Study on contamination and depuration of clam *Scapharca subcrenata* in the sea area of Qidong county in Jiangsu province, China

Étude de la contamination et de la purification du clam Scapharca subcrenata *sur le littoral du comté de Qidong dans la province de Jiangsu, Chine*

XIANJUN WU[1], SHANLIAN JU[2]

1. East China Sea Fisheries Research Institute, Chinese Academy of Fisheries Science, 300 Jun Gong Road, Shanghai 200090, P.R. China

2. Shanghai Medical University, 138 Yi Xue Yuan Road, Shanghai 200032, P.R. China

Abstract

A serious bio-pollution of virus in clam (*Arca*) broak out in Jiangsu coast water in 1988 winter season. About 400 thousand people were spreaded in Shangai, Jiangsu and Jiangjang provinces. The results of investigation and research showed that this serious event was caused by virus of hepatitis due to eating raw clam (*Arca*). The ecological and physical and chemistry oceanography investigation in water, intertide zone, sediment were carried out. The virus of hepatitis A was isolated and biossay and tested in laboratory of Shangai Medicine University. Some experiments of depuration of clam were done in Laboratory of East China Sea Fisheries Institute and mariculture experiment farm of Jiangsu coast water during 1988 to 1989. In general, after lasting 13 days of the experiment of adding special solution and feeding diatom, most of virus of hepatitis were killed. And then, after sterilizing of high pressure these cleaned clam still could be eaten safety. The expanding experiment in large scale of depuration still should be carried out in future.

Résumé

Une grave pollution biologique de type virale s'est produite sur des coquillages (*Arca*) dans les eaux littorales de Jiangsu pendant l'hiver 1988, affectant environ 400 000 personnes dans les provinces de Shanghai, Jiangsu et Jiangjang. Les résultats des études et des recherches démontrèrent que cet épisode grave était provoqué par le virus de l'hépatite suite à la consommation de clams crus. Les études écologiques, physico-chimiques et océanographiques furent réalisées sur l'eau, le long de la zone de marnage, et sur les sédiments. Elles permirent d'isoler le virus de l'hépatite A, après analyses et bioessais effectués en laboratoire à l'Université de médecine de Shanghai.

Plusieurs expériences de purification des clams ont été réalisées entre 1988 et 1989 au laboratoire de l'Institut de Recherche sur la Pêche de la Mer de Chine orientale, et dans un élevage conchylicole expérimental sur le littoral de Jiangsu. En règle générale, après ajout d'une solution spéciale et alimentation aux diatomées, la majorité des virus de l'hépatite ont disparu en l'espace de 13 jours. Après stérilisation à haute pression, les clams purifiés sont consommables sans danger pour la santé. Il conviendra cependant de réaliser ultérieurement des expériences de purification à grande échelle.

INTRODUCTION

With the development of economy, more concentration in heavy density of population and expanding of urbans in coast area, part hazardous pollutants are increasing greatly which have not been treated discharged into coast water because of administration problem. Marine environment was suffered by contamination. Inputting pathogenic bacteria of sewage is hazarding fisheries and public health, so that it has become one of social problems currently concerned.

Epidemic and outbreak of Hepatitis A due to eating raw shellfish have been reported in many countries since 1950's. It claimed that habitat environment of contaminated bivalve molluscs was suffered by sewage waste water. According to the literatures retrieved by the Index of Medline and ASFA, the contents of early reports were only limited within epidemiology, clinical data and experimental infection of marmoset. Virological researches for HAV including the achievement of cellular culture have not been successful until late 1970's. It was possible to isolate HAV from suspicious bivalve molluscs samples. Epidemics of Hepatitis A caused by clam have occurred in China in 1978, 1983 and 1988, respectively. Based on the epidemiology study, it was confirmed that those outbreaks were associated with contaminated clam *Scapharca subcrenata* and *Tegillarca granosa*. Study on effects of polluted environment on food consumption of shellfish was carried out. This paper put emphasis on HAV contamination of clam *Scapharca subcrenata (Lischke)* and its mechanism of depuration.

Status of environmental quality

Pathogen contamination

Detection of HAV

Specimens were collected from growing sea area of clams, the Xiaomiaohong sea area of Qidong County in Jiangsu Province, which caused the prevalence of HAV in Shanghai in spring of 1988. The suspension of gills and digestive glands of clams was extracted by phenol chloroform and precipitated by ethanol. After ultra-centrifugation, roundish virus particles of 27 nm in diameter were detected by immune electron microscope (IEM). HAV-Ag were detected by ELISA. cDNA-RNA hybridization was carried out by probe which was labelled by (^{32}P) PHAVLB 228, according to nick translation method. A great number of RNA of HAV were detected in specimens of clam. The samples were inoculated into FRhK-4 and PLC/PRF cells. After four to eight weeks incubation pinpoint-like granular HAV with fluorescence which could be absorbed by serum of HAV patients in recovering period and chimpanzee was found in cytoplasm of 90% cells by immunofluorescence (IFA) assay. Infection experiments by extracting viscera of clam were also carried out due to the similar sensitive dose of human and marmoset to HAV and their similar ill symptoms. Antibody reaction was appeared in marmoset after infection and then idio-anti HAV-IgM and IgG over a period of four to five weeks. And the original HAV could be re-obtained from faeces of marmoset. At the same time, bottom mud taken from the main estua-

ries of the sea area was inoculated in 2BS. HAV antigen could still be examined by Indirect Immunofluorescence method after secondary culture. It was proved by the idio-examinations mentioned above that the HAV contamination in the clams in this sea area was to a certain extent. In comparison with the conclusions obtained, a series of control tests were made with the clams from other sea areas and no HAV detected among them.

Detection of microbiology

Aerobic bacterial count, coliform bacteria, fecal coliform, *Salmonella, Vibrio El-Tor, Vibrio parahaemolyticus* were detected in both seawater in growing area and clams. In August and October of 1988, the number of aerobic bacterial count, coliform bacteria and fecal coliform were 52,000/ml, 23,800/l and 230/l, respectively. In sediment samples, the number of aerobic bacterial count was at the range of 2,300-230,000/g ($\bar{x}$=67,114/g); coliform bacteria, <90-2,300/kg ($\bar{x}$=575/kg); fecal bacteria <90-230/kg ($\bar{x}$=127/kg). In view of the distribution of indicator species of bacteria, the results showed that the sea water and sediment had been contaminated. According to the detection of clam, it revealed that the average number of aerobic bacteria count was 1,894/g (960-2,800); coliform bacteria was 618/100g (<30-4,600), 20% of them beyond the standard of selling clam in Italy (600/100g). Coliform bacteria specially concentrated in viscera organs (gill and digestive gland).

Environmental assessment

Water quality

As a result of data analysis in this sea area, it showed that the water of habiting sea area of clam have been contaminated and polluted greatly by organic pollutants. The area of Light-level contamination was 19.9%; the area of middle-level contamination was 7.8%, and the area of serious level contamination was 14.5%. The contamination grade of coliform bacteria in this area was also quite serious. For instance, the area of serious, middle and light level was 18.07%, 22.90% and 59.64%, respectively, in the investigation of august. However, in the investigation of october, the area of serious level contamination was significantly increased to 35.71%; and the middle-level and light-level contaminations occupied 17.86% seperatively; and that of clean water was 28.57%. There was non heavy metal contamination in the area.

Sediment quality

Based on the analysis of sediment quality of growing area of clam, it revealed that the indicator of bacteria in sediment was about a log grade higher than that in sea water. The distribution of bacteria was gradually decreased from the ports and coast-line towards the offshore. It showed that the contamination level of heavy metal on the beach was still light.

Clam

Parts of the Clam *Scapharca subcrenata (Lischke)* in the growing sea area of Xiaomiaohong was contaminated by HAV. And the concentration of heavy metals was still in the normal range.

Contaminated clams and their natural depuration

The indicators of artificial contaminated clams were *Shigella dysentery* (F 2a). *Poliovirus* (ILSC strain) and *hepatitis* A virus. They were representatives of enteric bacteria and viruses. The clams were exposed in different sea water containers, in which there were different kinds of pathogenic micro-organisms with certain concentrations. The count of *Shigella* accumulated in clams, that was 10^4 bacteria per gram digestive gland was less than the 10^7 bacteria count in sea water by the end of 24 hours. The titer of poliovirus in each clam was $2x10^3$ PFU. It was about 20 times of that in sea water. HAV had a rapid accumulating rate in clams. The titer in clams accumulated to $3.5x10^5$ TCD50 only in half an hour, three times of that in one hour and 29 times of the initial viral dose in sea water over a period of 24 hours (figure 1). It seems that clam accumulated pathogen from sea water directly and then become the transmitting vector of bacterial diseases as well as viral diseases. And it could also be inferred that eating contaminated clams was a transmission route of HAV in Shanghai in 1988.

A depuration test was carried out with contaminated clams mentioned above in a flow through system at a rate of ten litters per hour. The residual pathogen in clams was examined regularly and the result was showed in table I.

Table I indicated that the self-clean of the clams required certain times under natural conditions. The concentration of pathogen declined one log titter (90%) from one to two days and three logs (99.9%) from nine to 14 days, respectively. HAV could not be eliminated efficiently by relaying and depuration in flowing water at the end of 14 days. And it spent more time to do so in containers with still bottom for example in a depuration test the viral dose of polio virus in clams reduced by 90% in three days 99.9% in 14 days and it could still be isolated (depuration rate 99.9%) in 28 days.

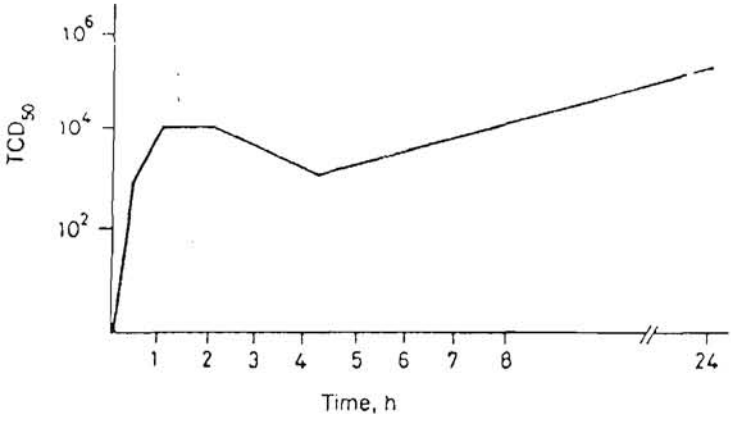

Figure 1: Accumulation of HAV in clam over a 24 hours period after contamination (Wang J.Q. *et al.*, 1990)

Table 1: Comparison of needed depurating time (day) of contaminated clams under conditions of flowing water at different degradation degree

Titer declined (log)	HAV	Pathogens	*Shigella B. (F2a)*
		Polio (I)	
1	2	1	1.5
2	5	10	2
3	14	<14	9

(Wang J.Q. *et al.*, 1990)

CONCLUSION

It could be confirmed by the aetiology study and the experimental infection in marmosets that HAV existed in the clams which caused the outbreak of HAV in Shanghai in early 1988. According to the standard of environmental pollution assessment, it could be claimed that the habitat of the clams was polluted by organic matters and colibacillus. So the catch and the sale of clams in this sea area have been prohibited by the Ministry of Agriculture of China since then.

Besides, it could be concluded after studies of contamination and depuration of pathogens in the clams that the clam could accumulate HAV in sea water and then become a transmitting vector of bacterial and viral diseases. In a natural depuration test, HAV could still be examined in clams although they had been relayed in flowing water for two weeks.

REFERENCES

Appleton H., M.S. Pereira, 1977. A possible virus aetiology in outbreaks of food poisoning from cockles. *Lancet,* 780-781.

Bostock A.D., P. Mepham, S. Phillips, S. Skidmore, M.H. Hambling, 1979. Hepatitis A infection associated with the consumption of mussels. *Journal of Infection*, **1**, 171-172.

Dienstag J.L., I.D. Gust, C.R. Lucas, V.C. Wong, J.R. Doris, R.H. Purcell, 1976. Mussel-associated viral hepatitis type A: serological confirmation. *Lancet,* 561-564.

Feingold A.O., 1973. Hepatitis from eating steamed clams. *Journal of the American Medical Association*, **225**, 526-527.

Flehmig B., 1980. Hepatitis A virus in cell culture: 1. Propagation of different Hepatitis A isolates in a fœtal rhesus monkey kidney cell line (FRhK-4). *Medical Microbiology and Immunology,* **168,** 239-248.

Gerba C.P., S.M. Goyal, 1978. Detection and occurrence of enteric viruses in shellfish; a review. *J. Food Prot.*, **41**, 743-754.

Millard J., H. Appleton, J.V. Parry, 1987. Studies on heat inactivation of hepatitis A virus with special reference to shellfish; Part 1. Procedures for infection and recovery of virus from laboratory-maintained cockles. *Epidem. Inf.*, **98**, 397-414.

Konno T., T. Chimoto, K. Taneichi, M. Deno, T. Yoshizava, O. Kimura, H. Sibaki, M. Konno, H. Kojima, 1983. Oyster associated hepatitis type A. Failure of certification of HAV in shellfish. Hokkaido *J. Med. Science*, **58,** 553-555.

Ohara H., H. Naruto, W. Watanabe, J. Ebisawa, 1983. An outbreak of hepatitis A caused by consumption of raw oyster. *J. Hyg. Cambridge*, **91**, 163-165.

O'Mahony M.C., C.D. Gooch, D.A. Smyth, A.J. Thrussell, C.L.R. Bartlett, N.D. Noan, 1983. Epidemic hepatitis A from cockles. *Lancet*, p.518-520.

Pietri C., B. Hugue, J.M. Crance, D. Puel, C. Cini, R. Deloince, 1988. Hepatitis A virus levels in shellfish exposed in a natural marine environment to the effluent from a treated sewage outfall. *Wat. Sci. Tech.*, **20**, 229-234.

Portnoy B.L., P.A. Mackoviak, C.T. Caraway, J.A. Walker, T.W. McKinley, C.A. Klein, 1975. Oyster-associated hepatitis: failure of the shellfish certification programs to prevent outbreaks. *Journal of the American Medical Association*, **233,** 1065-1068.

Provost P.J., M.R. Hilleman, 1979. Propagation of human hepatitis A virus in cell culture in vitro. Proceedings of the Society for Experimental Biology and Medicine. **160**, 213-221.

Roos B., 1956. Hepatitis epidemic conveyed by oysters. Svenska Lakartidningen, **53**, 989-1003.

Wang Jian-Yang, 1988. Études de séro-épidémiologie : l'explosion de l'hépatite A à Shanghai en 1988. *Revue académique à la faculté de médecine de Shanghai.*

You Hua-Shi, 1979. Rapport de l'enquête sur l'hépatite A apparue brutalement par ingestion de clams. *Revue de médecine préventive en Chine.*