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Vibrio parahaemolyticus in Sea Food in Northern Taiwan

Intmduction

Vibrio parahaemolyticus is one of the major pathogenic agents of bacterial gastroenteritis. They are more prevalent in the warm months[1,2]. In coastal countries such as Taiwan, Japan, the south-east Asian countries, the United Kingdom, the Netherlands and the United States, they are a common pathogenic agent of food poisoning[1,3-10]. The first incident of Vibrio parahaemolyticus-induced food poisoning was reported in 1950 in Osaka, Japan. It was a case of collective poisoning involving 272 victims after they had eaten fish contaminated by the pathogen. Of them, 20 had died of acute gastroenteritis. In 1953, Japanese scholars Fujino et al. succeeded in isolating Vibrio parahaemolyticus from feces of patients and cooked sardine. In 1963, the agent was named Vibrio parahaemolyticus by Sakazaki et a1[11].

Vibrio parahaemolyticus is gram-negative, straight or curved, highly active and halophilic bacterium (they grow in high concentration saline water, in 1-8% salt concentration though better under 3% concentration). They do not produce spores. They carry monopolar flagella. They grow better under 35° to 37 ℃, though can survive under 10° to 44℃. Growth is inhibited under pH values either lower than 5.0 or higher than 11.0. They are more often found in sea waters parasitizing on shellfish and fi5h¡¦23. Under favorable conditions, the number of pathogen can duplicate in 12 to 18 minutes[14]. Vibrio parahaernolyticus is more often found along off-shore sea waters and in sediment, suspensions, planktons and shellfish In spring and summertime, they parasitize on shellfish; in winter, they live in the sediment of sea water. With the floating of sediments, they can circulate again[15].

Vibrio parahaemolyticus has three antigens: somatic antigen O, capsular antigen K and flagellar antigen H. The specificities of H antigen are yet unknown, it therefore is not used in typing. 0 antigen and K antigen are known to be related. A strain containing certain K antigen also contains certain 0 antigen. Therefore, in practice, only K antigen and not 0 antigen is used for typing. Currently, there are 13 known 0 antigens and 65K serotypes.

Vibrio parahaenzolyticus K8 is more common in Taiwan's food poisoning outbreaks[17]. The "black Friday food poisoning outbreak" on 13 October 1995, Friday, involving 2,175 students of seven primary schools in Taipei City and County was caused by Vibrio parahaemolyticus K12[18]. There are sometimes simultaneous cross infections of Vibrio parahaernolvticus of various serotypes. The food poisoning incident at a country fair in Putai of Chiayi County in late October 1995 involved K8, K19, K21, K29 and K41 serotypes.

In the United States, Vibrio parahaemolyticus-induced food poisonings occur more often between May and November, and less so in winter. In Japan, they occur more often between June and October, particularly between August and September. In Taiwan, as in the US and Japan, more cases are reported between May and November[15] .

More cases in the US are caused by crustacean food, and some by food indirectly contaminated, plates and utensils contaminated by carrier sea food or during the process of food processing, for instance. Inadequate processing during cooking or eating contaminated sea food raw can result in food poisoning[19].

The pathogenicity of Vibrio parahaernolyticus is associated with hemolysin that causes β-hemolysis in human hemoglobin. Hemolysin is a heat-stable protein. The Kanagawa phenomenon refers to hemolysis by Vibrio parahaemolyticus on Wagatsuma agar that contains either human or rabbit blood. Experiment findings showed that no infection occurred when 10^{10} Kanagawa phenomenon negative Vibrio parahaenzolyticus were taken; however, diarrhea occurred when $2x10^5$-$3x10^7$ Kanagawa phenomenon positive Vibrio parahaemolyticus were eaten. Of the 2,720 strains of Vibrio parahaernolyticus isolated from the diarrheal patients, 96% were Kanagawa phenomenon positive; however, of the 650 strains isolated from sea fish, only 1% was Kanagawa phenomenon positive. These findings showed that the pathogenicity of Vibrio parahaemolyticus was significantly associated with hemo1ysin[20].

Zen-Yoji et al.[21] maintained that individuals who processed or cooked sea food more often would have more chances of eating sea food, they were more likely to be carriers of Vibrio parahaernolyticus, though they might not become ill. Zen-Yoji et al. examined 200 sushi cooks of no enteritis symptoms to find Vibrio parahaernolyticus in 14 (7%) of them. This rate was nine times higher than what would normally be found in ordinary people. The finding indicated that Vibrio parahaernolyticu.c could be transferred by cooks to customers (secondary infection). Peffers et al.[22] noted that the temperature at the central part of crab meat was only 63℃ even after 15 minutes of boiling. Special care should be taken in cooking.

Statistics in Japan for 25 years (1961-1984) show that the number of deaths from Vibrio parahaernolytieus poisoning accounted for 30% of all bacterial deaths. It is fortunate that there has not had any death case yet in Taiwan. One should, however, stay alert.

There had been many food poisoning cases in Taiwan in 1997. There were on one day eight incidents of food poisoning. By September, there had been a recordhigh 200 food poisoning outbreaks. Particularly, more people switched to sea food after the outbreak of the foot-and-mouth disease. The food poisoning outbreaks in May for instance, with the exception of the one involving security guards of the Chungshan Hall which was caused by Staphylococcus aureus, the rest were caused by Vibrio parahaemolyticus. In Japan, also an island state, where the consumption of sea food is higher, Vibrio parahaemolyticus-induced food poisoning incidents account for more than a half of all accountable food poisoning incidents. It is also a major cause of sporadic diarrhea in summer.

To understand how the sea food on market was contaminated by Vibrio parahaemolyticus, sea food were bought from the Keelung area for investigation. As sea food are often kept live in water tanks for customers to choose, water specimens from water tanks were also collected for laboratory testings.

Materials and Method

1. Sea Food

   On 6 August 1997, sea food such as shrimps, crabs, shellfish and fish of all kinds were bought from three fish markets in the Keelung area of northern Taiwan: the Cheng-ping wholesale market, the Pa-tou-tze retail market, and the Ho-ping island retail market. As the sea food at the Cheng-ping market were frozen, sterilized gauze dipped in saline water was used to swab the skin surface of the fish. The gauze was then placed in 20 mL culture fluid with polymyxin. Specimens in cold storage and polymyxin-contained specimens under room temperature were sent for laboratory testings.

2. Isolation of Strains

   About 50 g each of the specimens was placed in peptone water with 4% NaCl or alkaline peptone water under 37℃ for multiplication for 15-18 hours. One on two loops of colony was then taken and swabbed on Thiosulfate-Citrate-Bile Salt Sucrose (TCBS) agar. After isolation, the specimen was placed for culture under 37 ℃ for 18-24 hours[23].

3. Identification of Strains

   Colonies on TCBS were observed. Some green colonies were transferred onto TSI (Triple-Sugar-Iron agar), LIA (Lysine-Iron agar), SIM (Sulfide-Indol-Motility medium) or LIM (Lysine-Indol-Motility medium) under 37 ¢XC for 18-24 hours to observe their biochemical reactions. When colonies on TSI: did not decompose sucrose (the red slant), decomposed glucose (the upper yellow), did not produce gas, did not produce sulfur hydride, and showed Lysine (+), Indol (+), Motility (+), Oxidase (＋), they were placed in alkaline peptone water with 0%, 3%, 8% and 10% NaCl under 37℃ for 18-24 hours to observe their salt tolerance. Vibrio parahaernolyticus can grow in peptone water with 6% and 8% NaCl; they cannot grow in peptone water with 0% and 10% NaCI. Bio-tests No 1 and No 2 of the Eikenshia, Japan, can be used for biochemical reactions. They can be identified with code numbers[23].

For the testing of heat-resistant hemolysin, strains were planted on agars containing 5% human or rabbit blood under 37℃ for 48 hours to see if there was any Kanagawa phenomenon. Qualitative and quantitative analyses could also be done with the KAP-RPLA method developed by the Seikenshia of Japan. This method was used in the present study[23].

4. Identification of Serotypes

Some saline water was added to the colonies on TSI to make antigen fluid. First, use Vibrio parahaenzolyticus anti-K serum to induce on slide agglutination reactions by multivalence anti-serum. Then, use monovalence antiserum of each group for agglutination reactions to decide their K serotypes[23].

Results and Discussion

1. Vibrio parahaemolyticus by Sea Food

As the sea food at the Cheng-ping wholesale market were frozen and not for retail, only swab specimens were collected. Frozen conditions are inhibitive and disinfective to the growth of Vibrio parahaenzolvticus. Therefore, untypable Vibrio parahaernoivticus was isolated in only three out of the 17 specimens (Table 1). Thus, as far as Vibrio parahaenzolyricus is concerned, frozen sea food are safer than sea food that have been defrosted or are refrigerated only.

Of the 18 sea food and water specimens collected at the Pa-tou-tze retail market, eight were found to be contaminated by Vibrio parahaernolyticus (Table 2), at a high rate of 44.4%. By sea food, as is noted in the literature¡¦9, more shellfish (63.6%) than fish (14.3%) were contaminated. Generally speaking, dead sea food are more contaminated than the live ones. Vibrio parahaenzolyticus can also grow in water tanks. One type of Vibrio parahaenzolvticus in three of the eight contaminated specimens, two types in four specimens, and three types in one specimen were isolated.

Fang et al.[24] had also collected sea food specimens from eight coastal markets in Taiwan for Vibrio parahaernolvticits testings. They found that 45.7% of the sea food were contaminated by Vibno parahaerno1vticus 40.0% of fish, 22.3% of raw fish (sashimi), 44.4% of shrimps and 47.8% of crabs

Of the six sea food and water specimens collected at the Ho-ping retail market, four were found to be contaminated by Vibrio parahaernolvticus (Table 3), at a high rate of 66.7%. One type and two types of Vibrio parahaeinolvneus were isolated in two each of the four contaminated specimens

2. Vibrio parahaernolvticus by Typing

Of the 23 strains isolated from 15 specimens, 12 were untypable, five were of K7, two were of K42, and one each was of K4, K48. K53 and K54

3. Vibrio parahaenzolvticus by Serotype

Between January 1983 and December 1993, of the 1,610 specimens of food poisoning outbreaks and sporadic diarrhea cases sent for laboratory testings by medical care institutions in the northern part of Taiwan. Vibrio parahaemolyticus had been isolated in 786 of them involving 42 serotypes. Of them, 20 were new serotypes isolated after 1983; K20, K28, K36 and K45 though had been isolated before 1983, had never appeared again until then. By frequency, the top ten were K8 (36.8%), K15 (10.8%), K12 (8.7%), K56 (7.9%), K63 (4.7%), K4 (4.2%), K41 (3.8%), K7 (2.9%), K54 (2.8%) and K29 (2.5%). They were distributed throughout the year, fewer in January to April and November to December, and more in May through October[17]

When the serotypes of Vibrio parahaemolyticus isolated in the present study were compared to the top ten serotypes isolated in the ten years time, K4, K7 and K54 were noted in both cases. The K6 type most frequently isolated in the food poisoning outbreaks of 1997 was not isolated in this study.

4. Pathogenic Agents of Food Poisoning in Some Countries

Table 5 shows the number and percentages of food poisoning outbreaks by pathogenic agents in Taiwan for the last ten years. As can be noted, in the last ten years, either by number of outbreaks or number of cases, Vibrio parahaemolyticus was the most important pathogenic agent of food poisoning2527.

Table 6 shows the number of food poisoning outbreaks by pathogenic agents for the US, Japan and Taiwan. Salmonella was the first leading cause of food poisoning in the US; in Japan and Taiwan, it was Vibrio parahaemolyticus.

5. Vibrio parahaemolyticus-induced Food Poisoning in Japan

Table 7 gives the number of food poisoning outbreaks, number of cases and number of deaths in Japan for 1994-1995. In the two years, though Vibrio parahaemolyticus accounted more for food poisoning outbreaks, Salmonella was the major cause of deaths. Seven people had died in two years from food poisoning; of them, five were from bio-toxins, and one each from Salmonella and Staphylococcus aureus[28].

Table 8 shows Vibrio parahaenzolvticus-induced food poisoning outbreaks of larger scale (involving more than 10 persons) in Japan for 1994-1995. Seventy- seven percent of the outbreaks involved 50 and less people; 16% involving 50-100 people; and 7% involving 100 and more people28.

6. Prevention and Treatment

As Vibrio parahaemolyticus is the most important pathogenic agent of food poisoning outbreaks in Taiwan, how to prevent and control food poisoning outbreaks induced by this agent is most essential. For detailed methods of prevention and treatment, please refer to relevant literature[29].

Prepared by: Pan TM, Wang TK, Tsai CL (Division of Bacteriology, National Institute of Preventive Medicine, Department of Health)

Table 1. Vibrio parahaemolyticus in Specimens Collected at Cheng-ping Wholesale Market

Food Item	Isolation of *Vibrio parahaemolyticus*
Shrimp 1	--
Shrimp 2	--
Shrimp 3	--
Small soft crab	--
Feet of small soft crab	--
Crab	--
Head of slipper lobster	--
Yellow sea bream	--
Cuttle fish	VP*
Red bulleye	VP*
Squid (large)	--
Squid (small)	--
Flat fish	--
Japanese butterfish (dead)	--
Swordfish	--
Lizard fish	VP*
Small shark	--

Notes: -- for "no Vibrio parahaeniolyticus isolated"
* VP for "untypahle Vibrio parahaemolvticus"

Table 2. Vibrio parahaemolylicus in Specimens Collected at the Pa-tou-tze Retail Market

Food Item	Isolation of *Vibrio parahaemolyticus*
Clam (dead)	VP K42
Clam (live) 1	VP*
Clam (live) 2	--
Shrimp	--
Oyster (live)	VP K7, VP*
Oyster (dead)	VP K7, VP K42, VP K53
Water of oyster	VP K7, VP*
Soft crab	--
Feet of soft crab 1	VP K4, VP*
Feet of soft crab 2	--
Water of soft crab	VP K48, VP*
Swordfish	--
Yellow scads	--
Hairtail	--
Golden-thread	VP K54
Porgy	--
Kuokuang fish	--
Squid	--

Notes: -- for "no Vibrio parahaemolyticus isolated"
VP for "untypable Vibrio parahaemolyhticus"

Table 3. Vibrio parahaemolyticus in Specimens Collected at the Ho-ping Retail Market

Food Item	Isolation of *Vibrio parahaemolyticus*
Clam (live)	--
Water of clam	VP*
Oyster (dead)	VP K7, VP*
Water of oyster	VP K7, VP*
Feet of soft crab	VP*
Water of Feng snail	--

Notes: -- for "no *Vibrio parahaemolyticus* isolated"
*VP for "untypable *Vibrio parahaemolyticus*"

Table 4. Serotypes of Vibrio parahaemolyiicus in Sea Food Collected in Northern Taiwan

Serotype	No. isolated
Untypable	12
K7	5
K42	2
K4	1
K48	1
K53	1
K54	1

Table 5. Food Poisoning Outbreaks in Taiwan by Pathogenic Agents, 1986-1995

Pathogenic Agent	Food Poisoning Outbreak		Case	
	No.	%	No.	%
Vibrio parahaemolyticus	197	35.3	8,967	38.5
Staphylococcus aureus	169	30.5	6,651	28.6
Bacillus cereus	104	18.7	4,844	20.8
Escherichia coli	36	6.5	1,391	6.0
Salmonella	31	5.6	1,038	4.5
Clostridium botulinum	10	1.8	19	<0.1
Others*	8	1.4	360	1.5
Total	555	100 0	23,270	100.0

*Others include: *V. cholerae* non-O1, *Plesiomonas shigelloides*, and *Aeromonas hydrophila*
(Source: J Clin Microbiol 35; 1260-1262, 1997)

Table 6. Food Poisoning Outbreaks in US, Japan and Taiwan

Pathogenic Agent	Order (%)		
	USA	Japan	Taiwan
Salmonella	1 (28%)	2 (29%)	4 (8%)
Staphylococcus aureus	2 (13%)	3 (11%)	2 (20%)
Clostridium botulinum	3 (8%)		
Clostridium perfringens	4 (7%)	7 (3%)	
Shigella	5 (4%)		
Bacillus cereus	6 (2%)	6 (3%)	3 (15%)
Campylobacter	6 (2%)	5 (5%)	
Escherichia coli	7 (<1%)	4 (5%)	5 (<1%)
Vibrio parahaemolyticus	7 (<1%)	1 (32%)	1 (57%)

Data for USA, 1973-87; for Japan and Taiwan, 1994
(Source: J Formos Med Assoc, 95; 417-120, 1996)

Table 7. Food Poisoning in Japan, 1994-1995

Year	No. of outbreaks	No. of cases	No. of deaths
1994			
Total	830	35,735	2 (bio-toxins)
Agents known	709	29,894	
V. parahaemolyticus	224 (32%)	5,849 (20%)	
Salmonella	205 (29%)	14,410 (48%)	
1995			
Total	699	26,325	5 (3: bio-toxins 1: *Salmonella* 1: *S. aureus*)
Agents known	627	22,660	
V. parahaemolyticus	245 (39%)	5,515 (24%)	
Salmonella	179 (29%)	7,996 (35%)	

(Source: IASR, 17; 151-152, 1996)

Table 8. Vibrio parahaemolyticus-induced Food Poisoning Outbreaks of Larger Scale, Japan, 1994-1995

Year	No. of cases in each outbreak			Total
	More than 101	100-50	49-10	
1994	8	11	60	79
1995	3	14	61	78
Total	11 (7%)	25 (16%)	121 (77%)	157 (100%)

(Source: IASR, 17; 151-152, 1996)

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