Annu. Rev. Nutr. 1997. 17:255–75

EMERGING ISSUES IN MICROBIOLOGICAL FOOD SAFETY

J. Meng
The Center for Food Safety and Quality Enhancement and the Department of Food Science and Technology, University of Georgia, Griffin, Georgia 30223, and the Department of Nutrition and Food Science, University of Maryland, College Park, Maryland 20742; e-mail: JM332@umail.umd.edu

M. P. Doyle
The Center for Food Safety and Quality Enhancement and the Department of Food Science and Technology, University of Georgia, Griffin, Georgia 30223-1797; e-mail: MDoyle@cfsqe.griffin.peachnet.edu

KEY WORDS: Shiga toxin-producing *Escherichia coli*, *Listeria monocytogenes*, *Helicobacter*, *Cryptosporidium*, *Cyclospora*

ABSTRACT

Many microorganisms previously unrecognized as food-borne or harmful are emerging as human pathogens transmitted by food. This is a result of recent acquisition of key virulence factors, detection by newly developed isolation procedures, or astute detective-like laboratory skills of microbiologists. Six microbial pathogens, including Shiga toxin-producing *Escherichia coli*, *Listeria monocytogenes*, *Arcobacter butzleri*, *Helicobacter pylori*, *Cryptosporidium parvum*, and *Cyclospora*, have become recognized as significant causes of human illness. Although the ecology and epidemiology of illness caused by some of these pathogens have not been fully elucidated, food has the potential of being an important vehicle in their dissemination. Existing technologies and new approaches such as irradiation and hazard analysis critical control point (HACCP) programs are useful tools in the control of food-borne hazards. However, because of ever-changing products, processes, food-handling practices, societal habits, and pathogens, emerging food-borne diseases will continue to be an important public health concern.

CONTENTS

0199-9885/97/0715-0255$08.00

INTRODUCTION

Within the past decade, the epidemiology of microbial food-borne diseases has changed, not only because of a human population increasingly susceptible to diseases and changing life styles—including more adventurous eating, more convenience foods, and less time devoted to food preparation—but also because of the emergence of newly recognized food-borne pathogens (3, 9). This article focuses on six microorganisms that have recently emerged as, or have the potential to be, significant food-borne pathogens. Shiga toxin-producing *Escherichia coli* (STEC), first recognized as food-borne pathogens in 1982, have recently been identified as major causes of severe illness. Although not all STEC are likely to cause human illness, several serotypes, including *E. coli* O157:H7 and O111:NM, are responsible for many of the food-borne outbreaks of bloody diarrhea and hemolytic uremic syndrome worldwide. *Listeria monocytogenes* causes very severe manifestations of illness, primarily in immunocompromised individuals, including pregnant women and the elderly. *Arcobacter butzleri* can cause persistent diarrhea, and *Helicobacter pylori* has been identified as the etiologic agent of gastritis and as a major contributing factor in development of peptic gastroduodenal ulcers. However, the significance of *A. butzleri* and *H. pylori* as food-borne pathogens is unclear. Parasites *Cryptosporidium parvum*

and *Cyclospora* both cause watery diarrhea. The former was the causative agent of several large, waterborne outbreaks of cryptosporiosis, whereas the latter was identified as the cause of a recent multistate outbreak associated with raspberries involving more than 850 cases in the United States and Canada.

SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI*

Background

Pathogenic *E. coli* that produce Shiga toxin(s) were first recognized in 1982 when *E. coli* serotype O157:H7 was associated with two outbreaks of bloody diarrhea (39). Since then, *E. coli* O157:H7 has caused, in the United States and many other countries worldwide, a series of food-borne outbreaks with severe manifestations of illness, including hemorrhagic colitis, hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (39). A 1993 multistate outbreak of *E. coli* O157:H7 infection associated with undercooked ground beef in the western United States involving 732 cases (55 developed HUS and 4 died) brought national and international attention to this pathogen (8). Major outbreaks caused by STEC O157:H7 and other serotypes continue to be reported in North America, Europe, Japan, Australia, and southern Africa (19–21, 39). A recent epidemic of *E. coli* O157:H7 infection in Japan involved more than 9500 cases and resulted in 11 deaths.

Cytotoxins produced by *E. coli* are genetically related to Shiga toxin produced by *Shigella dysenteriae* type 1 (64). Hence, these toxins have been named Shiga toxins (ST) after the prototype of the family, Shiga toxin (Stx) (16). The term Shiga toxin-producing *E. coli* is now used for strains of *E. coli* that elaborate Shiga toxin, without reference to their association with a clinical syndrome. The term enterohemorrhagic *E. coli* (EHEC) refers to those serotypes of *E. coli* that cause a clinical illness similar to that caused by *E. coli* O157:H7, that produce one or more Shiga toxins, that possess a 60-MDa virulence plasmid, and that produce attaching-effacing lesions in an animal model (39). Hence, EHEC are a defined subset of STEC (39).

Over 100 non-O157 STEC serotypes have been isolated from humans, but not all of these serotypes have been shown to cause illness (1, 39). Some of those most frequently isolated from persons with diarrhea are O26:H11, O103:H2, O111:NM (NM denotes nonmotile strain), and O113:H21. Serotypes O26:H11 and O111:NM are also among the most common serotypes of classic enteropathogenic *E. coli* (EPEC) (39). However, only a small proportion of classic EPEC O111 and O26 strains produce Shiga toxin(s). *E. coli* O157:H7 is the most important serotype of STEC in North America, although studies revealed that non-O157 STEC have been isolated from stools of North American

children (7, 14). Isolation of non-O157:H7 STEC requires techniques not generally available in clinical laboratories; hence these bacteria are rarely sought or detected in routine practice. Small outbreaks of non-O157 STEC could easily go undiagnosed and unrecognized. Recent recognition of non-O157 EHEC in disease outbreaks necessitates identification of other serotypes of STEC in persons with bloody diarrhea and/or HUS, and in implicated food. The increased availability in clinical laboratories of techniques such as testing for ST or their genes, and techniques for identification of other virulence markers unique for EHEC, may enhance the detection of disease attributable to non-O157 STEC.

Food-Associated Outbreaks of STEC of Serotypes Other than E. coli *O157:H7*

Most food-associated outbreaks of STEC in North America and Europe have been associated with serotype O157:H7, but several were caused by STEC of serotypes other than O157. Outbreaks caused by O111:NM and O26 have been reported in Italy (17–18). *E. coli* O103:H2 was isolated from patients suffering from HUS in France (57). In Japan, STEC serotypes O?:H19, O111:NM, and O145:NM were the cause of several outbreaks (52). Studies in Australia indicated that O157:H7 was uncommon, but other less-well-recognized serotypes such as O111:NM, O6:H31, O98:NM, and O48:H7 were responsible for hemorrhagic colitis and HUS (35). In most outbreaks caused by non-O157 STEC, the mode of transmission was unknown; however, two recent outbreaks of non-O157 STEC have been linked to food products.

OUTBREAK OF ACUTE GASTROENTERITIS ATTRIBUTABLE TO E. COLI O104:H21 From February through April 1994, in Helena, Montana, 11 confirmed and 7 suspected cases of *E. coli* O104:H21 infection were identified (21). Manifestations of illness included abdominal cramping, diarrhea, bloody diarrhea, vomiting, and fever. Four persons were hospitalized. Milk was implicated as the vehicle of transmission. Inspection of the dairy plant that processed the milk revealed that for 12 days during the months of February through May 1994, the coliform count exceeded the state regulation limit in milk products.

OUTBREAK OF HEMOLYTIC UREMIC SYNDROME ATTRIBUTABLE TO E. COLI O111:NM In January and February 1995, 23 cases of HUS among children <16 years of age in South Australia were reported (19). Sixteen patients required dialysis; one died. *E. coli* O111:NM was isolated from stool specimens from 16 of these patients. Mettwurst, an uncooked fermented meat product, was identified as the vehicle of infection. Of 10 mettwurst samples taken from the homes of patients, eight were positive for STs 1 and 2 by polymerase chain reaction (PCR) assay; *E. coli* O111:NM was isolated from four of these samples. Of 47 additional

sausage samples from the same manufacturer and retail stores, 18 were PCR positive; 3 yielded *E. coli* O111:NM.

ACIDIC FOOD ASSOCIATED WITH E. COLI O157:H7 OUTBREAKS A recent *E. coli* O157:H7 outbreak in the United States was associated with a dry fermented sausage product (20). Dry fermented sausage such as salami often is not cooked but is produced by fermentation followed by drying. An inoculation study revealed that *E. coli* O157:H7 survives during fermentation, drying, and storage for 2 months at 4°C (33). This organism can survive at pH as low as 3.6 for an extended period of time (90, 91). Outbreaks of *E. coli* O157:H7 infection associated with other acidic foods, including mayonnaise (90) and apple cider (10), have also been reported. Similar to serotype O157:H7, O111:NM is acid tolerant and has a low infectious dose.

Significance as Food-Borne Pathogens

Cattle are a major reservoir of EHEC, including O157:H7 and other serotypes. However, these bacteria generally do not cause illness among cattle (23, 88). Contamination with EHEC during slaughter is a primary route by which the pathogens enter the food supply. Although most EHEC outbreaks have been associated with the consumption of undercooked ground beef, EHEC has also been transmitted in milk, roast beef, dry fermented sausage, apple cider, mayonnaise, venison jerky, and lettuce, as well as by water and by direct person-to-person and cattle-to-person contact (39, 71, 83). In many cases, the ultimate source of infection has been traced to cattle; hence, cattle have been the focus of many studies to determine their involvement in transmitting EHEC. Currently, most attention is focused on O157:H7; however, the potential for zoonotic transmission of STEC other than O157:H7 also should be recognized.

SURVEYS OF STEC IN ANIMALS AND FOODS The United States Department of Agriculture (USDA) conducted in 1991–1992 the first national survey of cattle for carriage of *E. coli* O157:H7, isolating the pathogen from only 0.36% of 6894 preweaned calves in 19 (1.8%) of 1068 herds sampled in 28 states. However, a follow-up study of many of these herds and others using more sensitive detection procedures revealed that *E. coli* O157:H7 prevalence in calves was much higher than was previously reported (92). The pathogen was isolated from 31 of 965 calves (3.2%), and 18 of 64 (28%) herds were positive.

A study of STEC infection on dairy farms in Canada revealed evidence of carriage of STEC in a randomly selected human population not linked epidemiologically to diarrheal illness or HUS (1). The prevalence of STEC infection in this population was significant: 20% of families and 6.3% of family members were positive for STEC. All 80 herds, including 36% of cows and 57% of calves, were STEC-positive. On four farms, the same STEC serotypes were

identified in cattle and humans. Seven animals (0.45%) on four farms (5%) were positive for *E. coli* O157:H7.

Sheep recently have also been identified as carriers of *E. coli* O157:H7 (53). This pathogen has also been isolated from deer on a ranch in Texas (73). In Germany, a study on prevalence of STEC in domestic animals revealed that STEC were isolated from 208 out of 720 animals (28.9%), most frequently from sheep (66.6%), goats (56.1%), and cattle (21.1%), and less frequently from pigs (7.5%), cats (13.8%), and dogs (4.8%); none was found in chickens (11, 12). *E. coli* O157:H7, however, was not detected in this study. Experimentally, chickens have been readily colonized following peroral administration of small populations (25 cells) of *E. coli* O157:H7 (79). However, surveys of 50 poultry farms did not detect *E. coli* O157:H7 in any of 500 individual birds. Hence, it appears that currently poultry is not a primary source of STEC.

Several studies have shown the presence of STEC in foods. Doyle & Schoeni (28) isolated *E. coli* O157:H7 from beef, pork, poultry, and lamb samples obtained from retail stores in Wisconsin and Alberta, Canada. A survey on STEC in retail fresh seafood, beef, lamb, pork, and poultry in Seattle, Washington, revealed 17% of 294 food samples were positive using Stx DNA probe(s) (78).

STRAIN DIFFERENCES IN PATHOGENICITY Virulence heterogeneity among serotypes and strains of the same serotype is not clear. Interestingly, serotypes O157:H7 and O111:NM have caused major outbreaks more often and have led to higher rates of systemic disease than have any of the other serotypes (19, 39). Factors such as a lower infectious dose with these serotypes, higher production of toxin, greater gastrointestinal colonization, or enhanced delivery of toxins to endothelial cell targets may account for some of these differences (1). A two-year prospective study of stool specimens from 5415 patients in Canada revealed that 130 patients were infected with O157:H7, whereas 29 patients were infected with a broad range of non-O157 STEC strains (1). Severity of disease varied from one strain to another, with O157:H7 consistently being the most virulent. Surveys in the United States also indicate that many non-O157 strains are present in patients with severe diarrhea (7, 14). But the non-O157 strains were associated with bloody diarrhea less frequently than were the O157:H7 strains.

The vast majority of strains of serotype O157:H7 produce Shiga toxin 2 (Stx2) with or without Shiga toxin 1 (Stx1). In a study of 88 sporadic *E. coli* O157:H7 isolates in the United States in 1987, 76% had both Stx1 and Stx2 sequences, 20% had only Stx2, and 3% had only Stx1. Studies of humans suggest that Stx2 is a more important virulence factor than Stx1 is for progression of *E. coli* O157:H7 infection to HUS.

LISTERIA MONOCYTOGENES

Background

Listeria monocytogenes, largely an opportunistic pathogen, has been recognized as an important food-borne pathogen since the early 1980s (30, 32). Although listeriosis can occur in otherwise healthy adults and children, immunocompromised individuals, including the immunosuppressed, the elderly, pregnant women, and persons suffering a range of underlying diseases, are primarily at risk (30). The spectrum of disease is broad, ranging from asymptomatic infection and carriage to uncommon cutaneous lesions and flu-like symptoms, to miscarriage, stillbirth, sepsis, and meningitis (30). Although listeriosis can be treated with antimicrobial drugs, there is considerable mortality. Cases of mild gastrointestinal illness following the ingestion of *L. monocytogenes* also have been documented (74).

L. monocytogenes is widely distributed in soil, sewage, and fresh-water sediments and effluents and is frequently carried in the intestinal tract of animals and humans (30). The carriage of listeriae by healthy human and animals is well documented. Recent studies on the prevalence of *L. monocytogenes* in humans revealed that 2–6% of people were positive (76). More frequent fecal carriage (10–50%) has been documented in animals, including cattle, poultry, and pigs (76). The bacterium grows well at refrigeration temperature and in minimal nutrients and is able to survive and even to multiply in plants, soil, and water (30). Its widespread nature allows easy access to food products during various phases of production, processing and manufacture, and distribution.

The incidence of listeriosis in the United States is relatively low. Studies by the US Center for Disease Control and Prevention (CDC) in the mid-to-late 1980s revealed an estimated 1965 cases of listeriosis annually, with 481 deaths (84). A recent study revealed that the number of estimated cases is now less, about 1092 annually, with about 248 deaths (84). Considering the widespread presence of *L. monocytogenes* in the environment, in households, and in foods, most of the human population frequently ingest listeriae without ill effects. Hence, some unique host factors apparently predispose certain individuals to listeriosis, with most persons apparently resistant to listeric infection.

Recent Food-Borne Outbreaks and Epidemiology

RECENT OUTBREAKS Since the first documented outbreak in 1981, several outbreaks of food-borne listeriosis have been reported in North America and Europe. Two major outbreaks occurred in France in 1992–1993. The first involved 279 cases, including 22 spontaneous abortions and 63 deaths, whereas 39 cases were associated with the second (48, 76). Both outbreaks were linked to processed, ready-to-eat pork products. All cases were caused by serovar 4b,

phagovar 2389/2425/3274/2671/47/108/340, a clone of *L. monocytogenes* that has been described previously in other outbreaks (48). Investigations revealed that cross contamination between raw and cooked products was an important factor (77). In 1995, an outbreak associated with raw milk soft cheese was also reported in France.

Outbreaks of a milder form of listeriosis also have been reported. An outbreak of gastroenteritis occurred in Italy in June 1994, involving 39 cases (74). Of these, 70% had gastroenteritis and 30% had a flu-like illness. Rice salad was implicated as the source, and *L. monocytogenes* serotype 1/2a was the causative agent. A third recent outbreak occurred at a dairy cattle convention in Illinois in July 1994, with 52 of 64 otherwise healthy individuals developing mild gastrointestinal illness (26). Chocolate milk was identified both epidemiologically and by culture as the vehicle of infection. The chocolate milk had been temperature abused and was determined to contain approximately 10^9 cells of *L. monocytogenes*/ml (26).

REDUCTION IN THE INCIDENCE OF LISTERIOSIS IN THE UNITED STATES Studies by the CDC on the incidence of human listeriosis in the United States suggested that invasive disease due to *L. monocytogenes* decreased by 44% from 1989 through 1993 (84). Listeriosis-related deaths declined by 48% during the same period. These estimates were based on projections from diverse areas under surveillance (19.1 million, 8% of the US population), where incidence rates declined uniformly during the study period. Efforts by the US food industry and regulatory agencies to control listeriosis were likely in part responsible for this reduction (84). Dietary recommendations for consumers issued in 1992 also may have contributed to the decline in incidence.

Significance as a Food-Borne Pathogen

HIGH-RISK FOODS *L. monocytogenes* has been found in a variety of food products, including fresh vegetables (11%), raw meats (13%), raw milk (3–4%), dairy products (3%), eggs, and seafood products (38, 80, 89). Despite widespread occurrence, epidemic illnesses largely were linked to refrigerated, processed (ready-to-eat) foods consumed without prior cooking or reheating, including coleslaw, pasteurized milk, soft cheeses, pâte, pork tongue in jelly, shrimp, and smoked mussels. Some foods appear to be of greater risk than others do. Foods of highest risk are those that can support the growth of *L. monocytogenes*, are stored at refrigeration temperature for a long period, and are ready-to-eat. These include low-acid soft cheese—such as Camembert, Brie, and Mexican-style white cheeses—and pâte (56). In contrast, ready-to-eat foods that do not support the growth of *L. monocytogenes* to large populations within reasonable shelf lives are of low risk.

ZERO TOLERANCE FOR CERTAIN FOODS A zero-tolerance policy for *L. monocytogenes* in all ready-to-eat food products was established in 1986 by US federal agencies (Food and Drug Administration and the USDA) in response to a food-associated outbreak of listeriosis. The policy requires ready-to-eat foods to be negative for *L. monocytogenes* in two 25-g samples of food product. This policy was established based on minimal information about food-borne *Listeria*. Most scientific data available at that time addressed the veterinary aspects of *L. monocytogenes* as an animal pathogen. Hence, regulatory decisions were made without the benefit of a data base on the food-related aspects of the bacterium. The processed food industry has made great strides in mitigating the occurrence of *L. monocytogenes* in processing plant environments. However, the reality of the matter is that it is not possible to completely eliminate the bacterium from such facilities and still provide affordable products. Considering the frequent occurrence of *L. monocytogenes* in household kitchens and refrigerators, today's consumers are more responsible for providing conditions under which listeriosis can be acquired than is the food processor. It would appear that the point has been reached where further major reductions in the incidence of listeriosis will require behavior changes in food handling, preparation, and storage practices in the home. With a more complete understanding of the occurrence, transmission, and control of *L. monocytogenes* as a food-borne pathogen, many microbiologists believe that it is time to reevaluate the zero-tolerance policy and establish tolerances for *L. monocytogenes* in low-risk, e.g. acidic, foods that do not allow growth of the bacterium. The International Commission on Microbiological Specifications for Foods, based on the best available expert judgment, has recommended as an acceptable level 100 *L. monocytogenes* per g in certain foods that are consumed by low-risk populations (46).

STRAIN DIFFERENCES IN PATHOGENICITY In addition to host factors and exposure to specific foods, it is likely that certain microbial characteristics are important risk factors for disease (41). Specific virulence factors that influence the infectious dose could affect the occurrence and course of infection. Presently, all strains of *L. monocytogenes* with phenotypic characteristics of this species are considered to be pathogenic. However, it is important to recognize that *L. monocytogenes* isolates from patients represent a selected population derived from infective foci that consequently have been proved as able to cause infection in a host, whereas many *L. monocytogenes* isolates from food or the environment differ from most clinical strains (41). Subtyping of *L. monocytogenes* isolates, including serotyping and molecular typing, have revealed a wide array of differences within the species *L. monocytogenes* among strains frequently associated with illness versus those that are not (13, 15, 47, 58, 63). Most of the strains isolated from patients during outbreaks, as well as 45–70%

of strains responsible for sporadic cases, belong to serovar 4b, to two major profiles of isoenzymes, and to one major ribovar. In contrast, serogroup 1/2 strains accounted for most food and environmental isolates, with serovar 1/2c being a frequent contaminant of meat.

ARCOBACTER

Background

The genus *Arcobacter* was proposed in 1991 for bacteria previously identified as aerotolerant campylobacters or campylobacter-like organisms (24, 55). *Arcobacter* spp. primarily differ from campylobacters in their ability to grow under aerobic conditions at an optimal temperature of 30°C (27). These spiral organisms were found in bovine mastitis, in aborted fetuses from cows and pigs, and in nonhuman primates (24, 27). They have also been isolated from domestic animals, humans, poultry, ground pork, and drinking water (24, 87). Two species of *Arcobacter* (*A. cryaerophilus* and *A. butzleri*) have been associated with human disease. Most human isolates are *A. butzleri* (50, 55). Information regarding its clinical significance, pathogenicity, and epidemiology is limited.

Association with Human Illness

Evidence that *Arcobacter* spp. are pathogenic is based on their more frequent recovery from aborted pig litters and from infertile sows with vaginal discharge than from healthy animals (55). In addition, *A. butzleri* has been cultured from humans with enteritis who were otherwise healthy and from patients suffering from diarrhea with chronic underlying disease. Knowledge of the clinical importance of these bacteria is still limited. A recent study revealed that more than 50% of patients with *A. butzleri* infection suffered from persistent diarrhea accompanied by abdominal pain, nausea, and fever (55). Ten Italian school children involved in a 1983 outbreak experienced recurrent abdominal cramps without diarrhea (86).

Potential as a Food-Borne Pathogen

The most frequent risk factor associated with *Arcobacter* infection is exposure to contaminated water. Although to date food has not been directly associated with *Arcobacter* infection, the fact that the bacterium causes diseases in domestic animals and diarrheal illness in humans and has been isolated from meat products and water indicates that *Arcobacter* is likely a food-borne pathogen.

HELICOBACTER PYLORI

Background

Since its identification in 1982, *Helicobacter pylori*, formerly *Campylobacter pylori*, has been implicated as the etiologic agent of gastritis and as a major contributing factor in the development of peptic gastroduodenal ulcers (75, 85). It has also been linked to an increased risk of gastric cancer. *H. pylori* infection occurs worldwide and is frequently asymptomatic, particularly in children and young adults. In developed countries among healthy people over 50 years of age, *H. pylori* seroprevalences of 30–60% are commonly observed, whereas this infection is much less frequent in children. In developing countries, however, the infection occurs more frequently at younger ages, with prevalences of 70% to over 90% in some regions (75, 85).

Association with Human Illness

H. pylori infection, once acquired, persists for many years. Follow-up of infected individuals one to two years postdiagnosis indicates that the infection is stable, with little change in histologic grading of gastritis or fluctuation of antibody titer (85). Prolonged elevation of serum antibody titers also suggests that infection persists for years, for decades, or possibly for life (85). Spontaneous eradication of infection with gastric healing may occur but is probably uncommon. Eradication of *H. pylori* usually results in a healing, or at least a pronounced improvement, of gastritis in adults and children. Chronic superficial gastritis caused by *H. pylori* may be either symptomatic or asymptomatic. Early in the course of infection there may be changes in acid secretion. Later, infection may be associated with non-ulcer dyspepsia, peptic ulcer disease, type B atrophic gastritis, or gastric carcinoma (54, 85). The progression of *H. pylori*-induced chronic superficial gastritis to one of these later syndromes may require cofactors, such as genetic predisposition, smoking, alcohol, or diet. Differences in the virulence properties of *H. pylori* strains may also be a factor for disease progression.

Potential as a Food-Borne Pathogen

Humans were originally thought to be the only natural host of *H. pylori* (54, 81). However, more recently the bacterium has been isolated from nonhuman primates and very recently it has been isolated from cats (46), which suggests that the organism may also be a zoonotic pathogen, with transmission occurring from animals to humans. However, epidemiologic studies have revealed that animal exposure is not associated with *H. pylori* infection (5).

H. pylori is fragile under laboratory conditions, suggesting that the bacterium does not survive well outside the host. Although the pathogen has been detected in drinking water and vegetables using PCR and ELISA (36, 42), currently available cultural techniques have had little success in isolating viable *H. pylori* from environmental sources or foods. There is suggestive evidence of waterborne transmission (45). *H. pylori* could survive for more than one year in coccoid forms in a river-water microcosm and remain viable and culturable for more than 10 days in river water at 4°C (36). The food-borne disease potential of *H. pylori* needs further elucidation.

CRYPTOSPORIDIUM

Background

The protozoan parasite *Cryptosporidium parvum* has been recognized as a human pathogen since 1976 (2, 29). From 1976 to 1982, the disease was rarely reported and primarily occurred in immunocompromised persons. However, in 1982, the number of reported cases began to increase dramatically as part of the AIDS epidemic. Initially, illnesses were associated with immunocompromised persons; however, with the aid of newly developed laboratory diagnostic techniques, outbreaks in immunocompetent persons began to be recognized. Cryptosporidiosis in immunocompetent persons is manifested as an acute, self-limiting diarrheal illness lasting for 7–14 days and is often accompanied by nausea, abdominal cramps, and low-grade fever (2, 29). Cryptosporidiosis in patients with AIDS is generally chronic and more severe than in immunocompetent persons; the voluminous watery diarrhea is often debilitating and may be accompanied by severe abdominal cramps, weight loss, anorexia, malaise, and low-grade fever (68). *Cryptosporidium* is associated with diarrheal illness in most areas of the world. Studies have documented its presence in more then 40 countries of six continents.

Outbreaks

Several outbreaks of cryptosporidiosis attributed to drinking water have been recognized in the United States, including an outbreak in Milwaukee in 1993 that affected 403,000 persons (51). The source of municipal drinking water in these outbreaks included surface water (lakes, rivers, streams), well water, and spring water. Outbreaks also have been associated with swimming pools and amusement park wave pools or water slides (51, 82). Only one documented outbreak has been associated with food (60).

LARGEST OUTBREAK The outbreak of cryptosporidiosis in Milwaukee was the largest documented waterborne disease outbreak in the United States (51).

An estimated 403,000 persons became ill, of whom 4,400 were hospitalized. The outbreak was associated with water that had been filtered and chlorinated after it was obtained from Lake Michigan. Deterioration in raw-water quality and decreased effectiveness of the coagulation-filtration process led to an increase in the turbidity of treated water and to inadequate removal of *C. parvum* oocysts. Although the treated water met all state and federal quality standards that were then in effect, *C. parvum* oocysts were found in ice blocks that were made during the outbreak period. The etiologic agent and the waterborne nature of the outbreak were not identified until at least two weeks after the onset of the outbreak. Thereafter, a boil-water advisory was issued. The original environmental source of the oocysts was not definitively determined.

OUTBREAK IN NEVADA An outbreak of cryptosporidiosis associated with drinking water in Clark County, Nevada, which includes Las Vegas, lasted approximately seven months (from December 1, 1993, to June 30, 1994), with 103 laboratory-confirmed cases, and was first recognized among persons infected with HIV (34, 51). Although *Cryptosporidium* also caused illness in non-HIV–infected persons in this outbreak, no estimates of the total number of persons in the general population who had outbreak-related illness are available. This outbreak was associated with water obtained from Lake Mead that was filtered and chlorinated during treatment, and water quality was much better than that required by current national standards. No direct evidence found the lake water to be contaminated, nor was a specific source of contamination identified. The occurrence of cases of cryptosporidiosis over an extended period in conjunction with the failure to detect oocysts in water samples collected suggested low-level, probably intermittent, contamination of the water, and the potential for low-level, waterborne transmission may be greater than was previously recognized.

FOOD-BORNE OUTBREAK In October 1993, an outbreak of cryptosporidiosis associated with fresh-pressed apple cider occurred among students and staff attending a one-day school agricultural fair in Maine (60). In a survey of 611 attendees, 160 persons were identified as primary cases. *Cryptosporidium* oocysts were detected in the stools of 50 of 56 primary and secondary case patients tested. Epidemiologic studies revealed that persons drinking apple cider that was hand pressed during the fair were at high risk for cryptosporidiosis. The parasite was also detected in the apple cider, on the cider press, and in the stool specimen of a calf on the farm that supplied the apples.

Epidemiology

PREVALENCE IN ANIMALS *C. parvum* is capable of infecting all species of mammals, including humans (2, 49). Other species of *Cryptosporidium* that

infect birds (*C. meleagridis* and *C. baileyi*), rodents (*C. muris*), reptiles (*C. serpentis*), and fish (*C. nasorum*) are not generally considered to be infectious for humans (29). To date there have been no confirmed instances of *C. parvum* transmission from infected household pets (2, 49).

In contrast, *C. parvum* transmission from calves to humans is unequivocal (61). It is estimated that 50% of dairy calves shed oocysts and that the parasite is present on more than 90% of dairy farms (4, 6). Calves are a source of human infection, with up to 44% of dairy farmers in one study having serologic evidence of past infection, and contact with animals is frequently reported in sporadic cases. Drinking raw milk has been implicated as a mode of transmission.

WATERBORNE TRANSMISSION There is considerable circumstantial evidence to indicate that low-level transmission of *Cryptosporidium* species through drinking water may be occurring throughout the United States. However, the health risk associated with consumption of filtered or unfiltered public drinking water contaminated with small numbers of *C. parvum* oocysts is unknown. Recent studies indicate that *Cryptosporidium* oocysts are present in 65–97% of surface waters (rivers, lakes, etc) tested throughout the country (49, 51). Because *Cryptosporidium* species are highly resistant to chemical disinfectants used in the treatment of drinking water, physical removal of the parasite from contaminated water by filtration is an important component of the water treatment process. However, a filtration system, especially one that is not well maintained and operated, may not afford absolute protection. Documented waterborne outbreaks of cryptosporidiosis to date have occurred in communities where water utilities met state and federal standards for acceptable quality of drinking water, and in all three of the outbreaks that involved surface water supplies, a filtration system had been used (34, 51). Data from the outbreaks suggest that compliance of utility companies with state and federal standards for water treatment may not be adequate to protect citizens from waterborne cryptosporidiosis. Moreover, recent surveys for the occurrence of *Cryptosporidium* oocysts in fully treated (disinfected and filtered) municipal water demonstrate that small numbers of oocysts were able to breach filters and were present in tap water in 27–54% of the communities evaluated (51).

PERSON-TO-PERSON TRANSMISSION There is growing evidence that person-to-person transmission from fecal-oral spread is an important means of transmission. Children wearing diapers who attend day care centers are at high risk for this form of transmission, either through intimate play or because of careless diaper-changing practices. Infections acquired by children in the day care setting are often transmitted to care-givers at the facility and to older children and adults who come in contact with the infected child at home (25). Any

sexual practice that brings a person into oral contact with the feces of an infected person is also considered a high risk for exposure to *Cryptosporidium* species. It is not known how many patients with HIV infection or AIDS acquire cryptosporidiosis by this route of transmission.

Significance as a Food-Borne Pathogen

Cryptosporidium oocysts have been found in fresh vegetables, raw milk, sausage, and tripe (43, 62, 69). Food contaminated with feces from infected persons or animals has always been considered to be a theoretical risk factor for cryptosporidiosis. It is also possible that oocysts are present in raw milk and may be present on processed animal carcasses. Uncooked foods may be vectors for transmission of oocysts to consumers. Although oocysts do not survive cooking, infected food handlers may unwittingly transmit the infection by fecal contamination of beverages, green salads, or other foods that are not cooked or heated after handling (43, 69). A study on survival of *C. parvum* in beverages revealed that 85% of the oocysts died in beers, sodas, and fruit juices after 24 h at 4° or 22°C, whereas only 11% and 30% reductions occurred in baby formula and tap water, respectively, after 24 h at each temperature (67).

CYCLOSPORA

Background

The coccidia *Cyclospora* species (previously called cyanobacterium or blue-green algae-like organism) is a newly recognized enteric pathogen. Although the presence of the organism in stool samples was first reported in 1977 (66), it has been reported with increasing frequency since the 1980s, perhaps because of the increasing use of acid-fast strains to identify other coccidia in stool specimens and the recognition that similar organisms of different sizes represent different genera (37, 66). *Cyclospora* shares some features with other coccidian human pathogens, such as *Microsporidia*, *Isospora*, and *Cryptosporidia* (37). The diameter of *Cyclospora* oocysts is 8–10 μm, approximately twice that of *C. parvum.* Phylogenetic analysis of rDNA sequences revealed that *Cyclospora* was closely related to members of the *Eimeria* genus (72). The acute clinical presentations of *Cyclospora* and *Cryptosporidium* infection are similar: frequent watery, nonbloody stools accompanied by crampy abdominal pain, nausea, and fatigue. Fever is usually absent. Unlike cryptosporidiosis in the normal host, which usually lasts about 10–14 days, illness caused by *Cyclospora* is often prolonged, with an average duration of 43 days (65). Symptoms may relapse.

Although *Cyclospora* is transmitted by the fecal-oral route, direct person-to-person transmission is unlikely because excreted oocysts require days to weeks under favorable environmental conditions to become infectious (i.e. sporulate)

(66). Warm temperatures and high humidity facilitate sporulation. Some evidence suggests that infection is most common in spring and summer. Organisms of the genus *Cyclospora* have previously been found in reptiles, myriapods, insectivores, and one murine host (66). The oocysts reported in those animals, however, are different in size from those reported in humans, which warrants classifying the human *Cyclospora* as a new species. Whether animals serve as sources of infection for humans is unknown; the complete life cycle and epidemiologic features of this newly recognized parasite are also unknown (37). Persons of all ages are at risk of infection. Although travelers to tropical countries may be at increased risk, the infection can also be acquired in such countries as the United States and Canada (31).

Outbreaks

FIRST OUTBREAK IN THE UNITED STATES Sporadic cases of *Cyclospora* infection have been reported worldwide. The first reported outbreak of diarrheal illness associated with *Cyclospora* in the United States occurred at a hospital in Chicago in July 1990 (44). Twenty-one cases were identified. Symptoms typically occurred in a distinctive cycle of remissions and exacerbations lasting up to several weeks. Microscopic examination of stool specimens from 11 ill persons revealed *Cyclospora* organisms. The organisms disappeared within nine weeks after onset of illness in seven patients. Epidemiologic studies implicated tap water from a physicians' dormitory as the most likely source of the outbreak.

RECENT OUTBREAK In May and June 1996, social event–related clusters of cases and/or sporadic cases of *Cyclospora* infection involved at least 14 states and the District of Columbia in the United States and Ontario, Canada (22). Approximately 1400 total unconfirmed and 850 laboratory-confirmed cases were reported: 14% of all confirmed cases were from Ontario, Canada; nearly all (99%) of the other confirmed cases were reported from states east of the Rocky Mountains. Most cases occurred in immunocompetent adults. Fifteen patients were hospitalized, but no deaths were reported. Because *Cyclospora* is not life threatening and is not a reportable illness in some states, the actual number of cases was likely much higher. Epidemiologic evidence indicated that raspberries were the vehicle of transmission and that some regions of Guatemala were the most likely sources.

Significance as a Food-Borne Pathogen

Cyclospora is transmitted through infected feces by the fecal-oral route. The parasite may be transmitted by a person swallowing oocysts found in contaminated water or food. Potential sources of infection include fruits and vegetables that originate from different domestic and international locations at different

times of the year. The complex distribution routes and handling of these foods complicate trace backs and other key aspects of epidemiologic investigations. How common the various modes of transmission and sources of infection are is not known, nor is it known whether animals can become infected and serve as sources of infection for humans.

CONCLUDING REMARKS

The emergence of newly recognized food-borne pathogens and changes in the epidemiology of food-borne diseases are continuing occurrences that further complicate the difficulties of controlling food-borne illness. Although the significance of *E. coli* O157:H7 and *L. monocytogenes* as food-borne pathogens has been well established, more research is needed to fully elucidate the ecology and epidemiology of illness caused by pathogens like *Arcobacter*, *Helicobacter pylori*, *Cryptosporidium parvum*, and *Cyclospora*. It is also important to be able to differentiate microbial strains of the same species or the same serotype that are pathogenic for humans from those that are nonpathogenic, as in the case of STEC.

Control of food-borne disease is important at all levels, including food production, preparation, and delivery to consumers (59). Each link presents unique food-borne disease hazards and control opportunities. Existing technologies and new approaches such as irradiation and hazard analysis and critical control point (HACCP) programs are useful tools in the control of food-borne hazards. However, because of ever-changing products, processes, food handling practices, societal habits, and pathogens, emerging microbiological food safety issues will continue to be an important public health concern.

Literature Cited

1. Acheson DWK, Keusch GT. 1996. Which Shiga toxin-producing types of *E. coli* are important? *ASM News* 62:302–6
2. Adal KA, Sterling CR, Guerrant RL. 1995. *Cryptosporidium* and related species. In *Infections of the Gastrointestinal Tract*, ed. MJ Blaser, PD Smith, JI Ravdin, HB Greenberg, RL Guerrant, pp. 1107–28. New York: Raven
3. Altekruse SF, Swerdlow DL. 1996. The changing epidemiology of foodborne diseases. *Am. J. Med. Sci.* 311:23–29
4. Anderson BC, Hall RF. 1982. Cryptosporidial infection in Idaho dairy calves. *J. Am. Vet. Med. Assoc.* 181:484–85
5. Ansorg R, Heintschel E, Heinegg V, Rechlinghausen GV. 1995. Cat owners' risk of acquiring a *Helicobacter pylori* infection. *Zentralbl. Bakteriol.* 283:122–26
6. Aurich JE, Dobrinski I, Grunert E. 1990. Intestinal cryptosporidiosis in calves on a dairy farm. *Vet. Rec.* 127:380–81
7. Begue RE, Neill MA, Papa EF, Dennehy PH. 1994. A prospective study of Shiga-like toxin-associated diarrhea in a pediatric population. *J. Pediatr. Gastroenterol. Nutr.* 19:164–69
8. Bell BP, Goldoft M, Griffin PM, Davis

MA, Gordon DC, et al. 1994. A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. *JAMA* 272:1349–53
9. Berkelman RL. 1994. Emerging infectious diseases in the United States, 1993. *J. Infect. Dis.* 170:272–77
10. Besser RE, Lett SM, Weber JT, Doyle MP, Barrett TJ, et al. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. *JAMA* 269:2217–20
11. Beutin L, Geier D, Steinruck H, Zimmermann S, Karch H. 1995. Virulence markers of Shiga-like toxin-producing *Escherichia coli* strains originating from healthy domestic animals of different species. *J. Clin. Microbiol.* 33:13–21
12. Beutin L, Geier D, Steinruck H, Zimmermann S, Scheutz F. 1993. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. *J. Clin. Microbiol.* 31:2483–88
13. Boerlin P, Piffaretti JC. 1991. Typing of human, animal, food, and environmental isolates of *Listeria monocytogenes* by multilocus enzyme electrophoresis. *Appl. Environ. Microbiol.* 57:1624–29
14. Bokete TB, O'Callahan CM, Clausen CR, Tang NM, Tran N, et al. 1993. Shiga-like toxin-producing *Escherichia coli* in Seattle children. *Gastroenterology* 105:1724–31
15. Brosch R, Catimel B, Milon G, Buchrieser C, Vindel E, et al. 1993. Virulence heterogeneity of *Listeria monocytogenes* strains from various sources (food, human, animal) in immunocompetent mice and its association with typing characteristics. *J. Food Prot.* 56:301–12
16. Calderwood SB, Acheson DWK, Keusch GT, Barrett TJ, Griffin PM, et al. 1996. Proposed new nomenclature for SLT (VT) family. *ASM News* 62:118–19
17. Caprioli A, Luzzi I, Minelli F, Benedetti I, Tozzi AE, et al. 1994. Hemolytic uremic syndrome and verotoxin-producing *Escherichia coli* infection in Italy, 1988–1993. In *Recent Advances in Verocytotoxin-Producing Escherichia coli Infections*, ed. MA Karmali, AG Goglio, pp. 29–32. Amsterdam: Elsevier
18. Caprioli A, Luzzi I, Rosmini F, Resti C, Edefonti A. 1994. Community-wide outbreak of hemolytic uremic syndrome associated with non-O157 verocytotoxin-producing *Escherichia coli*. *J. Infect. Dis.* 169:208–11
19. Centers for Disease Control and Prevention. 1995. Community outbreak of hemolytic uremic syndrome attributable to *Escherichia coli* O111:NM—South Australia, 1995. *MMWR* 44:550–58
20. Centers for Disease Control and Prevention. 1995. *Escherichia coli* O157:H7 outbreak linked to commercially distributed dry-cured salami—Washington and California, 1994. *MMWR* 44:157–60
21. Centers for Disease Control and Prevention. 1995. Outbreak of acute gastroenteritis attributable to *Escherichia coli* serotype O104:H21—Helena, Montana, 1994. *MMWR* 44:501–3
22. Centers for Disease Control and Prevention. 1996. Outbreaks of *Cyclospora cayetanensis* infection—United States and Canada, 1996. *MMWR* 45:459–61
23. Chapman PA, Wright DJ, Norman P, Fox J, Crick E. 1993. Cattle as a possible source of verotoxin-producing *Escherichia coli* O157:H7 infections in man. *Epidemiol. Infect.* 111:439–47
24. Collins CI, Wesley IV, Murano EA. 1996. Detection of *Arcobacter* spp. in ground pork by modified plating methods. *J. Food Prot.* 59:448–52
25. Cordell RL, Addiss DG. 1994. Cryptosporidiosis in child care settings: a review of the literature and recommendations for prevention and control. *Pediatr. Infect. Dis. J.* 13:310–17
26. Dalton CB, Austin C, Sobel J, Hayes P, Bibb B, et al. 1995. Outbreak of gastroenteritis from *Listeria monocytogenes*. In *Proc. 44th Annu. Epidemic Intell. Serv. Conf.*, pp. 19. Atlanta: CDC
27. De Boer E, Tilburg JJHC, Woodward DL, Lior H, Johnson WM. 1996. A selective medium for the isolation of *Arcobacter* from meats. *Lett. Appl. Microbiol.* 23:64–66
28. Doyle MP, Schoeni JL. 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. *Appl. Environ. Microbiol.* 53:2394–96
29. Dubey JP, Speer CA, Fayer R. 1990. *Cryptosporidiosis of Man and Animals.* Boston: CRC Press
30. Farber JM, Peterkin PI. 1991. *Listeria monocytogenes*, a foodborne pathogen. *Microbiol. Rev.* 55:476–511
31. Gascon J, Corachan M, Bombi JA, Valls ME, Bordes JM. 1995. *Cyclospora* in patients with traveller's diarrhea. *Scand. J. Infect. Dis.* 27:511–14
32. Gellin BG, Broome CV, Bibb WF, Weaver RE, Gaventa S, et al. 1991. The epidemiology of listeriosis in the United States—1986. *Am. J. Epidemiol.* 133:392–401

33. Glass KA, Loeffelholz JM, Ford JP, Doyle MP. 1992. Fate of *Escherichia coli* O157:H7 as affected by pH or sodium chloride in fermented, dry sausage. *Appl. Environ. Microbiol.* 58:2513–16
34. Goldstein ST, Juranek DD, Ravenholt O, Hightower AW, Martin DG, et al. 1996. Cryptosporidiosis: an outbreak associated with drinking water despite state-of-art water treatment. *Ann. Intern. Med.* 124:459–68
35. Goldwater PN, Bettelheim O. 1994. The role of enterohaemorrhagic *E. coli* serotypes other than O157:H7 as causes of disease. In *Recent Advances in Verocytotoxin-Producing Escherichia coli Infections*, ed. MA Karmali, AG Goglio, pp. 57–60. Amsterdam: Elsevier
36. Goodman KJ, Correa P. 1995. The transmission of *Helicobacter pylori*. A critical review of the evidence. *Int. J. Epidemiol.* 24:875–87
37. Goodman RW. 1996. Understanding intestinal spore-forming protozoa: *Cryptosporidia, Microsporidia, Isospora, and Cyclospora. Ann. Intern. Med.* 124:429–41
38. Greenwood MH, Roberts D, Burden P. 1991. The occurrence of *Listeria* species in milk and dairy products—a national survey in England and Wales. *Int. J. Food Microbiol.* 12:197–206
39. Griffin PM. 1995. *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*. In *Infections of the Gastrointestinal Tract*, ed. MJ Blaser, PD Smith, JI Ravdin, HB Greenberg, RL Guerrant, pp. 739–61. New York: Raven
40. Handt LK, Fox JG, Dewhirst FE, Fraser GJ, Paster BJ, et al. 1994. *Helicobacter pylori* isolated from the domestic cat: public health implications. *Infect. Immun.* 62:2367–74
41. Hof H, Rocourt J. 1992. Is any strain of *Listeria monocytogenes* detected in food a health risk? *Int. J. Food Microbiol.* 16:173–82
42. Hopkins RJ, Vial PA, Ferreccio C, Ovalle J, Prado P, et al. 1993. Seroprevalence of *Helicobacter pylori* in Chile vegetables may serve as one route of transmission. *J. Infect. Dis.* 168:222–26
43. Hoskin JC, Wright RE. 1991. *Cryptosporidium*: an emerging concern for the food industry. *J. Food Prot.* 54:53–57
44. Huang P, Webber T, Sosin DM, Griffin PM, Long EG, et al. 1995. The first reported outbreak of diarrheal illness associated with *Cyclospora* in the United States. *Ann. Intern. Med.* 123:409–14
45. Hulten K, Han SW, Enroth H, Klein PD, Opekun AR, et al. 1996. *Helicobacter pylori* in the drinking water in Peru. *Gastroenterology* 110:1031–35
46. Int. Comm. Microbiol. Specific. Foods. 1994. Choice of sampling plan and criteria for *Listeria monocytogenes*. *Int. J. Food Microbiol.* 22:89–96
47. Jacquet C, Bille J, Rocourt J. 1992. Typing of *Listeria monocytogenes* by restriction polymorphism of the ribosomal ribonucleic acid gene region. *Zentralbl. Bakteriol.* 276:356–65
48. Jacquet C, Catimel B, Brosch R, Buchrieser C, Dehaumont P, et al. 1995. Investigations related to the epidemic strain involved in the French listeriosis outbreak in 1992. *Appl. Environ. Microbiol.* 61:2242–46
49. Juranek DD. 1995. Cryptosporidiosis: sources of infection and guidelines for prevention. *Clin. Infect. Dis.* 21:S57–61
50. Kichlbauch JA, Brenner DJ, Nicholson MA, Baker CN, Patton MP, et al. 1991. *Campylobacter butzleri* sp. nov. isolated from humans and animals with diarrheal illness. *J. Clin. Microbiol.* 29:376–85
51. Kramer MH, Herwaldt BL, Craun GF, Calderon RL, Juranek DD. 1996. Surveillance for waterborne disease outbreaks—1993–1994. *MMWR* 45:1–33
52. Kudoh Y, Kai A, Obata H, Kusunoki J, Monma C, et al. 1994. Epidemiological surveys on verotoxin-producing *Escherichia coli* infections in Japan. In *Recent Advances in Verocytotoxin-Producing Escherichia coli Infections*, ed. MA Karmali, AG Goglio, pp. 53–56. Amsterdam: Elsevier
53. Kudva IT, Hatfield PG, Hovde CJ. 1996. *Escherichia coli* O157:H7 in microbial flora of sheep. *J. Clin. Microbiol.* 34:431–33
54. Lee A. 1994. The microbiology and epidemiology of *Helicobacter pylori* infection. *Scand. J. Gastroenterol.* 29(Suppl. 201):2–6
55. Lerner J, Brumberger V, Preac-Mursic V. 1994. Severe diarrhea associated with *Arcobacter butzleri*. *Eur. J. Clin. Microbiol. Infect. Dis.* 13:660–62
56. Linnan MJ, Mascola L, Lou XD, Goulet V, May S, et al. 1988. Epidemic listeriosis associated with Mexican-style cheese. *N. Engl. J. Med.* 319:823–28
57. Mariani-Kurkdjian P, Denamur E, Milon A, Picard B, Cave H. 1993. Identification of a clone of *Escherichia coli* O103:H2 as a potential agent of hemolytic uremic syndrome in France. *J. Clin. Microbiol.* 31:296–301

58. McLauchlin J. 1990. Distribution of serovars of *Listeria monocytogenes* isolated from different categories of patients with listeriosis. *Eur. J. Clin. Microbiol. Infect. Dis.* 9:210–13
59. Meng J, Doyle MP, Zhao S, Zhao T. 1994. The detection and control of *Escherichia coli* O157:H7 in foods. *Trends Food Sci. Technol.* 5:179–85
60. Millard PS, Gensheimer KF, Addiss DG, Sosin DM, Houck-Jankoski A, Hudson A. 1994. An outbreak of cryptosporidiosis from fresh-pressed apple cider. *JAMA* 272:1592– 96
61. Miron D, Kenes J, Dagan R. 1991. Calves as a source of an outbreak of cryptosporidiosis among young children in an agricultural closed community. *Pediatr. Infect. Dis. J.* 10:438–41
62. Monge R, Chinchilla M. 1996. Presence of *Cryptosporidium* oocysts in fresh vegetables. *J. Food Prot.* 59:202–3
63. Norrung B, Skovgaard N. 1993. Application of multilocus enzyme electrophoresis in studies of the epidemiology of *Listeria monocytogenes* in Denmark. *Appl. Environ. Microbiol.* 59:2817–22
64. O'Brien A, Tesh V, Donohue-Rolfe A, Jackson M, Olsnes S, et al. 1992. Shiga toxin: biochemistry, genetics mode of action and role in pathogenesis. *Curr. Top. Microbiol. Immunol.* 180:65–94
65. Ooi WW, Zimmerman SK, Needham CA. 1995. *Cyclospora* species as a gastrointestinal pathogen in immunocompetent hosts. *J. Clin. Microbiol.* 33:1267–69
66. Ortega YR, Sterling CR, Gilman RH, Cama VA, Diaz F. 1993. *Cyclospora* species—a new protozoan of humans. *N. Engl. J. Med.* 328:1308–12
67. Patten KJ, Rose JB. 1995. Viability of *Cryptosporidium parvum* oocysts in beverages: correlation of in vitro excystation with inclusion or exclusion of fluorogenic vital dyes. *Abstr. Annu. Meet. Int. Assoc. Milk Food Environ. Sanit.*, p. 67. Des Moines, IA: Int. Assoc. Milk, Food & Environ Sanit.
68. Petersen C. 1992. Cryptosporidiosis in patients infected with the human immunodeficiency virus. *Clin. Infect. Dis.* 15:903–9
69. Petersen C. 1995. *Cryptosporidium* and the food supply. *Lancet* 345:1128–29
70. Pinner RW, Schuchat A, Swaminathan B, Hayes PS, Deaver KA, et al. 1992. Role of foods in sporadic listeriosis. II. Microbiologic and epidemiologic investigation. *JAMA* 267:2046–50
71. Reida P, Wolff M, Pohls HW, Kuhlmann W, Lehmacher A, et al. 1994. An outbreak due to enterohemorrhagic *Escherichia coli* O157:H7 in a children day care center characterized by person-to-person transmission and environmental contamination. *Zentralbl. Bakteriol.* 281:534–43
72. Relman DA, Schmidt TM, Gajadhar A, Sogin M, Cross J, et al. 1996. Molecular phylogenetic analysis of *Cyclospora*, the human intestinal pathogen, suggests that it is closely related to *Eimeria* species. *J. Infect. Dis.* 173:440–45
73. Rice DH, Hancock DD, Besser TE. 1995. Verotoxigenic *E. coli* O157:H7 colonization of wild deer and range cattle. *Vet. Rec.* 132:88
74. Riedo FX, Pinner RW, Tosca MD, Cartter ML, Graves LM, et al. 1994. A point-source foodborne listeriosis outbreak: documented incubation period and possible mild illness. *J. Infect. Dis.* 170: 693–96
75. Riegg SJ, Dunn BE, Blaser MJ. 1995. Microbiology and pathogenesis of *Helicobacter pylori*. In *Infections of the Gastrointestinal Tract*, ed. MJ Blaser, PD Smith, JI Ravdin, HB Greenberg, RL Guerrant, pp. 535–50. New York:Raven
76. Rocourt J. 1994. *Listeria monocytogenes*: the state of the science. *Dairy Food Environ. Sanit.* 14:70–82
77. Salvat G, Toquin MT, Michel Y, Colin P. 1995. Control of *Listeria monocytogenes* in the delicatessen industries: the lessons of a listeriosis outbreak in France. *Int. J. Food Microbiol.* 25:75–81
78. Samadpour M, Ongerth JE, Liston J, Tran N, Nguyen D, et al. 1994. Occurrence of Shiga-like toxin-producing *Escherichia coli* in retail fresh seafood, beef, lamb, pork, and poultry from grocery stores in Seattle, Washington. *Appl. Environ. Microbiol.* 60:1038–40
79. Schoeni JL, Doyle MP. 1994. Variable colonization of chickens perorally inoculated with *Escherichia coli* O157:H7 and subsequent contamination of eggs. *Appl. Environ. Microbiol.* 60:2958–62
80. Schuchat A, Deaver KA, Mascola L, Wenger JD, Plikaytis BD, et al. 1992. Role of foods in sporadic listeriosis. I. Case-control study of dietary risk factors. *JAMA* 267:2041–45
81. Seymour C, Lewis RG, Kim M, Gagnon DF, Fox JG, et al. 1994. Isolation of *Helicobacter* strains from wild bird and swine feces. *Appl. Environ. Microbiol.* 60:1025–28
82. Sorvillo FJ, Fujioka K, Nahlen B, Tormey MP, Kebabjian R, Mascola L. 1992. Swimming-associated cryptosporidiosis. *Am. J. Public Health* 82:742–44

83. Swerdlow DL, Woodruff BA, Brady RC. 1992. A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. *Ann. Intern. Med.* 117:812–19
84. Tappero JW, Schuchat A, Deaver KA, Mascola L, Wenger JD. 1995. Reduction in the incidence of human listeriosis in the United States—effectiveness of prevention efforts? *JAMA* 273:1118–22
85. Taylor DN, Parsonnet J. 1995. Epidemiology and natural history of *Helicobacter pylori* infection. In *Infections of the Gastrointestinal Tract,* ed. MJ Blaser, PD Smith, JI Ravdin, HB Greenberg, RL Guerrant, pp. 551–63. New York: Raven
86. Vandamme P, Pugina P, Benzi R, Van Etterijck R, Vlaes L, et al. 1992. Outbreak of recurrent abdominal cramps associated with *Arcobacter butzleri* in an Italian school. *J. Clin. Microbiol.* 30:2335–37
87. Wesley IV. 1995. *Arcobacter* and *Helicobacter* —risks for foods and beverages. *J. Food Prot.* 59:1127–32
88. Whipp SC, Rasmussen MA, Cray WC. 1994. Animals as a source of *Escherichia coli* pathogenic for human beings. *J. Am. Vet. Med. Assoc.* 204:1168–75
89. Wilson IG. 1995. Occurrence of *Listeria* species in ready to eat foods. *Epidemiol. Infect.* 115:519–26
90. Zhao T, Doyle MP. 1994. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in commercial mayonnaise. *J. Food Prot.* 57:780–83
91. Zhao T, Doyle MP, Besser RE. 1993. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in apple cider with and without preservatives. *Appl. Environ. Microbiol.* 59:2526–30
92. Zhao T, Doyle MP, Shere J, Garber L. 1995. Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. *Appl. Environ. Microbiol.* 61:1290–93