Summary of the First International Workshop on Human Caliciviruses

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The First International Workshop on Human Caliciviruses was held from 29 to 31 March 1999 at the Centers for Disease Control and Prevention in Atlanta. The attendance of over 100 scientists specializing in diverse fields (including civilian and military clinical medicine, basic research, epidemiology, public health, food and water safety, environmental protection, and veterinary medicine) from 18 countries underscored the increasing interest in these diarrheal pathogens.

The prototype human calicivirus strain (Norwalk virus) was discovered in 1972. In the nearly 3 decades since then, enteric caliciviruses have been linked to sporadic cases and epidemic outbreaks of gastroenteritis in humans. However, the magnitude of the medical importance of Norwalk virus and an increasing number of related caliciviruses (known collectively as the “human caliciviruses”) has been recognized only recently. In part, this delayed recognition has been attributed to the extraordinary technical difficulties in working with these viruses, which, even now, cannot be grown in cell culture. The cloning of the Norwalk virus genome in 1990 and the development of molecular biologic methods were an important advance that enabled rapid progress in nearly all areas of human calicivirus research in the past decade. The purpose of the First International Workshop on Human Caliciviruses was to provide a forum in which this progress could be reviewed in order to evaluate what is currently known about the role of human caliciviruses in diarrheal disease and to consider future directions of research.

A predominant theme in the workshop was the remarkably high frequency with which human caliciviruses are associated with acute gastroenteritis worldwide. New data collected from several large-scale epidemiologic studies (one involving an entire country, The Netherlands), community studies, and gastroenteritis outbreak investigations confirmed that calicivirus infection and disease are common. Environmental surveys found that caliciviruses are ubiquitous and apparently stable in the environment, providing a ready source of virus for potential infection. It is now estimated that as many as 95% of nonbacterial gastroenteritis outbreaks, many of which are associated with the ingestion of contaminated food or water, are caused by human caliciviruses related to the Norwalk-like viruses. Furthermore, these viruses may cause gastroenteritis episodes that number in the millions per year in individual countries.

This considerable disease burden is undoubtedly expensive to society in terms of the cost of outbreak management and loss of productivity. However, of even greater importance, were several reports in this workshop showing an association of human caliciviruses with severe gastroenteritis that required medical intervention; this suggested that the impact of this disease may extend well beyond the economic burden. These new data are important because they contrast with the usual observation that human calicivirus illness is mild and self-limiting. Continued assessment of the precise overall burden of the morbidity and mortality associated with human calicivirus disease in all age groups, including infants and young children, will be essential in the decision-making process for the development of future prevention and control strategies.

The striking genetic diversity among the human caliciviruses was recognized as a major research challenge. The development of effective diagnostic tests, such as a broadly reactive reverse transcriptase–polymerase chain reaction (RT-PCR) assay or an immunoassay that recognizes all circulating strains, has been problematic. However, analysis of the rapidly growing sequence database for the human caliciviruses may explain this difficulty. There are now 2 distinct genera containing human caliciviruses in the family Caliciviridae that are provisionally named “Norwalk-like viruses” and “Sapporo-like viruses.” Within each genus, there is additional genetic diversity, and it is likely that even more diversity will be identified as molecular epidemiologic studies progress. For example, caliciviruses recently identified in swine and cattle were found to be genetically related to the human caliciviruses, which raises the possibility of animal-to-human transmission (or vice versa).

Defining the extent of genetic relatedness among human and animal caliciviruses was recognized as an area that should be studied further to identify a potential natural reservoir for the caliciviruses that cause disease in humans. In addition, it may be possible to develop a surrogate diarrheal disease model with an enteric animal calicivirus. A significant implication of the marked genetic diversity among the human caliciviruses is that it raises the possibility for considerable antigenic variation. Although the relationship between the genetic and antigenic characteristics of many human caliciviruses has not been established, data presented at this workshop showed that a battery of recombinant, self-assembled, virus-like particles (VLPs) de-
veloped by expression of the capsid protein from several different viruses holds great promise as a tool to study antigenic relationships. Knowledge of the antigenic relationships among the human caliciviruses will undoubtedly be crucial in understanding the epidemiology, immunity, and biology of these viruses.

The identification of a permissive cell culture system for the human caliciviruses remains an important goal. Without this system it will be impossible to define serotypes by neutralization or to assess the role of neutralizing antibodies in immunity. The ability to quantitate virus by titration in cell culture would facilitate environmental studies designed to assess risk factors for gastroenteritis resulting from exposure to contaminated food or water in which the viability of viruses detected by RT-PCR is not clear. The basic mechanisms of calicivirus replication will remain largely unknown until a cell culture system is available. Meanwhile, molecular biology–based studies using recombinant proteins and cDNA clones will certainly lead to major new findings, as evidenced by several elegant molecular biology studies presented at this workshop. For example, a complete proteolytic cleavage map was generated for the Southampton virus nonstructural polyprotein from studies that were performed entirely in vitro with cDNA clones. Studies such as this may give insight into the development of antiviral drugs, a potential intervention strategy.

An atomic resolution structure of the Norwalk virus capsid obtained from x-ray crystallography analysis of Norwalk VLPs was presented, and this major accomplishment is leading to a deeper level of understanding of the topology and function of the intact virion. Furthermore, these same Norwalk VLPs were immunogenic when administered orally to adult volunteers, suggesting that this technology may yield a potential vaccine candidate, if vaccines are ultimately established as a prevention strategy for calicivirus illness.

The findings presented in this workshop demonstrated the astounding progress in the human calicivirus field over the past few years. However, there is still much to be learned. For example, it was clear that a justification for the development and evaluation of calicivirus vaccines could be made, especially in settings where it is already apparent that individuals may be at particular risk for serious consequences, such as deployed military personnel or the elderly in nursing homes. However, what type of vaccine would be best? Would it need to protect against several serotypes? Who would receive the vaccine, and at what age? What constitutes protective immunity? What is the risk/benefit ratio for calicivirus vaccine development? These questions alone extend to almost every area of calicivirus research and illustrate the need for continued studies.

An encouraging sign from this workshop was the expressed willingness of calicivirus researchers to find ways to coordinate and share information on a global level. Two satellite meetings were held following the workshop. One meeting discussed the organization of a computer networking effort called “CaliciNet.” The goal of CaliciNet would be to aid the tracking of calicivirus strains as they spread globally, by sharing nucleotide sequences associated with current outbreak strains via a special World Wide Web site. This information would be especially useful to public health officials who are interested in preventing the spread of outbreaks caused by virus-contaminated food and water vehicles in the context of the increasing global market.

The second satellite meeting focused on the standardization and sharing of calicivirus diagnostic reagents. An agreement was made among interested scientists to perform a comparative study of their VLP-based immunoassays and RT-PCR primer pairs in order to identify the most effective methods for identification of the diverse array of circulating caliciviruses associated with disease. Diagnostic assays for human caliciviruses are not widely available. This is partly due to the fact that broadly reactive reagents have not been developed for practical use. Coordinated efforts similar to these and continued research give promise for lessening the impact of diarrheal disease associated with human caliciviruses, which should be an important public health goal.

Note

This summary reviews presentations made by the following workshop participants: David Brown, Ian Clarke, Mary Estes, Albert Kapikian, Marion Koopmans, Morris Potter, Mark Sobsey, and Duncan Steele.