Meeting Summary

The 2nd International Symposium of the European Study Group on Enterohemorrhagic *Escherichia coli* (EHEC)

A total of 193 participants from 15 European countries, Canada, and China gathered in Brussels, Belgium, on April 16-17, 1999, to discuss progress in EHEC epidemiology and surveillance, origin of infection, virulence factors, and pathomechanisms, as well as diagnostics, molecular characterization, and typing methods. Alberto Tozzi (Italy); Helge Karch, Herbert Schmidt, Lothar Zimmerhackl, Matthias Pulz, and Jochen Bockemühl (Germany); Gad Frankel, Henry R. Smith, and Frederick J. Bolton (United Kingdom); Vincent Leclerc (France); and David Karpman (United States) provided overviews and poster session summaries.

Overview of EHEC Infections and Hemolytic Uremic Syndrome (HUS) in Europe (1)

In continental Europe, the incidence of Shiga-toxin producing *Escherichia coli* (STEC) is low ($<1/10^5$) and the incidence of HUS is $1.9/10^5$, while in the United Kingdom the incidence of STEC infections has been as high as $2.7/10^5$ (in 1997). The vehicles of transmission are often unknown, although EHEC infections in the United Kingdom were foodborne and (in contrast to those in continental Europe) associated with the prototype of EHEC O157:H7. In Germany (1992 and 1993) and in France (1992), person-to-person transmission seems to be predominant, while in Spain (1995) waterborne infections were reported. In the Netherlands (1993), Finland (1997), and Spanish Islands (1986, 1994, and 1997), infections due to swimming in lakes were reported. Continental Europe was faced with emerging non-O157-infections (e.g., O111, O103, and O26), although non-O157 STEC were isolated from every European country. Prevalence of O157 EHEC-induced HUS was 69% (Belgium, 1996), 61% (Germany, 1996-98), 58% (France, 1997-98), and 38% (Italy, 1996-98). STEC infections are more frequent in Northern Europe.

The introduction and establishment of Enter-Net (International Network for Surveillance of Enteric Infections, Salmonella, and STEC) should overcome the problem of "not speaking the same language" in Europe and contribute to better understanding and successful communication between countries regarding enteric and STEC outbreaks, with the benefit of a coordinated approach to international STEC standardized surveillance. Enter-Net started with 15 European countries, and the participants are now negotiating communicative exchange with the United States, South Africa, Japan, and Asia. The objectives include monitoring of *Salmonella* sp. antimicrobial resistance and STEC O157 typing-method harmonization. This concerted action will lead to an international *Salmonella* database. The STEC file specifications are reference, microbiologic, and epidemiologic data and data transfer. Long-term surveillance will identify ongoing changes in the epidemiologic situation in participating countries. As an example, a 20% increase in *Salmonella enteritidis* infections in Western Europe in 1995 to 1998 reflects successful Enter-Net communication. In addition, international outbreaks are being recognized. Thus, the coordinated approach will provide an international STEC surveillance.

Model of the Evolution and Origin of EHEC (2)

Grouping of at least four EHEC clone complexes, all related to an ancestral enteropathogenic *E. coli* (EPEC)-like strain O55:H7, harboring the locus of enterocyte effacement (LEE) pathogenicity island, was suggested on the basis of multilocus enzyme electrophoresis, DNA fingerprinting, polymerase chain reaction (PCR)-based techniques, and sequence analysis data. Additional characteristics, such as the ability to ferment sorbitol (SOR+), express ß-D-glucuronidase (GUD+), and express either Shiga toxin 1 (Stx 1+), Shiga toxin 2 (Stx 2+), or both were also considered. These four clone complexes are 1) O157:H7/H- and O55:H7; 2) O26:H11 and O111:H8; 3) O113:H21 and 4O91:H21; and 4) O103:H2 and O45:H2. While O157:H7 is found worldwide, the sorbitol fermenting group (SOR+) of O157:H- seems to be geographically restricted to Germany and the Czech Republic. O157:H- *E. coli* are important pathogens in Germany, with most infections occurring during winter. Compared with the prototype-like *E. coli* O157:H7, where infections occurred during summer, the SOR+ O157:H- strains are often negative for hemolysin (Hly-), negative for the

catalase-peroxidase (Kat P-), negative for the serin protease (Esp P-), and sensitive to tellurite. Microevolutional events (exchange of amino acids of individual genes, e.g., housekeeping genes) and macroevolutional events (horizontal gene transfer of plasmids, phages, and LEE) may have contributed to the evolution of STEC.

Pathogenic Mechanism of *E. coli* Leading to Diarrhea (3)

The attaching and effacing (A/E) lesions on epithelial cells caused by either EHEC or EPEC are mediated by binding of intimin (gene: *eae*) to its receptor (Tir) on the host cell. Both genes are located in LEE. Frankel and colleagues introduced four different classes of intimin (intimin α, ß, γ, and δ) on PCR-based data, which seem to exhibit different Tir/cell-binding properties, as shown by gel overlay and by the yeast two-hybrid system. As a hypothesis and a conclusion from the experiments presented, Frankel et al. suggested two binding activities involving a host cell receptor and Tir and binding of EHEC O157 to human intestinal mucosa. These interactions can produce A/E lesions in the Peyers Patches but not in all parts of the mucosa.

Virulence Factors of EHEC (4)

The most important characteristic elements of EHEC are located on mobile genetic elements—Shiga toxin (phage-encoded); the gene for hemolysin (*ehly*); and serin protease (*esp P*, plasmid-encoded), which is flanked by IS (Insertion) elements. LEE, which consists of a cluster of genes involved in A/E lesions, is located on a 35.5-kb fragment and inserted at 82 min in the *E. coli* chromosome (5). Besides the capability of expressing prophage-encoded Shiga toxins, the expressions of large plasmid (pO157)-encoded genes, such as hemolysin (*ehly*), *etp* (transporter of type II secretion pathway), *kat P* (bifunctional catalse-peroxidase), and *esp P* (serin protease) are considered additional or potentially important virulence factors. While *E. coli* O157 is associated to 100% with hemolysin and Etp expression and to 66% with Kat P and Esp P expression, 95% of the non-O157 strains express hemolysin, 52% Etp, 38% Kat P, and 36% Esp P. These virulence factors are not found in EPEC, diffuse enteroaggregative *E. coli,* and enteroaggregative *E. coli*. The hemolysin is a 107-kd pore-forming protein. It lyses eucaryotic cells, vero cells, and sheep red blood cells and is closely related to the α-hemolysin. It is cell-associated, exported in low amounts, and reacts with reconvalescent-phase sera from HUS patients. The ability of expressing hemolysin is proven after 20 hours of incubation at 37°C on indicator agar. The bifunctional catalase-peroxidase is located in the periplasm and interacts with the host cell defense mechanism on the reactive oxygen intermediate level. The serin protease (Esp P) is temperature regulated, expressed during infection, and cleaves the human coagulation factor V. The Shiga toxins consist of 1 A subunit and 5 B subunits (A1B5), inserted in the same region in the phage, and are most probably not randomly integrated in the *E. coli* chromosome.

A European and International Standard for the Detection of *E. coli* O157 in Food (6)

This is an approach to harmonize O157 STEC diagnostics. Precise recommendations are given on how to set up such a diagnostic procedure. The inquiry on the launch began in early 1999 among members of the European Committee for Standardization and the International Organization for Standardization.

HUS Surveillance in Europe (7)

HUS is a complex of symptoms (including anemia, acute renal failure, and thrombocytopenia) with multiple etiologies. Data from a prospective study indicate that 85% of the HUS cases in Germany in 1997 were associated with EHEC infections and thus involved postinfectious HUS. Most cases were due to eating contaminated food, but person-to-person transmission seems to be a prominent vehicle of transmission in Germany. Also, only a minority of European countries have a mandatory EHEC reporting system (Austria, since 1996; Finland, since 1994; Sweden, since 1996; and Germany, since 1998). In addition to a standardized definition, EHEC also requires standardized diagnostic procedures. Early diagnosis is a prerequisite for fewer deaths and fewer patients on dialysis.

Prevalence and Molecular Characterization of STEC in Asymptomatic Children (8)

In 1997, an increase in EHEC infections was observed after raw milk consumption in an EHEC-endemic area in Germany (Weser-Emsland). Four children contracted HUS after drinking raw milk. A total of 1,697 asymptomatic

children from 27 kindergartens were screened for STEC in stool specimens with enzyme immunoassay, and positive results were confirmed by PCR. The prevalence was 0.8% (15 cases), but the infectivity of these asymptomatic carriers was low, since during a 4-week monitoring phase of five children who were still shedding virus no new infection occurred. Nine of these 15 cases were positive for *eae* and *hly*, *as* well as *stx*. The predominant serotype found was *E. coli* O111:H-.

New Aspects on the Antibody Response in Children with Diarrhea and HUS (9)

The anti-LPS antibodies that can be screened for comprise a group of approximately 25 *E. coli* serotypes. Other *E. coli* proteins, such as Esp A and Esp B (secretory proteins) and intimin, can mount an antibody response. Although Western blot analysis did not show significant changes in pattern of acute- and convalescent-phase sera, further investigation is warranted for this *E. coli*-serotype-independent method of detecting EHEC infections.

Since diagnostics of EHEC from clinical specimens (stool samples) of patients with EHEC-induced diarrhea, hemorrhagic colitis, or HUS may fail (10), a further focus on serologic diagnostics could be one approach to meet the need for additional reliable diagnostics of the biochemical heterogeneous *E. coli* strains belonging to the STEC/EHEC group.

Overview of EHEC Infections and Detection in the Laboratory

Although progress has been made in harmonizing STEC/EHEC diagnostic procedures, in-vitro diagnostic manufacturers still concentrate on *E. coli* O157 detection systems. Outer membrane serotyping (OMS) has problems along with its advantages; for example, there are 1-173 O-groups (three times as many as for *Salmonella*), polysaccharides covering the O-specific side chain, heating is required for agglutination (boil strains for 1-2 hours), and cross-reactions between O- and H-antigens (1-53) serogroups might occur.

Typing and Characterization of EHEC

While a number of new molecular techniques are useful for analysis of clonal diversity and characterization of virulence factors, the optimal method should always be a combination of at least two methods (Tschäpe [Germany], Grimont [France]). These methods include such phenotyping procedures as phage-, colicin-, and serotyping and antibiotic resistance pattern, but also outer membrane protein pattern, LPS pattern, and multilocus enzyme electrophoresis. Genotyping procedures are plasmid profile analysis, restriction fragment length polymorphism, methods, such as ribotyping, virulence-associated gene probes, macrorestriction by pulse-field gel electrophoresis, PCR-based methods, multilocus sequence typing, and random amplified polymorphism DNA PCR fingerprinting (11).

Bacteriologic and Immunologic Techniques for Detecting EHEC in Food and Water (12)

Since *E. coli* O157 is usually present in low numbers (<10 cfu/g), methods of detection and selective enrichment procedures are required. Many factors affect the efficacy of protocols: the enrichment broth or agar used, the temperature of incubation, the choice of method used (e.g., immunomagnetic separation [IMS] for *E. coli* O157 only), and the subculturing methods. The detection level (number of bacteria) for IMS after enrichment is 1.7×10^1, for direct IMS 3.0×10^2, for direct culture 1.7×10^4, and for culture after enrichment 3.4×10^1. As a standard protocol, starting with 25 g food/ml broth for enrichment is recommended; water should be filtered first and may need to be concentrated. Bile salts as supplements often do not allow recovery and may inhibit the PCR reaction (A. Lehmacher, Germany). Also, the following incubation times should be used for different types of foods: raw meat, 6 hours; milk, 20 to 24 hours; cheese, 20 to 24 hours; and potable water, 20 to 24 hours. There are now suitable commercially available enzyme immunoassays that allow sensitive and specific detection of *E. coli* O157.

Conclusions

The European study group on EHEC has made enormous progress in the last 2 years in the surveillance of STEC/EHEC and efforts in harmonizing detection methods, above all the *E. coli* O157-specific techniques. Europe must confront the emerging group of non-O157 *E. coli*, although some European countries do not seem to have this problem, perhaps because of lack of recommended procedures and techniques for detecting non-O157 *E. coli*. For example, the Federal Republic of Germany is successfully

facing the problem of independently diagnosing STEC/VTEC serotype (13). There is still much to do, but we are now gaining a better understanding of this complex field.

Abstracts of other presentations from the Symposium of the European Study Group on EHEC were published in Acta Clinica Belgica, 1999, 54-1, pages 33-52.

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10th International Rabies in the Americas Meeting
November 14-19, 1999

The 10th International Rabies in the Americas Conference is scheduled for November 14-19, 1999, at the Town and Country Resort Hotel, San Diego, CA. Sponsors include the California Association of Public Health Laboratory Directors and the Viral and Rickettsial Disease Laboratory Branch and the Veterinary Public Health Section of the California Department of Health Services. Rabies in the Americas, an annual meeting that has been held since 1990 in the United States, Canada, Mexico, and South America, highlights current issues and research advances in rabies control in the Americas.

A call for papers is proposed in the following areas: animal populations susceptible to rabies; vaccination of animals; rabies transmission by bats, wildlife, and domestic animals; rabies diagnosis and epidemiologic surveillance; human rabies prophylaxis; clinical aspects of rabies and rabies pathology; molecular epidemiology; health education and rabies prevention; and the legal and economic aspects of rabies control. A special all-day session arranged by Dr. Hilary Koprowski, entitled "Rabies 2000," will be held November 19, 1999.

The proposed deadline for paper submission and registration is September 1, 1999. A late fee may apply to registrations after September 1. A registration discount will be given to presenters. To obtain registration materials or submit papers, please contact Donna Taclindo, California Department of Health Services, Viral & Rickettsial Disease Laboratory, 2151 Berkeley Way, Room 454, Berkeley, CA, 94704; phone (510) 540-2830, or e-mail dtaclind@dhs.ca.gov or bsun@dhs.ca.gov. Program and registration information is also available at the following website: http://www.caphld.org.

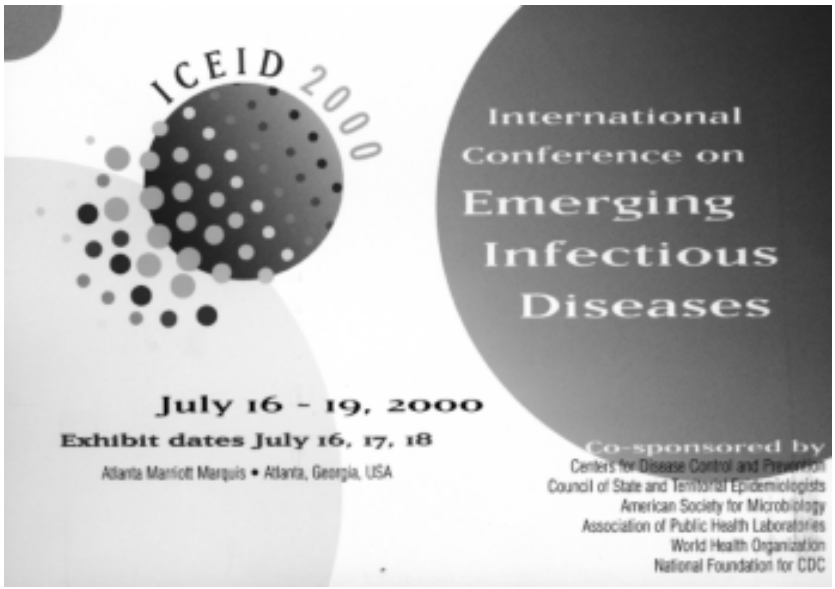
The 10th International Symposium on Viral Hepatitis and Liver Disease
Atlanta, Georgia, USA, April 9–13, 2000

Topics will include virology, epidemiology, diagnosis, treatment, and prevention. For more information contact: Organizing Secretariat, MediTech Media Ltd., Tower Place 100, 3340 Peachtree Road, Suite 550, Atlanta, GA 30326, USA; telephone: 404-233-4490; fax: 404-233-7464; e-mail: info@Hep2000.com.

Erratum Vol. 5, No. 3

In the article "Bacterial Vaccines and Serotype Replacement: Lessons from *Haemophilus influenzae* and Prospects for *Streptococcus pneumoniae*," by Marc Lipsitch, there is an error in the figure legend on page 339. Controls are incorrectly identified as black bars; however, in the text and the figure itself, controls are correctly represented by white bars and vaccine recipients by black bars. We regret any confusion this error may have caused.

ICEID 2000



Hold the dates of July 16–19, 2000, for the International Conference on Emerging Infectious Diseases, a meeting of 2,500 specialists in infectious diseases. The program will include plenary sessions and symposia with invited speakers, presentations on emerging infections activities, and oral and poster presentations. Major topics will include current work on surveillance, epidemiology, research, communication and training, as well as prevention and control of emerging infectious diseases, both in the United States and abroad. Abstracts are invited and will be accepted beginning in October 1999.

The Call for Abstracts and Preliminary Program will be mailed in October 1999.

For more information, call ICEID management at 202-942-9248, e-mail meetinginfo@asmusa.org, or www.cdc.gov/ncidod/iced2k.htm.