Summary: Nagasaki Enterohemorrhagic Escherichia coli Meeting and Workshop

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In Nagasaki on 14–16 November 1996, the Japanese Panel on Cholera and Related Diarrheal Diseases of the US-Japan Cooperative Medical Science Program hosted the first international conference on enterohemorrhagic Escherichia coli (EHEC) and other diarrheal diseases since the devastating 1996 outbreaks in Japan and the United States. There were a series of presentations on the ecology, detection, genetics, epidemiology, pathogenesis, and impact of and vaccine developments for Vibrio cholerae, E. coli, Salmonella, Shigella, and Yersinia infections. These were followed by 1.5 days of presentations on the EHEC outbreaks in Japan and in the United States and the clinical manifestations, pathogenesis, and potential intervention strategies, including immunoprophylaxis. There was also a workshop on new directions and potential basic and clinical research collaborations relevant to EHEC and other diarrheal diseases.

Presentations

Hideshi Michino (Tokyo Ministry of Health and Welfare) and Hisao Fukushima (Sakai Municipal Hospital) presented their investigations of the large-scale outbreak of E. coli O157:H7 infections among schoolchildren in Sakai City, first reported on 12 July 1996. On 14 July, E. coli O157:H7 was isolated from the stools of 13 symptomatic patients, and on 23 July and 6 August, 2 schoolgirls (10 and 12 years old) died with hemolytic-uremic syndrome (HUS). Although 2 patients had illness onsets on or before 9 July, the initial peak of diarrheal illnesses (often bloody) occurred in the north and east of Sakai City on 11 July and in the central and southern areas on 12 July. Uncooked radish sprouts from a specific farm served in school meals on 8 and 9 July were epidemiologically implicated. The possibility that E. coli O157 could spread from the roots to the upper parts of the plant during cultivation in contaminated water was also demonstrated. Summaries of clinical presentations, secondary cases, asymptomatic infections, treatment with fosfomycin or norfloxacin and lactobacilli, and transient carriage of the organisms with or without antimicrobial agents were presented.

Hidemasa Izumiya (National Institute of Health [NIH], Tokyo) presented the results from analysis of >200 patient, food, and cattle isolates of E. coli O157:H7 from Japan and the United States during 1990–1996 and noted the predominance of Shiga toxin (STX) 1 and 2 production. Pulsed field gel electrophoresis data showed three different groups of E. coli O157:H7 (two involved in the 1996 outbreaks in May–June in Okayama and one in July in Osaka and Sakai City). Furthermore, they reported evidence by random amplified polymorphic DNA analysis and plasmid profile analysis showing >30 subtypes of E. coli O157:H7 in Japan and the United States since 1990.

Tae Takeda (National Children’s Medical Research Center, Tokyo) then summarized stool polymerase chain reaction (PCR) evidence (for the Shiga/Vero toxin gene) for association of E. coli O serotypes 157, 11, 165, 18, 128, 26, 2, and 145 with the cases of HUS in Japan since 1983.

Paul Mead (Centers for Disease Control and Prevention [CDC], Atlanta) then emphasized the critical importance of prompt outbreak investigation and public health responses in preventing many cases and deaths, often on the basis of epidemiologic data long before the incriminated vehicle yields the organism (if it ever does). He cited the estimated 800 cases of hemorrhagic colitis prevented in the four-state western US outbreak in January 1993 that involved >700 ill persons, 178 hospitalizations, 56 cases of HUS, and 4 deaths. On the basis of a definitive early case-control study, the incriminated hamburger patties were recalled 12 days before the organism was isolated from the hamburger. Mead also cited a similar prompt response in the apple juice outbreak in several western states in the United States that had occurred in the 3 weeks previous to the meeting and workshop. He also noted increasing concerns about potentially contaminated uncooked products, such as lettuce used in salads.

The United States has two passive surveillance systems for E. coli O157:H7 infections, one physician-based and one laboratory-based. Data from these two passive systems are published weekly in Morbidity and Mortality Weekly Report (MMWR). In addition, the United States has recently initiated an active, population-based surveillance system in five states. This system depends on regular telephone calls to laboratories and periodic surveys of physicians and the public. Active surveillance data are not published in MMWR. Also discussed was the sweeping US Department of Agriculture (USDA) Pathogen...
Reduction/Hazard Analysis Critical Control Point (PR/HACCP) system for culturing generic E. coli (not just E. coli O157:H7) and Salmonella in animal carcasses and identifying potential hazards from the farm to the kitchen.

Differential lipopolysaccharide (LPS) and cytokine signaling pathways involved in regulating Shiga toxin receptors on human vascular endothelial cells were described by Thomas Obrig (University of Rochester, New York). The movement of Shiga toxin across T-84 and CaCo-2 cell monolayers in vitro and its induction of interleukin-8 message and product in T-84 cells was reported by David Acheson (New England Medical Center, Boston).

In studies of mouse and rabbit models, Shin-ichi Yoshida (University of Occupational and Environmental Health, Kitakyushu) reported that intravenous STX-2 disrupts the blood-brain barrier, reaches central nervous system neurons, and causes fatal neurotoxicity and periventricular changes on magnetic resonance imaging similar to those seen in patients with EHEC infections. Philip Tarr (University of Washington, Seattle) described studies of the genetic regulation of the LPS O side chain of E. coli O157:H7, suggesting that it was acquired independently by this organism and may reduce its acid susceptibility, possibly contributing to its apparent low infectious dose.

A pathogenicity island (locus of enterocyte effacement), including a 94-kDa outer membrane protein intimin and a type III protein secretion system with products essential for attaching to and effacing target cells, was reported for E. coli O157:H7 analogous to that for enteropathogenic E. coli by James Kaper (University of Maryland, Baltimore). In addition, these secreted proteins were recognized by HUS patient sera. Gerald Keusch (New England Medical Center) presented data on the immunogenicity in rabbits of the Shiga toxin B subunit expressed on a plasmid electroporated into the live oral cholera vaccine strain CVD 103-HgR and the immunogenicity in mice of toxoid produced from formalin-treated STX-1 and -2 holotoxins.

Shinji Yamasaki (International Medical Center of Japan, Tokyo) described work to develop a potential live oral E. coli O157:H7 cattle vaccine using a double mutation at the glutamic acid 167 and arginine 170 positions in Shiga-like toxin type IIv E167Q/R170L with substantially reduced toxicity. Finally, Alison O’Brien (Uniformed Services University for the Health Sciences, Bethesda, Maryland) presented intriguing data on using the outer membrane product EaeA (or intimin, found to be absolutely required for tissue culture cell colonization or for the attaching and effacing lesion in gnotobiotic pigs) to produce antibody to block E. coli O157:H7 colonization in calves and piglets. She also described plans for active immunization of sows and cows intranasally or orally, perhaps with a plant-based (canola or alfalfa) intimin-expressing vaccine.

Workshop

At the very stimulating workshop that followed the meeting, several key questions were identified.

How best should surveillance for E. coli O157:H7 be conducted, including epidemiologic criteria for causality, and what would constitute appropriate rapid responses? The necessity for rapid public health responses based on sound epidemiologic data, such as using case-control studies often before sophisticated etiologic data are available, was stressed. The importance of clinical case definitions in both US and Japan surveillance systems as well as active surveillance was emphasized. An active surveillance system perhaps would require proactive telephone contact to sentinel physicians on a weekly basis and rapid information availability via the internet (with data available via MMWR from the CDC and from the Japanese NIH at http://www.NIH.go.jp/yoken/index.html).

What is a case definition? Clinical definitions and suspected or confirmed case definitions were discussed. The importance of using these to define the natural history and any clinical, serologic, or etiologic predictors of resolution or development of HUS or other complications was emphasized. The importance of establishing standard case definitions was noted, as was the necessity to develop international training opportunities in field epidemiology as well as basic laboratory methods at sites such as the US CDC, Japan NIH, and elsewhere.

What methods of diagnosis are available or needed for detection of EHEC in food, water, or fecal samples? The limitations of simply testing sorbitol-negative isolates for serotype and stx genes by PCR or for toxins by reversed passive latex agglutination and the potential importance of examining for Shiga toxin produced by organisms other than E. coli O157 were noted. Preliminary data from a hospital in Virginia suggested that non-O157 EHEC strains may rival E. coli O157:H7 in frequency of occurrence. Standard protocols and procedures for diagnosis and serologic or toxin testing would therefore be extremely helpful.

What therapies or combinations might be effective in reducing illness severity or the frequency of complications such as HUS? Options include receptor analogue Gb3 derivatives, antitoxic immunoglobulin, antibody to O antigens or other surface antigens, and antibiotics. Careful double-blind efficacy and cost-effectiveness studies are badly needed. Due to the widely scattered and often sporadic nature of EHEC infections, international collaboration in the conduct of clinical trials should be developed.

What are the risk factors for HUS? The roles of genetics and age may be important.

What preventive measures are effective? Options include reservoir control by regulating food safety such as the new USDA PR/HACCP system, vaccines for cattle (using STX-1, STX-2, or intimin), and means to prevent spread or disease in humans. In outbreak or high-risk situations, vaccines administered to family members, elderly patients, or day care center children might help limit secondary cases.
What basic pathogenesis studies are most needed? Studies suggested were those that would determine the efficacy of immunization in human volunteers using stx-negative mutants of *E. coli* O157:H7 or possibly using intimin mutants as a candidate vaccine. The importance of international collaborations and the development of international support for improved and standardized surveillance methods, double-blinded control treatment and prevention trials, and exchange of serum and stool samples and bacterial isolates were emphasized.

It is hoped that this workshop provided the stimulus for further, more effective international collaboration in controlling this increasingly important emerging enteric infection around the globe.