Journal of Food Protection, Vol. 44, No. 4, Pages 294-296 (April 1981)

Demonstration of Viral Contamination of Oysters Responsible for an Outbreak of Viral Gastroenteritis

M. J. EYLES[1], G. R. DAVEY[2] and E. J. HUNTLEY[3]

Department of Veterinary Pathology, University of Sydney, N.S.W. 2006, Australia; Division of Analytical Laboratories, Health Commission of N.S.W., Lidcombe, N.S.W. 2141, Australia and Food Inspection Branch, Health Commission of N.S.W., Wagga Wagga, N.S.W. 2650, Australia

(Received for publication August 27, 1980)

ABSTRACT

Two viruses, echovirus type 8 and a reovirus, were isolated from a batch of oysters responsible for an outbreak of gastroenteritis. Characteristics of the illness, detection of Norwalk virus in the feces of one of the victims and other factors indicated strongly that the illness was due to infection with Norwalk virus. Examination of the implicated oysters and a fecal specimen from a victim failed to provide evidence of the involvement of any other causative agent. Thus laboratory evidence of human enteric virus contamination of a batch of food responsible for a viral illness has been provided.

Epidemiological studies have demonstrated that numerous outbreaks of viral hepatitis have been caused by contaminated foods, particularly shellfish (*5,7*). Human enteric viruses have been detected in shellfish during several laboratory studies (*7*) and, on rare occasions, enteroviruses have been isolated from other foods (*14*). Viruses infective for man have not previously been isolated from a batch of food responsible for a foodborne outbreak of viral disease. In 1978, a series of outbreaks of gastroenteritis occurred in Australia which was linked to consumption of oysters. Murphy et al. (*11*) and Linco and Grohmann (*9*) have shown that the outbreaks were caused by Norwalk virus, a known cause of non-bacterial gastroenteritis not previously associated with foodborne gastroenteritis. However, they were unable to demonstrate the presence of human enteric viruses in the oysters. Thus, as in previous investigations of foodborne outbreaks of viral disease, there was no laboratory evidence that the oysters had been exposed to viral contamination. Norwalk virus cannot be detected in environmental or food samples using methods presently available (*11*). Most of the oysters consumed in Australia are cultivated in various estuaries along the coast of the state of New South Wales (N.S.W.), of which the Georges River estuary and Port Stephens are the most important. Sewage pollution of oyster growing areas in the Georges River is believed to be the cause of the outbreaks (*11*). This report describes the isolation of enteric viruses infective for man from a batch of oysters which had caused an outbreak of Norwalk virus gastroenteritis during the series of outbreaks mentioned above.

MATERIALS AND METHODS

Investigation of the outbreak

Where possible, the victims of the outbreak were interviewed to obtain details of the illness, food consumption histories and other relevant information. A fecal specimen was obtained from one of the victims a few days after the onset of the illness and submitted to the Institute of Clinical Pathology and Medical Research, Westmead, N.S.W. for bacteriological and virological examination by the methods described by Murphy et al. (*11*). Unopened containers of oysters from the batch that had caused the outbreak were obtained from one of the victims the day after the onset of illness and examined as described below. The oysters which caused the outbreak were bottled oysters. Bottled oysters are fresh oysters which have been removed from their shells and packaged in glass jars with a small amount of potable water. No preservatives are added and the oysters receive no further processing. They are then stored under refrigeration for no more than a week or two. Oysters are usually eaten raw or very lightly cooked in Australia.

Virological examination of oysters

The method of Metcalf and Stiles (*10*) was used to prepare a sample consisting of 10 oysters for virological examination. The extract obtained was inoculated into cultures of primary monkey kidney cells derived from Cynomolgus monkeys (Commonwealth Serum Laboratories, Melbourne) and the human diploid fibroblast cell line, MRC-5. The cultures were incubated at 37 C and examined daily for 2-3 weeks for cytopathic effects. A portion of the extract was negatively stained with 4% phosphotungstic acid (pH 7.2) and examined with a Philips EM 201 electron microscope. The virus isolates were identified by routine virological procedures (*8*), including examination of cell culture fluids by electron microscopy as described above.

Bacteriological examination of oysters

About 15 oysters and a small quantitiy of liquid (total weight approximately 200 g) were removed from a container and homogenized at high speed for 60 sec in an MSE Atomix blender. The methods prescribed by the Standards Association of Australia (*2*) were used to determine the aerobic plate count of the homogenate and the Most Probable Number (MPN) of fecal coliforms (presumptive *Escherichia*

[1]*University of Sydney. Present address: CSIRO Division of Food Research, P.O. Box 52, North Ryde, N.S.W. 2113, Australia.*
[2]*Division of Analytical Laboratories.*
[3]*Food Inspection Branch.*

coli) and *E. coli* present in the homogenate. Portions of 100 g and 50 g of the homogenate were examined for the presence of *Salmonella* and *Vibrio parahaemolyticus* respectively (*2*).

Chemical analysis of oysters

Five subsamples each consisting of 12 oysters were tested. Each subsample was homogenized in a Waring blender at high speed for 60 sec. Two g of each homogenate were digested using a $HNO_3/HClO_4$ (7:1) mixture. When oxidation was complete, the digest was evaporated almost to dryness. The residue was then dissolved in 5 ml of 20% HNO_3 and analysed for Cu and Zn, using a Varian AA6 atomic absorption spectrophotometer.

RESULTS

Investigation of the outbreak

Five cases of gastroenteritis from two separate incidents were traced to a single batch of bottled oysters. A number of bottles of the implicated oysters had been purchased from a retailer by a woman. Some of these oysters were eaten by the purchaser and two others at a social gathering. All three subsequently suffered gastroenteritis. A fourth person present did not eat any oysters and remained well. The affected persons consumed other foods and beverages at the gathering but no item other than oysters was consumed by all three. The purchaser had also given a bottle of the oysters to a friend who, with her husband, suffered gastroenteritis after eating the oysters. The couple had eaten common meals before but not after eating the oysters because shortly afterwards the husband had departed on a business trip. Consumption of oysters was the only likely cause of gastroenteritis in this case and was the only identifiable factor common to both incidents. Remaining unopened bottles of oysters were collected from the purchaser's home for use in the laboratory investigations. The records maintained within the oyster industry at the time of the outbreak were very imprecise; however, the information available indicated that this particular batch of oysters had probably been harvested from the Georges River.

Details of the illness were obtained from four of the victims. All had suffered diarrhea, two had experienced nausea, two had experienced abdominal cramps and two reported vomiting. The incubation periods measured from the time of consumption of the oysters were 31 and 28 h for two victims of the first incident and between 24 and 48 h for victims of the second incident. Recovery had occurred within a few days.

The fecal specimen submitted to the Institute of Clinical Pathology and Medical Research for examination was obtained from the woman who had originally purchased the oysters. Norwalk virus was detected in the specimen by electron microscopy and echovirus type 5 was isolated in cell cultures. No salmonellae, shigellae, staphylococci, enteropathogenic *E. coli, V. parahaemolyticus, Clostridium perfringens* or *Yersinia enterocolitica* were isolated from the specimen.

Examination of oyster samples

Two viruses were isolated from the oysters, echovirus type 8 and a reovirus. Both were isolated in primary monkey kidney cell cultures and produced cytopathic effects without the necessity of a blind passage. Direct examination by electron microscopy of the oyster extract which yielded the virus isolates did not reveal any viral particles.

The aerobic plate count of the oyster sample tested was 2.2×10^4/g and the fecal coliform count was 500/100 g. The fecal coliforms present were confirmed as *E. coli*. No salmonellae or *V. parahaemolyticus* were detected.

The five subsamples of oysters analysed chemically contained 29-40 and 340-410 ppm (wet weight) of Cu and Zn, respectively.

DISCUSSION

This represents the first reported occasion on which a batch of food responsible for an outbreak of viral disease has been shown to have been contaminated with human enteric viruses. The batch of oysters from which viruses were isolated was clearly incriminated as the vehicle for the illness, and, when considered in relation to the results of Murphy et al (*11*) and Linco and Grohmann (*9*), the evidence presented above indicates strongly that the illness was due to infection with Norwalk virus. The characteristics of the illness were consistent with those described by Murphy et al (*11*) and Linco and Grohmann (*9*) for oyster-associated Norwalk virus gastroenteritis and Norwalk virus was present in the feces of one of the victims. Both salmonellae and *V. parahaemolyticus* are potential causes of gastroenteritis, and have been isolated from N.S.W. oysters on many occasions, (*6*). Neither was detected in the implicated oysters and no bacterial pathogens were isolated from the feces of a victim of the outbreak. The applicable microbiological standard for oysters specifies that they must not contain more than 230 (MPN) fecal coliforms/100 g (*13*). The oysters responsible for the outbreak exceeded this limit and therefore were not legally marketable. The concentrations of copper and zinc found in the oysters are unlikely to cause illness (*12,15*).

The Norwalk virus cannot be cultivated in the laboratory, using techniques presently available. It is detected in clinical specimens by electron microscopy. Electron microscopy is not a sufficiently sensitive technique to detect viruses in samples where low numbers of virus particles are present, therefore it has not been possible to detect Norwalk virus in oysters or in environmental samples such as sewage or estuary water (*11*). The cultural isolation of other human enteric viruses from food or water has sometimes been questioned because of the possibility of cross-contamination during the lengthy procedures used for isolation of viruses from such samples (*4*). Cross-contamination can be disregarded in this instance because neither of the viruses isolated from the oyster sample had ever previously been handled in these laboratories.

It is unlikely that the echoviruses and reovirus isolated from samples associated with this outbreak contributed to the observed illness. Echoviruses and reoviruses have been isolated from patients with a variety of clinical

syndromes. However, the involvement of the virus in the disease is often unclear and most infections with echoviruses and reoviruses are clinically inapparent (*1*). Both echovirus 8 (*3*) and reoviruses (*10*) have previously been isolated from shellfish that were not associated with cases of illness.

The series of gastroenteritis outbreaks which occurred throughout Australia in 1978 and of which the outbreak described here was a part were almost certainly the result of sewage pollution of oyster growing areas in the Georges River (*11*). Sewage, even treated sewage, may contain a wide variety of enteric viruses. In most virological surveys of shellfish from estuaries polluted with sewage, a number of different enteric virus types have been found (*7*), as would be expected with pollution from such a source. Therefore a mixture of virus types was to be expected in the oysters which caused the present outbreak and in the feces of the victims. A total of four different enteric viruses was present in the samples associated with the outbreak, two in the oysters and two in the fecal sample. Murphy et al. (*11*) examined acute fecal specimens from 72 patients during their investigations and found Norwalk virus in 28, an unidentified 22-25 nm virus-like particle in 36, enteroviruses in 11 and other enteric viruses in a small number of specimens. The proportion of the enteric viruses isolated by Murphy et al. (*11*) that the victims might originally have acquired from oysters is unknown.

The virus most probably responsible for the outbreak described here was not detected in oysters; however, human enteric virus contamination of a batch of food responsible for an outbreak of viral disease has been confirmed by laboratory investigations.

ACKNOWLEDGMENTS

We thank the following for providing the results of their investigations: Mr G. S. Grohmann, virological examination of the fecal samples, Mr R. Chiew, bacteriological examination of the fecal sample, Mr E. Peel, chemical analysis of the oysters. We also thank Dr. M. Cloonan for serological typing of the echovirus and Dr D. N. Love for assistance with electron microscopy.

REFERENCES

1. Andrewes, C., H. G. Pereira, and P. Wildy. 1978. Viruses of vertebrates, 4th ed. Bailliere Tindall, London.
2. Australian Standard 1766. Methods for the microbiological examination of food. Standards Association of Australia, Sydney.
3. Bendinelli, M., and M. Ruschi. 1969. Isolation of human enterovirus from mussels. Appl. Microbiol. 18:531-532.
4. Clarke, N. A., E. W. Akin, O. C. Liu, J. C. Hoff, W. F. Hill, Jr., D. A., Brashear, and W. Jakubowski. 1975. Virus study for drinking-water supplies. J. Am. Water Works Assoc. 67:192-197.
5. Cliver, D. O. 1971. Transmission of viruses through foods. Crit. Rev. Environ. Control. 1:551-579.
6. Davey, G. R. Unpublished information.
7. Gerba, C. P., and S. M. Goyal. 1978. Detection and occurrence of enteric viruses in shellfish: a review. J. Food Prot. 41:743-754.
8. Lennette, E. H., and N. J. Schmidt. 1964. Diagnostic procedures for viral and rickettsial diseases, 3rd ed. American Public Health Association Inc., New York.
9. Linco, S. J., and G. S. Grohmann. 1980. The Darwin outbreak of oyster-associated viral gastroenteritis. Med. J. Aust. 1:211-213.
10. Metcalf, T. G., and W. C. Stiles. 1968. Enteroviruses within an estuarine environment. Am. J. Epidemiol. 88:379-391.
11. Murphy, A. M., G. S. Grohmann, P. J. Christopher, W. A. Lopez, G. R. Davey, and R. H. Millsom. 1979. An Australia-wide outbreak of gastroenteritis from oysters caused by Norwalk virus. Med. J. Aust. 2:329-333.
12. National Health and Medical Research Council. 1974. Appendix XI. Heavy metal contaminants in seafoods - cadmium and zinc. Nat. Health Med. Res. Counc. (Canberra) Rep. Session. 77:100-108.
13. New South Wales. Pure Food Act 1908. As amended 1976. Regulation 19B (8A), Oysters.
14. Sullivan, R., A. C. Fassolitis, and R. B. Read, Jr. 1970. Method for isolating viruses from ground beef. J. Food Sci. 35:624-626.
15. Thrower, S. J., and I. J. Eustace. 1973. Heavy metal accumulation in oysters grown in Tasmanian waters. Food Technol. Aust. 25: 546-553.

Moon, *con't. from p. 293*

29. Whittenbury, R., P. McDonald, and D. G. Bryan-Jones. 1967. A short review of some biochemical and microbiological aspects of ensilages. J. Sci. Food Agric. 18:441-444.
30. Wirahadikusumah, S., O. Rajala, S. Lingren, and R. Nilson. 1972. Development of lactic acid bacteria during early stages of fermentation of fish silage. Arch. Microbiol. 82:95-100.
31. Woolford, M. K. 1975. The significance of *Propionibacterium* species and *Micrococcus lactolyticus* to the ensiling process. J. Appl. Bacteriol. 39:301-306.
32. Yamasato, K., D. Okuno, and T. Ohtomo. 1973. Preservation of bacteria by freezing at moderately low temperatures. Cryobiology 10:453-463.