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

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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 (6), including examination of cell culture fluids by electron microscopy as described above.

Bacteriological examination of oysters

About 15 oysters and a small quantity 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

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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 $2.2 \times 10^4$ 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 (I3). 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 (I2,15).

The Norwalk virus cannot be cultivated 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 (I1). 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 (I). Both echovirus 8 (I) and reoviruses (II) 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 (I). 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. (II) 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. (II) 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.

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