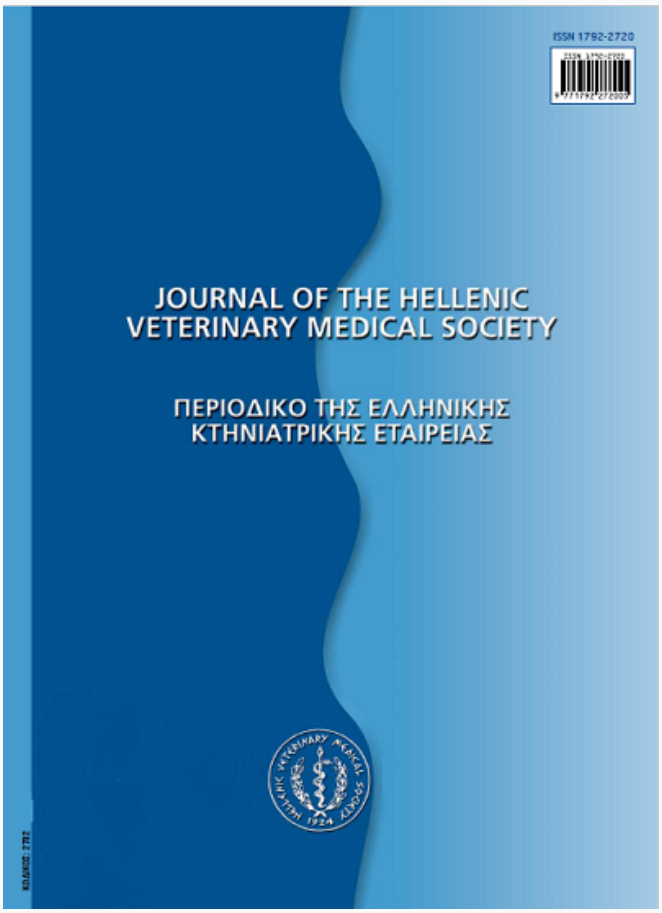
Περιοδικό της Ελληνικής Κτηνιατρικής Εταιρείας

Τόμ. 63, Αρ. 1 (2012)



Το Vibrio parahaemolyticus σε τροφιμογενείς λοιμώξεις αλιευμάτων

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doi: 10.12681/jhvms.15398

J HELLENIC VET MED SOC 2012, 63(1): 54-62
ΠΕΚΕ 2012, 63(1): 54-62

Review article
Ανασκόπηση

Vibrio parahaemolyticus in seafood – associated outbreaks

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Το *Vibrio parahaemolyticus* σε τροφιμογενείς λοιμώξεις αλιευμάτων

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ABSTRACT. Among the 30 species of the genus *Vibrio*, only 13 of them are pathogenic to humans. All pathogenic vibrios have been reported to cause foodborne diseases, although *V. parahaemolyticus* is considered the most important pathogenic *Vibrio*. *V. parahaemolyticus* is a halophilic bacterium that occurs naturally in aquatic environments worldwide. The pathogen caused sporadic diarrhoea mainly associated with the consumption of raw or undercooked seafood up to recent years. Since 1996, the incidence of *V. parahaemolyticus* infections has increased dramatically. *V. parahaemolyticus* is the leading cause of seafood-associated bacterial gastroenteritis in the United States and of the half foodborne outbreaks in some Asian countries. This increase in incidence has been related to the emergence of the O3:K6 serovar. The pathogenic *V. parahaemolyticus* strains can produce a thermostable direct hemolysin or a thermostable direct hemolysin-related hemolysin, which are encoded by the *tdh* and *trh* genes, respectively. *Vibrio parahaemolyticus* has not been included in the microbiological criteria of E.U. Food legislation, probably because the risk by this pathogen was considered rather low in Europe. However, climate changes favour the growth of the pathogen in seawater. Recent studies in Spain and France have shown that *V. parahaemolyticus* infections from seafood consumption have been increased. The emergence of the pathogen in Europe is of public health concern and emphasizes the importance of microbiological surveillance and control programs for *V. parahaemolyticus*.

Keywords: *Vibrio parahaemolyticus*, seafood, fish, outbreaks

ΠΕΡΙΛΗΨΗ. Μέχρι σήμερα έχουν καταγραφεί 30 είδη *Vibrio* (δονάκια), από τα οποία 13 είναι παθογόνα για τον άνθρωπο. Όλα τα παθογόνα δονάκια έχουν αναφερθεί ότι σχετίζονται με τροφιμογενείς λοιμώξεις, αλλά το *V. parahaemolyticus* είναι το σημαντικότερο παθογόνο δονάκιο. Το *V. parahaemolyticus* είναι ένα αλλόφιλο βακτήριο που βρίσκεται σε υδάτινα οικοσυστήματα σε όλη την υφήλιο. Μέχρι το 1996, το παθογόνο σχετίζονταν με σποραδικά περιστατικά τροφιμογενούς γαστρεντερίτιδας μετά από την κατανάλωση νωπών αλιευμάτων ή αλιευμάτων που είχαν υποστεί ατελή θερμική επεξεργασία. Από το 1996, ο αριθμός των καταγεγραμμένων τροφιμογενών λοιμώξεων από το *V. parahaemolyticus* έχει αυξηθεί σημαντικά με αποτέλεσμα να αποτελεί το κυριότερο αίτιο τροφιμογενών λοιμώξεων από κατανάλωση αλιευμάτων σε ορισμένες ασιατικές χώρες και στις Η.Π.Α. Η αύξηση αυτή αποδίδεται στην εμφάνιση του ορότυπου O3:K6 του βακτηρίου. Τα παθογόνα στελέχη του *V. parahaemolyticus* παράγουν μια θερμοανθεκτική τοξίνη (Kanagawa ή TDH) ή μια αιμολυσίνη παρόμοια με την τοξίνη Kanagawa (TRH) που σχετίζονται με τα γονίδια *tdh* και *trh*, αντίστοιχα. Το *V. parahaemolyticus*

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Submission date: 06.02.2012
Acceptance date: 19.03.2012

Ημερομηνία υποβολής: 06.02.2012
Ημερομηνία αποδοχής: 19.03.2012

δεν περιλαμβάνεται στα μικροβιολογικά κριτήρια ασφαλείας για τα αλιεύματα που ισχύουν στην Ευρωπαϊκή Ένωση, αφού μέχρι πρόσφατα θεωρούνταν χαμηλή η συχνότητα εμφάνισης λοιμώξεων από το παθογόνο στην Ευρώπη. Όμως, οι κλιματικές αλλαγές του πλανήτη μας ευνοούν την ανάπτυξη του παθογόνου στο θαλάσσιο νερό. Πρόσφατες μελέτες στην Ισπανία και τη Γαλλία αναφέρουν ότι οι λοιμώξεις αυτές παρουσιάζουν σημαντική αύξηση. Στη παρούσα ανασκόπηση αναφέρονται οι μεγαλύτερες επιδημίες που έχουν προκληθεί παγκοσμίως από *V. parahaemolyticus* μετά από την κατανάλωση αλιευμάτων. Η αυξανόμενη καταγραφή της παρουσίας του παθογόνου σε αλιεύματα στην Ευρώπη παρουσιάζει ιδιαίτερη σημασία για τη Δημόσια Υγεία και υπογραμμίζει την ανάγκη ένταξης του *V. parahaemolyticus* στα προγράμματα επιτήρησης και ελέγχου.

INTRODUCTION

The *Vibrio parahaemolyticus* is an ubiquitous Gram-negative bacterium that naturally occurs in marine coastal waters and is recognized as the most common causative agent of seafood - associated gastroenteritis, worldwide. *V. parahaemolyticus* infections result from ingestion of raw or improperly cooked seafood (Johnson et al. 2008). Shellfish that are frequently consumed whole and raw can serve as carriers of pathogenic Vibrios. Especially, bivalves (oysters, clams and mussels), which filter water through their digestive system to obtain food, accumulate and concentrate microorganisms (Su and Liu 2007) such as *V. parahaemolyticus*.

European Union Regulation 2073/2005, which sets out the microbiological criteria for foods, makes no provision for *Vibrio* control in seafood within the European Union. However, there is a growing concern that *V. parahaemolyticus* may pose an important and increasing problem for seafood safety in Europe (Baker-Austin et al. 2010, Ottaviani et al. 2008). The isolation of this pathogen in recent studies from areas, including Europe, where it was rarely found, highlights its geographical and seasonal expansion (Martinez – Urtaza et al. 2010, Su and Liu 2007). The present review is focussed on the importance of *V. parahaemolyticus* in seafood safety.

Vibrio parahaemolyticus

The genus *Vibrio* is classified within the family *Vibrionaceae*. There are 30 species in the genus *Vibrio*, 13 of these are considered pathogenic to humans, including *V. cholerae, V. mimicus, V. fluvialis, V. parahaemolyticus, V. alginolyticus, V. cincinnatiensis, V. hollisae, V. vulnificus, V. furnissii, V. damsela, V. metshnikovii,* and *V. carchariae*. All pathogenic vibrios have been reported to cause foodborne diseases, but it has been considered that *V. cholerae* O1, *V. parahaemolyticus* and *V. vulnificus* are the most significant causative agents (Atlas 1997). Members of the *Vibrio* genus are straight or curved Gram-negative, non spore-forming rods, 0.5 to 0.8 μm in width and 1.4 to 2.6 μm in length. Vibrios are motile by a single polar flagellum and are aerobic or facultative anaerobic. Most species produce oxidase and catalase and ferment glucose without producing gas (McLaughlin 1995).

In 1950, a new bacterium was identified as the cause of a large outbreak in Japan, which was associated with the consumption of a local fish product "shirasu" (Fujino et al. 1953). Initially, the new species was named *Pasteurella parahaemolyticus* and later designated as *Vibrio parahaemolyticus* (Hugh and Sakauki 1975). *V. parahaemolyticus* is a moderately halophilic (salt-requiring) enteric pathogen that can grow in NaCl concentrations of up to 8%, but the optimum growth is observed at 2 to 3% NaCl (Yang et al. 2010). It has a very short generation time (<10 min) at the optimum growth temperature of 37 °C (Miles et al. 1997). The pathogen grows at a minimum temperature of 15 °C and a maximum temperature of 44 °C. All strains of *V. parahaemolyticus* produce H2S in triple sugar iron (TSI) medium (Su and Liu 2007).

Serotyping of *V. parahaeomolyticus* is based on antigens of O (somatic) and K (capsular polysaccharide). All strains of the pathogen share a common H (flagellar) antigen. Up to date, 12 O antigen types and over 76 K antigen types have been described, although serotypes of many strains have not been identified (Kaysner and DePaola 2004).

Virulence Factors

V. parahaemolyticus strains isolated from human gastroenteritis cases are characterized by a variety of virulence factors. Typically, virulent strains of the pathogen can lyze red blood cells on Wagatsuma blood agar plates. This haemolytic activity is named Kanagawa phenomenon (Kp) and has been associated with the production of a thermostable direct haemolysin (TDH). This protein is not inactivated by heat (100 °C for 10 min) and its haemolytic activity is not

enhanced by the addition of lecithin, which suggests direct activity on erythrocytes (Nishibuchi and Kaper 1995). Almost all clinical isolates are Kp positive, therefore TDH production is considered as the major virulent factor of *V. parahaemolyticus* (Su and Liu 2007, Takeda 1983). Occasionally, Kp negative strains of the pathogen have been isolated from human cases of gastroenteritis associated with seafood consumption. Such strains produced a protein, similar but not identical to TDH (TDH related haemolysin - TRH) (Honda et al. 1988, 1987). The genes of both types of haemolysin share several biological properties such as haemolytic activity, enterotoxicity and cytotoxigenicity (Park et al. 2004).

Since only 1 – 2 % of the environmental strains carry the genes encoding TDH (*tdh*) and TRH (*trh*), the presence of *tdh* or/and *trh* genes has been considered as molecular indicators for distinguishing pathogenic and non pathogenic strains (Oliver and Kaper 2007). However, it has been reported that *V. parahaemolyticus* strains lacking both *tdh* and *trh* genes were associated with severe cases of foodborne infection, many of which required hospitalization (Yu et al. 2006). In Japan, 215 clinical isolates of *V. parahaemolyticus* were examined for the presence of *tdh* and *trh* genes and it was found that 52 strains (24.3%) carried only the *trh* gene (Shirai et al. 1990).

The analysis of the complete genome of *V. parahaemolyticus* (Makino et al. 2003) revealed more complex pathogenic mechanisms than previously considered. High adherence capability, some enzymes, urea hydrolysis and more recently, a heat-labile protein (serine protease) have been also identified as potential virulence factors (Lee et al. 2002, Iida et al. 1997).

Pathogenesis

V. parahaemolyticus infections are very common worldwide and are considered as the leading cause of bacterial illnesses associated with seafood consumption in Asia and USA (Nordstrom et al. 2007). The virulence and pathogenicity of *V. parahaemolyticus* are less pronounced than those of other Vibrios like *V. cholerae* or *V. vulnificus* and deaths associated with this bacterium are rare. Symptoms include headache, nausea, acute abdominal pain, low grade fever, vomiting and diarrhoea (watery or bloody). The stool is described as "meat washed" due to the presence of blood (Yang et al. 2008, Honda and Lida 1993). The infectious dose is usually about 2×10^5 to 3×10^7 CFU. Incubation period is about 15 h (4 – 96 h) and symptoms typically resolve in less than 72 h. *V. parahaemolyticus* colonize the intestine and produce toxins that cause cell damage as well as loss of fluids and electrolytes. Though the *V. parahaemolyticus* infection induces a strong immune response, the detailed mechanism of pathogenesis is still not well known (Levin 2006). Unlike other *Vibrios*, person to person transmission of *V. parahaemolyticus* has not been reported yet and foodborne outbreaks are almost always associated with seafood consumption (Martinez-Urtaza et al. 2004, Joseph et al. 1982).

The gastroenteritis caused by *V. parahaemolyticus* infection is often self-limited, but in some cases the infection may cause septicaemia that is life-threatening to people having underlying medical conditions such as liver disease or immune disorders. Two deaths were reported among three cases of wound infections caused by *V. parahaemolyticus* in USA after the Hurricane Katrina in 2005 (Centers for Disease Control and Prevention - CDC 2005).

Emerging strain of *Vibrio parahaemolyticus*

Prior to the 1970, almost all infections caused by *V. parahaemolyticus* were reported in Japan. Food-borne outbreaks were typically associated with seafood consumption and represented about 70% of food-related cases during summer months. Throughout the 1970's, sporadic cases and outbreaks were also reported in Europe (Joseph et al. 1982). The first documented outbreak of *V. parahaemolyticus* in USA was reported in 1971 and was associated with contaminated crab products. The pathogenic strains isolated from patients and suspected seafood in USA were different than those isolated in outbreaks in Japan (Dadisman et al. 1972).

Since the mid 1990's an increased incidence of *V. parahaemolyticus* gastroenteritis cases was reported from many countries around the world, which was associated with a novel serotype (O3:K6) of the pathogen. This serotype was first isolated in 1996 in Calcutta, India from hospitalized patients (Okuda et al. 1997). The emerging Calcutta O3:K6 strain was different from other O3:K6 strains isolated in Asia in the previous decade. Additionally to the emergence of the new serovar, strains O4:K68 and O1:K untypeable (KUT) have also been associated with the increased incidence of *V. parahaemolyticus* infections worldwide

(Okura et al. 2003). Molecular techniques placed these serovars into a homogenous group with clearly differentiated virulence traits (*tdh*$^+$, *trh*$^-$) from previously recovered strains. The clonal clustering of the strains and their distinctive genetic characteristics, led to this group being named a "new O3:K6 clone" (Chowdhury et al. 2000, Okuda et al. 1997). During the next year the new clone was isolated from *V. parahaemolyticus* infections throughout Asia. In June of 1997 the O3:K6 strain spread to Peru, for the first time beyond Asia and later that year it was implicated in an outbreak in Chile (Martinez – Urtaza et al. 2005). In 1998, a large outbreak was reported in the USA, which was associated with consumption of raw contaminated oysters (Daniels et al. 2000). A retrospective study compared O3:K6 strains from North America and Asia outbreaks using molecular techniques and indicated that these strains were indistinguishable (Matsumoto et al. 2000). This was the first definitive evidence of the *V. parahaemolyticus* pandemic and the term "pandemic clone" was designated (Gonzalez – Escalona et al. 2008, Chowdhury et al. 2000). Since then, a spread of this pandemic strain to Europe has been reported and it was isolated from outbreaks in France (Quilici et al. 2005), Spain (Martinez – Urtaza et al. 2005), Italy (Ottaviani et al. 2008), as well as from other countries like Russia (Nair et al. 2007) or Mozambique (Ansaruzzaman et al. 2005). In 2005, the largest *V. parahaemolyticus* outbreak caused by the pandemic O3:K6 strains (11000 human cases) was reported in Chile and it was associated with shellfish consumption (Fuenzalida et al. 2006).

Risk factors associated with the globalization of *V. parahaemolyticus*

Traditionally, *V. parahaemolyticus* illnesses have been associated with sporadic cases during the warmest months of the year in temperate and tropical areas (Bauer et al. 2006). Probably the most important change in the epidemiology of the pathogen has been the emergence of infections in many areas around the world, where this organism was rarely reported or was absent. Moreover, the recent global spread of larger outbreaks than those observed in the past, has been frequently related to the arrival of the pandemic clone to these regions (King et al. 2008). It is assumed that, when non – native strains of *V. parahaemolyticus* arrive to a new region, the lack of acquired immunity may be responsible for the sudden emergence of large number of infections among the exposed population (Baker – Austin et al. 2010).

Additionally, the consumption of shellfish has increased significantly the last decades worldwide. Recent estimates from the United Nations indicate that by 2030 (taking into account present-day consumption levels) will demand an additional 40 million tons of seafood globally (Gudmundsson et al. 2006). Such a global scale expansion in shellfish consumption is more likely to maximise the number of individuals exposed to pathogenic vibrios such as *V. parahaemolyticus* (United Nations 2002, Johnson et al. 1988).

Another factor that is gaining research interest the last years is the effect of global climate change (Martinez – Urtaza et al. 2010, Baker-Austin et al. 2010). It is well known that both temperature and salinity play important roles in the levels of *V. parahaemolyticus* (Drake et al. 2007). It has been suggested that the highest densities of *Vibrio* cells occur in waters with temperatures ranging from 20 to 30 °C (Tantillo et al. 2004). It has also been reported that the pathogen was isolated from USA coastal waters in the Pacific Ocean, only when water temperatures were above 17 °C and salinities were below 13 ppt (Kelly and Stroh 1988). In another study, *V. parahaemolyticus* was detected in 94.2% of oysters collected from waters with temperatures well above 25 °C, but only in 14.9% of samples harvested from waters below 10 °C (Cook et al 2002). Similar results were reported from other researchers, who found that the mean density of *V. parahaemolyticus* was 13000 CFU/ 100 g of shellfish when water temperature at harvest was more than 20 °C (April through December), whereas, when water temperatures were lower than 20 °C (January through March), the mean density was significantly lower (Gooch et al. 2002). Even a small increase of temperature (approximately 5 °C) has resulted in an increase of infections of human populations with pathogenic Vibrios (Huq et al. 2005). Some of the larger *V. parahaemolyticus* outbreaks are believed to be the result of temperature anomalies during which warm waters transported hundreds or thousands of kilometres away from the outbreak region. Satellite data confirmed the presence of unusually warm seawater during the onset of outbreaks in Chile and Peru in 1997, in Spain in 1999 and in Alaska in 2004 (Martinez-Urtaza et al. 2010, Baker-Austin et al. 2010, Martinez-Urtaza et al. 2008). A retrospective study of the oceanographic conditions during the

appearance of *V. parahaemolyticus* illnesses in Peru revealed that the emergence of cases was concurrent with the arrival of two episodes of El Niño to the coastal waters of this country (Martinez-Urtaza et al. 2008).

Indirect and not obvious effects associated with the climate change, like the increase of water temperatures and the decrease in salinity due to rainfall events, may have implicated in the global expansion of *V. parahaemolyticus*. For example, the pathogen grows preferably in low salinity marine and brackish environments (< 30 ppt NaCl). Flooding, which is associated with sea-level changes, can increase significantly the estuarine and brackish environments and consequently cause the expansion of the geographical areas in which *V. parahaemolyticus* can flourish (Garcia et al. 2009, Martinez-Urtaza et al. 2008).

The effect of other factors (i.e. cargo ships, aquatic wildlife and zooplankton) in the global spread of the pandemic complex has been also investigated. For example, the dissemination of *V. parahaemolyticus* from endemic areas has been attributed by some researchers to the discharge of ballast water from cargo ships (Niimi 2004, Ruiz et al. 2000). The outbreaks in Texas in the USA and Coruña in Spain were associated with the discharge of ballast water (Martinez-Urtaza et al. 2008, Daniels et al. 2000). These areas were close to international ports. The pandemic strains were not detected in both areas prior to these episodes (Rodriguez-Castro et al. 2010, DePaola et al. 2000).

Vibrio parahaemolyticus and seafood outbreaks

Consumption of raw or undercooked seafood, particularly shellfish, contaminated with *V. parahaemolyticus* may lead to development of acute gastroenteritis. This pathogen is considered as the main cause of human gastroenteritis associated with seafood consumption in Asia and the United States and an important seafood-borne pathogen worldwide (Kaysner and DePaola 2001).

Chiou et al. (2000) reported that 542 out of 850 (63.76%) outbreaks in Taiwan were caused by *V. parahaemolyticus* in the years 1995 to 1999. Su et al. (2005) reported 2057 cases of *V. parahaemolyticus* infections in northern Taiwan in the years 1995 to 2001. The majority (99.4%) of *V. parahaemolyticus* strains could be identified by K serotyping, with 55.2% representing the K6 serovar. *V. parahaemolyticus* was the leading cause of food poisoning (1710 episodes with 24.373 cases) in Japan between 1996 and 1998 (Infectious Disease Surveillance Center 1999) and 31.1% of 5770 foodborne outbreaks occurred in China between 1991 and 2001 (Liu et al. 2004). In a recent study, Chao et al. (2009) examined 574 aquatic food samples including freshwater fish, shrimp, mud eel, marine fish, clam etc. from retail markets, hotels and restaurants in the Jiangsu providence in China, for the presence of *V. parahaemolyticus*. The results showed that the prevalence of the pathogen was 47.2% and the prevalence of *tdh*$^+$ and *trh*$^+$ strains was 8.5% and 1.5%, respectively. In the same study, 205 fecal samples from patients from 7 foodborne outbreaks in the same region were also examined for the presence of the pathogen. *V. parahaemolyticus* was isolated from 24.9% of the samples and interestingly 96.1% of the clinical strains were *tdh*$^+$.

In the US, recent data indicate that *V. parahaemolyticus* infections have increased since 2000, while the relative rates of infections from other major foodborne pathogens have decreased (CDC 2009). Between 1973 and 1998, approximately 40 outbreaks of *V. parahaemolyticus* infections were reported to the CDC (Daniels et al. 2000). Recently, 62 people in Alaska were reported to develop foodborne gastroenteritis after eating raw oysters in 2004 (McLaughlin et al. 2005). More recently, an outbreak of *V. parahaemolyticus* involving 177 cases was recorded in 2006 and was linked to the consumption of contaminated oysters (CDC 2006). Twenty three *V. parahaemolyticus* strains were isolated using molecular methods from oysters and mussels, collected from aquatic environments, fish markets and restaurants in São Paulo, Brazil, between February 1989 and January 1990(Rojas et al. 2011). In this study, the *tlh* gene was present in 100% of isolates, the *tdh* gene was identified in two (10.5%) isolates, whereas the gene *trh* was not detected.

In contrast to Asian countries and the USA, *V. parahaemolyticus* outbreaks are not often reported in Europe and is mainly associated with travel to endemic areas (Anonymous 2001). Moreover, *V. parahaemolyticus* is not a notifiable illness in Europe since it is not included in the European Network for Epidemiologic Surveillance and Control of Communicable Diseases or in the Microbiological Surveillance System for Infectious Gastroenteritis. Thus, laboratory test techniques for the organism is not widespread in several European countries. One of

the first outbreaks in Europe was recorded in the UK in 1973 and involved the consumption of contaminated crabs (Hooper et al. 1974). Between 2004 and 2005, 57 cases of *V. parahaemolyticus* infection were reported in the UK (Wagley et al. 2008). In a recent study (Wagley et al. 2008), 161 samples comprised of oysters, mussels and crabs were tested in the UK for the presence of *V. parahaemolyticus* and 30% of the samples were found positive for the pathogen, while the *tdh* gene was identified in 12% of the samples. The *trh* gene was not detected in any of the samples.

Eight cases of foodborne gastroenteritis caused by the pathogen and associated with contaminated fish or shellfish ingestion were reported in Spain in 1989 (Molero et al. 1989). The first large *V. parahaemolyticus* outbreak with 64 cases occurred in 1999 in Spain and it was associated with the consumption of raw oysters (Lozano-León et al. 2003). A serious outbreak affecting 44 people associated with consumption of shrimps occurred in France in 1997 (Robert-Pillot et al. 2004). Another serious outbreak with 100 patients caused by imported mussels was also recorded in France (Hervio-Heath et al. 2005). Likewise, a large outbreak implicating 80 cases of *V. parahaemolyticus* infection among guests attending weddings in a restaurant was recorded in Spain in 2004 (Martinez-Urtaza et al. 2005). More recently, 2 new cases of acute gastroenteritis by *V. parahaemolyticus* occurred in Central Italy in 2008 (Ottaviani et al. 2010). Both patients consumed local mussels purchased from different hawkers and cooked before consumption. However, mussels are often consumed without sufficient cooking (Ottaviani et al. 2010).

Numerous studies have isolated pathogenic *V. parahaemolyticus* (*tdh*$^+$ and/or *trh*$^+$) strains in seafood and water across numerous European countries in the last years (Martinez – Urtaza et al. 2010, Ripabelli et al. 1999). Collin and Rehnstam-Holm (2011) during a 4 month study, analyzed mussels from the Baltic Sea for the presence of *V. parahaemolyticus*. The results showed that 79% of samples were positive for *V. parahaemolyticus* (of which 47% were *tdh*$^+$ and/or *trh*$^+$).

Seawater conditions (temperature, salinity, high nutrient concentration) in Mediterranean Sea are ideal for the growth of *Vibrios* (Alam et al. 2009, Martinez-Urtaza et al. 2008). Samples of mussels collected from coastal areas located on the Adriatic Sea (Central Italy), were examined for the presence of *V. parahaemolyticus* and pathogenic strains (Ottaviani et al. 2005). In this study, 35 *V. parahaemolyticus* strains were isolated out of 144 samples, while the *tdh* and *trh* genes were recorded in one and three isolates, respectively. Di Pinto et al. (2008) examined 144 mussels from Apulia (Italy), and *V. parahaemolyticus* were isolated in 47 shellfish samples (32.6%), the *tdh* was detected in 7 samples only, whereas *trh* was not highlighted at all. In a recent study in Turkey (Terzi et al. 2009), 120 fresh and processed fish and mussel samples collected from Middle Black Sea and analyzed for the presence of *V. parahaemolyticus*. Thirty two isolates were identified as *V. parahaemolyticus* by bacteriological methods and confirmed by molecular techniques. Of them, 13 isolates were found positive for *tdh* gene, 6 isolates for *trh* gene and 13 isolates for both genes by multiplex PCR.

Davies et al. (2001) reported the incidence of the pathogen in various fish from Greece (anchovy, bogue, mackerel, mussels, picarel, mullet), in a relatively high incidence (14%). In another study in Greece (Papadopoulou et al. 2007), 360 samples, including 105 marine fish, 25 prawns, 50 squid, 50 octopus, 30 mussels and 100 freshwater fish were examined for the presence of potentially pathogenic microorganisms, while two isolates of *V. parahaemolyticus* were found in the marine fish bogue. Yagnisis et al. (2007) reported the isolation of seven hundred twenty nine *Vibrio* bacteria, potentially human pathogenic (696 from diseased farmed and wild fish and 33 from seawater). The most frequent *Vibrio* species found was *V. alginolyticus* (61.4%), followed by *V. parahaemolyticus* (18.6%). *V. parahaemolyticus* was isolated from sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*) and snarpsnout sea bream (*Puntazzo puntazzo*).

Conclusions

V. parahaemolyticus is the leading cause of seafood-associated bacterial gastroenteritis in the United States and causes approximately half of the foodborne outbreaks in some Asian countries. Probably a reconsideration of E.U. Food Legislation for microbiological criteria is needed concerning *V. parahaemolyticus*. In recent years, *V. parahaemolyticus* infections have also increased in Europe. Thus, the emergence of the pathogen in Europe is a public health concern and emphasizes the importance of microbiologic surveillance and control programs of *V. parahaemolyticus* in seafood and particularly, bivalves.

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