1980). Multiplication of *Vibrio cholerae* in earthworms. In *Current Chemotherapy and Infectious Disease: Proceedings of the 11th International Congress of Chemotherapy and the 19th Interscience Conference on Anti-Microbial Agents and Chemotherapy*, eds. Nelson, J. D. and Grassi, C., pp. 936–937. Washington, D.C.: American Society of Microbiology.

Neogy, K. N., (1965). Viability of *V. cholerae* and *V. cholerae* El Tor in food and water. *Bulletin of the Calcutta School of Tropical Medicine*, **13,** 10–11.

Ohwada, S. (1924). Destination of cholera vibrios in the sewage water of Tokyo city. *Journal of the Keio Medical Society*. **3,** 11–12.

Pandit, C. G., Pal, S. C., Murti, G. V., Misra, B. S., Murty, D. K. and Shrivastav, J. B. (1967). Survival of *V. cholerae* biotype El Tor in well water. *Bulletin of the World Health Organization*, **37,** 681–685.

Pesigan, T. P., Plantilla, J. and Rolda, M. (1967). Applied studies on the viability of El Tor vibrios. *Bulletin of the World Health Organization*, **37,** 779–786.

Pollitzer, R. (1959). *Cholera*. Monograph 43. Geneva: World Health Organization.

Prescott, L. M. and Bhattacharjee, N. K. (1969) Viability of El Tor vibrios in common foodstuffs found in an endemic cholera area. *Bulletin of the World Health Organization*, **40,** 980—982.

Read, W. D. B., Gurkirpal Singh, J., Seal, S. C. and Bose, S. (1939). Growth and survival of *V. cholerae* with special reference to growth and survival in water. *Indian Journal of Medical Research*, **27,** 1–40.

Read, W. D. B. and Pandit, S. R. (1941). Distribution of *V. cholerae* and El Tor type strains in certain rural areas in India. *Indian Journal of Medical Research*, **29,** 403–415.

Sack, G. H., Pierce, N. F., Hennessey, K. N., Mitra, R. C., Sack, R. B. and Mazumder, D. N. G. (1972). Gastric acidity in cholera and non cholera diarrhoea. *Bulletin of the World Health Organization*, **47,** 31–36.

Sanyal, S. C., Singh, S. J., Tiwari, I. C., Sen, P. C., Marwah, S. M., Hazarika, U. R., Hardas Singh, Shimada, T. and Sakazaki, R. (1974). Role of household animals in maintenance of cholera infection in a community. *Journal of Infectious Diseases*, **130,** 575–579.

Sayamov, R. M. and Zaidenov, A. M. (1978). Survival and properties of cholera vibrios cultivated in mineral water. *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii*, **11,** 66–70.

Shoda, T., Koreyeda, T. and Otomo, T. (1934). The viability of cholera vibrios in the human excreta. *Journal of the Public Health Association of Japan*, **10,** 1–9.

Shousha, A. T. (1948). Cholera epidemic in Egypt (1947): a

preliminary report. *Bulletin of the World Health Organization*, **1**, 353–381.

Shrewsbury, J. F. D. and Barson, G. J. (1957). On the absolute viability of certain pathogenic bacteria in a synthetic well-water. *Journal of Pathology and Bacteriology*, **74**, 215–220.

Sinha, R., Deb, B. C., De, S. P., Abou-Gareeb, A. H. and Shrivastava, D. L. (1967). Cholera carrier studies in Calcutta in 1966-67. *Bulletin of the World Health Organization*, **37**, 89–100.

Smith, H. L., Freter, R. and Sweeney, F. J. (1961). Enumeration of cholera vibrios in fecal samples. *Journal of Infectious Diseases*, **109**, 31–34.

Sochard, M. R., Wilson, D. F., Austin, B. and Colwell, R. R. (1979). Bacteria associated with the surface and gut of marine copepods. *Applied and Environmental Microbiology*, **37**, 750–759.

Sommer, A. and Mosley, W. H. (1973). Ineffectiveness of cholera vaccination as an epidemic control measure. *Lancet*, **1**, 1232–1235.

Taylor, J. and Ahuja, M. L. (1938). Incidence and characters of vibrios in waters in northern India. *Indian Journal of Medical Research*, **26**, 1–32.

Threlfall, E. J., Rowe, B. and Huq, I. (1980). Plasmid-encoded multiple antibiotic resistance in *Vibrio cholerae* El Tor from Bangladesh. *Lancet*, **1**, 1247–1248.

Towner, K. J., Pearson, N. J., Mhalu, F. S. and O'Grady, F. (1980). Resistance to antimicrobial agents of *Vibrio cholerae* El Tor strains isolated during the fourth cholera epidemic in the United Republic of Tanzania. *Bulletin of the World Health Organization*, **58**, 747–751.

Towner, K. J., Pearson, N. J. and O'Grady, F. (1979). Resistant *Vibrio cholerae* El Tor in Tanzania. *Lancet*, **2**, 147–148.

Van de Linde, P. A. M. and Forbes, G. I. (1965). Observations on the spread of cholera in Hong Kong, 1961–1963. *Bulletin of the World Health Organization*, **32**, 515–530.

Venkatraman, K. V., Krishnaswami, A. K. and Ramakrishnan, C. S. (1941). Occurrence of vibrio El Tor in natural sources of water in the absence of cholera. *Indian Journal of Medical Research*, **29**, 419–424.

West, P. A., Knowles, C. J. and Lee, J. V. (1980). Ecology of *Vibrio* species, including *Vibrio cholerae*, in waters of Kent, United Kingdom. *Society for General Microbiology Quarterly*, **7**, 80.

WHO Scientific Working Group (1980). Cholera and other vibrio-associated diarrhoeas. *Bulletin of the World Health Organization*, **58**, 353–374.

Yasukawa, Y. (1933). Experiments on sea water and *Vibrio cholerae*. *Japanese Journal of Experimental Medicine*, **11**, 119–127.

Zaidenov, A. M., Sayamov, R. M., Maloletkov, I. S., Lazorenko, N. F., Bichul, K. G., Milyutin, V. N., Titenko, M. T., Popov, G. M., Kotov, V. K., Galtseva, G. V., Goldberg, A. M., Kiseleva, V. I., Stepanets, V. I., Seliverstov, V. I., Goryachkina, T. Ya., Reznikov, L. G., Lukash, V. O., Arendarskaya, L. S. and Azykovskaya, I. S. (1976). Prolonged survival of El Tor cholera vibrio in naturally infected sewage. *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii*, **12**, 61–69.