OCCURRENCE OF BACTERIA IN COCKLES, *ANADARA GRANOSA* LINNÉ IN J AKARTA BAY, INDONESIA

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ABSTRACT

Fecal indicator and pathogenic bacteria were isolated from *Anadara granosa* L., water, and sediment during October and November 1997. Samples were taken from 5 stations located in the mouth of Kalarantua River, part of Jakarta Bay. In most cases, the bacteria levels in cockles and sediment were higher than in the water. Samples taken in November showed a higher concentration of bacteria compared with samples taken in October. The results of Spearman's rank correlation exhibited a direct correlation between *E. coli* and *Salmonella*, and also between *E.coli* and *Shigella*. No correlation could be established between *E. coli* and *Vibrio*.

INTRODUCTION

Boiled cockles, *Anadara granosa* L., is one of the favorite street foods in Jakarta and surroundings. Most of the cockles sold at "street-restaurants" are collected from Jakarta Bay waters. It is unfortunate that Jakarta Bay might have been affected by bacterial contamination coming from both point and non-point sources of discharge.

As a filter feeder the cockles pump large amounts of water through their gills and filter out their microscopic-sized food particles. When the waters or sediments are contaminated with bacteria, they also filter out bacteria from the overlying water. Whilst pathogens may not directly harm the cockles, they can be harmful to humans, sometimes with deadly consequences.

In some countries many prime shellfish beds had been closed for harvesting because the level of bacterial contamination is not safe for human consumption (Gales & Baleux 1992; Busse 1998). Other studies have also aimed at viral contamination of shellfish (Richards 1985, 1987; Jehl-Pietri *et al.* 1991; Dupont *et al.* 1992; Beril *et al.* 1996).

Much research on bacterial contamination has been carried out in Jakarta Bay (Thayib & Suhadi 1974; Thayib & Listiawati 1977; Thayib & Martoyudo 1977; Thayib & Ruyitno 1981; Ruyitno & Thayib 1994), but no research has been done after the intensive River Clean Act entered into force during the period 1989-1997.

E. coli as recommended by US Environmental Protection Agency (1986) is one of the most widely used indicator bacteria, which is used to asses the sanitary quality of water and potential public health risk from waterborne disease. The rationale behind using *E. coli* as indicator is for their potential to indicate the presence of human fecal matter and hence the possible presence of disease-causing bacteria (Faigenblum 1988). The aim of this work was to detect the occurrence of faecal indicator (*Escherichia coli*) and pathogenic bacteria (*Salmonella*, *Shigella* and *Vibrio*) from cockle samples, seawater, and sediments to judge

if the situation had become better in the coastal area of Jakarta Bay.

MATERIALS AND METHODS

Study site:

The study was conducted in the mouth of Kalarantua river, part of Jakarta Bay (Fig. 1). Five stations were sampled on 26 October and 15 November 1997. Characteristics of the water are compiled in Table 1.

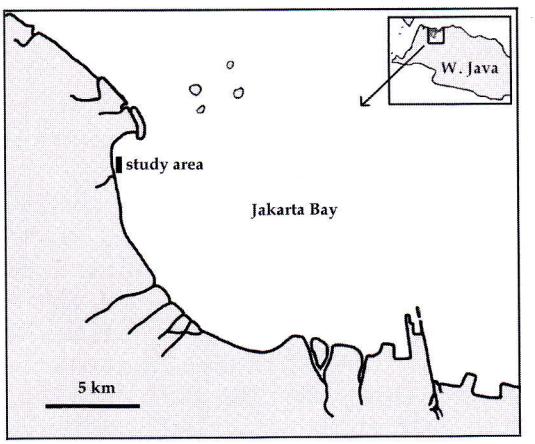

Fig. 1. Study site.

Collection of cockles, seawater, and sediment

Two market-sized cockles (shell length > 3 cm) were collected by hand. Water samples from about 50 cm above the bottom were collected by means of sterile plastic bottles, while sediment samples were collected from the surface in thick plastic bags. The cockles, sea water and sediment samples were kept in closed-sterilized cool container to maintain freshness during transportation to the Laboratory of Fish Pathology, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University. All samples were processed within 24 hours.

Table 1. Ranges of selected environmental variables in the study site.

Property of water	Unit	Range
Temperature	°C	31.0-31.5
Salinity	‰	32.5-34.0
pH	-	7.00-7.38
Dissolved Oxygen	mg/l	4.40-4.80

Media and culture procedure

All flesh of cockles except the mantle was separated from the shell and weighed. They were minced using a sterile pestle and mortar, and were then suspended in sterile seawater (1:10). 0.1 ml of the suspension was used to analyse each type of bacteria. Sediment with a given weight was diluted with sterile sea water and shaken to homogenise the mixture. 0.1 ml of the suspension was spread on an agar plate. For the sea water samples, 0.1 ml of was directly spread on the agar plates. The method used was as described by Thayib & Suhadi (1974).

Isolation of *E. coli* comprised two steps; i.e. presumptive test and confirmation test respectively. In presumptive test LTB (Lauryl Tryptose Broth) media was used, while EMB (Eosin Methylene Blue) agar was used in confirmation test (Standar Nasional Indonesia (SNI) 1991[a]). Most Probable Number (MPN) of *E. coli* was achieved by the technique proposed by APHA (1976).

Isolation of *Salmonella* and *Shigella* was conducted in accordance with SNI (1991[b]). This method consists of selective enrichment (24 hours at 37 °C) using SCB (Selenite Cystine Broth) and plate isolation on SS (*Salmonella-Shigella*) agar, incubated for 24 hours at 37 °C. Colony forming unit (CFU) per milligram of cockles and sediment samples and CFU/ml for sea water samples were recorded according to Alifuddin (1996).

Isolation of *Vibrio* from cockles, sediment and seawater samples were determined according to the method recommended by AOAC (1975). TCBS (Thiosulfate Citrate Bile salts Sucrose) agar was used in this method. CFU/mg for cockles and sediments samples and CFU/ml for seawater samples were recorded.

RESULTS

Tables 2 and 3 present the concentration of bacteria isolated from cockles, water and

Table 2. Bacteriological profile of cockles, water and sediment in October 1997. 0= not detectable. The unit of *E. coli* is MPN/100 ml for water and MPN/100 g for cockles and sediment. While the others are CFU/ml for water and CFU/g for cockles and sediment.

Sta.		*E. coli*	*Salmonella*	*Shigella*	*Vibrio*
1	Cockles	2.10×10^{2}	5.08×10^{10}	3.55×10^{11}	0
1	Water	0	0	0	1.04×10^{3}
1	Sediment	0	0	8.1×10^{4}	1.67×10^{5}
2	Cockles	0	1.20×10^{13}	6.51×10^{12}	0
2	Water	1.10×10^{2}	0	2.90×10^{2}	1.02×10^{3}
2	Sediment	2.00×10^{2}	0	1.58×10^{5}	8.04×10^{4}
3	Cockles	2.10×10^{3}	2.81×10^{12}	3.64×10^{12}	0
3	Water	1.10×10^{2}	0	2.04×10^{3}	1.16×10^{3}
3	Sediment	2.00×10^{2}	0	1.80×10^{5}	4.97×10^{5}
4	Cockles	2.00×10^{2}	1.10×10^{12}	2.24×10^{12}	0
4	Water	2.40×10^{4}	0	6.70×10^{2}	8.40×10^{2}
4	Sediment	1.20×10^{3}	0	1.92×10^{5}	3.94×10^{5}
5	Cockles	2.40×10^{4}	1.10×10^{8}	0	0
5	Water	2.10×10^{3}	4.00×10^{2}	5×10^{1}	1.84×10^{3}
5	Sediment	2.10×10^{3}	0	1.80×10^{3}	2.52×10^{4}

Table 3. Bacteriological profile of cockles, water and sediment in November 1997. 0= not detectable. The unit of *E. coli* is MPN/100 ml for water and MPN/100 g for cockles and sediment. While the others are CFU/ml for water and CFU/g for cockles and sediment.

Sta.		*E. coli*	*Salmonella*	*Shigella*	*Vibrio*
1	Cockles	70	6.59×10^{8}	1.41×10^{7}	0
1	Water	2.40×10^{4}	2.60×10^{2}	1.70×10^{2}	2.40×10^{3}
1	Sediment	2.40×10^{4}	0	1.38×10^{10}	9.72×10^{4}
2	Cockles	2.40×10^{4}	3.44×10^{8}	0	0
2	Water	2.40×10^{4}	0	5.90×10^{2}	1.01×10^{4}
2	Sediment	2.40×10^{4}	0	1.49×10^{14}	3.00×10^{3}
3	Cockles	2.40×10^{4}	4.69×10^{13}	1.39×10^{14}	0
3	Water	2.40×10^{4}	8.10×10^{2}	8.42×10^{14}	6.48×10^{3}
3	Sediment	2.40×10^{4}	3.12×10^{10}	2.00×10^{11}	3.66×10^{4}
4	Cockles	2.40×10^{4}	5.02×10^{9}	1.30×10^{10}	0
4	Water	2.00×10^{2}	1.80×10^{3}	8.72×10^{3}	2.05×10^{3}
4	Sediment	2.40×10^{4}	5.66×10^{6}	2.74×10^{8}	1.12×10^{5}
5	Cockles	2.40×10^{4}	1.42×10^{9}	1.63×10^{9}	0
5	Water	0	0	3.55×10^{5}	4.84×10^{3}
5	Sediment	2.40×10^{4}	3.25×10^{12}	2.10×10^{11}	5.82×10^{4}

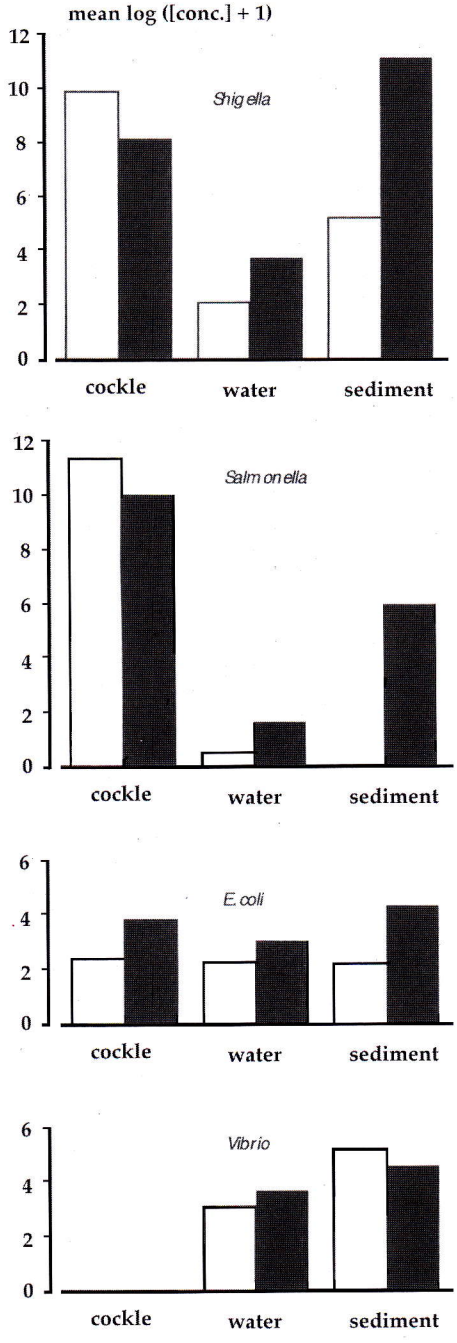

Fig. 2. Mean Log ([concentration of each bacteria]+1) of cockles, water and sediment isolated on October 1997 (white bar) and November 1997 (black bar).

sediment at each station of the study site. Most of the cockles contained *E. coli* except at Station 2 on October 1997.

With few exceptions, most of the cockles as well as the water and sediment contained *Salmonella* and *Shigella*. Yet, *Vibrio* could not be isolated at all from cockles neither in October nor in November. Only from water and sediment was *Vibrio* successfully isolated.

Fig. 2 shows the mean log ([bacterial concentration]+1). The bacterial concentration of cockles and sediment is in most cases higher than that of water. By comparing the time of sampling, bacterial concentrations of cockles, water and sediment taken in November 1997 is, with some exceptions, higher than in October 1997.

DISCUSSION

According to the standards from Ministry of Population and Environment in 1988, which is 1000 MPN/100 ml, *E. coli* of all water sample exceeded the standard except St. 1, St. 2 and St. 3 on October 1997 and St. 5 on November 1997. The mean concentration of *E. coli* in the water of the present study is lower than that of the study conducted by Thayib & Listiawati (1977). This might have something to do with the River Clean Act that was conducted intensively during the period 1989-1997. The same result, however, might not happen with *Vibrio* since it is a natural marine bacteria and probably unrelated to pollution.

E. coli in cockles and sediment, but not in the water, was directly correlated with the number of *Salmonella* (Spearman's rank correlation, 2-tailed test, $p < 0.02$) and of *Shigella* (Spearman's rank correlation, 2-tailed test, $p < 0.01$). No correlation could be established between *E. coli* and *Vibrio* (Spearman's rank correlation, 2-tailed test, $p>0.01$). This evidence lead us to conclude that if we find relatively high numbers of *E. coli* in the cockle and sediment of the study site we can assume that there is an increased likelihood of *Salmonella* and *Shigella* being

present as well. Strong association between *E. coli* and *Salmonella* was also found by Gales & Baleux (1992) in the Thau Lagoon, France. In the water the missing correlation between *E. coli* and *Salmonella* or *Shigella* might be caused by the dynamics of water at the study site. Brenner (1984) states *"Shigella* and *E. coli* strains are often extremely difficult to separate biochemically because there are aeroginac (gas-producing) *Shigella* and lactose-negative, anaerogenic, non-motile *E. coli"*.

E. coli can cause a dysentery-like diarrhea, so pathogenicity does not provide definitive separation. DNA relatedness between *E. coli* and *Shigella* is 70-100%. Since *Shigella* is closely related to *E. coli*, they possibly grow in the same environment. Wherever *E. coli* can grow, *Shigella* may grow as well.

As mentioned above, *E. coli* has no direct correlation with *Vibrio*. Several of the species can cause intestinal illness. *V. cholreae* and V. *parahaemolyticus* are two warm water species predominantly found in seafood. These bacteria have been associated with several severe cases of illnesses in Jakarta (Thayib & Suhadi 1974; Supraptini *et al.* 1998).

V. cholerae has been known to cause large epidemics of cholera in developing countries with poor sanitation. Sea food is an important vehicle for cholera but fecal contamination of drinking water and food is often responsible for the widespread and persistent nature of cholera outbreaks (Oliver & Kaper 1997; Nascumento *et al.* 1998). *V. parahaemolyticus* is also a common pathogen in Asia. It caused 197 outbreak and 8967 cases in Taiwan during 1986-1995 as well as the numerous outbreaks in Japan and some South East Asian countries (Okuda *et al.* 1997; Pan *et al.* 1997).

Setyobudiandi *et al.* (1998) found *Vibrio* bacteria in very low number in mussels taken from an area close to the study site. We failed to isolate *Vibrio* from the cockles. In comparison, Oliver & Kaper (1997) found 100% of the oyster tested contained detectable *V. parahaemolyticus*, and they found they were not positively correlated with indicators of fecal contamination. Therefore, *Vibrio* concentration is not necessarily higher in shellfish because of high *E. coli* counts (evidence of sewage contamination).

The mean log ([bacterial concentration]+1) of cockles and sediment is higher than that of water (Fig. 2). Since the cockle is filter-feeding, it accumulates particulate materials including pathogenic bacteria. That is probably why the bacteria level in cockles exceeded the level of water. Other studies have shown that the accumulation exceeded 1000 times those concentrations found in ambient water concentrations (Sound Conservancy, Inc. 1992).

The concentration of *Salmonella* and *Shigella* of the cockles in the present study was about 10^{10} and 10^{9} times the level of the surrounding water respectively, while *E. coli* was only 1-1.3 times higher. For comparison mean concentration of *Salmonella* and *Shigella* in market-size green mussel (*Perna viridis*) ranged from about 10^{9}-10^{11} and 10^{8}-10^{11} times the level of the surrounding water respectively, while *E. coli* was 1-24 times higher (Setyobudiandi *et al.* 1998).

In general it is believed that fecal indicators can not grow in natural environment, since they are adapted to live in the gastrointestinal tract. Sunlight, temperature, competition with other bacteria found naturally in the water, and predation by protozoa and other small organisms are all believed to prevent the survival of fecal bacteria and pathogens. However, if they can survive from a few hours up to several days in water, they may survive longer in sediment, where they may be protected from sunlight and predators. In addition, sediment provides more organic matter (Gales & Baleux 1992). This could be the reason why bacteria counts in sediment are higher than in water.

Fig.2 also shows that bacterial numbers were higher in November than in October. This may have relation to the season. Dur-

ing November rainfall is higher than during October. This interpretation agrees with Saitanu (1992) who found that bacterial levels in *Crassostrea lugubris* and *Anadara granosa* of Ban Don Bay, Thailand were higher during the rainy season than during the dry season. Gales & Baleux (1992) speculated that it was not only the rain as such (increased outlet through rivers) but also environmental factors at the time of rainfall (less light and lower water temperature) which would increase bacterial survival thereby significantly increasing the abundance of bacteria.

According to SNI (1992[a,b,c]) fresh and frozen seafood are acceptable for human consumption if the concentration of *E. coli* does not exceed 3 MPN/gram and no *Salmonella* nor *Vibrio cholerae* is to be found. By taking those criteria, the cockles collected from the study site are not safe to eat. However, they may be safe to eat after being purified. Microbial purification of shellfish has been reviewed by Richards (1988; 1991).

RECOMMENDATION

Even though in Indonesia there is no raw or undercooked shellfish-eating custom and no record of outbreaks of shellfish-associated enteric bacteria or virus illness yet, the results of the present study must be considered as a warning to people who like seafood, particularly shellfish. Shellfish must be boiled or steamed for adequate periods to ensure the total inactivation of all potential human pathogens.

ACKNOWLEDGMENTS

The first author (YW) would like to thank Prof. Jorgen Hylleberg who always encourages me in scientific work. This study was made possible by a grant from Bogor Agricultural University (OPF Research Fund 1997). Tulus, Dwi, Rini and Misra kindly assisted in collecting samples and laboratory work. M. Alifuddin provided laboratory facilities and some practical work. Mr. Ario Damar and Mr. A. Tarik provided supporting information. Mr. Manish of India corrected the English of the first manuscript. TMMP Danida is thanked for supporting all expenses during the 10th TMMP workshop in Hanoi, Vietnam.

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