

Decreasing *Vibrio parahaemolyticus* infections and analysis of seafood contamination in Japan

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Introduction

Since 1962, *Vibrio parahaemolyticus* infections have been a dominant cause of foodborne infections in Japan. There was a large epidemic of infections with a new clone with the serotype O3:K6 from 1997 to 2001; various other serotypes had been prominent before. While the incidence of *V. parahaemolyticus* infections has varied annually, the numbers of cases doubled (12,318 cases) and outbreaks quadrupled (839 outbreaks) in 1998. As a result, the control measures; the use of clean seawater for processing shellfish and finfish, storage at $\leq 10^{\circ}\text{C}$ for seafood and the standards for *V. parahaemolyticus* in seafood were taken by the Committee on Safe Seafood Management was organized under the Ministry of Health, Labor and Welfare (MHLW) from 1999. Between 1998 and 2009, the numbers of cases and outbreaks of *V. parahaemolyticus* infections decreased to 40-fold and 60-fold, respectively. The purpose of this study is to gain insights into changes in the prevalence and distribution of *V. parahaemolyticus* population during the past decade in relation to the sharp decrease of infections in Japan.

Materials and methods

Eight hundred and forty-two seafood samples of molluscan shellfish and fish were purchased from fresh seafood markets in various regions in Japan from May to November in 2007 to 2009. The following method was used in a 3-tube MPN format. A 3-step enrichment procedure was used and consisted of first enrichment in alkaline peptone water, second enrichment in salt polymyxin broth (SPB), and third enrichment in SPB. At this point an aliquot of SPB was tested by PCR for *tdh* and another was streaked to CHROMagar Vibrio for isolation of *V. parahaemolyticus*. The immunomagnetic separation method for K6 was also performed for effective isolation of TDH-producing *V. parahaemolyticus* O3:K6. PFGE (Pulsed-field gel electrophoresis) was performed with *NotI* and *SfiI* restriction enzymes. Dendrograms were constructed from the PFGE profiles. The data on serotypes in *V. parahaemolyticus* infections from 1998 to 2007 and strains from patients of *V. parahaemolyticus* outbreaks were collected from a part of local governments in Japan.

Results and discussion

Tdh-positive *V. parahaemolyticus* was detected in 65 (7.7%) of 843 samples. The frequency of *tdh*-positive samples from 2007 to 2009 did not change as compared to those in 2001 (10%). This suggests that the large reduction of *V. parahaemolyticus* infection was not the result of a change of *V. parahaemolyticus* contamination frequencies in seafood. Various serotypes including O3:K6 of TDH-producing *V. parahaemolyticus* were isolated from 18 samples. *V. parahaemolyticus* infections with serotypes other than O3:K6 were decreased as same as that with O3:K6. The PFGE profile patterns of O3:K6 isolates from patients over the last three years were coincident with those from domestic and imported seafood in 2008 and 2009. These results indicate that TDH-producing *V. parahaemolyticus* O3:K6 contamination of seafood still causes infections. The sharp decreases in outbreaks of infection with both O3:K6 and other serotypes may indicate that the control measures for reduction of *V. parahaemolyticus* infection have been successful.