Identification of the pandemic O3:K6 clone in cases of *Vibrio parahaemolyticus* related illness in the United Kingdom

Sariqa Wagley*
School of Biomedical and Molecular Sciences,
Faculty of Health and Medical Sciences, University of Surrey www.surrey.ac.uk

Rachel Rangdale
Centre for Environment, Fisheries & Aquaculture Science, Weymouth www.cefas.co.uk

Introduction

Vibrio parahaemolyticus is a halophilic bacterium that can be isolated from seawater, bivalve molluscs and crustacea (Figure 1). It is one of the causes of seafood-related gastroenteritis with symptoms including watery/bloody diarrhoea, vomiting, abdominal cramps, headaches, fever and nausea (Joseph *et al.*, 1982). The bacterium is found where sea temperatures are >13°C and where salinity ranges between 5 and 25ppt. Pathogenicity is associated with thermostable direct and related haemolysins (TDH/TRH), encoded by the *tdh* and *trh* genes respectively. These genes are present in up to 99% of clinical strains but rare (2-3%) in environmental isolates (Nishibuchi *et al.*, 1995).

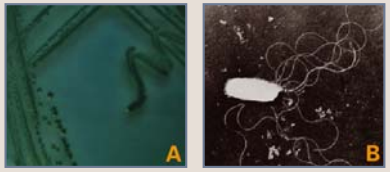

Figure 1: *V. parahaemolyticus* on thiosulphate citrate bile sucrose (TCBS) plate showing non-sucrose fermenting colonies (A) and a micrograph of *V. parahaemolyticus* cell (B).

Clinical isolates may be associated with diverse serotypes although in Asia since 1996 an O3:K6 serovar has accounted for an increased incidence of cases (Figure 2) (Okuda *et al.*, 1997). This serovar first emerged in Calcutta, India and was characterised as TDH positive and TRH negative. It was responsible for 50 to 80% of *V. parahaemolyticus* infections after February 1996 in the region (Okuda *et al.*, 1997). This newly recognized serovar had seven base differences in the *toxRS* operon. This polymorphism has been exploited to develop a group-specific PCR (GS-PCR), which has been used as a molecular marker for its identification (Matsumoto *et al.*, 2000).

In 2004 in Spain, Europe saw its first reported outbreak associated with the O3:K6 serovar where 76 cases were reported after infected crabs were consumed at a wedding in Galicia (Martinez-Urtaza *et al.*, 2005). To date there have been no reported outbreaks of O3:K6 *V. parahaemolyticus* in the UK (Table 1). Forty-six cases of *V. parahaemolyticus* were reported to the UK Health Protection Agency in 2004 – 2005 inclusive. However under reporting may mean that this figure is not a true representation of case numbers.

Figure 2: Location of outbreaks and cases of O3:K6 *V. parahaemolyticus* worldwide.

Table 1: Selected outbreaks caused by *V. parahaemolyticus* O3:K6 around the world demonstrating the pandemic status of this serovar.

Country	Year	Number of cases	Source
Niigata prefecture, Japan	1996	691	Boiled crabs
France	1997	44	Imported shrimp
Vladivostok, Russia	1997	27	Unknown
Khanh Hoa, Vietnam	1997-1999	256	Unreported
Texas, USA	1998	416	Oysters
Taiwan	1996-1999	2231	Seafood
Puerto Montt, Chile	1998 & 2004	1500	Seafood
Mozambique	2004	32	Uncooked Seafood
Spain	2004	76	Crabs

Objectives

In this study the significance of 4 clinical isolates of *V. parahaemolyticus* that had been submitted to the UK Health Protection Agency between 2000 and 2005 were examined. Three strains were isolated from individuals exhibiting *V. parahaemolyticus*-like disease on return to the UK following travel to the Far East (VP E154482, VP E155855 and VP E168143) while one strain was isolated from an individual with gastroenteritis after consumption of shellfish in the UK (F3305). Biochemical and molecular clonal relationships of these strains to the pandemic O3:K6 isolated from Spain and Japan, were analysed using PCR, serotyping and pulsed field gel electrophoresis (PFGE) techniques.

Results

- All *V. parahaemolyticus* strains were positive for species-specific molecular marker genes *toxR* and *tlh*.
- The pandemic O3:K6 strain has been characterised as TDH positive, TRH negative and GS positive. Strains VP E155855 and VP E168143 showed the same molecular characteristics.
- Figure 3 shows PFGE profiles of the clinical strains tested using *Not1* digestion. VP E155855 and VP E168143 form part of the same cluster group (C2, 100% similarity) as those isolated from the Spanish outbreak of 2004, illustrating further that these 2 UK strains are clones of the pandemic O3:K6 *V. parahaemolyticus* strain.

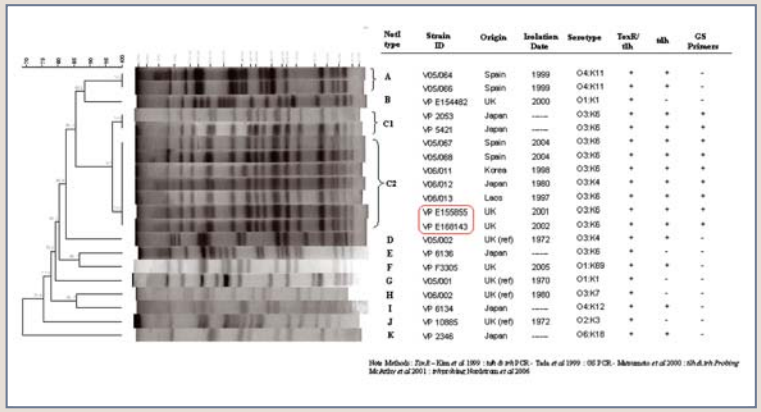

Figure 3: A dendrogram portraying PFGE profiles (using *Not1* digestion), of clinical isolates of *V. parahaemolyticus* from different locations. Molecular characteristics such as serotype and the presence of pathogenicity markers e.g. tdh are also shown. The *Not1* profiles show that VP E155855 and VP E168143 form part of the same cluster group (C2, 100% similarity) as other O3:K6 *V. parahaemolyticus* strains, illustrating that these two UK isolates are clones of the pandemic *V. parahaemolyticus* strain O3:K6 seen around the world.

Discussion

This study describes the first reported cases of the pandemic O3:K6 *V. parahaemolyticus* isolated in the UK. There have been two reported outbreaks of *V. parahaemolyticus* in the UK; the first in 1972, which occurred among airline crew travelling from Bangkok, Thailand to the UK (Peffers *et al.*, 1973) and the second in 1973 among holidaymakers on the south coast of England who had eaten prepared crabs caught locally (Hooper *et al.*, 1974). Sporadic travel-related cases of *V. parahaemolyticus* are more common and the true estimation of clinical cases indigenous to UK is unknown. For the few clinical cases that do occur in the UK, little epidemiological investigation is carried out. There is little or no patient history, little or no data on sources or vehicles of transmission and the presence of TDH/TRH and serotype are rarely determined. Thus, the information on clinical cases of *V. parahaemolyticus* in the UK is very limited.

The bacterium is routinely isolated from shellfish samples tested at Cefas during the summer season. The prevalence and density of *V. parahaemolyticus* in the environment and seafood products have been shown to be dependent on ambient water temperature with rapid proliferation occurring at sea water temperatures >16°C (Anon, 2001). In recent years, a number of reports have suggested that higher sea surface temperatures will result in a reduction of dissolved oxygen and increased bacterial metabolism. This would preferentially support the proliferation of pathogenic vibrios including *V. parahaemolyticus*. Sea surface temperature and air temperature over the sea within the mid-latitude North Atlantic and UK coastal waters have been rising by 0.2 – 0.6 °C per decade over the past 30 years. It is clear that if global climate change results in longer, hotter summers and elevation of ambient sea temperatures, it may also lead to an increase in incidence of seafood associated *V. parahaemolyticus* food poisoning in the community.

References

Anon 2001 Scientific Committee on Veterinary Measures relating to Public Health (SCVMPH).

Hooper WL., *et al.*, (1974). *Lancet.* Pg 1100-1102

Joseph SW., *et al.*, (1982) *Critical Reviews in Microbiology* **10**:77-124.

Kim YB., *et al.*, (1999) *Journal of Clinical Microbiology*, **37**:1173-1177.

Martinez-Urtaza J., *et al.*, (2005) *Emerging Infectious Diseases* **11**:1319-1320.

Matsumoto C., *et al.*, (2000) *Journal of Clinical Microbiology* **38**:578-585.

Nishibuchi M., *et al.*, (1995) *Infectious Immunity* **63**:2093-2099.

Nordstrom., *et al.*, (2006) *Journal Food Protection* **69**:2770-2772

Okuda J., *et al.*, (2001) *Infection and Immunity*, **69**:6091-6101

Peffers ASR., *et al.*, (1973). *Lancet* **20**:1(7795): 143-5

Tada J., *et al.*, (1992) *Mol.Cell Probe.* **6**:477-487.

sariqa.wagley@cefas.co.uk