

Laboratory Aspects on the Cause of the Cholera Outbreak Occurred in Japan, 1978

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Mr. Chairman, ladies and gentlemen:

It is indeed a great honor for me to be given this opportunity to present a paper on the occasion of the forty-third academic meeting of The Korean Society for Microbiology. Taking this opportunity, I would like to express my sincere appreciation to Dr. Yang, the President of the meeting and Dr. Ryu, Vice-Director General of

the National Institute of Health, who kindly invited me to the meeting.

According to the program on your hand, my topic to be presented here is entitled as "Problem of food-borne infections and bacterial food poisoning in Tokyo," but practically speaking, the present paper deals with the result of the microbiological examination and the analysis on the cause of the cholera outbreak which took place in Tokyo in a public wedding hall in November 1978. It is hoped that the study will contribute to an effective cholera control measure in the future.

Before going into the subject perhaps an outline introduction to the present status of gastrointestinal infection and bacterial food poisoning in Japan is in order.

The first figure indicates yearly change in incidence of typhoid fever, dysentery and bacterial food poisoning in Japan. As the figure shows case rate per population of one hundred thousand and for typhoid fever has been reduced gradually from the beginning of 1960s and has been maintained at a low rate of three to five tenths, recently. Dysentery reduction followed several years later. But it has been very drastic, and the case rate of this disease is also less than one, today.

In contrast, food poisoning has not been reduced. This is to say that Japan's environmental hygiene has been improved to the level of effective

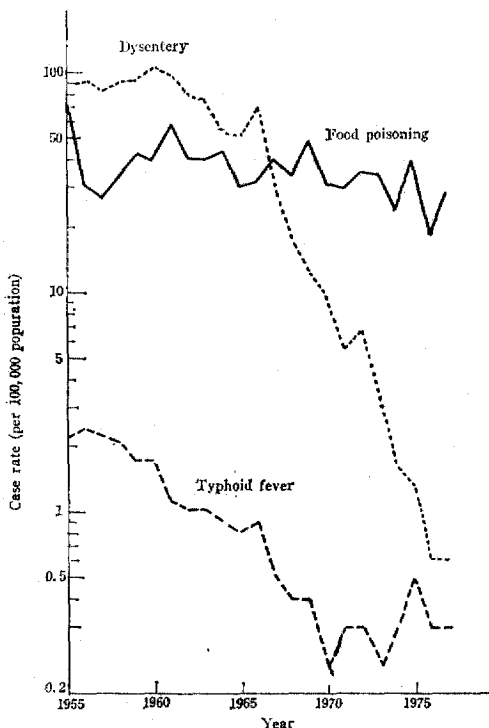


Fig. 1. Yearly change in case rate of dysentery, typhoid fever and food poisoning in Japan, 1955-1977.

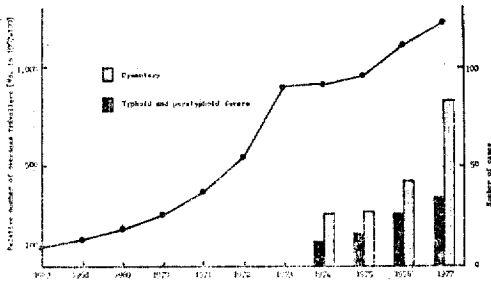


Fig. 2. Increasing trend for imported cases of dysentery, and typhoid and paratyphoid fevers in the last decade.

tively controlling typhoid fever and dysentery but not the food poisoning. Perhaps it reflects the increased trend for eating outside and using cooked fast foods rather than eating at home, as well as for spread of mass food supply system in School and offices(Fig. 1).

In addition, it must be noted that there has been higher rate of gastrointestinal infections imported into the country by overseas travellers. This figure shows the increasing trend for imported dysentery and typhoid and paratyphoid fevers. The number of the Japanese travelling abroad has increased twelve fold between 1967 and 1977. The cases of dysentery and typhoid and paratyphoid fevers imported have rapidly

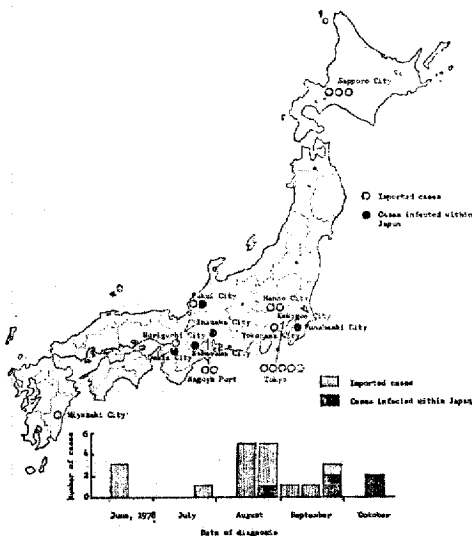


Fig. 3. Occurrence of sporadic cholera cases in Japan, 1978.

increased in the same ten year period, suggesting the correlation between overseas travel(Fig. 2). As you have already known, in 1977 the cholera outbreak took place in Arida city, which involved about one hundred patients and carriers. At that time the city fell into a panic. The outbreak has been considered to be originated from an unrecognized patient who suffered from the disease in a cholera-infected area abroad.

In 1978, we also had many sporadic cases of cholera in Japan which are shown on this figure. Cases designated with open circles and stippled bars are those became ill immediately after returning from the cholera infected countries, suggesting the possibility of imported cases. However, those shown with solid circles and bars are cases with no experience of foreign travel suggesting that they had domestic source of infection(Fig. 3). Rightly, there was concern that cholera had settled in Japan, when the most recent outbreak took place in the last November.

A cholera patient with typical symptoms was found in Funabashi City, Chiba Prefecture on October thirteenth. The patient had no record of foreign travel. No other suspected cases of diarrheal patients were found around the patient, and the route of infection was unknown. Three weeks later, on November fourth another patient was found in the other part of Chiba prefecture, complaining of severe diarrhea and dehydration and was diagnosed as cholera. The health authority suspecting a possible outbreak, studied to see if there were common vehicle in the two cases, and found the fact that both had attended wedding parties held at the same public wedding hall in Ikenohata, Tokyo a few days prior to their onset, respectively.

The Bureau of Public Health of the Tokyo Metropolitan Government immediately took up the issue. On the following day, November fifth, it was found that there were others who attended the wedding celebration held on October twentyninth, and who complained of diarrhea. The complainers had all eaten festive food and/or

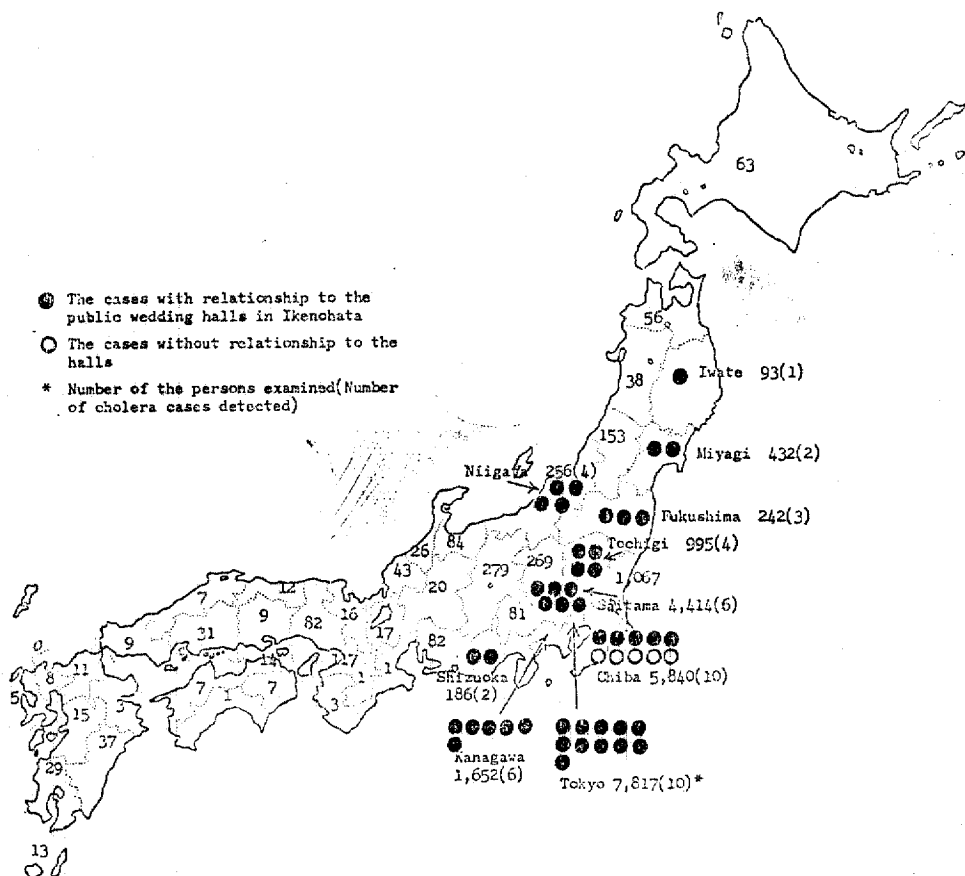


Fig. 4. Geographical distribution of the cases and the persons at risk which were involved in the cholera outbreak originated from Ikenohata, Tokyo, 1978.

packed take-home-food served at the hall. Within the same day, fecal specimens of diarrheal patients and the staff at the catering service of the wedding hall were collected for bacteriological examination and were sent to our laboratory.

On the following day, it was revealed that specimens from three diarrheal patients were positive for cholera vibrio, suggesting the case of a cholera outbreak.

Since it was likely that the food served at the wedding and the food taken home and eaten were the vehicle of the outbreak, fecal examination was conducted for all persons at risk (Fig. 4)

In Tokyo, the priority was placed on those who attended wedding celebrations at the public hall in question and those who had shared the take-home-food during the period of two weeks between October twentyfourth and November

sixth. The reason for omitting the prior period, in spite of occurrence of one case in October, was that excretion of cholera vibrio is limited mostly to a period of about two weeks and any

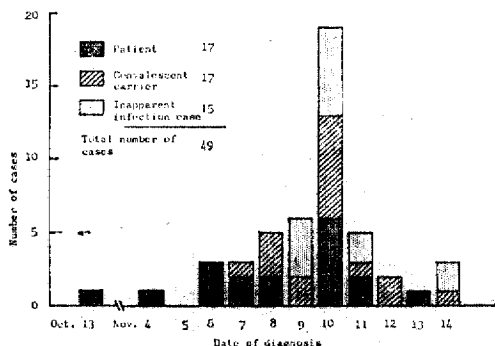


Fig. 5. Occurrence of the cases involved in the cholera outbreak originated from Ikenohata, Tokyo, 1978.

Table 1. Occurrence of cholera and other gastroenteritis cases by style of reception (28th October-6th November)

| Style of reception | Number of reception held | Number of reception with cholera cases | Number of reception with other gastroenteritis cases* |
|--------------------------------------|--------------------------|--|---|
| Japanese style | | | |
| Course A | 67 | 17(25.3%) | 22(32.8%) |
| Others | 33 | 2 (6.1%) | 2 (6.1%) |
| Chinese & European styles | | | |
| | 17 | — | 1 (5.9%) |

* Gastroenteritis cases due to *V. parahaemolyticus*, so-called NAG vibrio, enterotoxigenic *E. coli* and *Salmonella* were involved.

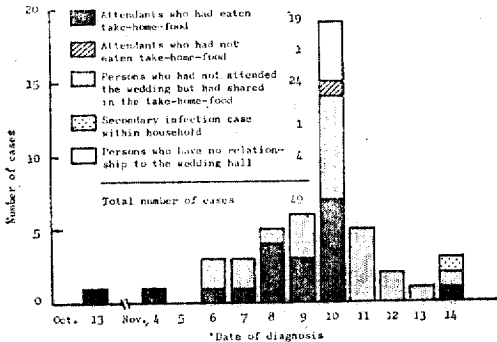


Fig. 6. Occurrence of cholera cases in terms of their relationship to the wedding hall.

attempt to include it would only tax the efforts with less effective results.

For those involving non-residents of Tokyo who had attended the weddings during the period in question, the health authorities of the respective prefectures have been responsible for the fecal examination according to their own policy. The results shown on this figure point out that the number of patients and carriers found out, in Tokyo and eight prefectures, had totalled forty-nine(Fig. 5).

The following figure shows the number of cases diagnosed for each day. Seventeen cases were patients, Other seventeen cases were found out in a convalescent carrier state. Fifteen were asymptomatic carriers or inapparent infection cases. No fatal case was seen(Fig. 6).

The break-down of the group in terms of their relationship to the weddings are as shown in this figure: there were nineteen cases of those who had attended the wedding in person and had

eaten both the food served at the hall and the take-home-food; one case of an attendee who had eaten only at the hall and not the food taken home; twenty-four cases who had not attended the wedding but had shared in the take-home-food; one suspected case of secondary infection within the household; and four cases in which the relationship to the wedding hall were unknown.

The number of wedding celebrations involved in the case totalled nineteen, all of which were Japanese style receptions. It was also revealed that there was a high concentration in occurrence of cholera cases on the eight thousand yen per head course or Course A in the table. As shown in the table, during the period between October twentyeighth and November sixth there were sixty-seven banquets in which eight thousand yen per head course were served. Of that seven-

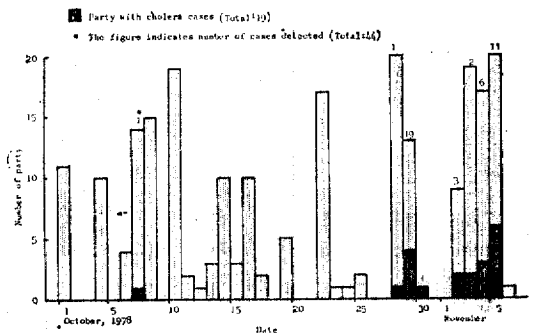


Fig. 7. Occurrence of cholera cases by party held in the public wedding hall in Ikenohata.

Table 2. Number of specimens examined in Tokyo Metropolitan Research Laboratory of Public Health

| Specimens | Number of specimens examined |
|-------------------------------------|------------------------------|
| Feces | |
| Diarrheal cases and persons at risk | 7,330 |
| Employees of the wedding hall | 487 |
| Sewage and waste water | 57 |
| Foods | 190 |
| Swabs of cooking utensils | 72 |
| Tap water | 14 |
| Total | 8,150 |

teen or 25.3 percent, banquets were responsible for the outbreak. On the other hand, only two out of thirty-three, or 6.1 percent, of the other courses were positive for cholera cases (Table 1).

Occurrence of cholera cases by date of banquets is shown in the figure. In general, the reception parties involving cholera cases seem to be more concentrated during the busy days (Fig. 6).

The main difference in the take-home-food

between the eight thousand yen per head course and other courses was the use of imported whole-cooked frozen lobsters in the former and of prawn in the latter.

In fact, before any attempt was made to analyze attack rates by type of food consumed by the patients and healthy controls, the knowledge that lobsters were imported from cholera infected country had put them in suspect. Since the lobsters were claimed to have been boiled, however, it became necessary to analyze the route of contamination. Possibilities included direct contact by unrecognized patient who may have been working in the kitchen or indirect transmission via the environment. The following are observations on some of the problem areas based on the examination results obtained in our laboratory.

During the outbreak we performed bacteriological examinations of about eight thousands specimens as shown in this table (Table 2).

This table shows the results of the fecal examination for the wedding participants and those who did not attend but ate the take-home-food, as well as for the employees at the hall. As previously mentioned, there were eleven cases of cholera among the participants of the wedding

Table 3. Results of bacteriological examination of fecal specimens performed in Tokyo Metropolitan Research Laboratory of Public Health.

| Subjects | Number of persons examined* | Number of persons positive | | | |
|--|-----------------------------|----------------------------|----------------------|--------------------|------------------|
| | | V. cholerae | So-called NAG vibrio | V. parahemolyticus | Kappa type phage |
| Attendants to the wedding | 2,350 | 3 | 7 | 4 | 1** |
| Persons who had shared in the take-home-food | 3,361 | 8 | 9 | 3 | 6** |
| Their family and contacts | 1,619 | — | — | 1 | — |
| Employees of the wedding hall | | | | | |
| Staffs in catering area | 143 | — | — | — | — |
| Others | 344 | — | — | — | — |

* Fecal specimens from 246 persons were subjected to the test for detection of kappa type phage. Selection of the subjects for the test was made giving priority to the employees working in catering area of the wedding hall and diarrheal patients.

** All the cholera vibrio positive specimens tested were proved to be positive for kappa type phage.

and/or who had enjoyed take-home-food in Tokyo. What is noteworthy is that, in addition, sixteen positive cases for so-called NAG vibrio and eight for *Vibrio parahaemolyticus* were found. There was one case which was positive for both *Vibrio cholerae* and *V. parahaemolyticus* and two cases of positive for *V. cholerae* and NAG vibrio. Isolated strains of *V. cholerae* were all biovar eltor serovar Inaba. Eleven cases of cholera with an exception of one, suffered diarrhea, but all were relatively mild and they did not required rehydration. Some complained of abdominal pain and fever which is believed to be due to mixed infection with cholera vibrio and other pathogen for example *V. parahaemolyticus*. The detection of pathogens other than *V. cholerae* strongly suggests simultaneous exposure to contaminated food or water rather than transmission by man-to-man direct contact. The results of the examination carried out in other prefectures also support this assumption showing positive for these pathogens. In addition, while vibrio cholerae isolated in Tokyo were all Inaba type, the non-Tokyo areas had in part Ogawa type isolates (Table 3).

The fecal examination was carried out also for all employees concerned, namely four hundred eighty seven persons and seven hundred twenty-five specimens. Three repetitive tests were carried out for those working in the cooking area during for about one week. However, the result was negative for all. None of them complained of diarrhea during the period in question, nor did any of them travel overseas after the month of October.

In order to improve the accuracy of the tests for employees, efforts were made to detect kappa type phage from their fecal specimen. This phage is a temperate phage of Celebes type eltro cholera vibrio. The phage is liberated at certain rate from the microbes during the proliferation state, and is known to be more stable in the natural environment than the vibrio itself. Therefore, the detection of this phage is useful as an auxillary for bacteria detection. That is

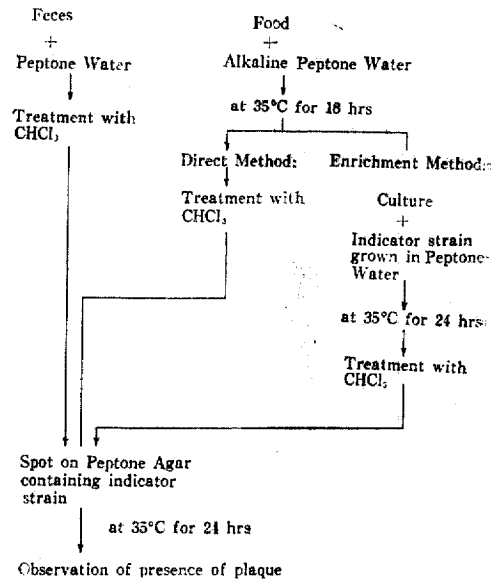


Fig. 8. Detection of Kappa type phage.

to say the presence of this phage predicts the positive case for cholera vibrio so that the test for detecting the vibrio can be carried out with more precision. Also the existence of this phage suggests that cholera vibrio may have been contained in the specimen in the very recent past even when detection of the vibrio was unsuccessful. However, despite all efforts, fecal specimens of employees remained negative for both the phage and the vibrio. Also the wastewater from the lavatory of the employees proved negative for both cholera vibrio and kappa type phage (Fig. 8).

By the way, I would like to show you an outline of the procedure for detecting the phage. The method was originated by Dr. Takeya, Professor of Kyushu University, and his colleagues. However, the procedure we used is not the same as the original one but somewhat modified. Of course, it has been confirmed that the isolates involved in the present outbreak are carrying kappa type phage.

In addition to the bacteriological findings on fecal specimens which were mentioned above, examination on the institutions and facilities of the wedding hall revealed that there existed no possibility of the cross connection between the

Table 4. Results of bacteriological examination of foods performed in Tokyo
Metropolitan Research Laboratory of Public Health.

| Specimens | Number of specimens examined | Number of specimens positive | | | | |
|--|------------------------------|------------------------------|----------------------|---------------------|------------------|------------------|
| | | Enteropathogen | | | V. alginolyticus | Kappa type phage |
| | | V. cholerae | So-called NAG vibrio | V. parahaemolyticus | | |
| Incriminated take-home-food | | | | | | |
| Whole contents including lobster | 12 | — | 1 | 1 | 10 | — |
| Lobster, whole cooked | 5 | — | 3 | — | 4 | — |
| Prawn, whole cooked | 8 | — | — | — | 6 | — |
| Others | 30 | — | — | — | 6 | — |
| Raw food materials confiscated | | | | | | |
| Lobster, whole cooked and frozen | 30 | — | 6 | — | 15 | — |
| Fish and shellfish, frozen | 27 | — | — | — | 3 | — |
| Others | 32 | — | — | — | — | — |
| Reference food, stock in whole seller | | | | | | |
| Lobster, whole cooked and frozen(A) | 23 | — | 5 | 4 | 19 | 2 |
| Lobster, whole cooked and frozen(B) | 7 | — | 2 | — | 4 | — |
| Others | 16 | — | — | 4 | 10 | — |

water supply system and the waste water system. Examination of the tap water taken at kitchens and other rooms showed that sufficient level of chlorine was maintained. No bacteria was detected.

From the above analyses it was concluded that contamination of food by cholera vibrio did not occur in the kitchen from the unrecognizable patient or chronic carriers among the employees directly or indirectly, but had occurred already before buying-in them.

The results of bacteriological examination of the food are shown on this slide. The leftover take-home-food was examined without delay. A total of fifty-five specimens were collected, of which four were positive for NAG vibrio, one for *V. parahaemolyticus*. From twenty-six specimens, *V. alginolyticus* was detected but this is not a pathogen. No cholera vibrio nor kappa type phage was detected. Existence of halophilic vibrios such as *V. parahaemolyticus* and *V. algi-*

nolyticus from cooked food suggests that the seafood was not sufficiently heated to kill such bacteria (Table 4).

In the same table, the results of tests for the confiscated frozen raw food materials from the kitchen of the wedding hall are shown. Noticeably six out of thirty cooked frozen lobsters were positive for NAG vibrio. From approximately half of the lobsters, *V. alginolyticus* was also detected.

Bacteriological examinations were also carried out on the stock of seafood materials at the wholeseller which had traded with the wedding hall. The lobsters designated as(A) in the table represents imported in the same package as those shipped to the catering outlet in question. All the specimens tested were negative for cholera vibrio. Out of the twenty-three specimens examined, however, NAG vibrio was isolated from four, *V. parahaemolyticus* from three and both of the vibrios concomitantly from one. Kappa

type phage was also detected from the lobster specimens from which NAG vibrio was isolated and from which both NAG and *V. parahaemolyticus* were detected. The lobsters in question were preboiled before they were packaged individually and then rapidly frozen at the producing site. Several tens lobsters were put in a cardboard box and sealed. In other words it was unlikely that contamination should take place during shipment.

After the lobsters were received by the catering outlets, they were taken out of the vinyl pack and defrozen in water tank. According to related sources all lobsters were re-boiled for thirty minutes and were washed and cooled with tapping water before being packaged into take-home box. However, the existence of halophilic vibrios in left over food specimens as previously mentioned would suggest that during busy catering time, proper boiling was neglected. Or possibly, secondary contamination took place through the defreezing tank, cooking utensils or fingers of the catering staffs after boiling.

The kitchen was quite small for the size of the restaurant and it seemed that food was sometimes cooked the day before to save preparation time. The kitchen for Japanese dishes was, however, independent from those for Chinese or European food so that it was considered that contamination was limited to that area. Since the prepared food was sometimes stored overnight, it seems likely that cholera vibrio was given good condition for multiplication. Again, since half a dozen to ten hours would easily pass before the take-home-food was consumed even if the food had been served immediately after preparation, the initially small number of the vibrio may have multiplied to an infectious levels.

Further investigation showed that the lobster in question had been imported in February 1978 and after small initial shipment to retail shops most of the balance had been shipped to the wedding hall since the end of the month. No definitive answer was available for the negative

period for occurrence of cholera cases due to the lobsters before October. However, it may be suggested that the contamination was limited to a small number of lobsters which were unequally distributed in the package and that the number of the contaminated bacteria was also small. The findings that only two out of several tens of specimen were positive for only kappa type phage with no detectable number of cholera vibrio may support the above assumption. Of course there may have been insidious infections undetected because of their mild symptom. The detection of four carriers in Chiba prefecture unrelated to the wedding and unexposed to patients involved in the outbreak may explain this in part.

In concluding, the results of investigation and findings obtained suggest the following mechanism for the cause of the outbreak. It is clear that the current cholera outbreak is due to the packed take-home-food served and provided by the wedding hall. Although no cholera vibrio was detected from the incriminated food, it seems likely that the outbreak was brought about by an unfortunate combination of a number of factors. In other words, a small number of the frozen lobsters had been contaminated with cholera vibrio at the producing site, while the bacteria multiplied and spread during the cooking process and then it was allowed to multiply further before the packed food was finally consumed.

In the past, perhaps too much emphasis was given in Japan to the infected travellers as the source of the cholera epidemic, especially after we experienced the outbreak in Arida City in 1977, but the present case suggests that attention should also be given to the imported fresh seafood materials. It was a very instructive experience for us.

In a shrinking world like today with fast and frequent travells and transportations of food, it is inevitable that some cases in incubation period or carriers of cholera as well as contaminated food may be brought from infected area passing

the quarantine undetected. In addition, it is not unusual that cholera infected individuals do not show any typical symptom. Many suffer only mild diarrhea or remain as a state of subclinical infection. Sometimes, the signs and symptoms will be modified by mixed infection with other pathogens than cholera vibrio.

It is difficult to check those persons at the quarantine, since subjecting all travellers without discrimination may be anti-human right if not physically impossible. Penetration of cholera via imported food from infected areas also cannot be wholly checked even under a strict system of inspection, since food safety is only confirmed through examination of a limited number of sampled specimens. That is to say cholera does travel even under strict inspection system. Perhaps it is too selfish to expect that one's country alone would be free from deadly infection like cholera. This suggests the need for an international cooperation to eradicate deadly communicable diseases from the world rather than atte-

tempt to save particular countries from contamination.

Domestic preventive efforts should be indispensable in each country at improving the environmental conditions to the level in which transmission of cholera will never be allowed. Enough basic rules for controlling food poisoning is also essential to prevent spread of cholera.

Finally, I would like to emphasize the importance of bacteriological examination for diarrheal patients before easily prescribing anti-biotics even when cholera is not suspected clinically. Because, sometimes clinical symptoms would be modified by mixed infection, and diagnosis of cholera demands a full bacteriological examination to differentiate it from other similar diseases such as gastroenteritis due to enterotoxigenic *E. coli*, NAG vibrio and others. No mild case is an excuse to neglect necessary examination, since that one case may cause an epidemic.

Thank you and Kam-sa hap-ni-da.