A REVIEW

Vibrio parahaemolyticus and Vibrio vulnificus in South America: Water, Seafood, and Human Infections

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Abstract

The bacterial species, *Vibrio parahaemolyticus* and *V. vulnificus*, are ubiquitous in estuaries and coastal waters throughout the world, but they also happen to be important human pathogens. They are concentrated by filter feeding shellfish which are often consumed raw or undercooked, providing an important potential route of entry for an infective dose of these bacteria. *V. parahaemolyticus* can cause abdominal cramping, nausea, diarrhea, vomiting, chills, and fever. *V. vulnificus* can cause similar gastrointestinal-related symptoms, but can also spread to the bloodstream, resulting in primary septicemia, and it can also cause disease via wound infections. The objective of this article is to summarize, for the first time, the incidence and importance of *V. parahaemolyticus* and *V. vulnificus* in South America (SA), in environmental waters and seafood, especially molluscan shellfish, as well as human infection cases and outbreaks. It appears that infections from *V. parahaemolyticus* have been more strongly related to shellfish ingestion and have been more frequently reported on the Pacific coast of SA. Conversely, *V. vulnificus* has been more frequently acquired by water contact with open wounds and its presence has been more heavily reported along the Atlantic coast of SA, and while documented to cause serious mortality, have been relatively few in number. The impacts of El Nino Southern Oscillation (ENSO) have been observed to cause an increase of *V. parahaemolyticus* outbreaks on the Pacific coast of SA. The implementation of a regulated monitoring approach, along with the use of faster, more accurate, and virulence-specific detection approaches, such as PCR confirmation, should be considered to detect the presence of pathogenic *Vibrio* strains in environmental and seafood samples for protection of public health. Furthermore, improved clinical surveillance with suspected cases should be implemented. This review highlights the need for more research and monitoring of vibrios in SA, in water, shellfish, and clinical samples.

Introduction

The proportion of food-borne disease derived from consumption of raw and undercooked seafood worldwide is considerable, reaching about 80,000 illnesses, 500 hospitalizations and 100 deaths each year in the United States (Altekruse et al. 2000; Iwamoto et al. 2010; Westrell et al. 2010; Schaeffer et al. 2013). While bacterial, viral, algal, and parasitic pathogens, particularly those stemming from fecal contamination, can contribute to seafood-borne illness, the pathogenic bacteria in the genus *Vibrio* have been garnering some recent headlines, as outbreaks and infections caused by these marine bacteria are increasing in number (Baker-Austin et al. 2012; Martinez-Urtaza et al. 2013), especially for raw and undercooked seafood.

In the United States, foodborne *Vibrio* infections are on the rise, even when numbers of infections from other foodborne bacterial pathogens are decreasing (CDC 2013a). The CDC produces an annual food safety report, which contains updates about foodborne infections including those caused by *Vibrio.* The most recent report presents some dour statistics. Most strikingly, the frequency rate of foodborne *Vibrio* infection, recorded during the 2013-2014 period has increased 173% as compared to the previous decade (CDC 2013a). Alarmingly, this trend is not restricted to the US. The geographic areas for which *Vibrio* diseases are being reported is expanding, even to locations unaccustomed to these infections, a phenomenon most likely brought about by warming ocean temperatures (Martinez-Urtaza et al. 2010; Baker-Austin et al. 2012; Levy 2015).

Vibrio spp. are ubiquitous along estuaries and coastal waters throughout the world (Urakawa and Rivera 2006). While the majority of these bacteria are harmless, several species can potentially infect humans or other animals and cause serious disease (Hotel 2007). Of those, *V. cholerae*, *V. vulnificus* and *V. parahaemolyticus* are the most important pathogens. The pathogens *V. vulnificus* and *V. parahaemolyticus* can cause waterborne diseases, but are particularly dangerous when combined with a filter-feeding vector, such as molluscan shellfish. Filter feeding mollusks pump the surrounding water over their gills, simultaneously obtaining oxygen and food. *Vibrio* spp. are often found attached to particles and as these particulates are passed over the sieve-like gills of filter feeding mollusks, they are strained out of the water and retained (Ward and Shumway 2004; Froelich et al. 2013). This filtration ultimately can concentrate the number of *V. vulnificus* and *V. parahaemolyticus* in shellfish up to 100-fold of that found in the overlaying water (DePaola et al. 2003). Because some seafood, especially oysters, is commonly eaten raw or only slightly cooked, this provides a route of entry for a significant dose of live, potentially pathogenic *Vibrio* bacteria. *Vibrio* pathogens can also be associated with other seafood, such as shrimp, fish, clams, mussels, or octopus (Oliver et al. 1983; Normanno et al. 2006; Yamamoto et al. 2008; García et al. 2009; Rodgers et al. 2014; Rodríguez-Camacho et al. 2014). In the US, even though *Vibrio* diseases are reportable to the CDC, there are a large

number of unreported cases, and approximately 84,000 Americans are estimated to contract a food-borne *Vibrio* infection every year (CDC 2013b).

V. parahaemolyticus infections cause symptoms that are typical of enteric viruses such as norovirus, and bacterial pathogens such as *Salmonella* spp. Symptoms of infection with *V. parahaemolyticus* can include abdominal cramping, nausea, diarrhea, vomiting, chills, and fever (Yeung and Boor 2004). Some *V. parahaemolyticus* strains are sufficiently virulent to cause outbreaks, in which large numbers of people can be affected (Martinez-Urtaza et al. 2005; Martinez-Urtaza et al. 2013). *V. vulnificus* can cause similar symptoms, but can also be far more morbid, with infections that can spread to the bloodstream, resulting in primary septicemia (Ratner 1987; Oliver 2006;). After a short incubation, in as little as 24 hours, the patient can experience dangerously low blood pressure, blistering skin lesions along the extremities, organ failure, and death (Jones and Oliver 2009). Interestingly, *V. vulnificus* can also cause serious infections through entry of the pathogen into an open wound. These types of infections predominate in subpopulations with compromised immune systems (such as people with liver disease, or diabetes), and can rapidly proceed to amputation of limbs or death (Horseman and Surani 2011). *V. vulnificus,* which has a fatality rate approaching 50%, is the most fatal foodborne pathogen in the US. Thus, *V. parahaemolyticus* results in the highest number of cases, while *V. vulnificus* cases are highly morbid and cause the most deaths.

While the USA has some of the best *Vibrio* disease epidemiology data available, reporting requirements only began in 2007. Other countries, with fewer historical infections, have large numbers of shellfish harvested and consumed on an annual basis but a relatively low level of data is available. In SA, for example, shellfish and finfish aquaculture are large and economically robust industries, corresponding to 7.5% of the world's production (FAO 2013). Yet, despite the occurrence of both *V. vulnificus* and *V. parahaemolyticus* along the Pacific and Atlantic coasts of SA, these bacteria are not part of any formal or official monitoring program for shellfish production in any of the SA countries. Only *V. parahaemolyticus* is regulated for seafood in Brazil and Peru, but not at shellfish production areas, as these regulations are focused on ready to eat products. There may be limited transmission of Vibrio-caused disease from non-molluscan

aquaculture and seafood products in South America, but the presentation of information here is focused on the well-known vectors of Vibrio-caused disease, molluscan shellfish. The objective of this article is to review and summarize the importance of *V. parahaemolyticus* and *V. vulnificus* in SA, across both environmental waters and seafood, as well as to document in a single publication, the reported cases and outbreaks caused by pathogenic *V. parahaemolyticus* and *V. vulnificus* pathogens. Through this summary, we present information indicating the importance of developing coordinated monitoring strategies for these pathogens into the future.

Shellfish Aquaculture in South America

SA is the fourth largest continent in the world with twelve countries and three major territories: the Falkland Islands, the Galapagos Islands, and the French Guiana. SA is surrounded by the Pacific and Atlantic Oceans, and by the Caribbean Sea. Bolivia and Paraguay are the only landlocked countries.

Aquaculture in SA produces a significant amount of food and contributes substantially to local economies. The continent contributes 7.6% (12,307,208 tons) of the world fishery production, with 80% of the total supplied by three countries: Peru, Chile and Brazil (FAO 2013). Bivalve mollusk production has always been remarkable in Chile and Peru, and in other countries, like Brazil, production has been on the rise (FAO 2013). Shellfish production is important for jobs and economic growth throughout the year and also serves as an alternative source of income for fishermen during closed fishing seasons. And even if *V. vulnificus* and *V. parahaemolyticus* are not known to be pathogenic to shellfish. The possible impact of *V. parahaemolyticus* on shrimp populations via early mortality syndrome and their pathogenicity to finfish has now been noted (De Schryver, Defoirdt, Scorgeloos, 2014).

***Vibrio parahaemolyticus* occurrence in South America**

In 1971, Argentina reported the occurrence of *V. parahaemolyticus* in mussels (Casellas et al. 1977), which was the first report in SA and until now it is the only report of the bacterium in that country. In 1975, Brazil reported the first human case of *V. parahaemolyticus* (Hofer 1983) in SA. Since then, many reports of *V. parahaemolyticus* in environmental samples, seafood, and

marine animals have been published, and isolated cases and outbreaks of *V. parahaemolyticus* have been observed in some SA countries.

The spread of *V. parahaemolyticus* in environmental samples (Figure 1) and the occurrence of human cases and years reported along the SA have been documented for the recent decades (Figure 2). It is important to observe that the spread of the disease coincides with ENSO years and route, reaching the Peruvian coast in 1993 and again in 1997, when the cases spread to Chilean coast (Figures 1 and 2).

Records of *V. parahaemolyticus* in SA marine water and animal samples are mainly found in Brazil, with only a few reports in Chile, Peru, Colombia, and Venezuela. Table 1 lists published reports, from 1971 until 2015, of *V. parahaemolyticus* findings in environmental and seafood samples from SA. Despite the existing reports of the bacterial presence in environmental and seafood samples, only Brazil and Peru have instituted national legislation establishing a maximum allowed number of *V. parahaemolyticus* in seafood. Peru requires non-detectable *V. parahaemolyticus* in 25 g for all seafood (Peru 2008), while Brazilian legislation established a maximum limit of 10^3 CFU g^{-1} in ready to eat seafood, which includes raw oysters (Brasil 2001), while the limit in USA is equal to or greater than 1×10^4 CFU g^{-1} (Kanagawa positive or negative) for ready to eat fishery products with minimal cooking by consumer (FDA 2011). Interestingly, even though numerous *V. parahaemolyticus* outbreaks have been reported, Chile does not establish maximum limits for *V. parahaemolyticus* in seafood, there is only a requirement for cold transportation of molluscan shellfish (Chile 1996).

Some strains of *V. parahaemolyticus* are especially virulent and instead of causing single sporadic cases, they are responsible for outbreaks (Martinez-Urtaza et al. 2004; Martinez-Urtaza et al. 2013), which happens when the occurrence of cases of a disease is above of what was expected in a defined community, geographical area or season (WHO, 2015). These strains are identified by serotyping of the capsular (K) and lipopolysaccharide (O) antigens (Parveen and Tamplin 2013). Two serotypes are of particular importance, O4:K12 and O3:K6 and have been implicated in recent outbreaks and have worldwide presence.

The first report of *V. parahaemolyticus* infection in SA occurred in 1975 in Brazil, and was reported as isolated watery diarrhea in a six-year-old child from Ceara, Brazil (Hofer 1983). The strain was serotyped as O5:K17, Kanagawa-positive. There is no epidemiological data available, except that the local population was known to eat salt-cured marine and freshwater fish (Hofer 1983; Santos and Vieira 2013). Even though there are other *V. parahaemolyticus* serotypes as the above mentioned O5:K17 in SA, O3:K6 and O4:K12 serotypes are worth mentioning as they are related to outbreaks in SA and also in other continents as cited before (Bhuiyan et al. 2002; Chao et al. 2011; Powell et al. 2013; Martinez-Urtaza et al. 2013).

Serotype O4:K12 and O4:KUT are of concern today in Europe and USA (Martinez-Urtaza et al. 2013; Haendiges et al. 2015). They have been shown to be more virulent than other pathogenic *V. parahaemolyticus* strains and they have caused large outbreaks in the USA in 1997, 2004 and 2013 (Martinez-Urtaza et al. 2013; Newton et al., 2014). In SA, except for a report in Brazil from an outbreak in 1989 (Magalhaes et al. 1991) and one report from Kanagawa negative environmental sample (Pereira et al. 2004), all findings from this serotype occurred on the Pacific Coast. Serotype O4:K12 has been recovered from environmental and seafood samples in SA since 1984, when it was found in ceviche samples in Peru (Guevara-Duncan et al. 1989). Since then it has been mainly found in outbreaks in Peru and Chile in 1997 and 2004, respectively at the same time when this serotype caused USA outbreaks (González-Escalona et al. 2005; Gil et al. 2007). The outbreak that occurred in Chile in 2004 affected approximately 1,500 people, mainly in Puerto Montt, a region characterized by cold coastal water and one of the main shellfish-producing areas in Chile (González-Escalona et al. 2005). It is important to mention that the Pacific coast was under the influence of ENSO phenomena in both the 1997-1998 and 2004-2005 years, with warmer water all along the coastline, favoring the growth of the bacteria.

Strain O3:K6 has been implicated in outbreaks worldwide until 1996 and has been typically found associated with other serotypes such as O1:K38, O3:K29, O4:K8, O2:K3, and O4:K8 (Okuda et al. 1997; Wong et al. 2000). This situation changed in 1996, after an atypical increase in *V. parahaemolyticus* O3:K6 infections in India (Velazquez-Roman et al. 2013) when the

serotype was detected alone causing disease. In the same year, this clone rapidly spread throughout Southeast Asian countries (Okuda et al. 1997; Chowdhury et al. 2000), and also to South America, particularly in Peru (Martinez-Urtaza et al. 2008). It was then reported in the following years in outbreaks and isolated cases in the Atlantic and Gulf coasts of the U.S. (Okuda et al. 1997; Matsumoto et al. 2000; Chowdhury et al. 2000). Recently, similar reports in Europe (Martinez-Urtaza et al. 2013), Africa (Ansaruzzaman et al. 2005), and North, Central and South America (Daniels and MacKinnon 2000; González-Escalona et al. 2005; Velazquez-Roman et al. 2013). Unfortunately, only a handful of reports from SA have included *V. parahaemolyticus* serotyping, which does not allow us to trace the frequency of outbreak strains along the SA coast temporally. Considering the serotyped samples, it is noted that serotype O3:K6 is still a public health concern in SA and it has been observed more frequently in outbreaks and environmental samples than O4:K12 serotype.

The first report of *V. parahaemolyticus* pandemic strain O3:K6 in SA was one isolated case in Trujillo, Peru in 1996 in a 6-month-old baby, and occurred simultaneously to the outbreak in Calcutta in February 1996. However, the first Peruvian outbreak related to the O3:K6 strain occurred in Lima, in 1998, which coincided with a strong ENSO occurrence and the strain was found to be similar to that found in Calcutta outbreak in 1996 (Gil et al. 2007). In Chile, the first report occurred in Antofagasta, in 1997 and the serotype has been present on the Chilean coast since then (González-Escalona et al. 2005; García et al. 2009; Dabanch P. et al. 2009). In 2005, there were nearly 11,000 cases reported by the Ministry of Health of Chile, with more than 95% of the cases caused by serotype O3:K6 (García et al. 2009). This strain was also found to cause gastroenteritis cases in Brazil in Pernambuco, Alagoas, and Ceara in 2002 (Leal et al. 2008). These states are within the tropical region, in northeast Brazil, characterized by warm waters and high tourism activity. Unfortunately, there is information about the number of cases only in Ceara outbreak, with 26 reported cases between guests of two hotels, and in which *V. parahaemolyticus* O3:K6 was found in 45% of the cases. Although the bacterium was not isolated from any food sample (FUNASA 2002), which could have been due to imprecise detection methodology or to inadequate food sample maintenance. This outbreak in Ceara

highlights the importance of an adequate surveillance system, not only to generate more accurate data about foodborne disease outbreaks, but also to ensure the correct treatment is administered. There were also outbreaks in 2003 and 2004 in Brazil, with no information available about location or serotype (Brasil 2014).

Notably, most of the recent reports do not discuss serotype information. Beyond the absence of information on *V. parahaemolyticus* serotypes in the reports referenced in Table 1, there is also a lack of information on salinity and seawater temperature. These two environmental parameters are critical to understanding the ecology of the species. *V. parahaemolyticus* growth is favored by warmer temperatures, between 5 and 43°C, with optimum temperature 37°C, and by moderate salinity, in a range of 0.5 to 10‰, with optimum growth between salinity of 1.5-3.0‰ (WHO and FAO 2011). These environmental parameters are often observed on SA coasts, especially in estuarine areas or after tropical rainfalls. Moreover, the ENSO oscillation that occurs along the Pacific coast, brings warm seawater to this area that can favor the presence of the bacteria on the SA west coast and increase the occurrence of cases and outbreaks.

A review of the data shows a broad variation in salinity and temperature in SA. Seawater temperature on the Atlantic coast varied between 14.4°C (Costa Sobrinho et al. 2010) to 35°C (Markman 2008), which is within the range of the survival and growth of the bacterium. Salinity ranged between 3‰ (Sousa et al. 2004) to 41.6‰ (Lira et al. 2001). The remarkably higher salinity data found (41.6‰) was logged in an estuarine area on the Brazilian northeast coast, with a water temperature of 30°C. The shellfish samples contained around 10^3 CFU g^{-1} of sucrose negative colonies on TCBS agar, which indicates the presence of *V. parahaemolyticus*. Approximately 60% of those isolates were confirmed to be *V. parahaemolyticus* by biochemical tests (Lira et al. 2001). It is important to note that even under extreme conditions, such as high salinities, researchers were able to collect the bacterium, showing that it can be a potential health hazard to molluscan shellfish consumers.

Since there is not any official monitoring program for *V. parahaemolyticus* in shellfish production area or seawater in SA countries, the data presented in this review are not uniform in respect to the methodology used, temporal or spatial coverage, and do not provide enough

information to establish relationships between bacterial abundance and environmental parameters. These factors are needed to perform predictive analyses.

Among the studies found in this review, two of them, from the same author, deserve to be mentioned for robust monitoring and the inclusion of environmental parameters, serotyping and PCR confirmation for thermostable direct hemolysin (*tdh*) and *tdh*-related hemolysin, *trh.* genes. The presence of one or both of these genes is typically associated with host cell cytotoxicity (Nishibuchi and Kaper 1995; Broberg et al. 2011). Ramos (2007; 2012), studied the prevalence of vibrios in oysters and seawater in Santa Catarina, the main oyster production area in Brazil. *V. parahaemolyticus* was found as the most prevalent *Vibrio* specie in both studies. Samples were examined for potential virulence by assaying for the presence of *tdh* and *trh* genes. There were positive strains for *tdh* and *trh* genes, but no positive strain was found on Wagatsuma agar. The hemolysis caused by some *V. parahaemolyticus* strains on this medium is called "Kanagawa phenomenon" and it is positively related to human pathogenicity. It was found that *tdh* is responsible for the Kanagawa phenomenon (Honda et al. 1980), although there are cases of Kanagawa negative and *tdh* positive strains (Vieira et al. 2011; Ramos 2012). Ramos (2012) found no correlation between environmental parameters and the presence of *V. parahaemolyticus* when the sampling occurred in a temperature range between ca. 21°C and 28°C, during summer and spring seasons. In another study, Ramos (2007) collected samples all year and there was a positive correlation with the incidence of *Vibrio* and seawater temperature, ranging from 18°C to 29°C. Ramos (2012) identified the presence of pandemic *V. parahaemolyticus* serotype O3:K6 among 83 *V. parahaemolyticus* strains collected and found ca. 37% as *trh* positive and 5% as *tdh* positive. The *trh* and *tdh* genes were found occurring simultaneously in 4.3% of oyster samples and in 5% of seawater samples. Although, all the strains found were Kanagawa negative.

Regrettably, none of the most recent reports published after 2009 serotyped *V. parahaemolyticus* in environmental sampling or in clinical cases. This information is crucial to trace pathogenic serotypes and to establish a control plan to reduce the number of cases and outbreaks. Table 2 shows outbreaks and isolated cases generated by *V. parahaemolyticus* in

SA countries in chronological order. The last report of disease caused by serotype O3:K6 in SA was in Chile (García et al. 2009) in 2009, the same year the serotype was found in environmental samples from Brazil (Ramos 2012). Although, the lack of serotyping information from the strains found after 2009, does not allow us to identify the presence this *V. parahaemolyticus* serotype in recent years in SA.

Although primary septicemia caused by *V. parahaemolyticus* is rare, it can occur in individuals with underlying chronic illness (Parveen and Tamplin 2013). In SA, Dabanch *et al.* (2009) reported the first case of septicemia due to *V. parahaemolyticus* in Puerto Montt, in Chile, which occurred in 2008.

V. parahaemolyticus human cases are more frequent on the Pacific coast of SA, and the majority of the reports coincide with ENSO occurrences. Furthermore, the spread of *V. parahaemolyticus* infections from Peru to Chile follows the same course observed during ENSO currents, with warm waters towards south, which is the opposite of normal Peru Current route, as seen in Figures 1 and 2. From 1993 until 1997, *V. parahaemolyticus* cases were concentrated on the Peruvian coast (Gil et al. 2007; Martinez-Urtaza et al. 2008). After 1997, the bacterium spread to Chile, where it is still causing outbreaks (Córdova et al. 2002; González-Escalona et al. 2005; García et al. 2009; Dabanch et al. 2009; Chile. 2014; Chile. 2015). These facts highlight the influence of ENSO on the spread and escalation of the disease along SA Pacific coast. Despite all of the cases and outbreaks in Peru and Chile, there are only a few publications about the presence of *V. parahaemolyticus* in environmental samples along the Pacific coast. Thus, the area in which the most cases have occurred is the region in which there is the least amount of available environmental data.

All of the cases reported above resulted from isolated research efforts, and most likely are a vast underrepresentation of the real number of infections. These data are an indication of the factors that can affect the patterns of disease in SA countries.

Strikingly, the disease caused by *V. parahaemolyticus* is not listed as a notifiable disease in any country in SA, although in contrast, all diarrheal and foodborne diseases are, and *V. parahaemolyticus* infections are included in this latter category. However, the epidemiological surveillance system on the continent is inaccurate, with a high percentage of foodborne diseases outbreaks classified as being from unspecific causes. Thus, cases of diarrhea caused by *V. parahaemolyticus* on the continent could be underestimated and underreported. This leads to weak food inspection programs regarding these bacteria as there are not enough data to justify costly control and warning systems.

Vibrio vulnificus occurrence in South America

The presence of *V. vulnificus* in environmental and seafood samples in SA is less documented than *V. parahaemolyticus*. It is mostly reported in studies focused on *V. parahaemolyticus*, in which they also detected *V. vulnificus,* all from the Atlantic and Caribbean coasts, as seen in Figure 3, where reports from environmental and human cases are shown.

V. vulnificus has been recorded in SA since 1982 when it was found in estuarine water samples in Rio de Janeiro State in Brazil, but there was no information on temperature or salinity (Rodrigues and Hofer 1986). However, as with *V. parahaemolyticus, V. vulnificus* samples taken from different sites and seasons, and analyzed through differing methodologies, do not allow analysis of its prevalence temporally in SA. Table 3 presents *V. vulnificus* records of environmental and seafood samples from SA countries. Seawater temperature and salinity ranges are listed when present in the article cited. The methodology used by different authors is rarely the same, and was potentially inadequate to detect the bacteria, especially since most of the studies were based only on microbiological and biochemical identification, without molecular confirmation.

Higher prevalence of *V. vulnificus* can be found in seawater with a salinity range between 5 and 20‰ (Parveen and Tamplin 2013), the consensus is that *V. vulnificus* has a maximum environmental salinity tolerance of about 25‰ (Kaspar and Tamplin 1993; Motes et al. 1998; Arias et al. 1999; Macián et al. 2000; Wetz et al. 2008; Froelich et al. 2012). While *V. vulnificus*

can survive at higher salinities, it becomes difficult to isolate and is considered to be rare (Froelich et al. 2012; Staley et al. 2013; Froelich et al. 2015). According to Froelich and Noble (2014), keeping oysters bathed in high salinity (>30‰) water is able to reduce the level of *V. vulnificus* in oyster meat. The relationship between vibrios and salinity has been identified by some studies (Johnson et al. 2010; Reyes-Velázquez et al. 2010; Igbinosa et al. 2011), while others did not (Singleton et al. 1982; Nigro et al. 2011; Costa Sobrinho et al. 2014), demonstrating that the relationship with salinity may be variable and complex (Johnson et al. 2012). Although, as seen in Table 3, salinity in SA is found to be, in general, higher than 25‰, which could explain the lower prevalence of *V. vulnificus* compared to the USA. In Brazil, Ramos (2007) found *V. vulnificus* in oyster and seawater samples with salinities ranging between 33 to 34‰. Silva (2003) and Ramos (2012) also found *V. vulnificus* in environmental samples in Brazil where salinity was as high as 36‰, which is above the normal upper limit of salinity expected to permit the presence of *V. vulnificus*, although none of the authors tested the presence of potentially pathogenic strains and there are no cases of infections reported for this area (Ramos et al. 2012; Froelich and Noble 2014).

Because of the difficulty in differentiating *V. vulnificus* from other *Vibrio* sp. on culture media, polymerase chain reaction (PCR) is typically employed to confirm presumptive colonies. The gene *vvhA* can be used to confirm *V. vulnificus* presence, but it does not differentiate potentially pathogenic and nonpathogenic strains. There are other genes, including the virulence correlated gene (*vcg*) (Warner and Oliver 2008) that provide some indication of potential pathogenicity. Only two studies used PCR to confirm the presence of *V. vulnificus* in SA environmental samples. Oliva (2012) used the gene *vvhA* to confirm *V. vulnificus* in mussels and Raszl *et al.* (2016) used the genes *vcg-E* and *vcg-C* and confirmed the presence of a pathogenic strain in an oyster sample from Brazil. All other studies were based on culture media alone, mainly Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS), and/or biochemical tests. Ramos (2012) analyzed oysters and seawater samples and collected data on salinity, temperature and rainfall, though there was no PCR confirmation for *V. vulnificus*. Ramos (2012) found a positive correlation between *V. vulnificus* presence in seawater samples and seawater temperature, and

also with weekly precipitation levels from the week antecedent to sampling, which agrees with studies from Høi et al. (1998), Strom and Paranjpye (2000), Lhafi and Khune, (2007) and Blackwell and Oliver (2008). But there was no correlation found between *V. vulnificus* and salinity in seawater samples as has been found previously in Brazil (Ramos 2007; Ristori et al. 2007) and in other areas of the world (Høi et al. 1998; Parveen et al. 2008; Blackwell and Oliver 2008).

In some studies, *V. vulnificus* was found to be highly prevalent, as in the study performed by Matté *et al.* (1994) who found *V. vulnificus* in 17% of mussels tested, and Garcia-Moreno and Landgraf (1997) found 55% of samples from mussels, oysters and shrimp tested. Moreover, Nascimento *et al.* (2001) found *V. vulnificus* in 35% of shrimp samples. Costa *et al* (2013) found 11.4% *V. vulnificus* presumptive in samples from frozen oysters, which were confirmed through biochemical tests. However, in other studies, the prevalence is low as 4% (Pereira et al. 2007; Ramos 2007). It is difficult compare quantitative data from the scientific articles reporting *V. vulnificus* in environmental samples because of variable methodologies.

Given the reports of *V. vulnificus* from environmental water and seafood samples, there are relatively few clinical cases reported in SA. Some countries that have had confirmed *V. vulnificus* cases, such as Uruguay, Ecuador and Peru, did not previously have any report from environmental samples or in seafood. Therefore, any correlative analysis between environmental and clinical samples is impossible. Infections cases of *V. vulnificus* in SA are reported only in Brazil, Uruguay, Ecuador and Peru. The first case was diagnosed in 1997 in Uruguay. All the reported cases in SA showed in Figure 3 are detailed in Table 4.

Severe sepsis has been described as the most common presentation of *V. vulnificus* infection. It is generally characterized by bacteremia without an evident focus of infection. Symptoms typically occur within 7 days, and they start with an abrupt onset of fever and chills, followed by metastatic infection characterized by cutaneous lesions, usually on the lower extremities or the trunk. Ibarra *et al* (1999) reported on *V. vulnificus* in three cases of acute diarrhea in Peru in 1998. One of the patients discussed had a co-infection with *V. cholera* O1, but there was no follow up information available and this it is not possible to know if the symptoms

evolved to sepsis. Diarrhea is not a common symptom caused by *V. vulnificus* but there are some reported cases of patients presenting gastroenteritis, characterized by vomiting, diarrhea, and abdominal pain, with a stool culture yielding *V. vulnificus*, negative blood cultures, and no skin lesions or septicemia caused by *V. vulnificus* (Klontz et al. 1988; Horseman and Surani 2011). Gastrointestinal symptoms often precede fever, chills, and cutaneous manifestations. Cutaneous lesions may progress to necrotic ulcers, necrotizing fasciitis, necrotizing vasculitis, or myonecrosis. Septic shock can occur in more than 50% of the cases. Hypotension during the first 12 h or leukopenia is often associated with a very poor prognosis. It has also been reported mental status changes characterized by lethargy or disorientation in half the patients (Horseman and Surani 2011).

The single case reported in Chile was recorded as being originated in El Salvador, a Central America country. A 53-year-old man, with diabetes and chronic liver disease, returned from a trip to El Salvador and had eaten raw oysters. Notwithstanding the rapid diagnosis, the man died after 68 hours of hospitalization (Poblete et al. 2002). Despite all the reports about *V. parahaemolyticus* in Chile, there is no information about the presence of *V. vulnificus* in environmental samples as well as in human cases, in this country, nor in El Salvador, from where the infection was supposed to be acquired.

In 2013, Ecuador had reported two cases of *V. vulnificus* that resulted in sepsis during summer season 2012/ 2013. Both patients were men with previously documented health disorders (diabetes and aplastic anemia, respectively). One of the patients presented a gastrointestinal disease prior to sepsis. Both cases resulted in death and there was no history of molluscan shellfish consumption, but it is intriguing that the cases occurred away from the coast (in Quito and in Ibarra). The authors confirmed the strains through the *rrs* gene (Villacrés et al. 2013).

In Brazil, regardless of the number of reports indicating the presence of *V. vulnificus* in seawater, in shellfish and in marine mammals (Garcia-Moreno and Landgraf 1997; Ramos 2007; Pereira et al. 2007; Ramos 2012; Costa et al. 2013); there are only three infection cases reported, and two of them occurred in 2004 in areas that are close to one another. The first case

reported in Brazil was related to an injury caused by a wood fragment, in a 53-year-old man, in Rio de Janeiro state. The case evolved to septicemia and the death occurred five days later (Brack et al. 2004). The second case was acquired by seafood ingestion (mussels and octopus) in the coast of Sao Paulo state, in an 86-year-old man, with a history of pancreatic, and hepatic and renal chronic disorders. The symptoms started with gastroenteritis and evolved to sepsis. The patient died after 12 days, due to fungemia caused by *Candida albicans* (de Araujo et al. 2007). The third case in Brazil occurred in Parana State, in 2013. The patient was a 39-year-old man, who was admitted to the hospital for elective liver transplantation due to an ulcerative colitis and sclerosing cholangitis. The man had been in the coast one day before being admitted in the hospital. Before the surgery, the symptoms of primary septicemia began and despite the diagnosis and the treatment applied, he died 32 hours after the onset of the symptoms (França et al. 2013).

Uruguay is the country in SA with the higher number of *V. vulnificus* cases, which occurred all the same area and were related to seawater contact. This country reported the first case of *V. vulnificus* infection in SA, in 1997, in the estuarine area of Rio de la Plata (Perreng and Luis 2001). The patient, a 60-year-old, and diabetic man, reported to be fishing at Rio de la Plata estuary the day before, letting both legs for hours into the water. The infection was evolved to sepsis and caused the death of the patient even with bilateral leg amputation. Another case occurred in 2000, in Maldonado Department, also in the estuarine area of Rio de la Plata, from a 57-year-old woman; with diabetes, using weekly immunosuppressive medication, and who had surgical wound not completely scarred; and had been in seawater 24 to 36 h before the first symptoms. She presented wound infections and also gastroenteritis, but she survived after 12 days of intensive treatment in hospital (Chicheff et al. 2001). Other cases occurred in Uruguay in 2005, 2007 and 2014/2015 summer season, all from the same area, having the cases from 2005 and 2007 evolved to sepsis and death, while in 2014/2015 summer season, two out of four patients died as consequence of the infection (Uruguay 2015). The information from 2005 and 2007 is not published, they were obtained from personal communication with Dr. T. Camou, from the Ministry of Public Health from Uruguay.

There were two outbreaks of *V. vulnificus* in SA, three cases occurring in Peru, without follow up of the patients (Ibarra et al. 1999) and another outbreak with four cases in Uruguay, as reported by the Ministry of Health in the 2014/2015 summer season in Punta Del Este, resulting in two deaths (Uruguay 2015). All cases from Uruguay occurred in the same area, an estuary that can present salinity rates around 20‰ and seawater temperature that can be as high as 25°C in summer seasons, as reported by Piola et al. (2003). There is no information about the presence of *V. vulnificus* in environmental samples from Uruguay, specifically from Rio de la Plata estuarine water where the infection cases occurred. Summer seawater temperature and salinity in Rio de la Plata are permissive for *V. vulnificus*, yet more studies are needed to determine if one or both parameters are related to these infections or if there are other factors that could influence the presence of pathogenic strains in the area. An increase in *V. vulnificus* cases in SA since 2012 has been observed, which could be related to an increase in the concentration of the bacteria, but it also could be evidence of increased surveillance (personal communication, T. Camou, Ministry of Public Health of Uruguay 2015).

Except for the gastrointestinal cases from Peru, all the reports of *V. vulnificus* human cases occurred on the Atlantic coast. Comparing the distribution maps from *V. parahaemolyticus* and *V. vulnificus* in SA (Figs. 1, 2 and 3), it can be seen that the only places where both bacteria were reported causing infection were Peru, Ecuador and Uruguay. It also appears that there could be some factor that affects the distribution of pathogenic strains of *V. parahaemolyticus* or *V. vulnificus* in some areas, which is not observed for environmental strain distribution. These factor could be related to seawater temperature, oceanic currents, salinity, or even with association of *Vibrio* pathogenic strains with other marine organisms, and also could be due to bacterial genetic characteristics, but it is difficult to establish since there is not a continuous monitoring and not all the countries had published environmental data, besides this is an assumption that needs to be further studied with environmental data and genotyping information.

Climate impact on *Vibrio* populations

V. parahaemolyticus and *V. vulnificus* occurrence in the estuaries and along the coastlines of SA is ubiquitous, and therefore, they are also commonly found in shellfish grown in these areas (Pereira et al. 2004; Markman 2008; Muñoz et al. 2008; Ramos et al. 2014; Aranda et al. 2015). In the United States, temperature and salinity have been determined to be fundamental predictors of *V. parahaemolyticus* and *V. vulnificus* abundance. For a recent review of how environmental factors affect the concentration of *V. parahaemolyticus* and *V. vulnificus* in oysters, see Froelich and Noble (2015). Salinity along the SA coast varies with precipitation and proximity to estuaries. For example, the Amazon River outflow can cause a decrease in the salinity that is observed more than 300 km from the mouth of the river (Dias 2007).

Moreover, ENSO, which happen every three to four years, cause an increase in seawater temperatures on the Pacific coast, as well as an increase in precipitation levels in tropical and equatorial areas on both coasts of SA. These temperature increases and precipitation associated with salinity decreases could play a major role in *V. parahaemolyticus* and *V. vulnificus* proliferation and, consequently, enhance the probability of cases and outbreaks generated by these bacteria. The hypothesis of ENSO role spreading waterborne diseases as a long-distance corridor between Asia and the Americas has also been presented by Martinez-Urtaza et al. (2016). Besides ENSO, decreases in sea surface salinity in the western and central equatorial Pacific can happens due to low salinity water that is brought in by anomalous eastward surface currents, and to a lesser extent due to excess rainfall in the Pacific Ocean (Zhu et al. 2014). According to Latif and Grötzner (2000) the effects of ENSO can also impact the Atlantic coast, often with a lag period of six months. The area also has another similar phenomenon, the Equatorial Atlantic oscillation, which happens ca. every 30 months. However, as water temperatures on the Atlantic coast are warm, the impact of ENSO and the Equatorial Atlantic oscillation on the Equatorial and Tropical Atlantic coast can be lesser than those observed for ENSO on the Pacific coast in relation to *Vibrio* sp.

Another consequence of ENSO is the spread of *V. parahaemolyticus* from other continents to the SA Pacific coast. According to Martinez-Urtaza (2011), the 1997 *V. parahaemolyticus* outbreak in Peru coincided with an ENSO phenomenon. The authors had analyzed data from *V. parahaemolyticus* strains from human cases in Peru between 1994 and 2005, and also studied the distribution and environmental parameters of these cases. The strains from the1997 outbreak were identified as being serotype O3:K6 and showed a close correspondence with the arrival and circulation of 1997 ENSO along the SA Pacific coast. Moreover, the increase in surface seawater temperature from 18°C - 23°C increased the risk of infection by 600-fold (Martinez-Urtaza et al. 2008).

ENSO also could explain *V. vulnificus* cases in Peru in 1998 (Ibarra et al. 1999; Martinez-Urtaza et al., 2016), a year notable for the elevated strength of ENSO (CPTEC/INPE 2015). More studies are needed, though, to confirm the influence of the Peru Current and/or ENSO on *V. vulnificus* abundance in SA to determine if it is not being monitored in the area, and it could be an underreport case. Adopting a policy of notification would allow countries to better monitor the prevalence of *V. vulnificus,* and to establish control plans in order to prevent and to inform people at high risk for infection. It also would permit doctors to better diagnose and to give safety recommendations to their patients.

Overall Conclusions

This review describes the presence and distribution of *V. parahaemolyticus* and *V. vulnificus* in seawater, shellfish and other marine animals, as well as clinical samples from SA. It appears that infections of *V. parahaemolyticus* have been more strongly related to seafood ingestion and have been more frequently reported on the Pacific coast. Conversely, *V. vulnificus* is more frequently acquired by water contact with open wounds and its presence has been more heavily reported along the Atlantic coast.

The impacts of ENSO have been observed as an increase of *V. parahaemolyticus* outbreaks on the Pacific coast of SA. Although, more studies are required to confirm the importance of environmental factors that are affecting the presence, concentrations and virulence of the bacteria in these areas of the continent.

V. vulnificus human cases have been restricted mostly to the southeastern and southern areas of the Atlantic coast of SA, with the only exception being the report of three gastroenteritis cases in Peru and two cases in Ecuador. Uruguay had the highest number of cases in SA, but the number of cases in all of SA is low compared with the numbers of reported infections in the USA, where, according to CDC (Ratner 1987), there are approximately 96 *V. vulnificus* cases reported annually.

Peru, Ecuador and Uruguay are the only countries where human cases from both *V. vulnificus* and *V. parahaemolyticus* were recorded. Even considering possible underreporting from other areas, and the concomitant presence of environmental strains of both bacteria along the Atlantic and Caribbean coasts, the distribution of pathogenic strains should be further studied. More research is necessary in order to determine if there are environmental or genetic factors that explain the distribution of *V. vulnificus* and *V. parahaemolyticus* pathogenic strains. It is evident that the continent experiences environmental conditions that are permissive for both *V. vulnificus* and *V. parahaemolyticus*. In the face of climate change, a formal monitoring program should be instituted to quantify *Vibrio* sp., and to develop models that permit predictions of environmental conditions that are favorable for the pathogenic forms of these bacteria. This monitoring would also serve to gather sufficient information to determine the impact of ENSO and other global climate phenomena on the abundance of *V. parahaemolyticus* and *V. vulnificus*. Eventually, this would allow for the establishment of control plans to minimize new cases and reduce the impact of outbreaks, especially for *V. parahaemolyticus* in the Pacific coast during years when ENSO is forecasted to be stronger than normal such as in the spring of 2016.

The use of faster, more accurate, and virulence specific detection approaches, such as PCR confirmation, should be considered to detect the presence of pathogenic *Vibrio* strains in environmental and seafood samples for protection of public health.

In summary, the published reports and the environmental characteristics from SA cannot be disregarded, there is a demonstrated risk of *V. parahaemolyticus* and *V. vulnificus* infection from the water and seafood. Although the total numbers of cases are very low, the morbidity, mortality, and close association of these cases with seafood production and water warrant an improved monitoring and surveillance framework for all countries in SA for *V. parahaemolyticus* and *V. vulnificus*, in order to predict protect public health and prevent outbreaks. This study highlights the need for more research and a formal monitoring program of *Vibrios* in SA, both in environmental and clinical samples, particularly in the countries with the worst sets of cases as Uruguay for *V. vulnificus,* and Chile for *V. parahaemolyticus*. A continent wide culture based monitoring program, with collection of salinity and temperature information would be a start, and then for the mentioned countries with the most cases, a pathogenic strain tracking system could be implemented initially in a basic way (conventional PCR). So, in order to maintain the economic, cultural, and societal roles of aquaculture, public health and food safety should be closely monitored and controlled.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figure legends

Figure 1: Distribution map of *V. parahaemolyticus* reports in SA environmental samples with tags showing the year of occurrence.

Figure 2: Distribution map of *V. parahaemolyticus* cases and outbreaks in SA, with tags showing the year of occurrence.

Figure 3: Distribution map of *V. vulnificus* reports from environmental samples (blue tags), and cases and outbreaks (red tags) in SA, with tags showing the year of occurrence.

Table 1 Reports of *Vibrio parahaemolyticus* in environmental and seafood samples in South America

Year	Country	State/ Region	Observation/ matrix	Serotype	Reference
1971	Argentina	Chubut	Mussel		Casellas et al. 1977
1976	Brazil	Sao Paulo	Oyster	O5:K17, 05:K47, O3:K45, O1:K32, O11:K5, O3:K33	Leitão et al. 1976
1979	Brazil	Sao Paulo	Oyster		Gelli et al. 1979
1979	Brazil	Ceara	Fish		Hofer and Silva 1974
1980	Peru	Trujillo	Crab and water		Bocanegra et al. 1981
1980	Brazil	Bahia	Mollusks, shellfish, crustaceans, fish		Franca et al. 1980
1982	Brazil	Rio de Janeiro	Oyster and water	O1:K32, O1:K33, 02:K28, O3:K29, O3:K*, O4:K13, O4:K*, O6:K61, O5:K*, O8:K20, O11:K15	Rodrigues and Hofer 1986
1984	Peru	Lima	Ceviche	O4:K33, O1:K33, O2:K28, **O4:K12**, O5:K17, O3:K30, O1:K33, O7:K19, O2:K22, O3:K33, O11:K61.	Guevara-Duncan et al. 1989
1986	Brazil	Rio de Janeiro	Fish	O2:K28	Hofer and Silva 1986

1988	Brazil	Rio de Janeiro	Squid		Lima et al. 1994
1990	Brazil	Ceara	Lobster	Serogroups K.	Vieira and Iaria 1993
1990	Brazil	Sao Paulo	Mussel Temp: 23-23.5°C Salinity: 32-33‰		Matté et al. 1994
1990	Brazil	Sao Paulo	Oyster		Rojas et al. 2011
1994	Brazil	Santa Catarina	Mussel Temp: 23-28.5°C Salinity: 35-36.5‰	O5:K30, OND:K30, OND:K17, OND:KND, OND:K39, OND:K22, O1:K30, O3:K17, O5:KND, OND:K13, OND:K34, OND:K11	Archer and Moretto 1994
1994	Brazil	Sao Paulo	Mussel		Matté et al. 2007
1998	Brazil	Rio de Janeiro	Oyster and mussel	O10:K*,O1:K*, O5:K17, O8:K*, O2:K28, O10:K69, O2:K3, O3:K57, O3:K72, O11:K*, O2:K*, O4:K*, O4:K42, O10:K52, O11:K19, O1:K32, O3:K33, O4:K34, O5:K47, O11:K34, O1:K12, O1:K33, O2:K25, O2:K30, O3:K*, O3:K5, **O3:K6**, O3:K30, O3:K31, O3:K36, **O4:K12**, O5:K25, O6:K*, O8:K11, O8:K39, O8:K41, O9:K*, O10:K7, O10:K25, O10:K31, O10:K60, O11:K22, O11:K36, O11:K40	Pereira et al. 2004
1999	Brazil	Pernambuco	Oyster and water Temp: 26-30°C Salinity: 27-42‰		Lira et al. 2001
1999	Brazil	Sao Paulo	Oyster and water Temp: 19-28°C Salinity: 16-21‰		Ristori et al. 2007
2001	Brazil	Maranhao	Clam and mussel Temp: 30-32°C Salinity: 9-23‰		Serra et al. 2001
2001	Brazil	Ceara	Oyster Salinity: 3‰		Sousa et al. 2004
2002	Brazil	Santa Catarina	Oyster, mussel and water Temp: 20-28°C Salinity: 29-36‰		Silva 2003
2003	Brazil	Ceara	Oyster		Barros et al. 2003
2003	Brazil	Ceara	Crab		Vieira et al. 2004

Year	Country	Region	Sample	Serotype	Reference
2004	Venezuela	Sucre	Mussel		Grau et al. 2004
2004	Brazil	Rio de Janeiro	Mussel		Lafisca et al. 2008
2004	Brazil	Sao Paulo	Tuna		Chen 2004
2004	Venezuela	Sucre	Clam and mussel		Muñoz et al. 2008
2005	Brazil	Rio de Janeiro	Marine mammals		Pereira et al. 2007
2005	Brazil	Rio Grande do Sul	Marine mammals		Pereira et al. 2007
2005	Brazil	Ceara	Shrimp and water		Costa 2006
2006	Brazil	Pernambuco	Shrimp and water		Mendes et al. 2009
2006	Colombia	Cartagena	Oyster		López et al. 2010
2007	Brazil	Rio de Janeiro	Mussel		Pereira et al. 2007
2007	Brazil	Santa Catarina	Oyster Temp: 23-24°C Salinity: 33-34‰		Ramos 2007
2007	Brazil	Rio Grande do Norte	Shrimp		Melo et al. 2011
2007	Brazil	Santa Catarina	Oyster Temp: 18-29°C		Ramos et al. 2012
2008	Brazil	Sao Paulo	Water Temp: 20-32°C Salinity: 19-32‰		Markman 2008
2008	Brazil	Parana	Water Temp: 16-35°C Salinity: 17-27‰		Markman 2008
2008	Brazil	Pernambuco	Water Temp: 23-29°C Salinity: 8-35‰		Markman 2008
2008	Venezuela	Sucre	Clam		Muñoz et al. 2008
2009	Peru	Lima	Fish Temp: 20°C	**O3:K6**	Aliaga et al. 2010
2009	Chile	Region de los Lagos	Molluscan shellfish	**O3:K6**, O3:KUT	García et al. 2009

Year	Country	Location	Sample	Serotype	Reference
2009	Brazil	Santa Catarina	Oyster and water Temp: 21-28°C Salinity: 31-34‰		Ramos et al. 2014
2009	Brazil	Santa Catarina	Oyster and water Temp: 24°C Salinity: 12-36‰	O1:K1, O1:K25, O1:K41, O1:K69, O1:KUT, O2:K3, O2:K28, **O3:K6**, O3:K30, O4:K34, O4:K63, O5:K61, O6:K4, O6:K6, O6:K18, O6:K46, O7:K7, O7:K19, O8:K20, O8:K39.	Ramos 2012
2009	Brazil	Bahia	Oyster		Rodrigues and Carvalho-Filho 2011
2009	Brazil	Ceara	Oyster		Vieira et al. 2011
2010	Brazil	Sao Paulo	Oyster Temp: 14-28°C Salinity: 5-30‰		Costa Sobrinho et al. 2010
2010	Brazil	Sao Paulo	Oyster		Costa Sobrinho et al. 2011
2010	Brazil	Ceara	Oyster		Vieira et al. 2010
2011	Brazil	Sao Paulo	Mussel, oyster		Rojas et al. 2011
2011	Brazil	Rio de Janeiro	Mussel		Oliva 2012
2012	Chile	Puerto Montt	Molluscan shellfish		Aranda et al. 2015
2014	Brazil	Piaui	Shrimp		Muratori et al. 2014

Table 2 Reports of *V. parahaemolyticus* incidence in isolated human cases and outbreaks in SA

Year	Country	State/ Region	Cases / Outbreak information	Strain identification/ serotype	Reference
1975	Brazil	Ceara	Isolated case	O5:K17	Hofer 1983
1989	Brazil	Pernambuco	Outbreak	**O4:K12**, O1:K56, O3:K5, O3:K58, O3:KUT, O4:K4, O4:K10, O4:K53, O5:KUT, O10:KUT	Magalhães et al. 1991
1993	Peru	Trujillo	Outbreak	O2:K3, O4:K8, OUT:KUT	Gil et al. 2007
1994	Peru	Lima, Trujillo	Outbreak	OUT:K3, O2:K3, O2:KUT, O4:K8	Gil et al. 2007
1995	Peru	Lima	Outbreak	**O4:K12,** OUT:K46	Gil et al. 2007
1996	Peru	Trujillo	Outbreak	**O3:K6**, O4:K8, OUT:K8.	Gil et al. 2007
1997	Peru	Lima, Arequipa	Outbreak	**O3:K6, O4:K12**	Gil et al. 2007
1997	Peru	Lima, Cajamarca, Lambayeque, Monqueagua	Outbreak	**O3:K6**	Martinez-Urtaza et al. 2008
1998	Peru	Lima	Outbreak	**O3:K6**	Martinez-Urtaza et al. 2008
1998	Chile	Antofagasta	Outbreak	**O3:K6**, O1:K56	González-Escalona et al. 2005
1998	Chile	Antofagasta	Outbreak		Córdova et al. 2002
1998	Peru	Lima, Trujillo	Outbreak	**O3:K6**, O3:K68, O3:K58, O4:K8, **O4:K12,** O11:KUT, O11:K15, OUT:KUT	Gil et al. 2007
1998	Peru	Lima	Outbreak		Ibarra et al. 1999
1999	Peru	Lima, Lambayeque	Outbreak	**O3:K6,** O3:KUT	Martinez-Urtaza et al. 2008
1999	Brazil	Maranhao	Wound		Rodrigues et al. 2001
1999	Peru	Lima	Outbreak	**O3:K6**	Gil et al. 2007
2000	Peru	Lima	Outbreak	**O3:K6, O4:K12**	Gil et al. 2007
2001	Peru	Lima, Lambayeque, Iquitos	Outbreak	**O3:K6**	Martinez-Urtaza et al. 2008
2001	Peru	Lima	Outbreak	O6:K18	Gil et al. 2007
2001	Brazil	Pernambuco	Outbreak	O3:KUT	Leal and Franco 2008
2002	Peru	Lima	Outbreak	**O3:K6**	Martinez-Urtaza et al. 2008
2002	Brazil	Pernambuco	Isolated case and outbreak samples	**O3:K6**	Leal et al. 2008;

			(26 cases/ 9 confirmed O3:K6, due to raw crab leg		FUNASA 2002
2002	Brazil	Ceara	Outbreak	**O3:K6**	Leal and Franco 2008
2002	Brazil	Alagoas	Isolated case	**O3:K6**	Leal and Franco 2008
2003	Peru	Lima, Cajamarca	Outbreak	**O3:K6**	Martinez-Urtaza et al. 2008
2003	Brazil		2 outbreaks		Brasil. 2014
2004	Brazil		1 outbreak		Brasil. 2014
2004	Chile	Puerto Montt	Outbreak	**O3:K6, O4:K12**	González-Escalona et al. 2005
2004	Chile	Region de los Lagos	Outbreak / 1,500 cases	**O3:K6**	García et al. 2009
2005	Chile	Region de los Lagos	Outbreak / 3,725 cases	**O3:K6**	García et al. 2009
2006	Chile	Region de los Lagos	Outbreak / 1,083 cases	**O3:K6**	García et al. 2009
2007	Chile	Region de los Lagos	Outbreak / 477 cases	**O3:K6**	García et al. 2009
2008	Chile	Puerto Montt	Outbreak / 1,153 cases	**O3:K6**, O3:KUT, OUT:KUT	García et al. 2009
2008	Chile	Puerto Montt	Isolated case, septicemia		Dabanch P. et al. 2009
2009	Ecuador		Isolated case	OUT:K29	Ottaviani et al. 2013
2009	Chile	Region de los Lagos	Outbreak / 441 cases	**O3:K6**, O3:KUT	García et al. 2009
2011	Brazil		1 outbreak		Brasil. 2014
2012	Brazil		2 outbreaks		Brasil. 2014
2013	Chile	Coquimbo, Valparaiso, Maule, Biobío, Los Ríos	31 outbreaks / 383 cases Jan-April)		Chile. 2014
2014	Chile	Arica y Parinacota, Atacama, Coquimbo, Metropolitana, Maule, Biobio	5 outbreaks / 26 cases and 3 isolated cases (Jan-April)		Chile. 2015
2014/ 2015	Uruguay	Rio de la Plata Estuary	3 cases wound infection (cellulitis)		Camou, T., 2015 pers.communication
2015	Chile	Arica y Parinacota, Tarapaca, Coquimbo, Valparaíso, Metropolitana, Maule, Los Rios	8 outbreaks / 60 cases and (Jan-April)		Chile. 2015

UT = untypeable

Table 3 Reports of *V. vulnificus* incidence in environmental and seafood samples in SA

Year	Country	State/ Region	Matrix	Salinity	Reference
1982	Brazil	Rio de Janeiro	Water		Rodrigues and Hofer 1986
1990	Brazil	Sao Paulo	Mussel	Temp.: 23-23.5ºC Salinity: 32-33‰	Matté et al. 1994
1995	Brazil	Sao Paulo	Molluscan shellfish, shrimp		Garcia-Moreno and Landgraf 1997
1999	Brazil	Sao Paulo	Oyster and water	Temp.: 19.2-28ºC Salinity: 16-21‰	Ristori et al. 2007
2001	Brazil	Ceara	Shrimp		Nascimento et al. 2001
2002	Brazil	Santa Catarina	Molluscan shellfish, water	Temp.: 19.2-28ºC Salinity: 29-36‰	Silva 2003
2002	Venezuela	Sucre	Mussel		Grau et al. 2004
2003	Brazil	Ceara	Oyster		Barros et al. 2003
2005	Brazil	Ceara	Water		Costa, 2006
2005	Brazil	Rio de Janeiro	Marine mammals		Pereira et al. 2007
2005	Brazil	Rio Grande do Sul	Marine mammals		Pereira et al. 2007
2006	Brazil	Pernambuco	Shrimp and water		Mendes et al. 2009
2006	Colombia	Cartagena	Oyster		López et al. 2010
2007	Brazil	Rio de Janeiro	Mussel		Pereira et al. 2007
2007	Brazil	Santa Catarina	Oyster	Temp.: 22.27-23.57ºC Salinity: 33.13-34.03‰	Ramos 2007
2007	Brazil	Santa Catarina	Oyster	Temp.: 18-29ºC	Ramos et al. 2012
2009	Brazil	Santa Catarina	Oyster and Water	Temp.: 20.9-27.5ºC Salinity: 30.7-33.9‰	Ramos et al. 2014
2009	Brazil	Santa Catarina	Oyster and Water	Temp.: 24.3ºC	Ramos 2012

			Salinity: 12-36‰	
2009	Brazil	Ceara	Oyster	Vieira et al. 2011
2010	Brazil	Ceara	Frozen oyster	Costa et al. 2013
2010	Brazil	Ceara	Oyster	Vieira et al. 2010
2011	Brazil	Rio de Janeiro	Mussel	Oliva 2012
2014	Brazil	Santa Catarina	*vcg*-C and *vcg*-E strains Temp.: 22.2°C Salinity: 34.18‰ (*Vcg*-C) 34.83‰ (*Vcg*-E)	Raszl et al., 2016

Table 4 Reports of *V. vulnificus* incidence in isolated human cases and outbreak in SA

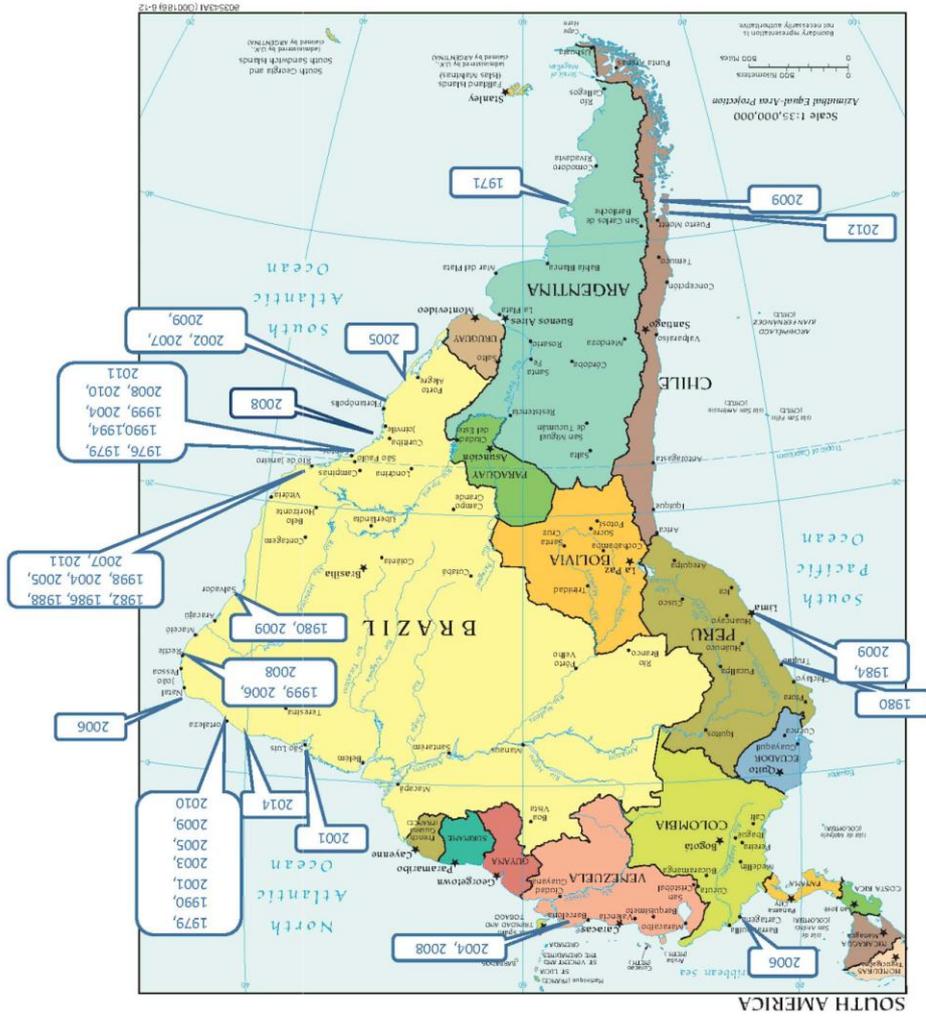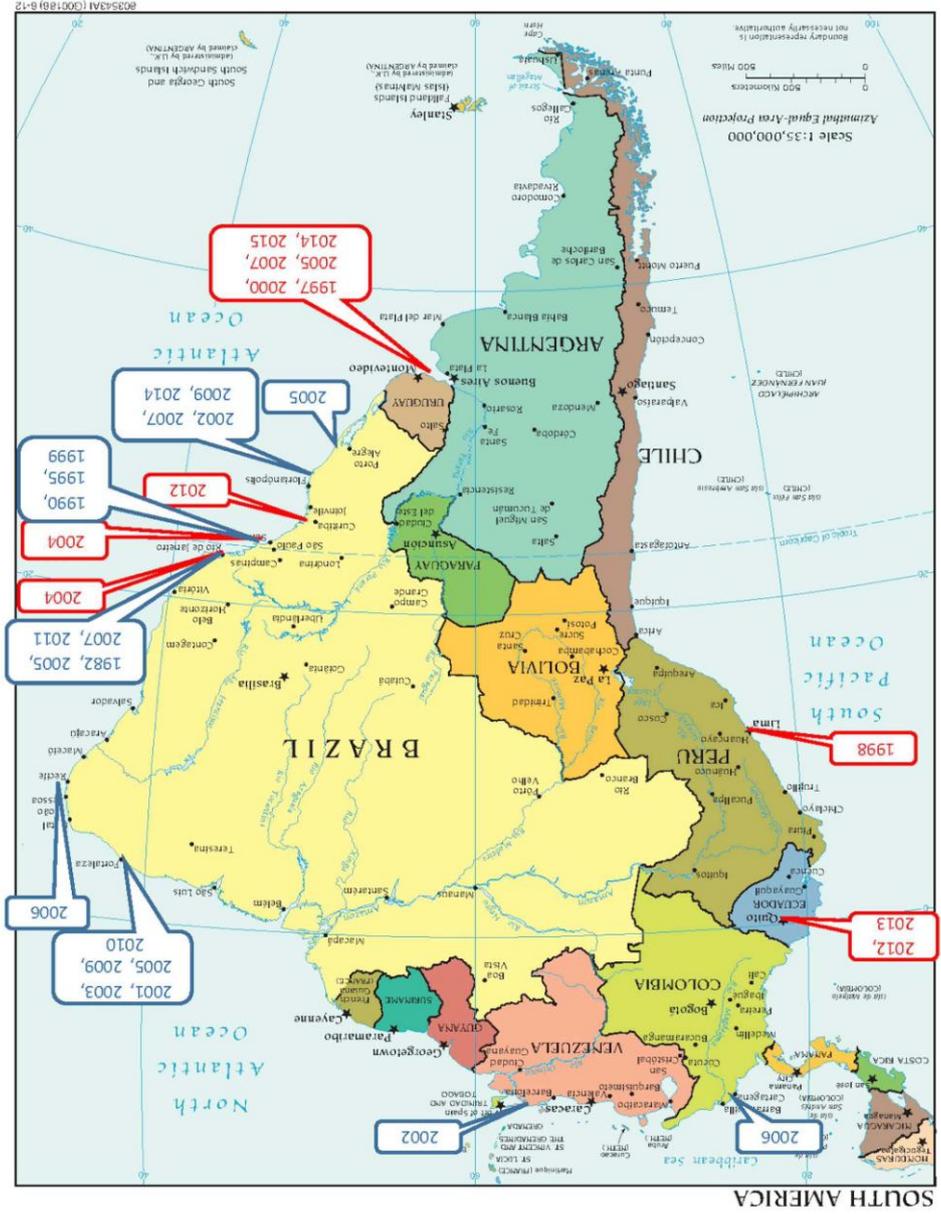| Year	Country	State/ Region	Case/ outbreak information	Reference
1997	Uruguay	Río de la Plata	Water contact, sepsis. Isolated case.	Carrerou-Perreng 2001
1998	Peru	Lima	Acute diarrhea. 3 cases.	Ibarra et al. 1999
2000	Uruguay	Maldonado	Water contact, sepsis. Isolated case.	Chicheff et al. 2001
2001	Chile	Imported case from El Salvador	Ingestion of raw oyster. Sepsis. Isolated case.	Poblete U. et al. 2002
2004	Brazil	São Paulo	Molluscan shellfish consumption. Isolated case.	de Araujo et al. 2007
2004	Brazil	Rio de Janeiro	Wound infection. Isolated case.	Brack et al. 2004
2005	Uruguay	Rio de la Plata Bay	Water contact. Wound infection. Isolated case.	Camou, T., 2015 (not published)
2007	Uruguay	Rio de la Plata Bay	Water contact. Wound infection. Isolated case.	Camou, T., 2015 (not published)
2012	Brazil	Paraná	Probably by molluscan shellfish consumption. Sepsis.	França et al. 2013
2012	Ecuador	Quito	Isolated case. Sepsis.	Villacrés et al. 2013
2013	Ecuador	Ibarra	Isolated case. Sepsis.	Villacrés et al. 2013
2014	Uruguay	Rio de la Plata Bay	Water contact. Wound infection. Isolated case.	Camou, T., 2015 (not published)
2014/2015	Uruguay	Punta del Este	Water contact. Outbreak, 4 cases, 2 deaths.	Uruguay 2015

Figure 1

Figure 2

Figure 3