doi.org/10.3406/bavf.2026.71170

Seasonal prevalence of *Vibrio parahaemolyticus* in raw shrimp in Morocco and comparative performance of real-time PCR and cultural method for its detection

Prévalence saisonnière de Vibrio parahaemolyticus *dans les crevettes crues au Maroc et performances comparatives de la PCR en temps réel et de la méthode culturale pour sa détection*

Mohammed Radouane Kriem[1], *Sakina Timouyas*[2], *Nour-Eddine Chaouqy*[3], *Abdelhaq El Marrakchi*[4]

Manuscrit initial reçu le 17 juin 2025, manuscrit révisé reçu et accepté le 31 octobre 2025, révision éditoriale le 23 mars 2026.

Abstract

Samples of raw shrimp (n=192), fished in the coastal waters of Agadir, Morocco, at different seasons and collected from the city's fish marketplace, were examined for the presence of pathogenic *Vibrio* bacteria using cultural, biochemical, and molecular methods, as well as Real Time Polymerase Chain Reaction (RT PCR). Microbiological analysis was performed according to the ISO 21872 1:2017 method, using the selective medium CHROMagar™ *Vibrio*. Presumptive positive colonies were identified by biochemical and conventional species specific PCR techniques to confirm the presence of *Vibrio* spp. and their pathogenicity factors. RT PCR performed directly on the samples revealed *V. parahaemolyticus* in 29 samples (15.10%), compared with 12 samples (6.25%) using the culture method. No genes encoding known virulence factors were detected, neither in the *V. parahaemolyticus* strains analyzed by conventional PCR, nor in the raw samples analyzed by RT PCR. Seasonal effects revealed a clear difference in shrimp contamination, depending on whether they were harvested in winter or summer.

Keywords *:* shrimp, pathogenic *Vibrio*, isolation medium, foodborne illness, real time PCR

1- Professor of Biology, Animal Ecology, and Microbiology at the Hassan II Institute of Agronomy and Veterinary Medicine, Rabat, Morocco
Courriel : m.kriem@iav.ac.ma
2- Doctor of Veterinary Medicine at the Hassan II Institute of Agronomy and Veterinary Medicine, Rabat, Morocco
3- Professor at the Regional Laboratory of Analysis and Research of the National Office of Food Safety in Agadir (LRARA/ONSSA), Morocco
4- Professor of Veterinary Medicine, former professor of microbiology and hygiene of foodstuffs of animal origin at the Hassan II Institute of Agronomy and Veterinary Medicine, Rabat, Morocco

Bull. Acad. Vét. France — 2026
https://academie-veterinaire.fr

Résumé

Des échantillons de crevettes crues (n=192), pêchées dans les eaux côtières d'Agadir, au Maroc, à différentes saisons, et collectées sur le marché aux poissons de la ville, ont été examinés pour la présence de bactéries Vibrio *pathogènes à l'aide de méthodes culturales, biochimiques et moléculaires, ainsi que par PCR en temps réel (RT PCR). L'analyse microbiologique a été réalisée selon la méthode ISO 21872 1:2017, en utilisant le milieu sélectif CHROMagar™ Vibrio. Les colonies présumées positives ont été identifiées par des techniques biochimiques et par des techniques de PCR conventionnelle spécifiques à l'espèce, afin de confirmer la présence de Vibrio spp. et de leurs facteurs de pathogénicité. La RT PCR réalisée directement sur les échantillons a révélé la présence de* V. parahaemolyticus *dans 29 échantillons (15,10 %), contre 12 échantillons (6,25 %) en utilisant la méthode culturale. Aucun gène codant des facteurs de virulence connus n'a été détecté, ni dans les souches de* V. parahaemolyticus *analysées par PCR conventionnelle ni dans les échantillons bruts analysés par RT PCR. Les effets saisonniers ont mis en évidence une nette différence dans la contamination des crevettes selon qu'elles étaient récoltées en hiver ou en été.*

Mots-clés : *crevette,* Vibrio *pathogène, milieu d'isolement, maladie d'origine alimentaire, PCR en temps réel*

Citation

Kriem MR, Timouyas S, Chaouqy N-E, El Marrakchi A (2026) Seasonal prevalence of Vibrio parahaemolyticus in raw shrimp in Morocco and comparative performance of real-time PCR and cultural method for its detection [*Prévalence saisonnière de Vibrio parahaemolyticus dans les crevettes crues au Maroc et performances comparatives de la PCR en temps réel et de la méthode culturale pour sa détection*] *Bulletin de l'Académie vétérinaire de France* 179: 71170. https://doi.org/10.3406/bavf.2026.71170

Introduction

Vibrios are Gram-negative, halophilic bacteria widely distributed in warm coastal waters (temperature>15 °C) and estuaries with low salinity (<25 ppt) (Baker-Austin *et al.*, 2010; Vezzulli *et al.*, 2012; Heng *et al.*, 2017). Species of the *Vibrio* genus are considered to be indigenous to the marine environment, where they are found in a free state or associated with various media, plankton, sediment, and marine organisms such as fish, crustaceans or mollusks (Huss, 1993; Huss *et al.*, 2004).

The *Vibrio* genus includes more than 150 species as listed in the List of Prokaryotic names with Standing in Nomenclature (LPSN, 2020). Thirteen of these species are associated with human infections, three of which are recognized as major pathogens: *Vibrio cholerae*, *V. parahaemolyticus*, and *V. vulnificus* (Castello *et al.*, 2022). *Vibrio* species are schematically subdivided into two groups: (i) cholera vibrios, namely *V. cholerae* serogroups O1 and O139, responsible for cholera in humans, and (ii) non cholera vibrios, comprising all other *Vibrio* species as well as *V. cholerae* isolates of serogroups other than O1 and O139, known as non O1/non O139 *V. cholerae*. Cholera vibrios, initially of marine origin, quickly adapted to humans, who became their main reservoir (Fournier & Quilici, 2007). *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*, whose habitat is exclusively the marine environment, are responsible for gastrointestinal and extra-intestinal diseases, septicemia, and skin infections, associated with two routes of exposure, ingestion of raw seafood and direct contact with seawater or the marine environment (Quilici & Robert-Pillot 2011; Fleischmann *et al.*, 2022).

Of these *Vibrio* species, *V. parahaemolyticus* has attracted attention as the cause of several epidemic outbreaks, particularly in Asian countries, including Japan (Su & Liu, 2007) and Taiwan (Pan *et al.*, 1997), and in the United States (Daniels *et al.*, 2000; De Paola *et al.*, 2000). In France, 44 patients were affected by episodes of gastroenteritis after eating shrimps imported from Asia (Lemoine et *al.*, 1999; Robert-Pillot *et al.*, 2004). The pathogenicity of this species is linked to the presence of two hemolysins, thermostable direct hemolysin (TDH), and TDH-related hemolysin (TRH). These toxins are not pre-formed in food but are produced in the intestine following colonization by these strains (Copin *et al.*, 2015).

Against this backdrop, the potential health risks to humans posed by these emerging pathogens warrant increased vigilance. Thus, the affected countries have drawn up and implemented surveillance plans to estimate the prevalence of this species in seafood.

It is important to emphasize that the incidence of non cholera *Vibrio* infections, including the most severe forms, is underestimated in Morocco, where these infections are underestimated because they are underrecognized by medical professionals and their microbiological identification can be challenging. Also, they are not reportable, except in the context of surveys on collective food poisoning. The present study aimed to estimate the prevalence of *V. parahaemolyticus* in commercial shrimp (*Parapenaeus longirostris*), which may be a source of human exposure, and to improve analytical methods for product analysis.
To enable sanitary control based on quantitative criteria, hygienists first developed methods for isolating this pathogen using culture techniques on agar media. The first medium used was TCBS, which showed its limitations for quantification and isolation. Chromogenic media have since been developed and have shown higher performance than TCBS for the isolation of pathogenic vibrios (Yukiko *et al.*, 2001; Kriem *et al.*, 2015). However, these chromogenic media were proved unsuitable for quantifying *V. parahaemolyticus*. In addition, all methods based on growth in culture media have the major disadvantage of being time consuming. Prior to isolation, one or two pre-enrichment steps in non selective broth are generally required, followed by isolation on a selective agar medium. Then colonies presumed to be *V. parahaemolyticus* must be cultivated on a non selective agar medium for confirmation of their identification, a process that can take seven days or more. A culture method developed by the US Food and Drug Administration (Bisha *et al.*, 2012), based on the determination of the most probable number, has proved to be tedious, costly, and inaccurate. The cultural method also has the disadvantage of providing false negative or false positive results, with confirmatory tests based on biochemical characteristics only (O'Hara *et al.*, 2003), and conventional polymerase chain reaction (PCR) tests are necessary for final confirmation of isolates.

Real-time PCR (RT-PCR) has proved satisfactory for confirming the identity of *V. parahaemolyticus*, quantifying the total number of strains, and targeting pathogenic strains carrying genes encoding the TDH or TRH hemolysins directly in food samples, without the need for a preliminary cultivation stage. This study compared the performance of the culture method using a selective chromogenic medium coupled with conventional PCR with that of the highly specific RT PCR method developed by Robert-Pillot *et al.* (2010) for the direct detection and quantification of *V. parahaemolyticus* in shrimp.

Materials and Methods

The study was carried out on shrimps (*Parapenaeus longirostris*) caught off the Agadir Coast, stored in boxes, treated with sodium bisulfite, and landed at the port of Agadir, where they are inspected and sold at the fish market. The time between capture and landing varies from 24 hours to three days. The catches of coastal fishing are mainly intended for local consumption but also for export to European Union countries.

Along the central Moroccan Atlantic coast, there are eight main fishing zones frequented by coastal trawlers, which land their catch at the port of Agadir for sale at the Agadir market (**Figure 1**).

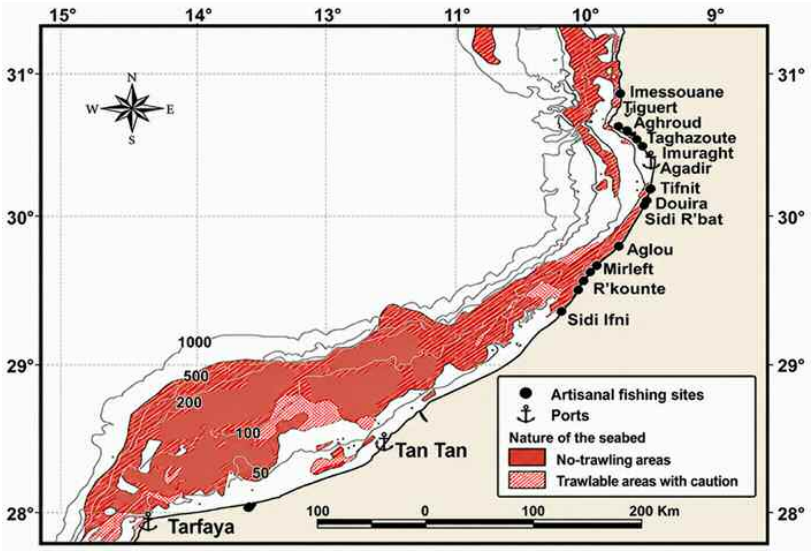

Figure 1. *Map of the fishing area frequented by coastal trawlers based at the port of Agadir (National Institute of Fisheries Research, Morocco [INRH])*

A total of 192 shrimp samples fished in the coastal waters of the Agadir region (Morocco) were randomly collected at the fish market in the port of this city. Samples of shrimp (300 g each) were rapidly put in sterile plastic bags using a sterilized sample collection clip and transported to the laboratory in ice boxes, where they were analyzed within 6 h of sampling.

Microbiological analysis

Two approaches were used:
- culture-based isolation techniques coupled with biochemical and molecular confirmation and characterization steps by conventional PCR.
- *Vibrio parahaemolyticus* detection and characterization by RT PCR method performed directly on the samples (**Figure 2**).

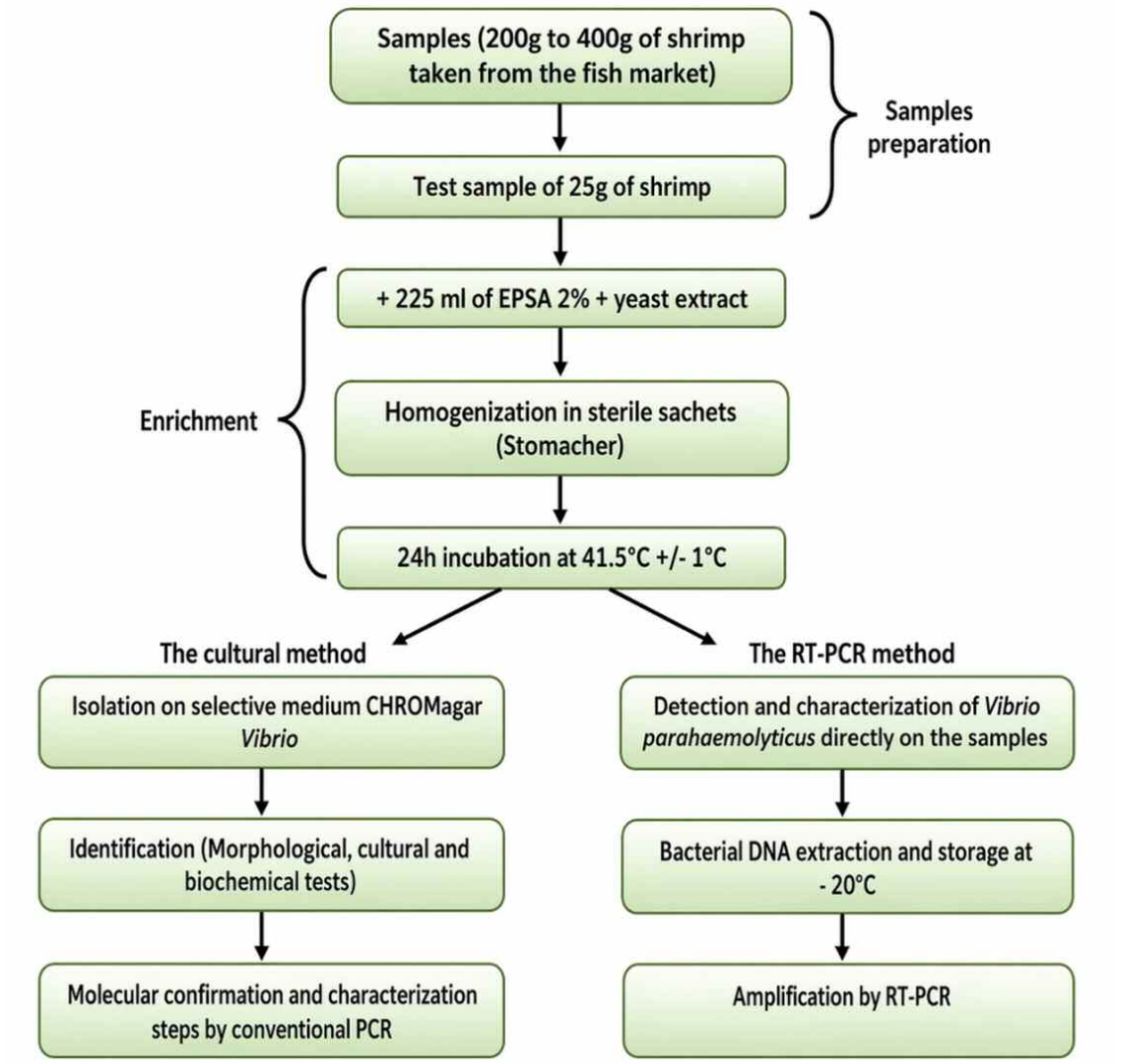

Figure 2. *The procedure used for sample analysis*

A portion of 25 g of sample was introduced into a sterile stomacher bag containing 225 mL of 2% NaCl alkaline saline peptone water (ASPW) and homogenized for 1 min before incubation at 41.5 °C for 18-24 h. From the enrichment broth, isolation on CHROMagar *Vibrio* medium (CHROMagar Microbiology, Paris, France), as described by Hara-Kudo *et al.* (2001), was used to differentiate *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* from other *Vibrio* species.

V. parahaemolyticus colonies are purple and *V. vulnificus*/*V. cholerae* have green blue to turquoise blue colonies, while *V. alginolyticus* colonies are colorless. After 24 h incubation at 37 °C, five typical colonies from each of the three morphological types found on CHROMagar *Vibrio* were randomly isolated for purity on 2% NaCl nutritive agar (Bio-Rad) and maintained on stock agar tubes (Bio-Rad) for 18-24 h at 37 °C before identification.

Bacteriological identification

All isolates were subjected to morphological, biochemical, and cultural tests, following the ISO 21872 1: 2017 protocol (ISO 2017) for the detection of pathogenic Vibrios. Gram stain, oxidase test, arginine dihydrolase (ADH) and lysine decarboxylase (LDC) (Bio-Rad) reactions were conducted on the isolates to ascertain their affiliation to the *Vibrio* genus. The oxidase-positive, Gram-negative, ADH negative, and LDC positive isolates were then speciated using the API 20E strips (Biomérieux, Marcy l'Étoile, France), and their ability to grow in alkaline peptone water with increasing salt concentrations (0, 3, 6, 8, and 10% NaCl) was tested (Robert Pillot *et al.*, 2002). Colonies testing positive for *Vibrio* by biochemical tests were selected for further analysis using molecular techniques.

Molecular analysis by conventional PCR

Genomic DNA was extracted from 18-24 h bacterial culture on alkaline nutritive agar using the InstaGene Matrix method (Bio-Rad Laboratories) according to the manufacturer's instructions. A loopful of culture was collected and triturated in 1 mL of sterile buffered saline solution previously dispensed into a 1.5 mL microcentrifuge tube. The mixture was vortexed, then centrifuged for 1 min at 12 000 rpm, and the supernatant was discarded. 200 µL of InstaGene Matrix was then added to the pellet, mixed by several pipettings with a wide tip cone (1 000 µL blue cone), and incubated in a water bath at 56 °C for 30 min. High speed vortexing of the microtube was performed for 10 s and the microtube was then placed in a heating block at 100 °C for 8 min. Another vortexing of the microtube at a high speed for 10 s, followed by centrifugation at 12 000 rpm for 3 min, was performed. Finally, 1 µL of supernatant containing the bacterial DNA was used in a 50 µL PCR reaction. PCR assays were conducted to confirm *Vibrio* species identification and, for strains identified as *V. parahaemolyticus*/*V. cholerae*, to further characterize them by detecting genes encoding virulence associated factors. The primers used in this study and the target genes are summarized in Table 1; detailed PCR protocols are available in the references cited for each primer.

Target gene	Primer name	Sequence (5'-3')	Interpretation	Amplicons' sizes	References
16S/23S	prVC-F prVC-M-R	TTA AGC STT TTC RCT GAG AAT G AGT CAC TTA ACC ATA CAA CCC G	Species identification (*V. cholerae*)	300 bp	Chun *et al.* (1999)
r72h	VP32 VP33	CGA ATC CTT GAA CAT ACG CAG C TGC GAA TTC GAT AGG GTG TTA ACC	Species identification (*V. parahaemolyticus*)	320 or 387 bp	Lee *et al.* (1995)
ctxA	CTX2 CTX3	CGG GCA GAT TCT AGA CCT CCT G CGA TGA TCT TGG AGC ATT CCC AC	*V. cholerae* virulence factor (Cholera Toxin A subunit)	564 bp	Fields *et al.* (1992)
ctxB	CTX7 CTX9B	GGT TGC TTC TCA TCA TCG AAC CAC GAT ACA CAT AAT TAG AAT TAA GGA TG	*V. cholerae* virulence factor (Cholera Toxin B subunit)	460 bp	Olsvik *et al.* (1993)
tdh	L.tdh R.tdh	GTA AAG GTC TCT GAC TTT TGG AC TGG AAT AGA ACC TTC ATC TTC ACC	*V. parahaemolyticus* (hemolysin TDH)	373 bp	Bej *et al.* (1999)
trh	L.trh R.trh	TTG GCT TCG ATA TTT TCA GTA TCT CAT AAC AAA CAT ATG CCC ATT TCC G	*V. parahaemolyticus* (hemolysin TRH)	250 bp	Bej *et al.* (1999)

Table 1. *Genes and primers used by conventional PCR for the identification and detection of virulence factors of* Vibrio parahaemolyticus *and* Vibrio cholerae.

Detection of Vibrio parahaemolyticus using RT PCR

Bacterial DNA was extracted from 1 mL of the 24 h enrichment ASPW broths incubated at 41.5 °C for all shrimp samples using the InstaGene Matrix kit (Bio Rad Laboratories, Marnes la Coquette, France), according to the manufacturer's recommendations. The RT PCR assay was performed using the same primer sequences as the conventional PCR (Table 1), hydrolysis detection probes, and optimal real time PCR conditions to detect and quantify total (*r72h* fragment) and pathogenic (*tdh* and *trh* genes) *V. parahaemolyticus*, as described by Robert Pillot *et al.* (2014).

Preparation of the DNA reaction mix

The 192 DNA extracts were distributed across three plates, with 64 samples per plate. Only the *r72 h* positive samples were used to search for the *tdh* and *trh* virulence genes.

Amplification by RT-PCR

Before transferring the plate to the Applied Biosystem 7 500 thermal cycler, the contents of the wells were homogenized in a refrigerated centrifuge. The data for the samples to be tested were then entered in a way that recorded the number of each well on the plate in order to ensure the traceability of the results, using the 7 500 system software installed on the computer linked to the thermal cycler. The volumes and concentrations of the reaction mixture components vary according to the gene tested and are calculated according to the instructions supplied with the kit. Each experiment included DNA dilutions of a positive control strain of *V. parahaemolyticus* (strain CNRVC010089 of the National Reference Center for Vibrios and Cholera, Institut Pasteur, Paris, France), which possesses the three target sequences (R72H fragment, *tdh* and *trh* genes), as well as two wells for negative controls and a well containing the reaction control mixture without DNA.

Results

Comparative performance of the cultural method and RT PCR for V. parahaemolyticus

The results are presented in Table 2. While the cultural method associated with conventional PCR detected only 12 positive samples out of 192 (6.25%), the RT PCR method detected an additional 17 positive samples, bringing the total to 29 (15.10%).

		Number of positive samples for *V. parahaemolyticus*	
Number of analyzed samples	Methods used	Molecular factor of species identification *r72h*	Molecular virulence factors *tdh/trh*
192	Cultural	12 (6.25%)	0 (0%)
	RT-PCR	29 (15.10%)	0 (0%)

Table 2. *Comparison between the cultural method and RT PCR in the detection of* V. parahaemolyticus

This result confirms the high performance of the RT PCR method, which is more sensitive, detecting low densities (5 CFU/g) of *V. parahaemolyticus* present in shrimps (Robert-Pillot *et al.*, 2010), and which offers the additional advantage of a shorter processing time (a few hours instead of a few days for culture). Tanxi *et al.* (2006) demonstrated that, out of 300 seafood samples analyzed, the detection of *V. parahaemolyticus* was more effective using RT PCR than the conventional cultural method, with 97 (32.3%) and 78 (26%) positive samples, respectively.

Only the samples positive for *V. parahaemolyticus* species, i.e. those with a Ct (Cycle threshold) value below 35 (Figure 3), were analyzed for the *trh* and *tdh* virulence genes. All the 29 positive samples were negative for these two genes.

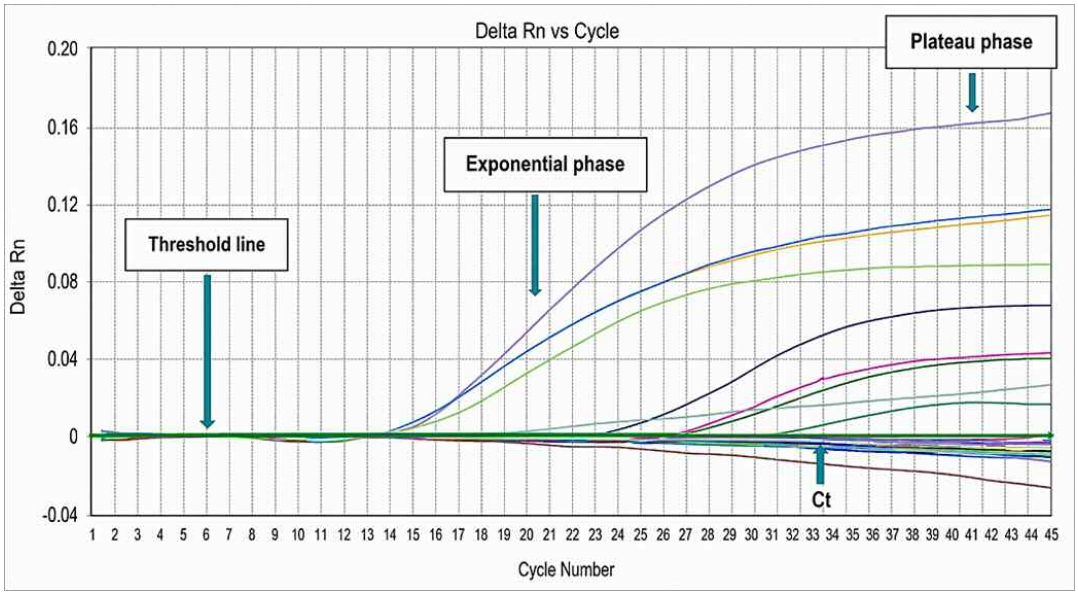

Figure 3. *Fluorescence emitted during amplification of the* r72h *gene by RT PCR*

All samples positive by the cultural method were positive with RT PCR. Of the 17 supplementary samples that tested positive using RT PCR, all tested negative using the cultural method, as none of them developed the purple colonies characteristic of *V. parahaemolyticus* on CHROMagar *Vibrio*. However, *V. parahaemolyticus* was shown to develop colorless colonies in this medium, which are usually associated with *V. alginolyticus*, and were therefore not studied during the cultural steps. Similarly, Gopal *et al.* (2005) reported several atypical biochemical characteristics associated with the *V. parahaemolyticus* species, and it is a general consensus that the study of cultural and biochemical characteristics is not always suitable for the identification of *Vibrio* strains isolated from the environment, due to the frequency of atypical biochemical characteristics (Dodin & Fournier 1992; Alsina & Blanch, 1994). Moreover, dormant or quiescent forms, corresponding to a viable but non culturable state, have also been reported for *Vibrio* (Rosec *et al.*, 2009; Aertsen & Michiels 2004; Wade 2004), which limits detection by culture methods in favor of PCR methods that directly target DNA.

Seasonality: total positive samples

The results show that 116 samples out of 192 (60.4%) were positive for *Vibrio* spp., of which 4/192 (2.08%) were positive for *V. cholerae*, 29/192 (15.10%) for *V. parahaemolyticus*, and 101/192 (52.60%) for *V. alginolyticus* (Table 3). In 21 samples, *V. parahaemolyticus* and *V. alginolyticus* were detected simultaneously, and in 4 other samples, the 3 species were isolated together.

Seasons	Number of analyzed samples	Samples positive for *Vibrio* spp. (%)	Number of positive samples for (%)		
			V. cholerae	*V. parahaemolyticus*	*V. alginolyticus*
Winter	34	11 (32.35%)	0 (0%)	3 (8.82%)	11 (32.35%)
Spring	52	17 (32.69%)	0 (0%)	5 (9,61%)	14 (26.92%)
Summer	65	56 (86.15%)	3 (4.62%)	12 (18.46%)	51 (78.46%)
Autumn	41	32 (78.05%)	1 (2.44%)	9 (21.95%)	25 (60.97%)
Total	192	116 (60.41 %)	4 (2.08%)	29 (15.10%)	101 (52.60%)

Table 3. *Incidence of* Vibrio *spp. in the analyzed samples*

Monitoring the evolution of the identified species confirms the seasonal variation in prevalence. Positive samples for *Vibrio* spp. were 56 out of 65 (86.15%) in summer, compared to 11/34 (32.35%) in winter. Regarding *V. parahaemolyticus*, samples containing this pathogen represented 3/34 (8.82%) in winter and 12/65 (18.46%) in summer (Table 3).

Using the cultural method, *V. alginolyticus* was isolated from 101 samples. The high presence of this species in the analyzed samples confirms that it is part of the native shrimp flora (Hosseini *et al.*, 2004). Its prevalence in Morocco is over 50% in both shellfish (mussels, oysters, and clams) (Bouchriti *et al.*, 1995) and shrimp (Kriem *et al.*, 2015).

Discussion and Conclusion

The results confirm the seasonal occurrence of *Vibrio* bacteria in marine environments and seafood products, primarily in relation to water temperature, as previously demonstrated (Deepanjali *et al.*, 2005; Ferchichi *et al.*, 2021; Fleischmann *et al.*, 2022). The influence of temperature on the seasonal prevalence of *V. parahaemolyticus* was first highlighted by Kaneto & Colwell (1973) in their study of the ecology of this pathogen in the Chesapeake Bay. They demonstrated that, at temperatures below 14°C, *V. parahaemolyticus* is no longer present in the water column because it nests in sediments, which act as ecological niches. Once the environmental temperature rises to 19 °C or above, the pathogen leaves the sediments and adheres to the zooplankton, allowing it to be isolated from the water column once again. Similarly, De Paola *et al.* (2003) observed that higher densities of *V. parahaemolyticus* were associated with temperatures above 20 °C and that the abundance of this pathogen was much more affected by temperature than by salinity. Previously, the same causal link was observed for *V. vulnificus* density levels, using oysters from the Gulf of Mexico as a matrix (Motes & De Paola, 1996). In tropical areas, where marine temperatures are consistently higher, *V. parahaemolyticus* is detected, with salinity being a major influencing factor (Deepanjali *et al.*, 2005). The high prevalence in summer coincides with the occurrence of *V. parahaemolyticus* gastroenteritis episodes during the warmer months (Bisha *et al.*, 2012). In recent years, the incidence of vibriosis has increased globally, likely due to sea warming caused by global climate change (Baker-Austin *et al.*, 2018).

The search for genes encoding the hemolysins TDH and TRH was undertaken to identify potentially pathogenic *V. parahaemolyticus* strains. It is well established that there is a strong correlation between the possession of these hemolysins and the ability of *V. parahaemolyticus* to cause severe gastroenteritis (Robert-Pillot *et al.*, 2004). The results of our study were negative (**Table 2**), which is consistent with those of other studies reporting a low prevalence of pathogenic strains in environmental populations (0–10%) compared with clinical populations, where the vast majority (up to 95%) possess hemolysins (Karunasagar & Karunasagar, 2018). However, the results of this study, carried out for the first time in Morocco on the prevalence of *V. parahaemolyticus*, a pathogen considered emerging, confirm its ubiquitous distribution in the marine environment and suggest that Morocco may be concerned by the risk associated with this pathogen. Assessing prevalence based solely on the presence or absence of *V. parahaemolyticus*, particularly using the cultural method, limits the value of the results for quantitative risk assessment (FAO, 2011). Therefore, it is necessary to refine surveillance using the RT PCR method, which has been proved to be more effective than conventional methods in terms of detection and quantification, and should be used instead of culture techniques thanks to the many advantages it offers (greater sensitivity and specificity, and detection of potentially pathogenic *V. parahaemolyticus*).

The choice of shrimp as the matrix for this study is justified by the fact that this fishery product is characterized by numerous anfractuosities and ciliated appendages that line the body and act as real microbial breeding grounds (Bhaskar *et al.*, 1998). This makes shrimp an appropriate matrix for biomonitoring studies of vibrios in fishery products. Given the seasonal prevalence, investigations should not be limited to a single matrix (in this case, shrimp), but should also be carried out in water and, most importantly, in sediments, which act as real breeding grounds during cold periods. Sediments offer an additional advantage because their *V. parahaemolyticus* densities are 200 times higher than in water, making them a useful matrix for a pathogen monitoring program (FAO, 2011). Finally, analyzing the physicochemical characteristics of the marine environment (temperature, salinity, dissolved oxygen, etc.) can provide more robust data on factors directly impacting the prevalence of *V. parahaemolyticus*, thereby improving long term monitoring.

Acknowledgements

We thank the National Reference Center for Vibrios and Cholera at the Institut Pasteur, Paris, France, and Marie-Laure Quilici for their support. This work has been conducted with the financial support of the PRAD projects 07/09.

Conflicts of interest

The authors declare they have no conflict of interest.

References

- Aertsen, A., & Michiels, C. W. (2004). Stress and how bacteria cope with death and survival. *Critical Reviews in Microbiology*, 30(4), 263-273.
- Alsina, M., & Blanch, A. R. (1994). A set of keys for biochemical identification of environmental *Vibrio* species. *Journal of Applied Bacteriology*, 76, 79-85.
- Baker-Austin, C., Stockley, L., Rangdale, R., & Martinez-Urtaza, J. (2010). Environmental occurrence and clinical impact of *Vibrio vulnificus* and *Vibrio parahaemolyticus*: A European perspective. *Environmental Microbiology Reports*, 2(1), 7-18.
- Baker-Austin, C., Oliver, J. D., Alam, M., Ali, A., Waldor, M. K., Qadri, F., & Martinez-Urtaza, J. (2018). *Vibrio* spp. infections. *Nature Reviews Disease Primers*, 4(1), 8.
- Bej, A. K., Patterson, D. P., Brasher, C. W., Vickery, M. C. L., Jones, D. D., & Kaysner, C. A. (1999). Detection of total and hemolysin-producing *Vibrio parahaemolyticus* in shellfish using multiplex PCR amplification of *tdh* and *trh*. *Journal of Microbiological Methods*, 36, 215-225.
- Bhashkar, N., Setty, T. M. R., Mondal, S., Joseph, M. A., Raju, C. V., Raghunath, B. S., *et al.* (1998). Prevalence of bacteria of public health significance in the cultured shrimp (*Penaeus monodon*). *Food Microbiology*, 15, 511-519.
- Bisha, B., Simonsen, D., Janes, M., Bauman, K., & Goodridge, L. D. (2012). A review of the current status of cultural and rapid detection of *Vibrio parahaemolyticus*. *International Journal of Food Science & Technology*, 47, 855-899.
- Bouchriti, N., & El Marrakchi, A. (1995). Occurrence of marine vibrios in Moroccan coastal waters and shellfish. *Microbiologie, Aliments, Nutrition*, 13, 381-387.
- Castello, A., Alio, V., Sciortino, S., Oliveri, G., Cardamone, C., Butera, G., *et al.* (2022). Occurrence and molecular characterization of potentially pathogenic *Vibrio* spp. in seafood collected in Sicily. Microorganisms, 11(53).
- Chun, J., Huq, A., & Colwell, R. R. (1999). Analysis of 16S-23S rRNA intergenic spacer regions of *Vibrio cholerae* and *Vibrio mimicus*. *Applied and Environmental Microbiology*, 65(5), 2202-2208.
- Copin, S., Robert-Pillot, A., Gay, M., & Quilici, M.L. (2015) Vibrio impliqués en pathologie humaine : une étude de leur répartition dans les produits de la mer consommés en France. *Bulletin Épidémiologique*, 68, 21-25. pasteur-01162421
- Daniels, NA., MacKinnon, L., Bishop, R., Altekruse, L., Ray, B., & Hammond, RM. (2000). *Vibrio parahaemolyticus* infections in the United States, 1973-1998. *Journal of Infectious Diseases*, 181(5), 1661-1666.

• De Paola, A., Kaysner, C. A., Bowers, J. C., & Cook, D. W. (2000). Environmental investigations of *Vibrio parahaemolyticus* on oysters after outbreaks in Washington, Texas, and New York (1997 and 1998). *Applied and Environmental Microbiology*, 66(10), 4649-4654.
• De Paola, A., Nordstrom, J. L., Bowers, J. C., Wells, J. G., & Cook, D. W. (2003). Seasonal abundance of the total and pathogenic *Vibrio parahaemolyticus* in Alabama oysters. *Applied and Environmental Microbiology*, 69(3), 1521-1526.
• Deepanjali, A., Kumar, S., & Karunasagar, I. (2005). Seasonal variation in abundance of total and pathogenic Vibrio parahaemolyticus bacteria in oysters along the southwest coast of India. *Applied and Environmental Microbiology*, 71(6), 3575-3580.
• Dodin, A., & Fournier, J.M. (1992). *Méthodes de laboratoire pour le diagnostic du vibrion cholérique et des autres vibrions*. Institut Pasteur. ISBN 13: 978 2901320067
• FAO/WHO. (2011). *Risk assessment of* Vibrio parahaemolyticus *in seafood: Interpretative summary and Technical report*. Microbiological Risk Assessment Series No. 16. https://www.fao.org/4/i2225e/i2225e00.pdf
• Ferchichi, H., St-Hilaire, A., Ouarda, T. B. M. J., & Lévesque, B. (2021). Impact of the future coastal water temperature scenarios on the risk of potential growth of pathogenic Vibrio marine bacteria. *Estuarine, Coastal and Shelf Science*, 250,1-10.
• Fields, P. I., Popovic, T., Wachsmuth, K., & Olsvik, O. (1992). Use of polymerase chain reaction for detection of toxigenic *Vibrio cholerae* O1 strains from the Latin American cholera epidemic. *Journal of Clinical Microbiology*, 30, 2118-2121.
• Fleischmann, S., Herrig, I., Wesp, J., Stiedl, J., Reifferscheid, G., Strauch, *et al.* (2022). Prevalence and distribution of potentially human pathogenic *Vibrio* spp. on German North and Baltic Sea coasts. *Frontiers in Cellular and Infection Microbiology*, 12, 1-15.
• Fournier, J.M., & Quilici, M.L. (2007). Choléra. *La Presse Médicale*, 36, 727-739.
• Gopal, S., Otta, S. K., Karunasagar, I., Nishibuchi, M., & Karunasagar, I. (2005). The occurrence of *Vibrio* species in tropical shrimp culture environments: Implications for food safety. *International Journal of Food Microbiology*, 102(2), 151-159.
• Hara-Kudo, Y., Nishina, T., Nakagawa, H., Konuma, H., Hasegawa, J., & Kumagai, S. (2001). Improved method for detection of *Vibrio parahaemolyticus* in seafood. *Applied and Environmental Microbiology*, 67(12), 5819-5823.
• Heng, S. P., Letchumanan, V., Deng, C. Y., Ab Mutalib, N. S., Khan, T. M., & Chuah, L. H. (2017). *Vibrio vulnificus*: An environmental and clinical burden. *Frontiers in Microbiology*, 8, 997.
• Hosseini, H., Cheraghali, M., Yazdani, R., & Vadood, R. (2004). Incidence of *Vibrio* spp. in shrimp caught off the south coast of Iran. *Food Control*, 15, 187-190.
• Huss, H. H. (1993). *Assurance of seafood quality*. FAO Fisheries Technical Paper No. 334. ISBN: 92 5 103446 X
• Huss, H. H., Ababouch, L., & Gram, L. (2003). *Assessment and management of seafood safety and quality*. FAO Fisheries Technical Paper. No. 444. ISBN: 92 5 104954 8
• ISO 21872 1. (2017). *Microbiologie de la chaîne alimentaire-Méthode horizontale pour la détermination des* Vibrio *spp. - Partie 1: Recherche des espèces de* Vibrio parahaemolyticus, Vibrio cholerae et Vibrio vulnificus *potentiellement entéropathogènes*. https://www.iso.org/fr/standard/74112.html
• Kaneko, T., & Colwell, R. R. (1973). Ecology of *Vibrio parahaemolyticus* in Chesapeake Bay. *Journal of Bacteriology*, 113(1), 24-32.
• Karunasagar, I., & Karunasagar, I. (2018). Ecology, virulence factors and global spread of *Vibrio parahaemolyticus*. *Asian Fisheries Science*, 31S, 15-28.
• Kriem, M. R., Banni, B., El Bouchtaoui, A., Hamama, A., El Marrakchi, A., Chaouqy, N., *et al.* (2015). Prevalence of *Vibrio* spp. in raw shrimps (*Parapenaeus longirostris*) and performance of a chromogenic medium for the isolation of *Vibrio* strains. *Letters in Applied Microbiology*, 61(3), 224-230.
• Lee, C. Y., Pan, S. F., & Chen, C. H. (1995). Sequence of a cloned pR72H fragment and its use for detection of *Vibrio parahaemolyticus* in shellfish with the PCR. *Applied and Environmental Microbiology*, 61(4), 1311-1317.
• Lemoine, T., Germanetto, P, & Giraud, P. (1999). Toxi-infection alimentaire collective à *Vibrio parahaemolyticus*. *Bulletin épidémiologique hebdomadaire*, 10, 37-38.
• LPSN. (2020). *List of Prokaryotic names with Standing in Nomenclature*. Accessed on March 23, 2026, at https://lpsn.dsmz.de/text/introduction
• Motes, M. L., & De Paola, A. (1996). Offshore suspension relay to reduce levels of *Vibrio vulnificus* in oysters (*Crassostrea virginica*). *Applied and Environmental Microbiology*, 62(10), 3875–3877.
• O'Hara, C. M., Sowers, E. G., Bopp, C. A., Duda, S. B., & Strockbine, N. A. (2003). Accuracy of six commercially available systems for identification of members of the family *Vibrionaceae*. *Journal of Clinical Microbiology*, 41(12), 5654–5659.
• Olsvik, O., Wahlberg, J., Petterson, B., Uhlen, M., Popovic, T., & Wachsmuth, K. (1993). Use of automated sequencing of polymerase chain reaction-generated products to identify types of cholera toxin subunit B in Vibrio cholerae O1 strains. *Journal of Clinical Microbiology*, 31(1), 22–25.
• Pan, T. M., Wang, T. K., Lee, C. L., Chien, S. W., & Horn, C. B. (1997). Foodborne disease outbreaks due to bacteria in Taiwan, 1986 to 1995. *Journal of Clinical Microbiology*, 35(5), 1260–1262.
• Quilici, M.L., & Robert-Pillot, A. (2011). Infections à vibrions non cholériques. In *Maladies infectieuses* (8-026-F-15). Elsevier (EMC). Accessed on March 23, 2026, at https://www.em-consulte.com/article/277344/infections-a-vibrions-non-choleriques
• Robert-Pillot, A., Guénolé, A., & Fournier, J. M. (2002). Usefulness of R72H PCR assay for differentiation between *Vibrio parahaemolyticus* and *Vibrio alginolyticus* species: Validation by DNA-DNA hybridization. *FEMS Microbiology Letters*, 215(1), 1-6.
• Robert-Pillot, A., Guénolé, A., Lesne, J., Delesmont, R.,

Fournier, J. M., & Quilici, M. L. (2004). Occurrence of the tdh and trh genes in *Vibrio parahaemolyticus* isolates from waters and raw shellfish collected in two French coastal areas and from seafood imported into France. *International Journal of Food Microbiology*, 91(3), 319-325.
• Robert-Pillot, A., Copin, S., Gay, M., Malle, P., & Quilici, M. L. (2010). Total and pathogenic *Vibrio parahaemolyticus* in shrimp: Fast and reliable quantification by real-time PCR. *International Journal of Food Microbiology*, 143(2), 190-197.
• Robert-Pillot, A., Copin, S., Himber, C., Gay, M., & Quilici, M. L. (2014). Occurrence of the three major *Vibrio* species pathogenic for humans in seafood products consumed in France using real-time PCR. *International Journal of Food Microbiology*, 189, 75-81.
• Rosec, J. P., Simon, M., Causse, V., & Boudjemaa, M. (2009). Detection of total and pathogenic *Vibrio parahaemolyticus* in shellfish: Comparison of PCR protocols using pR72H or toxR targets with a culture method. *International Journal of Food Microbiology*, 129, 136-145.
• Su, Y. C., & Liu, C. (2007). *Vibrio parahaemolyticus*: A concern of seafood safety. *Food Microbiology*, 24, 549-558.
• Tanxi, C., Luyan, J., Chengbo, Y., & Kehe, H. (2006). Application of real-time PCR for quantitative detection of *Vibrio parahaemolyticus* from seafood in eastern China. FEMS *Immunology & Medical Microbiology*, 46, 180-186.
• Vezzulli, L., Brettar, I., Pezzati, E., Reid, P. C., Colwell, R. R., Pezzuti, G., & Höfle, M. G. (2012). Long-term effects of ocean warming on the prokaryotic community: Evidence from the vibrios. *ISME Journal*, 6(1), 21-30.
• Wade, W. G. (2004). Non-culturable bacteria in complex commensal populations. Advances in *Applied Microbiology*, 54, 93-106.
• Yukiko, H. K., Tokuhiro, N., Hiroshi, N., Hirotaka, K., Junko, H., & Susumu, K. (2001). Improved method for detection of *Vibrio parahaemolyticus* in seafood. *Applied and Environmental Microbiology*, 67(12), 5819-5823.