Hot Topics in Infection and Immunity in Children V

Advances in Experimental Medicine and Biology

Recent Volumes in this Series

VOLUME 626 GENOMIC IMPRINTING
Edited by Jon F. Wilkins

VOLUME 627 TRANSGENESIS AND THE MANAGEMENT OF VECTOR-BORNE DISEASE
Edited by Serap Aksoy

VOLUME 628 BRAIN DEVELOPMENT IN *DROSOPHILA MELANOGASTER*
Edited by Gerhard M. Technau

VOLUME 629 PROGRESS IN MOTOR CONTROL
Edited by Dagmar Sternad

VOLUME 630 INNOVATIVE ENDOCRINOLOGY OF CANCER
Edited by Lev M. Berstein and Richard J. Santen

VOLUME 631 BACTERIAL SIGNAL TRANSDUCTION
Edited by Ryutaro Utsumi

VOLUME 632 CURRENT TOPICS IN COMPLEMENT II
Edited by John. D. Lambris

VOLUME 633 CROSSROADS BETWEEN INNATE AND ADAPTIVE IMMUNITY II
Edited by Stephen P. Schoenberger, Peter D. Katsikis, and Bali Pulendran

VOLUME 634 HOT TOPICS IN INFECTION AND IMMUNITY IN CHILDREN V
Edited by Adam Finn, Nigel Curtis, and Andrew J. Pollard

Adam Finn · Nigel Curtis · Andrew J. Pollard
Editors

Hot Topics in Infection and Immunity in Children V

Springer

Editors
Adam Finn
University of Bristol
UK
Adam.Finn@bristol.ac.uk

Nigel Curtis
University of Melbourne
Royal Children's Hospital
Melbourne, Australia
nigel.curtis@rch.org.au

Andrew J. Pollard
University of Oxford
Oxford, UK
andrew.pollard@paediatrics.ox.ac.uk

ISSN: 0065-2598
ISBN: 978-0-387-79837-0 e-ISBN: 978-0-387-79838-7
DOI 10.1007/978-0-387-79838-7

Library of Congress Control Number: 2008938672

Printed on acid-free paper

springer.com

Preface

Each of the chapters in this book is based on a lecture given at the fifth 'Infection and Immunity in Children' course held in June 2008 at St. Catherine's College, Oxford, UK. Thus, it is the fifth book in a series which collectively provide succinct and readable updates on just about every aspect of the discipline of paediatric infectious diseases.

The sixth course was scheduled for 23–25 June 2008 with another exciting programme and renowned speakers and we expect to produce a sixth edition of this book based on that course.

Paediatric infectious diseases continue to grow and flourish in Europe with centres now being accredited for training by the European Society for Paediatric Infectious Diseases on behalf of the European Academy of Paediatrics. Plans are also taking shape for a University of Oxford Diploma Course in Paediatric Infectious Diseases, towards which participation in the Oxford IIC course, as well as other ESPID-sponsored educational activities, will give credits.

We hope this book will provide a further useful contribution to the materials available to trainees and practitioners in this important and rapidly developing field.

UK, Australia

Adam Finn
Nigel Curtis
Andrew J. Pollard

Acknowledgments

We are indebted to all those who have contributed to the writing of manuscripts for this book. We are grateful to the staff of St. Catherine's College, Oxford, UK where the 2007 'Infection and Immunity in Children' course was held; many of the lectures of which form the basis for the chapters herein. Sue Sheaf has administered and run the course for several years now with enormous efficiency and effectiveness and we extend our sincere thanks to her on behalf of all the organizers, speakers, and delegates who have benefited from her labours. Lorraine Cantle patiently coaxed the authors and editors into action, carefully corrected and formatted the chapters and liaised with the publishers to ensure the book's efficient production and needs an extra special thanks for all her work.

We thank the European Society for Paediatric Infectious Diseases for consistent support and financial assistance for this and previous courses and for providing bursaries which have paid the costs of many young ESPID members' attendance. We also acknowledge the recognition given to the course by the Royal College of Paediatrics and Child Health.

Finally, we are grateful to several pharmaceutical industry sponsors who generously offered unrestricted educational grants towards the budget for the meeting.

Contents

Contributors

Christina K. Ahn, BS
Doris Duke Fellow, Washington University School of Medicine, 660 South Euclid Avenue, Campus Box 8208, St. Louis, MO 63110, USA, Ahn_C@kids.wustl.edu

Vinicius C. Antao
Centers for Disease Control and Prevention, 4770 Buford Highway NE, Mailstop F57, Atlanta, GA, USA, VAntao@cdc.gov

Louis Bont
Department of Pediatric Infectious Diseases, University Medical Center Utrecht, Rm KE4.133.1, POB 85090, 3508 AB Utrecht, l.bont@umcutrecht.nl

Professor Andrew J. Cant
Immunology and Infectious Diseases Unit, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, NE4 6BE

James D. Cherry, MD, MSc
Department of Pediatrics, David Geffen School of Medicine at UCLA, 10833 Le Conte Ave, MDCC 22-442, Los Angeles, CA 90095, jcherry@mednet.ucla.edu

Dr. Julia Clark
Consultant in Paediatric Immunology and Infectious Diseases, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, NE4 6BE, United Kingdom, Julia.clark@nuth.nhs.uk

Professor Ron Dagan
Pediatric Infectious Disease Unit, Soroka University Medical Center, P.O. Box 151, Beer-Sheva 84101, Israel, rdagan@bgu.ac.il

Professor Simon Dobson
Clinical Associate Professor, Division of Pediatric Infectious Diseases, Department of Pediatrics, University of British Columbia, British Columbia

Children's Hospital, 4480 Oak Street, Ambulatory Care Building – Rom K4-218, Vancouver, British Columbia, Canada, sdobson@cw.bc.ca

Dr. Andrew R. Gennery
Senior Lecturer in Paediatric Immunology, Ward 23, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, NE4 6BE, a.r.gennery@ncl.ac.uk

Beth Halperin,
Lecturer, Canadian Centre for Vaccinology, Dalhousie University, IWK Health Centre, 5850/5980 University Avenue, PO Box 9700, Halifax, Nova Scotia, B3K 6R8, Canada

William P. Hausdorff, PhD
Director, Epidemiology and Scientific Strategy, GlaxoSmithKline Biologicals s.a., Rue de l'Institut 89, B-1330 Rixensart, Belgium.
William.P.Hausdorff@gsk.com

Nicholas J. Holt, BS
Research Technician, Washington University School of Medicine, 660 South Euclid Avenue, Campus Box 8208, St. Louis, MO 63110, USA

David Isaacs
Clinical Professor, Department of Infectious Diseases, Children's Hospital at Westmead, Westmead, New South Wales 2145, Australia, DavidI@chw.edu.au

Sheldon L. Kaplan MD
Professor and Vice-Chairman for Clinical Affairs, Head, Section of Infectious Diseases, Department of Pediatrics, Baylor College of Medicine, Chief, Infectious Disease Service, Texas Children's Hospital, Feigin Center MC 3-2371, Suite 1150, 1102 Bates, Houston, TX 77030, skaplan@bcm.tmc.edu

Keith P. Klugman
William H Foege Professor of Global Health, Hubert Department of Global Health, Rollins School of Public Health, and Division of Infectious Diseases, School of Medicine, Emory University, 1518 Clifton Road, N.E - Room 720 Atlanta, GA 30322 USA, keith.klugman@emory.edu

Noni MacDonald
Professor of Pediatrics, Canadian Centre for Vaccinology, Dalhousie University, IWK Health Centre, 5850/5980 University Avenue, PO Box 9700, Halifax, Nova Scotia, B3K 6R8, Canada, noni.macdonald@dal.ca

Ben J. Marais
Department of Paediatrics and Child Health, Faculty of Health Sciences, Stellenbosch University, PO Box 19063, Tygerberg, 7505 South Africa, bjmarais@sun.ac.za

George H. McCracken, Jr. MD
Division Director, GlaxoSmithKline Distinguished Professor of Pediatric Infectious Disease, Department of Pediatrics, Division of Pediatric Infectious Diseases, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas, George.McCracken@UTSouthwestern.edu

Dr. E. David G. McIntosh
Medical Director Infectious Diseases (Europe, the Middle East and Africa), Wyeth Europa Limited, Vanwall Road, Maidenhead, Berkshire, SL6 4UB, United Kingdom, mcintod@wyeth.com

Shelly McNeil
Assoc. Professor of Medicine, Canadian Centre for Vaccinology, Dalhousie University, IWK Health Centre, 5850/5980 University Avenue, PO Box 9700, Halifax, Nova Scotia, B3K 6R8, Canada

Dr. Stéphane Paulus
Infectious Diseases Fellow, British Columbia Children's Hospital, 4480 Oak Street, Ambulatory Care Building – Room K4-218, Vancouver, British Columbia, Canada, scpaullus@gmail.com

Dr. Michael E. Pichichero
University of Rochester Medical Center, 601 Elmwood Avenue, Box 672, Rochester, New York 14642, USA, Michael.pichichero@urmc.rochester.edu

Dr. F. Andrew I. Riordan
Consultant in Paediatric Immunology and Infectious Diseases, Royal Liverpool Childrens' Hospital (Alder Hey), Liverpool L12 2AP, Andrew.riordan@rlc.nhs.uk

Phillip I. Tarr, MD
Melvin E. Carnahan Professor of Pediatrics, Professor of Molecular Microbiology, Washington University School of Medicine, 660 South Euclid Avenue, Campus Box 8208, St. Louis, MO 63110, USA

Mirjam van der Burg
Department of Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands, m.vanderburg@erasmusmc.nl

Jacques J.M. van Dongen
Department of Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands,

Menno C. van Zelm
Department of Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

Dr. Thomas N. Williams
Reader in Tropical Medicine, University of Oxford; Honorary Consultant Paediatrician, Department of Paediatrics, Oxford Radcliffe NHS Trust, Oxford, UK; Wellcome Senior Research Fellow in Clinical Science, KEMRI/Wellcome Trust Collaborative Programme, Centre for Geographic Medicine Research Coast, PO Box 230; Kilifi, Kenya & Honorary Consultant Paediatrician, Kilifi District Hospital, Kilifi, Kenya, twilliams@kilifi.kemri-wellcome.org

Shiga-Toxin Producing *Escherichia coli* and the Hemolytic Uremic Syndrome: What Have We Learned in the Past 25 Years?

Christina K. Ahn, Nicholas J. Holt, and Phillip I. Tarr

1 Introduction

Escherichia coli that belong to the serotype O157:H7 and produce Shiga toxins are important and challenging human pathogens. This organism can cause quite severe human enteric illnesses, including diarrhea and bloody diarrhea. Most notably, *E. coli* O157:H7 is the predominant cause of the hemolytic uremic syndrome (HUS) worldwide. HUS consists of nonimmune hemolytic anemia, thrombocytopenia, and acute renal failure, and disproportionately affects children. Both *E. coli* O157:H7 infections and HUS are relatively rare. According to 2007 estimates from the Centers for Disease Control (CDC) (McNabb, Jajosky et al. 2007), there are only *c*. 2600 cases of culture-proven *E. coli* O157:H7 infection annually in the entire United States. Based on these data, we estimate that there are about 400 cases of HUS per year, and half or more of the cases of HUS are in children less than 10 years of age. HUS is a thrombotic illness (Upadhyaya, Barwick et al. 1980; Inward, Howie et al. 1997; Tsai, Chandler et al. 2001), and it is quite likely that ischemic renal injury secondary to these thrombi leads to acute renal failure (Bellomo, Kellum et al. 2007). It is also probable that the thrombotic injury begins early in the illness, well before azotemia ensues. This is a challenge because interventions that target the infecting bacteria are probably futile, but if patients are identified in a timely manner, there is an opportunity to maintain renal perfusion as thrombi evolve. Because of the rarity of *E. coli* O157:H7 infections, their serious consequences and their epidemiological importance, it is critical to have good community-based microbiology diagnosis close to point of presentation, and to utilize syndrome profiling to identify infected patients accurately and expeditiously.

C.K. Ahn (✉)
Washington University School of Medicine, 660 South Euclid Avenue, Campus Box 8208, St. Louis, MO 63110, USA

A. Finn et al. (eds.), *Hot Topics in Infection and Immunity in Children V*,
DOI 10.1007/978-0-387-79838-7_1,

2 History

HUS was first described in 1955 by von Gasser et al. who reported five children, all of whom died, with small-vessel renal thrombi, thrombocytopenia, and Coombs-negative hemolytic anemia (Gasser C 1955). In 1975, Kaplan et al. described simultaneous cases of HUS within families and suggested that their cause was environmental and probably infectious (Kaplan, Chesney et al. 1975). In 1983, Karmali et al. linked childhood HUS with a cytotoxin produced by *E. coli* in stool (Karmali, Steele et al. 1983). Some of these toxin-producing *E. coli* were of serotype O157:H7. One week later, a publication by Riley et al. described two outbreaks in adults with painful bloody diarrhea, which used the term hemorrhagic colitis (Riley, Remis et al. 1983). In both outbreaks, undercooked hamburgers were eaten, and many of the patients had *E. coli* O157:H7 in their stools. That same year, O'Brien and colleagues established that *E. coli* O157:H7 produced a toxin similar to that produced by *Shigella dysenteriae* serotype 1 (O'Brien, Lively et al. 1983). These studies underlie our current understanding of the pathophysiology of *E. coli* O157:H7 infections. They also form the basis for our understanding of HUS as a toxemic systemic consequence of a nonbacteremic enteric infection.

3 Epidemiology of *E. coli* O157:H7 Infections and HUS

Time of year: The most common times of year for *E. coli* O157:H7 infections and HUS to occur are the summer and autumn. It is not clear if this pattern is related to seasonal environmental or food exposures, or to seasonally varying levels of contamination from these or other vehicles of transmission.

Location E. coli: O157:H7 infections and HUS seem to be more prevalent in the Northern Hemisphere and in latitudes farther from the equator (Tarr and Hickman, 1987; Jernigan and Waldo, 1994; Slutsker, Ries et al. 1997; Cummings, Mohle-Boetani et al. 2002). However, there are notable exceptions. Buenos Aires, Argentina, although below the equator, has a high incidence of HUS cases (Lopez, Diaz et al. 1989). In countries that lack the laboratory resources to diagnose the infection, rates of *E. coli* O157:H7 infections are probably underestimated.

Rural versus urban: Rural locations have a higher incidence of diagnosed *E. coli* O157:H7 infections and HUS than do urban populations. A possible explanation for this difference is that environmental rather than food-borne exposures are responsible, which is also inferred in a recent study from rural Scotland (O'Brien, Adak et al. 2001).

Outbreaks of E. coli: Contrary to popular belief, *E. coli* O157:H7 infections are largely sporadic, or occur in small clusters, such as within households. Large, well-publicized outbreaks are the exception and not the rule. Absence

of associated cases should not discount the possibility that a patient is infected with *E. coli* O157:H7.

Vehicle of transmission: While people can certainly be infected through poorly cooked, ground beef, there are many other modes of infection that are often overlooked. In fact, in aggregate, nonbeef sources for this pathogen might be more prominent. Such nonhamburger vehicles include municipal (Swerdlow, Woodruff et al. 1992) and swimming water (Keene, McAnulty et al. 1994), deer jerky (Keene, Sazie et al. 1997), unpasteurized milk (Keene, Hedberg et al. 1997), spinach (Uhlich, Sinclair et al. 2007), salami (Tilden, Young et al. 1996), lettuce (Ackers, Mahon et al. 1998), bovine contact (Crump, Sulka et al. 2002), radish sprouts (Michino, Araki et al. 1999), unpasteurized apple cider (Besser, Lett et al. 1993), and salmon roe (Terajima, Izumiya et al. 2002). Also, the infection can be spread by person-to-person contact and via air-borne routes (Varma, Greene et al. 2003).

4 Clinical Course of *E. coli* Infection

Figure 1 illustrates the typical course of an *E. coli* O157:H7 infection. According to data accumulated from a large epidemic in Seattle, the median time between ingestion of the bacteria and onset of diarrhea was 3 days (Bell, Goldoft et al. 1994). The first day of illness is most appropriately defined as the first day that a patient has diarrhea. Other prodromal symptoms can precede or accompany the diarrhea (e.g., fatigue, headache, abdominal pain, vomiting, muscle pain, fever, etc.), but they are nonspecific, and variably experienced and reported. Furthermore, the date of onset of these symptoms is difficult to assign. For these reasons, we identify the first day of diarrhea as the first day of illness. Diarrhea caused by *E. coli* O157:H7 usually lasts for about 1 week (Ostroff, Kobayashi et al. 1989). Bloody diarrhea is noted in about 85% of bacteriologically confirmed cases, and occurs about 2–5 days into illness (Ostroff,

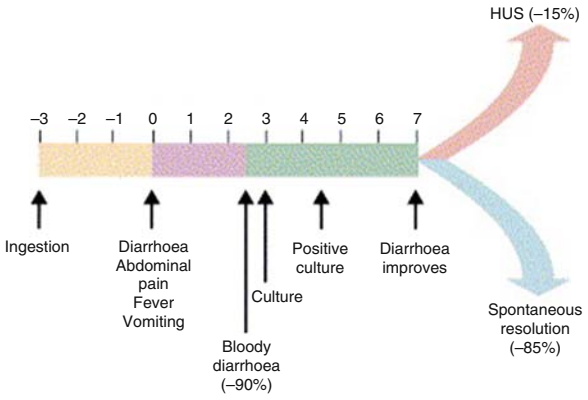

Fig. 1 Progression of *E. coli* O157:H7 infections in children (Tarr et al. 2005)

Kobayashi et al. 1989). There is a spectrum of severity, but most culture-confirmed cases are quite ill.

Some clues help distinguish children and adults infected with *E. coli* O157:H7 from those infected with other pathogens that cause bloody diarrhea. First, patients infected with *E. coli* O157:H7 generally have no fever at their initial presentation (Wong, Jelacic et al. 2000), though it is important to note that about half of all patients report having a fever earlier in the illness. Second, only half of fecal samples contain leukocytes and when leukocytes are present they are rarely abundant (Slutsker, Ries et al. 1997; Klein, Stapp et al. 2002). Furthermore, the abdominal pain experienced is usually greater than that associated with gastroenteritis, and more painful during defecation. Physicians often document tenderness on palpation of the abdomen of infected patients (Slutsker, Ries et al. 1997; Klein, Stapp et al. 2002).

5 Pathophysiology of *E. coli* O157:H7 Infections

The central virulence factor for Shiga-toxin producing *E.coli* (STEC) is its ability to produce Shiga toxin or toxins. Shiga toxin (Stx) is the main extracellular cytotoxin produced by *Shigella dysenteriae* serotype 1, but only rarely by other shigellae. Stx is classified as an A_1B_5 toxin. The A subunit, an N-glycosidase, halts protein synthesis by disrupting the large eukaryotic ribosomal subunit (Endo, Tsurugi et al. 1988), while the B subunit attaches to a glycosphingolipid on eukaryotic cell surfaces. Because of these properties, Shiga toxins induce renal cell death among other adverse effects (Karpman, Hakansson et al. 1998; Taguchi, Uchida et al. 1998). The type of toxin that is carried by *E. coli* O157:H7 might determine the virulence; most *E. coli* O157:H7 carry the *Shiga toxin 2* gene (Stx2) (Slutsker, Ries et al. 1997; Klein, Stapp et al. 2002). For reasons that are unclear, *E. coli* O157:H7 that generate both Stx1 and Stx2 are paradoxically less virulent than those that produce only Stx2.

6 Diagnostic Considerations for Pathogenic STEC

The clinical importance of making a rapid and accurate diagnosis of *E. coli* O157:H7 cannot be understated. Children who are diagnosed early in illness are at lower risk of severe consequences than those diagnosed later (Ake, Jelacic et al. 2005). Also, establishing a microbiological diagnosis can provide clinical clarity in cases that can be confusing, and avert unnecessary or risky additional diagnostic efforts.

However, the optimal diagnostic methodologies for the detection of *E. coli* O157:H7 and of other STEC are not yet delineated (Bielaszewska, Kock et al. 2007). Clearly the best way to recover *E. coli* O157:H7, in the context of current technology, is to plate all submitted stools on sorbitol–MacConkey (SMAC)

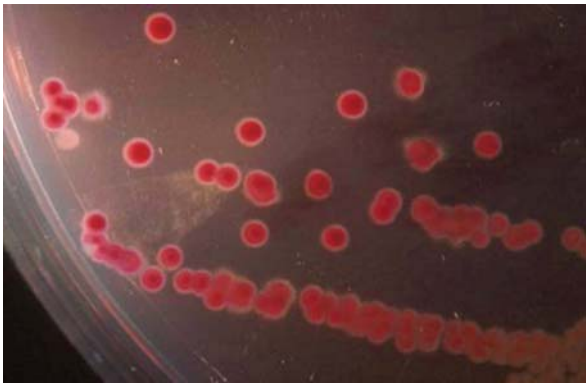

Fig. 2 *E. coli* O157:H7 on a sorbitol–MacConkey agar plate. *Arrow* indicates a distinctive colorless *E. coli* O157:H7 colony (Tarr et al. 2005)

agar (March and Ratnam 1986), which uses sorbitol as a carbon source, and not lactose. Unlike most human fecal *E. coli*, O157:H7 strains cannot ferment sorbitol rapidly and appear as colorless colonies on SMAC agar (Fig. 2).

Another test, which is available commercially, is the Stx detection assay, which is performed on a broth culture of the stool. The advantage of this technique is that a positive signal will be obtained from non-O157:H7 STEC as well as *E. coli* O157:H7. However, Stx assays do not tell the clinician if the strain producing the toxin is an *E. coli* O157:H7 or a non-O157:H7 STEC. This latter group of organisms is considerably less likely to cause HUS or to be associated with outbreaks, and the value of their detection to clinicians and disease control authorities is nowhere near as great as is the value of detecting *E. coli* O157:H7. Furthermore, for unexplained reasons, Stx assays are not always as sensitive as SMAC agar screening for the detection of *E. coli* O157:H7 (Fey, Wickert et al. 2000; Klein, Stapp et al. 2002; Carroll, Adamson et al. 2003; Park, Kim et al. 2003; Teel, Daly et al. 2007). More specifically, SMAC agar can sometimes detect *E. coli* O157:H7 when simultaneously performed toxin assays do not. Toxin assays should not be performed directly on stool as this approach lacks sensitivity (Cornick, Jelacic et al. 2002).

We believe that current best microbiological practice throughout the world is to plate all submitted stools onto SMAC agar, whether or not the medical provider requests detection of *E. coli* O157:H7. The stool should also be inoculated into a nutrient broth, and the broth should be tested the next day for Stx presence. Regrettably, resources might not permit such simultaneous culture and nonculture diagnosis. In that case, because of *E. coli* O157:H7's enduring epidemiologic predominance, its strong linkage with HUS, and its repeatedly demonstrated ability to cause outbreaks, we believe that plating of the stools on SMAC agar should take precedence over toxin assays because culture is accurate and recovers *E. coli* O157:H7 expeditiously.

We do not believe that observation of blood in the stool by microbiology staff should be used as a criterion to seek *E. coli* O157:H7, because about 15% of patients infected with *E. coli* O157:H7 will have nonbloody diarrhea (Wong,

Jelacic et al. 2000; Klein, Stapp et al. 2002), and laboratory staff often fail to perceive the red color in submitted specimens (Klein, Stapp et al. 2002). We also believe that all stools should be plated on receipt in the laboratory, 24 h a day, 7 days a week. This practice reduces the time needed to identify positive specimens that contain this pathogen and the more rapid the diagnosis, the stronger the association with a good case outcome (Ake, Jelacic et al. 2005). Finally, we believe that microbiologists should notify the requesting physician as soon as a presumptive *E. coli* O157:H7 is identified, even before confirming the organism as an *E. coli*, or determining the presence or absence of the H7 antigen. Once a sorbitol nonfermenting colony is found that reacts with a serologic reagent that detects the O157 lipopolysaccharide antigen, the provider should be alerted that a presumptive *E. coli* O157:H7 has been identified. Strains should be forwarded to the appropriate public health authorities for typing as expeditiously as possible.

7 Management of Patients with Confirmed or Suspected *E. coli* O157:H7 Infection

If a patient is suspected of having an *E. coli* O157:H7 infection, we recommend admission to hospital to mitigate infection risk in the household, to expand circulating volume, and to control pain.

Infection control: Patients infected with *E. coli* O157:H7 are highly contagious in the early diarrheal phase of the illness, and they should be placed on contact precautions and removed from the community where they can continue to spread the infection. In many hospitals, the placement of these patients on contact precautions is inconsistent. The current recommendations are to continue contact precautions until the diarrhea resolves and two consecutive stool cultures are negative for *E. coli* O157:H7 (Pediatrics 2006). Some authorities have recommended admission as a form of community infection control (Seto, Soller et al. 2007; Werber, Mason et al. 2008), and we believe that this measure is justified for both outbreak and sporadic cases (Ahn, Klein et al. 2008). Indeed, Werber et al. calculated that the number needed to isolate (NNI) in order to prevent one HUS case was 95 (95% CI 38–200), while the number needed to treat to prevent one secondary household case of meningococcal disease was appreciably more at 200 (Werber, Mason et al. 2008).

Volume expansion: Recent data suggest that circulating volume expansion early in illness with isotonic crystalloid, and not hypotonic fluids, is associated with less severe courses of HUS (Ake, Jelacic et al. 2005). The risk of developing HUS in children under age 10 years who are infected with *E. coli* O157:H7 is appreciable: over 20% of children presenting on or before day 4 of illness will develop this complication (Wong, Jelacic et al. 2000), and we believe that all such patients should be considered to be at risk. As mentioned above, we

consider the first day of diarrhea to be the first day of illness, and base decisions on that time point.

We usually administer a bolus of at least 20 mL of normal saline/kg of body weight at the first opportunity, and then maintain intravenous fluid infusion at maintenance volume. We recommend infusion of repeated boluses of normal saline if there is any suggestion of oliguria or pain (see below). Such patients should be assiduously monitored, and before administering boluses or continuing fluids, they should be evaluated for hypertension and 'central' volume overload. Peripheral edema, without signs of central overload or hypertension, should not deter fluid infusion.

The typical endpoint of intravenous fluid infusion is either a rising platelet count, which is usually not difficult to identify, or a patient whose platelet count is stable and whose symptoms have abated. This resolution rarely occurs before the fourth day of illness. Potassium can be added to the fluids if the serum potassium concentration is normal or low. Hyperkalemia is surprisingly rare as HUS ensues despite the renal insufficiency and hemolysis. Daily laboratory tests should consist of a complete blood count (CBC), electrolytes, blood urea nitrogen (BUN), and creatinine. Urinalysis should not be obtained; the daily creatinine concentration is sufficient to assess renal function. Furthermore, the urinalysis can be misleading, especially if collected from a patient with diarrhea, where contamination is likely. Urinary catheterization should be also avoided, principally to avoid infection risk in the setting of diarrhea.

We recognize that the patient who is experiencing a rising creatinine, but who is still passing urine, poses a management challenge. On the one hand, if anuria is inevitable, then fluid restriction is probably prudent. On the other hand, anuria cannot be predicted, and any fluid restriction is likely to diminish renal blood flow, increase renal ischemia (Bellomo, Kellum et al. 2007) and is ill advised. We prefer to continue the fluids, monitor the patient extremely closely, and restrict fluids for persistent hypertension or clinical signs of cardiopulmonary overload.

Pain control: Patients infected with *E. coli* O157:H7 are often in quite severe pain. Narcotics and antimotility agents are associated with increased risks of HUS (Bell, Griffin et al. 1997). We encourage liberal use of fluid boluses in attempts to address the pain because it is plausible that intestinal ischemia from the underlying thrombotic process exacerbates the pain. A benzodiazepine such as lorazepam can be used to reduce anxiety and facilitate sleep. We discourage use of nonsteroidal anti-inflammatory drugs because they can diminish renal blood flow. Acetaminophen (paracetamol) is probably not harmful, but from our observations, it is not a very effective analgesic in this infection.

Antibiotics: Patients with suspected or confirmed *E. coli* O157:H7 should not be given antibiotics because the evidence does not suggest any benefit for them and, in fact, may suggest that antibiotics increase the risk of HUS. In an analysis of the large outbreak of *E. coli* O157:H7 in Washington State in 1993, antibiotics were given early in the illness but failed to decrease the risk of HUS (Bell, Griffin et al. 1997). In another study, administration of antibiotics probably

increased risk of HUS in children infected with O157:H7 (Wong, Jelacic et al. 2000). A trend toward worse outcome has also been seen in adults infected with *E. coli* O157:H7 (Dundas, Todd et al. 2001). A possible mechanism for this observation is that antibiotics might lead to bacterial lysis, which increases the availability of Stx for systemic absorption (Grif, Dierich et al. 1998). Also, bacteriophages that contain the *stx* genes might be stimulated by the antibiotics, leading to increased Stx generation (Kimmitt, Harwood et al. 1999). Even though a flawed meta-analysis suggested that antibiotics were not associated with a higher risk of HUS (Safdar, Said et al. 2002), we are unaware of any reports that demonstrate that the rate of HUS among patients treated with antibiotics is lower than those not treated.

Platelet transfusion: Platelet transfusions should not be given to children infected with *E. coli* O157:H7 because platelets might exacerbate the thrombotic process that underlies HUS. Clinically significant hemorrhage as a consequence of HUS is very rare. However, if an invasive procedure that has a risk of bleeding is considered, platelets might be needed to prevent hemorrhage.

8 HUS and the Course of *E. coli* O157:H7 Infections

HUS is defined by nonimmune hemolytic anemia (packed cell volume $< 30\%$ and evidence of erythrocyte destruction on peripheral blood smear), thrombocytopenia (platelets $< 150 \times 10^9$/L), and azotemia (serum creatinine above the upper limit for age). HUS occurs in about 15% of culture-proven childhood *E. coli* O157:H7 infections in children less than 10 years of age (Ostroff, Kobayashi et al. 1989; Bell, Griffin et al. 1997; Rowe, Orrbine et al. 1998; Wong, Jelacic et al. 2000; Chandler, Jelacic et al. 2002). HUS almost always develops between the 5th and 13th day of illness, and the median time of its onset (defined as when children meet the above case definition) is 7–8 days (Wong, Jelacic et al. 2000; Chandler, Jelacic et al. 2002). About 60% of patients with HUS become anuric. HUS patients who continue to pass urine through day 10 of illness rarely become anuric.

E. coli O157:H7 infections follow a remarkably stereotypical course in most patients. The relation of the laboratory tests to the day of illness is most helpful in managing such infections. Figure 3 portrays the four patterns of thrombocytopenia, anemia, and azotemia that are observed in infected children.

Pattern 1 is seen in *c*. 70% of cases. Pattern 2 (hemolysis requiring transfusion) is seen in about 5% of cases; this patient required a transfusion of erythrocytes. There is no azotemia, so, strictly speaking, this is not HUS. Pattern 3 is nonanuric HUS, which did not require dialysis, and occurs in about 5–10% of cases. Pattern 4 is anuric HUS, requiring dialysis. Anuric HUS has a worse long-term prognosis than nonanuric HUS (Siegler, Milligan et al. 1991; Garg, Suri et al. 2003), and occurs in about 10–15% of infected cases.

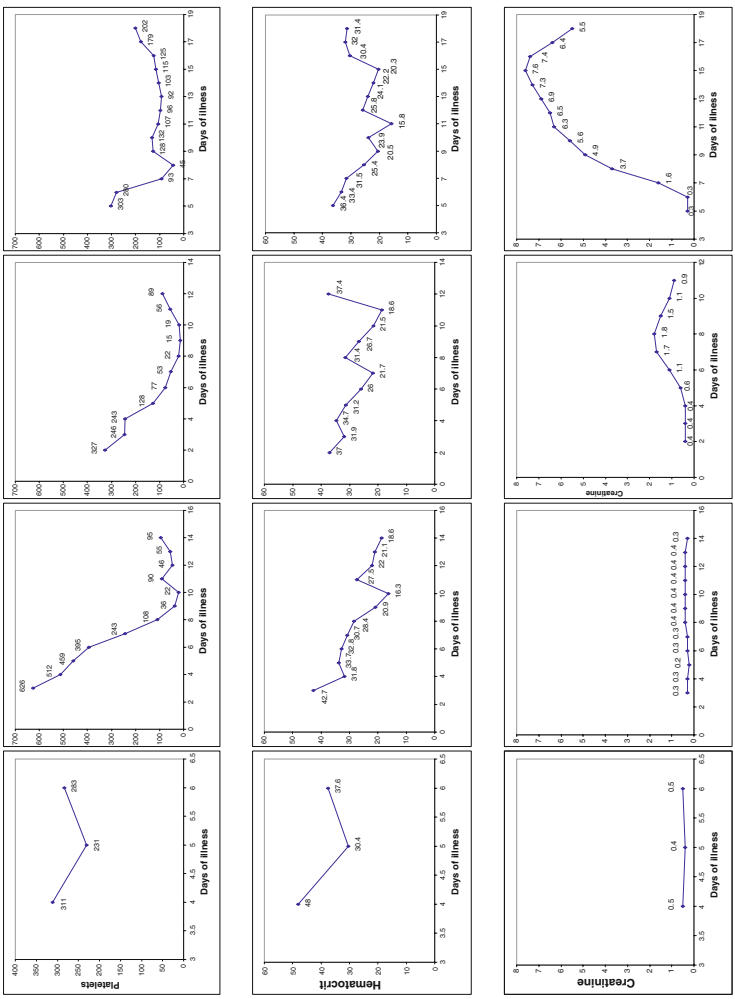

Fig. 3 Typical laboratory courses of *E. coli* O157:H7 infections. Pattern 1 represents a completely uncomplicated enteric infection. Note the temporary drop in the platelet count. Pattern 2 represents partial HUS, and portrays the profound vascular injury that can occur even in the absence of renal insufficiency. Pattern 3 is nonanuric HUS. Pattern 4 is anuric HUS

In most centers, HUS is best managed by pediatric nephrologists, and a detailed discussion of support of such patients is beyond the scope of this chapter. We direct interested readers to a recent thorough review of management of HUS (Loirat and Taylor 2003). Thrombocytopenia is generally the first abnormality to resolve in patients with HUS. Hemolysis can be prolonged, and patients can require erythrocyte transfusions well after renal recovery is underway. We find it helpful periodically to reculture inpatients so that contact precautions may be discontinued if appropriate. In any case, infection control measures should be in compliance with institutional policies, and public health guidelines and regulations.

9 Pathophysiology of HUS

Bacteremia is rare in STEC infections. It is plausible that the enteric symptoms, including the colitis, are the result of vascular injury from systemic toxemia, and not the direct effects of *E. coli* O157:H7 or its toxin on epithelial cells. Indeed, colitis in animals is induced by parenteral administration of toxin (Ritchie, Thorpe et al. 2003; Siegler, Obrig et al. 2003).

Figure 4 proposes a model for the host response to *E. coli* O157:H7 infection that leads to HUS. Tables 1 and 2 review common myths about this infection and provide some helpful hints related to management of these patients.

Stxs bind to the glycosphingolipid globotriosylceramide (Lingwood 2003), which is found on a wide variety of renal cells, including those of glomerular, endothelial, mesangial, and tubular epithelial cells (Boyd and Lingwood 1989; Takeda, Dohi et al. 1993; Lingwood 1994; Robinson, Hurley et al. 1995). Differences in organ damage might be attributed to the varying expression of this glycosphingolipid. Current data suggest that early in illness, possibly even before clinical presentation, circulating (absorbed) Stx injures the vascular endothelium, and this damage generates thrombin, which causes fibrin to be

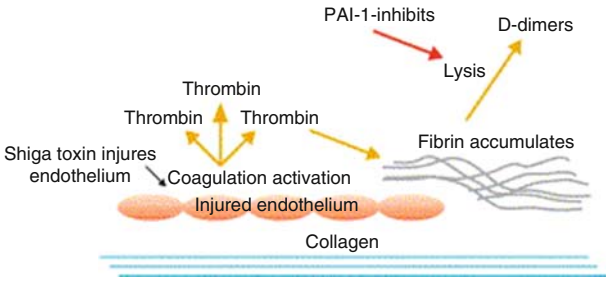

Fig. 4 Proposed model for pathological coagulation response leading to HUS (Tarr et al. 2005)

Table 1 Myths and facts about *E. coli* O157:H7 and Shiga toxin-producing *E. coli*

MYTH: As a group, non-O157:H7 STEC cause diseases that are as serious, and are at least as common, as *E. coli* O157:H7
FACT: Non-O157:H7 STEC as a group is less virulent than *E. coli* O157:H7. Individual infections are, on average, much less likely to lead to HUS or to be associated with epidemics. They are, in aggregate, about as common as *E. coli* O157:H7 or slightly less common, in most of the world (Klein, Stapp et al. 2002; Jelacic, Damrow et al. 2003; Brooks, Sowers et al. 2005). A small subset of non-O157:H7 infections will lead to HUS.
MYTH: Toxin assays will detect *E. coli* O157:H7 as well as or better than SMAC agar screening.
FACT: SMAC agar screening is more accurate and quicker at detecting *E. coli* O157:H7 than toxin assays. If you have to choose one detection method, choose SMAC agar (Fey, Wickert et al. 2000; Klein, Stapp et al. 2002; Carroll, Adamson et al. 2003; Park, Kim et al. 2003; Teel, Daly et al. 2007).
MYTH: Most cases are caused by eating poorly cooked hamburger, and epidemics are common.
FACT: Ground beef is associated with relatively fewer cases in recent years, while fresh fruits and vegetables, recreational water, and animal contact are emerging as bigger risk factors (Swerdlow, Woodruff et al. 1992; Besser, Lett et al. 1993; Keene, McAnulty et al. 1994; Tilden, Young et al. 1996; Keene, Hedberg et al. 1997; Keene, Sazie et al. 1997; Ackers, Mahon et al. 1998; Michino, Araki et al. 1999; Crump, Sulka et al. 2002; Terajima, Izumiya et al. 2002; Uhlich, Sinclair et al. 2007). Most cases are sporadic.

Table 2 Helpful hints for physicians caring for patients with confirmed or suspected *E. coli* O157:H7 infections

1.	Daily Laboratory tests: CBC, electrolytes, BUN, creatinine.
2.	Urinalysis is not helpful and could be counterproductive.
3.	Keep hydrating with isotonic crystalloid until trend in platelet counts is apparent (unequivocal rise, or stability with resolving symptoms).
4.	Obtain a CBC the day after discharge to confirm continued improvement.
5.	Give intravenous boluses with isotonic crystalloid for abdominal pain.
6.	Afebrile, bloody, painful diarrhea, especially if the blood appears after several days of nonbloody diarrhea, is suspicious for *E. coli* O157:H7 infection.
7.	If the bloody diarrhea ceases immediately after admission, patients are very unlikely to be infected with *E. coli* O157:H7.
8.	Fecal leukocytes, if present, are rarely abundant.
9.	About half of all infected patients report a fever in the days before presentation, but fever is rarely documented at presentation.
10.	Oral rehydration is not adequate.
11.	Children who continue to pass urine through day 10 of illness (day 1 is the first day of diarrhea) rarely become anuric.
12.	Platelet transfusions should not be given.
13.	Potassium can be added to the IV fluids if serum potassium is normal or low.
14.	If you are highly suspicious that a patient is infected with *E. coli* O157:H7, such as a household contact of known positive case, you should obtain a stool culture. If the child is, for whatever reason, not admitted, obtain a CBC, as this will provide a useful reference value for the platelet count if the stool is reported positive. If the count is rising, and the patient is doing well, then you might be able to avert an admission.
15.	Hospitalization of infected children at the height of their symptoms could be an important community infection control strategy.

deposited in the microvasculature. At the same time, circulating plasminogen activator inhibitor 1 (PAI-1) activity increases, which inhibits fibrinolysis. Fibrin further accumulates, and the thrombotic injury is compounded (Chandler, Jelacic et al. 2002)

Patients infected with *E. coli* O157:H7 rarely exhibit a fever once bloody diarrhea begins. In fact, before HUS develops, they do not seem to have a classic systemic inflammation response as is seen, for example, in septic shock. Despite this finding, pro-inflammatory cytokines and chemokines probably do injure host cells (Proulx, Seidman et al. 2001), but the assessment of local effects of cytokine-mediated injury in the living human is quite difficult.

Long-term sequelae: Most long-term sequelae of HUS relate to renal function. After the large outbreak in 1993, most survivors had good renal function 5 years after infection (Brandt, Joseph et al. 1998). Risk factors for long-term sequelae include an initial white blood cell count $> 20 \times 10^3/\mu L$ with neutrophilia, a high serum creatinine or urea concentration, central nervous syndrome symptoms such as coma or seizures, ischemic colitis, hypertension, anuria, and the need for dialysis (Siegler, Milligan et al. 1991; Garg, Suri et al. 2003).

Non-O157:H7 STEC: Non-O157:H7 STEC can certainly be human pathogens, and they can cause HUS (Klein, Stapp et al. 2002; Jelacic, Damrow et al. 2003; Brooks, Sowers et al. 2005). Moreover, without a toxin assay or nucleic acid hybridization testing, such organisms can be overlooked in diagnostic protocols (Bielaszewska, Kock et al. 2007). However, even when technology has been applied that would detect this group of organisms, *E. coli* O157:H7 remains the predominant human pathogenic STEC (Pai, Ahmed et al. 1988; Jelacic, Damrow et al. 2003; Manning, Madera et al. 2007; Teel, Daly et al. 2007). Non-O157:H7 STEC are quite common in food, and *E. coli* O157:H7 is rare. The infrequency with which non-O157:H7 are isolated in stool cultures compared to *E. coli* O157:H7 suggests that as a group they are considerably less pathogenic than *E. coli* O157:H7. While non-O157:H7 STEC as a subset can cause serious and even epidemic human disease, screening technology should not be directed at non-O157:H7 at the expense of recovering *E. coli* O157:H7. Optimally, clinical laboratories will seek both sets of pathogens (*E. coli* O157:H7 and non-O157: H7 STEC) in parallel.

10 Conclusions

Since the 1980s, much has been discovered about HUS; however, specific treatments do not exist. Specific therapies are unlikely to emerge, because available data suggest that the vascular lesion that leads to HUS is well underway by the time infected patients present to medical attention. Aggressive isotonic volume expansion, especially early in illness, appears associated with a diminished risk of anuria if HUS ensues. The best way to prevent HUS is to

prevent primary infections with *E. coli* O157:H7. Additional reported measures are syndromic recognition of this rather rare event, and early and accurate microbiological detection of infected patients. Early illness recognition will lead to careful monitoring and volume expansion at an earlier stage of illness, with isolation.

References

Ackers, M. L., Mahon, B. E., Leahy, E., Goode, B., Damrow, T., Hayes, P. S., Bibb, W. F., Rice, D. H., Barrett, T. J., Hutwagner, L., Griffin, P. M. and Slutsker, L. (1998). An outbreak of Escherichia coli O157:H7 infections associated with leaf lettuce consumption. *J Infect Dis* **177**(6): 1588–93.

Ahn, C. K., Klein, E. and Tarr, P. I. (2008). Isolation of patients acutely infected with Escherichia coli O157:H7: low-tech, highly effective prevention of hemolytic uremic syndrome. *Clin Infect Dis* **46**(8): 1197–9.

Ake, J. A., Jelacic, S., Ciol, M. A., Watkins, S. L., Murray, K. F., Christie, D. L., Klein, E. J. and Tarr, P. I. (2005). Relative nephroprotection during Escherichia coli O157:H7 infections: association with intravenous volume expansion. *Pediatrics* **115**(6): e673–80.

Bell, B. P., Goldoft, M., Griffin, P. M., Davis, M. A., Gordon, D. C., Tarr, P. I., Bartleson, C. A., Lewis, J. H., Barrett, T. J., Wells, J. G. and et al. (1994). A multistate outbreak of Escherichia coli O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. The Washington experience. *JAMA* **272**(17): 1349–53.

Bell, B. P., Griffin, P. M., Lozano, P., Christie, D. L., Kobayashi, J. M. and Tarr, P. I. (1997). Predictors of hemolytic uremic syndrome in children during a large outbreak of Escherichia coli O157:H7 infections. *Pediatrics* **100**(1): E12.

Bellomo, R., Kellum, J. A. and Bagshaw, S. M. (2007). Normotensive ischemic acute renal failure. *N Engl J Med* **357**(21): 2205; author reply 2205–6.

Besser, R. E., Lett, S. M., Weber, J. T., Doyle, M. P., Barrett, T. J., Wells, J. G. and Griffin, P. M. (1993). An outbreak of diarrhea and hemolytic uremic syndrome from Escherichia coli O157:H7 in fresh-pressed apple cider. *JAMA* **269**(17): 2217–20.

Bielaszewska, M., Kock, R., Friedrich, A. W., von Eiff, C., Zimmerhackl, L. B., Karch, H. and Mellmann, A. (2007). Shiga toxin-mediated hemolytic uremic syndrome: time to change the diagnostic paradigm? *PLoS ONE* **2**(10): e1024.

Boyd, B. and Lingwood, C. (1989). Verotoxin receptor glycolipid in human renal tissue. *Nephron* **51**(2): 207–10.

Brandt, J. R., Joseph, M. W., Fouser, L. S., Tarr, P. I., Zelikovic, I., McDonald, R. A., Avner, E. D., McAfee, N. G. and Watkins, S. L. (1998). Cholelithiasis following *Escherichia coli* O157:H7-associated hemolytic uremic syndrome. *Pediatr Nephrol* **12**(3): 222–5.

Brooks, J. T., Sowers, E. G., Wells, J. G., Greene, K. D., Griffin, P. M., Hoekstra, R. M. and Strockbine, N. A. (2005). Non-O157 Shiga toxin-producing Escherichia coli infections in the United States, 1983–2002. *J Infect Dis* **192**(8): 1422–9.

Carroll, K. C., Adamson, K., Korgenski, K., Croft, A., Hankemeier, R., Daly, J. and Park, C. H. (2003). Comparison of a commercial reversed passive latex agglutination assay to an enzyme immunoassay for the detection of Shiga toxin-producing *Escherichia coli*. *Eur J Clin Microbiol Infect Dis* **22**(11): 689–92.

Chandler, W. L., Jelacic, S., Boster, D. R., Ciol, M. A., Williams, G. D., Watkins, S. L., Igarashi, T. and Tarr, P. I. (2002). Prothrombotic coagulation abnormalities preceding the hemolytic-uremic syndrome. *N Engl J Med* **346**(1): 23–32.

Cornick, N. A., Jelacic, S., Ciol, M. A. and Tarr, P. I. (2002). *Escherichia coli* O157:H7 infections: discordance between filterable fecal shiga toxin and disease outcome. *J Infect Dis* **186**(1): 57–63.

Crump, J. A., Sulka, A. C., Langer, A. J., Schaben, C., Crielly, A. S., Gage, R., Baysinger, M., Moll, M., Withers, G., Toney, D. M., Hunter, S. B., Hoekstra, R. M., Wong, S. K., Griffin, P. M. and Van Gilder, T. J. (2002). An outbreak of *Escherichia coli* O157:H7 infections among visitors to a dairy farm. *N Engl J Med* **347**(8): 555–60.

Cummings, K. C., Mohle-Boetani, J. C., Werner, S. B. and Vugia, D. J. (2002). Population-based trends in pediatric hemolytic uremic syndrome in California, 1994–1999: substantial underreporting and public health implications. *Am J Epidemiol* **155**(10): 941–8.

Dundas, S., Todd, W. T., Stewart, A. I., Murdoch, P. S., Chaudhuri, A. K. and Hutchinson, S. J. (2001). The central Scotland *Escherichia coli* O157:H7 outbreak: risk factors for the hemolytic uremic syndrome and death among hospitalized patients. *Clin Infect Dis* **33**(7): 923–31.

Endo, Y., Tsurugi, K., Yutsudo, T., Takeda, Y., Ogasawara, T. and Igarashi, K. (1988). Site of action of a Vero toxin (VT2) from *Escherichia coli* O157:H7 and of Shiga toxin on eukaryotic ribosomes. RNA N-glycosidase activity of the toxins. *Eur J Biochem* **171**(1–2): 45–50.

Fey, P. D., Wickert, R. S., Rupp, M. E., Safranek, T. J. and Hinrichs, S. H. (2000). Prevalence of non-O157:H7 shiga toxin-producing *Escherichia coli* in diarrheal stool samples from Nebraska. *Emerg Infect Dis* **6**(5): 530–3.

Garg, A. X., Suri, R. S., Barrowman, N., Rehman, F., Matsell, D., Rosas-Arellano, M. P., Salvadori, M., Haynes, R. B. and Clark, W. F. (2003). Long-term renal prognosis of diarrhea-associated hemolytic uremic syndrome: a systematic review, meta-analysis, and meta-regression. *JAMA* **290**(10): 1360–70.

Gasser C, G. E. Steck A. (1955). Hamolytisch-uramishe syndrome: Bilaterale Ni.

Grif, K., Dierich, M. P., Karch, H. and Allerberger, F. (1998). Strain-specific differences in the amount of Shiga toxin released from enterohemorrhagic Escherichia coli O157 following exposure to subinhibitory concentrations of antimicrobial agents. *Eur J Clin Microbiol Infect Dis* **17**(11): 761–6.

Inward, C. D., Howie, A. J., Fitzpatrick, M. M., Rafaat, F., Milford, D. V. and Taylor, C. M. (1997). Renal histopathology in fatal cases of diarrhoea-associated haemolytic uraemic syndrome. British Association for Paediatric Nephrology. *Pediatr Nephrol* **11**(5): 556–9.

Jelacic, J. K., Damrow, T., Chen, G. S., Jelacic, S., Bielaszewska, M., Ciol, M., Carvalho, H. M., Melton-Celsa, A. R., O'Brien, A. D. and Tarr, P. I. (2003). Shiga toxin-producing *Escherichia coli* in Montana: bacterial genotypes and clinical profiles. *J Infect Dis* **188**(5): 719–29.

Jernigan, S. M. and Waldo, F. B. (1994). Racial incidence of hemolytic uremic syndrome. *Pediatr Nephrol* **8**(5): 545–7.

Kaplan, B. S., Chesney, R. W. and Drummond, K. N. (1975). Hemolytic uremic syndrome in families. *N Engl J Med* **292**(21): 1090–3.

Karmali, M. A., Steele, B. T., Petric, M. and Lim, C. (1983). Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. *Lancet* **1**(8325): 619–20.

Karpman, D., Hakansson, A., Perez, M. T., Isaksson, C., Carlemalm, E., Caprioli, A. and Svanborg, C. (1998). Apoptosis of renal cortical cells in the hemolytic-uremic syndrome: in vivo and in vitro studies. *Infect Immun* **66**(2): 636–44.

Keene, W. E., Hedberg, K., Herriott, D. E., Hancock, D. D., McKay, R. W., Barrett, T. J. and Fleming, D. W. (1997). A prolonged outbreak of *Escherichia coli* O157:H7 infections caused by commercially distributed raw milk. *J Infect Dis* **176**(3): 815–8.

Keene, W. E., McAnulty, J. M., Hoesly, F. C., Williams, L. P., Jr., Hedberg, K., Oxman, G. L., Barrett, T. J., Pfaller, M. A. and Fleming, D. W. (1994). A swimming-associated outbreak

of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and Shigella sonnei. *N Engl J Med* **331**(9): 579–84.

Keene, W. E., Sazie, E., Kok, J., Rice, D. H., Hancock, D. D., Balan, V. K., Zhao, T. and Doyle, M. P. (1997). An outbreak of *Escherichia coli* O157:H7 infections traced to jerky made from deer meat. *JAMA* **277**(15): 1229–31.

Kimmitt, P. T., Harwood, C. R. and Barer, M. R. (1999). Induction of type 2 Shiga toxin synthesis in Escherichia coli O157 by 4-quinolones. *Lancet* **353**(9164): 1588–9.

Klein, E. J., Stapp, J. R., Clausen, C. R., Boster, D. R., Wells, J. G., Qin, X., Swerdlow, D. L. and Tarr, P. I. (2002). Shiga toxin-producing *Escherichia coli* in children with diarrhea: a prospective point-of-care study. *J Pediatr* **141**(2): 172–7.

Lingwood, C. A. (1994). Verotoxin-binding in human renal sections. *Nephron* **66**(1): 21–8.

Lingwood, C. A. (2003). Shiga toxin receptor glycolipid binding. Pathology and utility. *Methods Mol Med* **73**: 165–86.

Loirat, C. and Taylor, C. M. (2003). Hemolytic Uremic Syndromes. *Pediatric Nephrology*. E. D. Avner, W. E. Harmon and P. Niaudet, Lippincott Williams and Wilkins: 887–915.

Lopez, E. L., Diaz, M., Grinstein, S., Devoto, S., Mendilaharzu, F., Murray, B. E., Ashkenazi, S., Rubeglio, E., Woloj, M., Vasquez, M. and et al. (1989). Hemolytic uremic syndrome and diarrhea in Argentine children: the role of Shiga-like toxins. *J Infect Dis* **160**(3): 469–75.

Manning, S. D., Madera, R. T., Schneider, W., Dietrich, S. E., Khalife, W., Brown, W., Whittam, T. S., Somsel, P. and Rudrik, J. T. (2007). Surveillance for Shiga toxin-producing Escherichia coli, Michigan, 2001–2005. *Emerg Infect Dis* **13**(2): 318–21.

March, S. B. and Ratnam, S. (1986). Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *J Clin Microbiol* **23**(5): 869–72.

McNabb, S. J., Jajosky, R. A., Hall-Baker, P. A., Adams, D. A., Sharp, P., Anderson, W. J., Javier, A. J., Jones, G. J., Nitschke, D. A., Worshams, C. A. and Richard, R. A., Jr. (2007). Summary of notifiable diseases — United States, 2005. *MMWR Morb Mortal Wkly Rep* **54**(53): 1–92.

Michino, H., Araki, K., Minami, S., Takaya, S., Sakai, N., Miyazaki, M., Ono, A. and Yanagawa, H. (1999). Massive outbreak of *Escherichia coli* O157:H7 infection in schoolchildren in Sakai City, Japan, associated with consumption of white radish sprouts. *Am J Epidemiol* **150**(8): 787–96.

O'Brien, A. O., Lively, T. A., Chen, M. E., Rothman, S. W. and Formal, S. B. (1983). *Escherichia coli* O157:H7 strains associated with haemorrhagic colitis in the United States produce a Shigella dysenteriae 1 (SHIGA) like cytotoxin. *Lancet* **1**(8326 Pt 1): 702.

O'Brien, S. J., Adak, G. K. and Gilham, C. (2001). Contact with farming environment as a major risk factor for Shiga toxin (Vero cytotoxin)-producing *Escherichia coli* O157 infection in humans. *Emerg Infect Dis* **7**(6): 1049–51.

Ostroff, S. M., Kobayashi, J. M. and Lewis, J. H. (1989). Infections with *Escherichia coli* O157:H7 in Washington State. The first year of statewide disease surveillance. *JAMA* **262**(3): 355–9.

Pai, C. H., Ahmed, N., Lior, H., Johnson, W. M., Sims, H. V. and Woods, D. E. (1988). Epidemiology of sporadic diarrhea due to verocytotoxin-producing Escherichia coli: a two-year prospective study. *J Infect Dis* **157**(5): 1054–7.

Park, C. H., Kim, H. J., Hixon, D. L. and Bubert, A. (2003). Evaluation of the duopath verotoxin test for detection of shiga toxins in cultures of human stools. *J Clin Microbiol* **41**(6): 2650–3.

Pediatrics, A. A. O. (2006). Isolation Precautions. In: Pickering LK, ed. Red Book: 2006 Report of the Committee on Infectious diseases. *Red Book*. Elk Grove Village, IL: American Academy of Pediatrics: 154–160.

Proulx, F., Seidman, E. G. and Karpman, D. (2001). Pathogenesis of Shiga toxin-associated hemolytic uremic syndrome. *Pediatr Res* **50**(2): 163–71.