

Seasonal variations in the occurrence of *Vibrio vulnificus* along the Dutch coast

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SUMMARY

The seasonal variation in the occurrence of *V. vulnificus* in relation to water temperature and salinity was studied along the Dutch coast. In two consecutive years *V. vulnificus* strains could be isolated in August when the water temperature was highest. The indole-positive strains isolated from North Sea water samples were identical to most strains isolated from human disease and from the environment. However, strains isolated from four of five patients living in countries around the North Sea were different from the North Sea isolates in that they were indole-negative and have a lower NaCl tolerance.

INTRODUCTION

Vibrios are one of the most common organisms in surface waters in the world. Thirty-four *Vibrio* species are currently recognized in the genus, a third of which are known to be pathogenic for humans [1]. The halophilic *Vibrio* species, *V. parahaemolyticus*, *V. alginolyticus* and *V. vulnificus* are known to be involved in intestinal as well as in extra-intestinal human infections [2]. *V. parahaemolyticus* and *V. alginolyticus* strains have been isolated from seawater samples and from brackish inshore water collected along the Dutch, the Belgian and the British coasts and a seasonal effect in their occurrence has been demonstrated [3–5]. In the summer of 1991 we isolated *V. vulnificus* in seawater from 3 of 11 sampling sites along the coast of The Netherlands [6]. The purpose of this study was to obtain more data concerning the seasonal variations of the occurrence of *V. vulnificus* along the Dutch coast in relation to water temperature and salinity.

MATERIALS AND METHODS

At 4-week intervals in June, July, August and September 1992 water samples were taken from three sites along the coast of The Netherlands, where *V. vulnificus* had been isolated in August 1991 (Zandvoort, Katwijk and Scheveningen). In addition a sample was taken from one site in the marine lake Grevelingen. The

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samples were always taken on the same site one metre from the shore at a depth of 10–20 cm.

Bacteriological investigations

To each 250 ml flask containing a sample of 20 ml seawater, 100 ml peptone water, pH 8.9, containing 1.5% NaCl was added. After overnight incubation at 35 °C, $\pm 10 \mu\text{l}$ from the upper layer of the broth was subcultured on well-dried Thiosulphate Citrate Bile Salt Sucrose agar plates (Oxoid, CM 333) and incubated for 2 days at 35 °C. The inoculated agar plates were checked daily for bacterial growth. Bacterial colonies suspected of being *Vibrio* species were identified as described by Farmer and colleagues [7]. Identification of strains as *V. vulnificus* was confirmed by Dr Ir. K. van Soest, Faculty of Agricultural Sciences, University of Leuven, Belgium.

Biochemical investigations

Sodium and potassium were measured with a flame emission spectrophotometer, using lithium as an internal standard (Instrumentation Laboratory, UK). Chloride was determined with a coulometric technique (Marius Instruments, N.L.). Salinity was calculated using the formula: salinity % = $1.80655 \times \text{chlorinity \%}$ [8]. Salinity is slightly overestimated by the approximation that 1 litre seawater is the same as 1 kilogram.

Water temperature

At Noordwijk aan Zee, between Zandvoort and Katwijk, at 08.00 h the water temperature is measured at one metre depth daily throughout the whole year by the Tidal Waters Division of the Ministry of Transport, Public Works and Water Management.

RESULTS

The results of bacteriological and biochemical investigations are summarized in Table 1. *V. vulnificus* was isolated from three sea water samples and from none of the samples of the lake Grevelingen. All strains were indole-positive. All other 13 samples were negative for *V. vulnificus*. The positive samples were all taken on the same day in August. The salinity of the sea water samples varied from 25.8 to 31.7‰, the salinity of the water at Lake Grevelingen varied from 28.2 to 30.3‰. The water temperature at 08.00 h measured at Noordwijk aan Zee is shown in Fig. 1. *V. vulnificus* strains were isolated when the water temperature was highest in 1991 as well as in 1992 as indicated by arrows. *V. alginolyticus* was isolated from all water samples examined. *V. parahaemolyticus* was isolated from North Sea water samples in June, July and August.

DISCUSSION

In 1979 the name *V. vulnificus* was given to a *Vibrio* species of which the characteristics were described in 1976 [9, 10]. *V. vulnificus* has been isolated from numerous marine environments; the highest densities are generally found in areas of high temperature and low to moderate salinity [1]. An *in-vitro* study of growth characteristics of environmental isolates of *V. vulnificus* demonstrated salinity optima of 10–20% NaCl and a temperature optimum of 37 °C [11]. The inability to culture *V. vulnificus* from low-temperature environments is due not to cell death

Table 1. Monthly variations in *Vibrio* spp. and salinity along the coast of The Netherlands in 1992

	Zandvoort	Katwijk	Scheveningen	L. Grevelingen
29 June				
<i>V. vulnificus</i>	—*	—	—	—
<i>V. parahaemolyticus</i>	+	+	+	—
<i>V. alginolyticus</i>	+	+	+	+
Salinity ‰	29.5	29.4	25.8	30.8
26 July				
<i>V. vulnificus</i>	—	—	—	—
<i>V. parahaemolyticus</i>	+	—	+	—
<i>V. alginolyticus</i>	+	+	+	+
Salinity ‰	32.1	31.7	31.7	28.2
22 August				
<i>V. vulnificus</i>	+	+	+	—
<i>V. parahaemolyticus</i>	+	+	+	—
<i>V. alginolyticus</i>	+	+	+	+
Salinity ‰	29.1	27.9	28.1	30.1
24 September				
<i>V. vulnificus</i>	—	—	—	—
<i>V. parahaemolyticus</i>	—	—	—	—
<i>V. alginolyticus</i>	+	+	+	+
Salinity ‰	30.2	29.6	28.5	29.8

* +, present; —, absent.

but to a viable but non-culturable state. *V. vulnificus* can be resuscitated from this state by temperature upshifts [12]. Another problem in the isolation from environmental samples is overgrowth and masking of the presence of *V. vulnificus* by *V. alginolyticus*, *V. parahaemolyticus* and other bacteria not members of the genus *Vibrio*. Incubation of test samples in alkaline peptone water, followed by isolation on TCBS agar, as used by us, is a standard approach. However, numerous colonies have to be checked due to the, often abundant, growth of other *Vibrio* spp. and non-vibrio bacteria. Overgrowth is a distinct possibility. A selective and differential medium for *V. cholerae* and *V. vulnificus* has been described (CPC-medium) [13]. However, few studies describe its use in the field. Recent methods to circumvent these problems are enzyme immunoassay [14] and polymerase chain reaction [15]. The last technique also enables the detection of viable but non-culturable cells.

V. vulnificus has been isolated from marine animals. Fish and all types of shellfish products may be involved including oysters, mussels, clams, shrimps and crabs. The risk of infection with *Vibrio* spp. is highest with filter-feeding bivalves since they concentrate contaminants in nature from surrounding water [16]. In humans wound infections are generally acquired from exposure to sea water or marine animals, while primary *V. vulnificus* septicaemias are most commonly associated with the consumption of raw oysters. Most of the cases of septicaemia are associated with underlying diseases [17]. *V. vulnificus* strains from widely separated marine regions and strains isolated from septicaemia or wound infections in man are phenotypically indistinguishable from each other and are highly genetically related [18, 19].

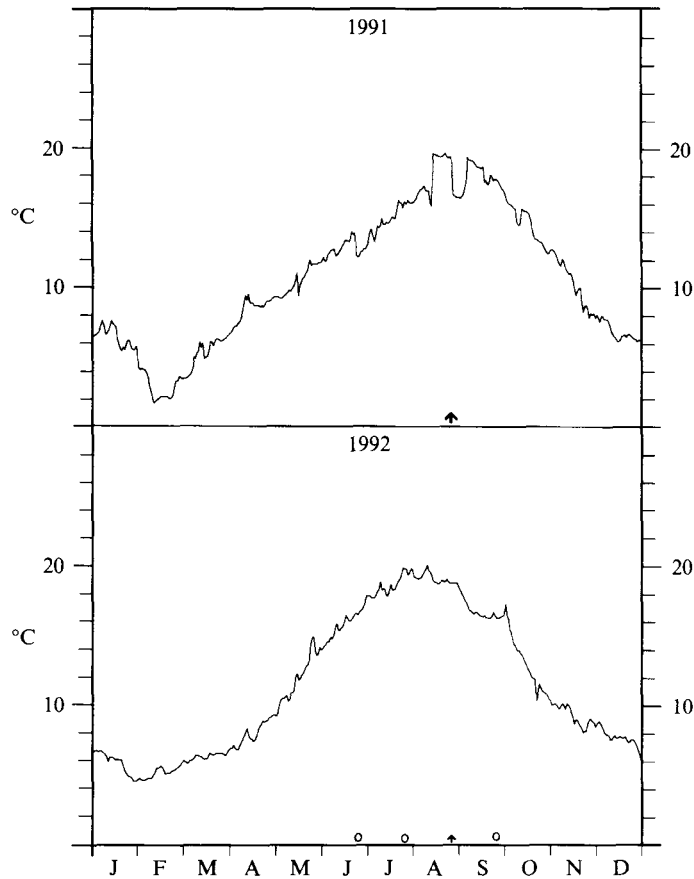


Fig. 1. Sea-water temperature 0.800 h, measured daily by the Tidal Waters Division, at Noordwijk aan Zee at one metre depth. Upper panel 1991. Lower panel 1992. Horizontal axis: months of the year. Vertical axis: water temperature in °C. Circles indicate the points in time of samples negative for *V. vulnificus*. Arrows indicate the points in time when samples positive for *V. vulnificus* were obtained.

Survey studies on the occurrence of *Vibrio* species along the North Sea coast in England and The Netherlands have been performed years before the species *V. vulnificus* was separated from the other *Vibrio* species [3, 5]. In a survey at the Belgian coast from August 1982 to February 1983 no *V. vulnificus* strains were isolated [4]. In two consecutive years, 1991 and 1992, we were able to isolate *V. vulnificus* from coastal water around The Netherlands in August, when the water temperature was at its highest. The limited period during which *V. vulnificus* could be isolated is most probably associated with the suboptimal environmental conditions for growth of *V. vulnificus*, a relatively high salinity and a low water temperature, during the rest of the year. The biochemical characteristics of the North Sea strains are similar to most strains isolated from human disease and from the environment. Five cases of *V. vulnificus* causing infection in human beings have been reported in countries around the North Sea (Belgium and The Netherlands) [20–22]. One patient was infected during the end of July, 3 in August and 1 in September. One of the 5 patients was in contact with brackish water

(Veerse Meer), 2 patients were probably infected while eviscerating living eels, in 2 patients the way of transmission could not be identified. In only one patient, with an unknown way of infection transmission, a strain biochemically identical to the North Sea strains was found (indole-positive). The biochemical characteristics of the strains isolated from 4 of the 5 Belgian and Dutch patients differ from most strains isolated from human disease and from the environment in that they are indole-negative and have a lower NaCl tolerance [20, 22].

In conclusion, *V. vulnificus* strains can be isolated along the North Sea coast in The Netherlands at the end of the summer when the water temperature is highest. The strains are identical to clinical and environmental isolates described in most of the literature, but until now in the countries along the North Sea only one clinical isolate with identical characteristics has been reported. The other four strains isolated from patients in The Netherlands and Belgium have a few biochemical characteristics (mainly a negative indole-reaction) which differ from the isolates from the North Sea.

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