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Identification of *Vibrio parahaemolyticus* by PCR Method in Fish and Water Samples from Tigris River, Turkey

Filiz ÖZCAN[1,a,*], Neval Berrin ARSERİM[2,b], Muzaffer Aydın KETANİ[3,c]

[1]Dicle University,Veterinary Faculty, Fisheries and Fisheries Diseases Department, Diyarbakır, Turkey .
[2]Dicle University, Veterinary Faculty**,** Department of Microbiology, Diyarbakır, Turkey.
[3]Dicle University, Veterinary Faculty**,** Department of Histology and Embryology, Diyarbakır, Turkey.
[a]ORCİD: 0000-0003-4767-9893; [b]ORCİD: 0000-0002-7097-0091; [c]ORCİD:0000-0002-1546-9747

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Abstract: Climate change has caused increasing *Vibrio parahaemolyticus* infections in areas unaffected by this pathogen. Water temperature is an important ecological factor. It is consequently of high importance to follow *V. parahaemolyticus* in aquatic environments and foods. The aim of this work was to detect the presence of *V. parahaemolyticus* in Tigris River from eight different sites namely Malabadi Bridge, Fiskaya, Ongözlü Bridge, Bağıvar, Pamukçay, Ambarçay, Seyrantepe and Silvan. Total 480 samples of water and fish were used for *V. parahaemolyticus* detection. Thiosulphatecitrate-bile salt sucrose (TCBS) Agar was employed for bacteria isolation for 24 h at 37◦C. The dominant uniform bacterial colonies were purified by streaking onto the TCBS agar plates three times. Identification of this pathogen was made by PCR. A total of 480 water and fish samples were analyzed. According to the results, *V. parahaemolyticus* was detected in 40 (6.6%) fish samples taken from the Seyrantepe region.
Keywords: *Fish, PCR, Vibrio parahaemolyticus, Water.*

Dicle Nehrindeki Balık ve Su Örneklerinde *Vibrio parahaemolyticus*'un PCR Yöntemi ile İdentifikasyonu

Özet: İklim değişikliğinin bir sonucu olarak artan sayıda *Vibrio parahaemolyticus* enfeksiyonu tespit edilmiştir. Su sıcaklığı çok güçlü bir ekolojik faktördür. Dolayısıyla su ortamlarında ve su ürünlerinde *V. parahaemolyticus'u* takip etmek önem arzetmektedir. Bu çalışmanın amacı, Dicle nehrinde bulunan Malabadi Köprüsü, Fiskaya, Ongözlü Köprüsü, Bağıvar, Pamukçay, Ambarçay, Seyrantepe ve Silvan olmak üzere sekiz farklı yerdeki *Vibrio parahaemolyticus*'un varlığını araştırmaktır. Bu amaçla toplam 480 su ve balık numunesi kullanılmıştır. Bakteri izolasyonu için örnekler Tiyosülfatsitrat-safra tuzu sukroz (TCBS) Agara ekim yapılmış, 37°C'de 24 saat inkübe edilmiştir ve bakteri kolonileri, TCBS agar plakalarına üç kez sürme yoluyla saflaştırılmıştır. Patojenin kesin tespiti PCR testi ile gerçekleştirilmiştir. Araştırmanın sonucunda Seyrantepe'deki 40 (6.6%) adet balık örneğinde patojenin varlığı tespit edilmiştir.
Anahtar kelimeler: *Balık, PCR, Su, Vibrio parahaemolyticus.*

Introduction

Infections with Vibrio spp. have increased worldwide by ongoing climate change (Logar-Henderson et al., 2019) *Vibrio parahaemolyticus* is a Gram-negative bacterium that inhabits estuarine and marine waters and grows in various temperatures. The highest density of this organism occurs when the water temperature is 20–30°C (Givens et al., 2014). The organism is a human pathogen and can cause gastroenteritis and septicemia because of consuming a variety of raw sea food (Constantin de Magny et al., 2009). This disease is usually observed in immunocompromised individuals (Froelich and Daines 2020).The surface temperature of rivers and estuaries has increased more quickly due to climate change than that of oceans (EEA 2012) High surface temperature in water provides optimum environmental growth conditions for Vibrio species (Vezzulli et al., 2013) The number of Vibrio cases has been found to rise in line with increased water surface temperature (Semenza et al., 2017). Water temperature is an essential part of chemical and biochemical processes in aquatic ecosystems. The Tigris River is one of the largest rivers in the Middle East and Diyarbakır is the greatest city and the largest urban place in the Tigris Basin in Turkey (Murib et al., 2017) It has been progressively developing through the southeastern Anatolia Project (GAP), and is one of the most important industry, agriculture and animal husbandry centers in the Tigris Basin.The Tigris River is an important water source for the city (Varol et al., 2010) Therefore, we felt the need to investigate of *Vibrio parahaemolyticus* of Tigris River in Diyarbakır. Accordingly, the present study was undertaken to identify *V.parahaemolyticus* and to assess.

Material and Methods

Sample Collection: This study was conducted in Turkey on Diyarbakır. Four hundred and eighty water and fish samples were obtained from selected eight areas along the Tigris River in Diyarbakır. The analyzed fish species were *Cyprinion macrostomum*, *Alburnus mossulensis*, *Capoeta trutta*, *Capoeta umbla*, *Garra variabilis* and *Garra rufa* . Fish weight was between 70 gr and 190 gr. Fish meat was analyzed. The water samples were collected from the water surface into sterile glass bottles. Fish samples were collected with a fishing net and transported to the laboratory in the ice box for analysis within two hours of collection. Laboratory work was conducted in the Department of Veterinary Medicine and Bioeks the STI R & D Technologies Ltd., Turkey.

Figure 1. Eight sampled points in Tigris River.

RINA ™ M14 for nucleic acid (NA) extraction from samples Nucleic Acid Isolation Kit (Cat No: RN-NA-14-120-192) and RINA ™ M14Nucleic Acid Extraction Robot (Cat No: RN-NA-302-100)) (Bioeks the R & D Technologies Ltd. Sti., Turkey) were used. Fish meat samples were homogenized by vortexing with four different chemicals. The homogenate (550 µL) was loaded into the robot. The water samples (550 µL) were loaded directly into the robot. A robot was loaded molecular-scale water (550 µL) as a negative control. Bio-peedy®vibrioparahaemolyticus Real-Time PCR Detection Kit (Cat No. BS-DTC-121-100) (Bioeks the STI R & D Technologies Ltd., Turkey) vecobas® TaqMan® 48 Analyzer (Roche Diagnostics, USA) were used. With COBAS® TaqMan® 48 Analyzer Software, threshold cycle numbers (Cq) were calculated and the shape of the propagation curves was examined. Non-sigmoidal curves were recorded as negative . Nucleic acid extraction was successfully recorded for reactions with IC threshold cycle number Cq <34. 37≤Cq is negative; A positive result was recorded if Cq <37. Samples that gave negative results in negative control reaction and positive results in *Vibrio parahaemolyticus* reaction were evaluated as *Vibrio parahaemolyticus* positive and the following results were obtained (THSK, 2019).

Results

A Total of 480 water and fish samples were analyzed (Fig. 2,3) According to the results *V. parahaemolyticus* was detected in 40 (6.6. %) fish samples taken from the Seyrantepe region (Table 1). The upwardly increased sigmoidal curve seen in Fig. 2 showed the sample of fish taken from Seyrantepe. It was an indication that *V. parahaemolyticus* was found in this sample.

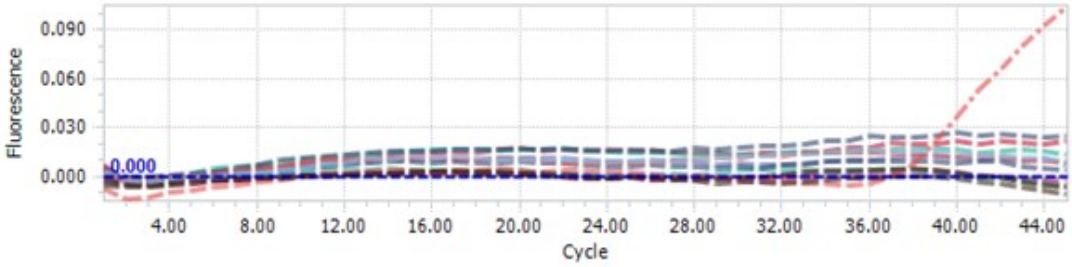

Figure 2. Growth curves were obtained from the tested samples. The sigmoidal curve show in the above graph yielded obtained from the Seyrantepe locality fish sample (40.92 Cq).

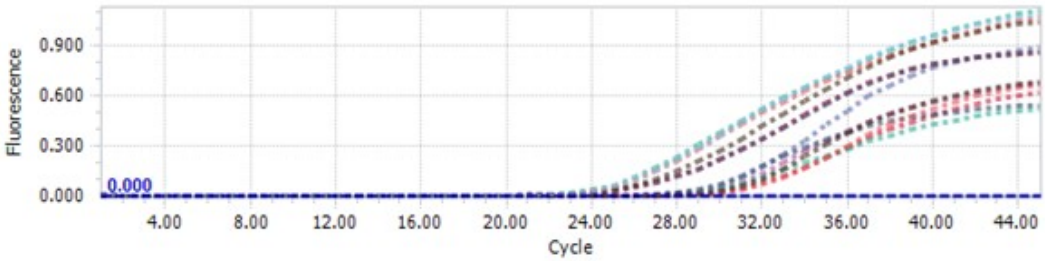

Figure 3. Internal control proliferation curves were obtained from the tested samples.

Table 1. Threshold cycle numbers (Cq) and test result of tested samples.

Sample	IC Cq	*V.parahaemolyticus* Cq	Extraction	V.parahaemolyticus
Negative Control	23.55	-	Successful	Negative
Ambarçay Water	25.46	-	Successful	Negative
Malabadi Bridge Water	24.86	-	Successful	Negative
Fiskaya Water	24.72	-	Successful	Negative
Fiskaya Fish	27.43	-	Successful	Negative
Pamukçay Water	25.13	-	Successful	Negative
Pamukçay Fish	26.14	-	Successful	Negative
Bağıvar Water	25.01	-	Successful	Negative
Bağıvar Fish	27.77	-	Successful	Negative
Silvan Water	25.08	-	Successful	Negative
Silvan Fish	26.93	-	Successful	Negative
Ongözlü Bridge Water	24.27	-	Successful	Negative
Ongözlü Bridge Fish	28.01	-	Successful	Negative
Seyrantepe Water	24.59	-	Successful	Negative
Seyrantepe Fish	27.75	40.92	UnSuccessful	Pozitive

Discussion and Conclusion

V. parahaemolyticus is a natural inhabitant in estuarine and marine environments worldwide (Mok et al., 2019a). Over the years, the epidemic of *V. parahaemolyticus* infections has been a significant public health problem for the number of its infections has increased in many countries (Hemmat et al., 2018). It has been observed in Chile, the United States, Asia, Europe and Peru during the last decades. These epidemics were linked to gradual water temperature increases (Velazquez-Roman et al., 2014). Various studies have shown that environmental factors such as temperature, turbidity, amounts of organic matter, water salinity cause *V. parahaemolyticus* infections (Siddique et al., 2021) *V. parahaemolyticus* causes at minimum 30000 infections per year most of them are gastroenteritis (Xie et al., 2015). The highest densities of this organism occur when the water temperature is 20–30°C (DePaola et al., 2003). *V. parahaemolyticus,* widely dispersed in estuarial and coastal marine waters has been detected from water, sediment, and different species of fish (Federici et al., 2018) The present results showed that *V. parahaemolyticus* was detected in fish samples (6.6%) taken from the Seyrantepe region (Table 1, Figure 2). The water temperature in this area was 32°C. These results suggest that it is correlated to water temperature as reported by other researchers (Adesiyan et al., 2021). Hemmat et al. (2018) reported that *V. parahemolyticus* spp. isolated from the examined fish samples were 8% and 12% for freshwater and marine water fish respectively. Conversely, Sanjeev (2002) informed

that the rate of *V. parahaemolyticus* in fresh, marine and brackish water fish varied from 35 to 55%. Terentjeva et al. (2015), said that Vibrio species could be found on the skin, chitinous shell, gills, intestinal tracts, kidney, and liver of fish or shellfish. Besides, the resource of the contamination may be human feces or sewerage Martin et al. (2004) stated that 24 % of catfish and 40 % of red tilapia samples were infected with *V. parahaemolyticus* in work from Malaysia (Noorlis et al., 2011). On the other hand, from water samples *V. parahaemolyticus* was detected in 15.6% (Guin et al., 2019). In the presented study, it was not detected in water samples. The reason for this may be salinity, stream, and depth. These water characteristics also vary temporally and spatially throughout the river. *V. parahaemolyticus* is positively connected with water temperature, negatively associated with water clarity (Kwit et al., 2019). For these reasons, it may not have been detected in water samples. Temperature, season, salinity, methods of identification, study area, source of samplesand temperature during storage or even transpo-rtation may affect the existence of *V. parahaeomolyticus*. Rivers are the most important freshwater resource for humans, plants and animals. Water is contaminated by industrial waste, sewerage, and human activities. These bacteria, which are closely related to human health, should be detected regularly. Ahmed et al. (2018) reported that from fresh water fish and human stool were detected in 5% and 3%. Many researchers have been studied to define the relationship between *V. paraheomolyticus* existence and environmental factors like temperature, dissolved oxygen, salinity and nutrients. Consequently, these water quality properties can be used in a predictive way to define when this pathogen may be present (Gayatri, 2011). In this study, the presence of *V. parahaemolyticus* was determined and its importance for public health was emphasized. In addition, it has been concluded that by carrying out these studies regularly, poisoning from these bacteria can be prevented, and the public should be informed.

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

FÖ and NBA planned, designed the research.The manuscript was written by FÖ and NBA.

All authors have interpreted the data, revised the manuscript for contents, and approved the final version.

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[*]**Correspondence:** Filiz ÖZCAN
Dicle University,Veterinary Faculty, Fisheries and Fisheries Diseases Department, Diyarbakır, Turkey
e-mail: filiz.ozcan@dicle.edu.tr