WHO/CSR/APH/98.8
Distr.: General
English only

ZOONOTIC NON-O157 SHIGA TOXIN-PRODUCING ESCHERICHIA COLI (STEC)

Report of a WHO Scientific Working Group Meeting

Berlin, Germany
23-26 June 1998

DEPARTMENT OF COMMUNICABLE DISEASE SURVEILLANCE AND RESPONSE
WORLD HEALTH ORGANIZATION

Contents

I. INTRODUCTION 1

II. CLINICAL MANIFESTATIONS AND MECHANISMS OF DISEASE 3

Clinical Manifestations 3
Mechanisms of Disease 3
Conclusions and Gaps in Current Knowledge 5

III. SURVEILLANCE AND FREQUENCY OF ISOLATION 7

Surveillance 7
Frequency of Isolation 7
Conclusions 9
Gaps in Current Knowledge 9

IV. SOURCES AND MODES OF TRANSMISSION 11

Sources of STEC 11
Modes of Transmission 13
Conclusions and Gaps in Current Knowledge 15

V. LABORATORY METHODOLOGIES 16

Detection 16
Isolation 17
Characterization of STEC 18
Criteria for Reporting Characteristics of Non-O157 Isolates from Clinical Samples 22
Conclusions and Gaps in Current Knowledge 22

VI. RECOMMENDATIONS 23

Clinical Manifestations 23
Mechanisms of Disease 23
Surveillance and Frequency of Isolation 24
Sources and Modes of Transmission 26
Laboratory Methodologies 26

LIST OF ABBREVIATIONS 28

LIST OF PARTICIPANTS AND OBSERVERS 29

AGENDA AND TIMETABLE 34

Tuesday, 23 June 1998 34
Wednesday, 24 June 1998 34
Wednesday, 24 June 1998 (continued) 35
Thursday, 25 June 1998 35
Friday, 26 June 1998 35

I. Introduction

Infections with Shiga toxin-producing *Escherichia coli* (STEC) bacteria are increasingly reported worldwide. Among STEC, O157:H7 is the classical serotype that was first associated with enterohaemorrhagic diseases in the early 1980's as a cause of serious outbreaks and sporadic cases of illness. However, over 100 different STEC serotypes, other than O157:H7, have now been associated with human illness. Non-O157 STEC, also first associated with human disease in the early 1980's, are only recently becoming recognized as important pathogens that cause a spectrum of disease in humans similar to that caused by serotype O157:H7. An increasing number of outbreaks and sporadic cases related to non-O157 have been reported.

In laboratories that routinely test for STEC in stool samples of patients with diarrhoea, STEC are usually found less often than *Salmonella* and *Campylobacter*, but more frequently than *Shigella* and *Yersinia*. Overall, non-O157 STEC are now found in 20 to 70 percent of patients with STEC infections. However, tremendous geographical differences have been recognized in the percentage of patients with STEC-associated disease who are infected with non-O157.

In general, the detection of STEC, particularly non-O157 STEC, is not widely practised in most microbiological laboratories worldwide. Screening for O157 strains is not done on a routine basis and even fewer laboratories are able to detect non-O157 strains. This is primarily because many non-O157 STEC strains lack the phenotypic characteristics of O157 STEC, such as delayed fermentation of sorbitol and haemolytic activity on enterohaemolysin agar, and therefore cannot be identified on the routinely used sorbitol-MacConkey agar. Consequently, the prevalence of infections with non-O157 strains is presently underestimated.

As with O157 STEC, human infection with non-O157 strains is potentially fatal and may be associated with serious complications such as haemolytic uraemic syndrome (HUS). There is no specific therapy for HUS and most patients require prolonged clinical and outpatient treatment. Healthcare costs associated with such infections are thus very significant.

Given the increasing impact of non-O157 STEC on human health, WHO convened a Scientific Working Group Meeting on Zoonotic Non-O157 STEC. The meeting was held in collaboration with the Robert Koch-Institut in Berlin, Germany, 23-26 June 1998.

The meeting was opened by Prof. Dr R. Kurth who welcomed the participants to Berlin on behalf of the Robert Koch-Institut. Dr K. Stöhr of the Zoonotic Diseases Unit of WHO then reviewed the objectives of the meeting. Those objectives were to:

- Review the knowledge on non-O157 STEC, with an emphasis on epidemiology and detection of infections;
- Identify research priorities in areas where public health action is needed on non-O157 STEC;
- Promote international cooperation and collaboration on the diagnosis and control of non-O157 STEC.

Dr M. Karmali and Dr L. Beutin were elected as Chairperson and Vice-Chairperson, respectively. Dr Patricia Desmarchelier was elected as Rapporteur. Following is a report of the findings, conclusions, gaps in current knowledge and recommendations made by the participants of the meeting in Berlin.

II. Clinical Manifestations and Mechanisms of Disease

Clinical Manifestations

Non-O157 STEC infections are found in 20 to 70 percent of patients with STEC-associated disease, depending on geographical location. The clinical course and outcome of infection with non-O157 STEC appears to be similar to that of infection with serotype O157:H7, although the latter may more frequently cause haemorrhagic colitis (HC). STEC infections are seen most frequently in infants, children and elderly patients. No gender differences have been observed.

Non-O157 associated intestinal disease includes watery diarrhoea, usually with painful cramps, and HC. Extraintestinal manifestations include HUS, or incomplete forms of the syndrome that consist of only one or two of the three features associated with it, namely thrombocytopaenia, haemolytic anaemia and acute renal failure. About 75% of patients with HUS caused by non-O157 STEC require peritoneal or haemodialysis. Uncommon complications of STEC infections include urinary tract infection and, particularly in women after pregnancy and in elderly patients, thrombotic thrombocytopaenic purpura (TTP).

Whether caused by O157 STEC or non-O157 STEC, treatment for severe complications of infection (HC, HUS, TTP) is non-specific and generally insufficient. Most patients with HUS require prolonged outpatient treatment, resulting in significant healthcare costs.

Mechanisms of Disease

As in the case of STEC serotype O157:H7, the major virulence characteristics of non-O157 STEC are the production of Shiga toxins and the ability to colonize the bowel. The mechanisms of bowel colonization are not well understood, however. The role of various other potential bacterial virulence factors and host factors remains to be elucidated.

Bacterial virulence factors

Shiga toxins

The production of Shiga toxin (STX) is the unifying feature of all non-O157 STEC and O157 STEC. Various types of STX are produced, but they fall into two main types: Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2). There are three serological or biological variants of Stx2 (2c, 2d and 2e) whose clinical significance is unknown. Stx2d, a feature of STEC serotype O91:H21, is activated by murine mucus. Stx2e, produced by porcine non-O157 serotypes, causes oedema disease in pigs. The majority of *STX* genes are bacteriophage encoded, thus allowing a level of interstrain and possibly interspecies mobility.

The presence of STX is thought to be the primary factor responsible for intestinal manifestations (bloody diarrhoea) and systemic complications (HUS). The toxin uptake mechanisms in the intestine have yet to be fully elucidated for all STEC.

Colonization factors and other potential virulence mediators

Locus for enterocyte effacement. As in the case of enteropathogenic *E. coli* (EPEC), O157:H7 STEC colonize the enterocytes of, probably, the large bowel with a characteristic attaching and effacing (A/E) cytopathology. The A/E ability is encoded by 41 genes that are present on a "pathogenicity island" referred to as the locus for enterocyte effacement (LEE). LEE encodes the adhesin called intimin (*eaeA* gene), as well as the machinery (*esc*/*sep* genes) and effector molecules (EspA, B and D) of a type III secretion system that are responsible for the genesis of the A/E lesion. LEE also encodes a receptor for intimin that is translocated to the eukaryotic cell membrane. This receptor is referred to as Tir (translocated intimin receptor) in EPEC and as EspE in O157:H7 STEC.

Some non-O157 STEC serotypes, notably O26:H11, O103:H2 and O111:NM, also cause LEE-mediated A/E cytopathology. In contrast, the colonization mechanisms of the large number of other non-O157 STEC serotypes are unknown.

Plasmid-mediated factors. All strains of O157:H7 STEC contain a highly conserved plasmid (pO157) of approximately 100 kilobases. A similar plasmid is found in many non-O157 STEC serotypes. Plasmid pO157 contains several genes that encode potential virulence factors. These genes include *espP* (extracellular serine protease, plasmid encoded), *katP* (catalase-peroxidase), *hlyCABD* (enterohemolysin) and *etp* (type II secretion proteins). The plasmid is also thought to encode fimbriae that may be involved in the initial colonization of enterocytes by O157 and non-O157 STEC. However the precise role of plasmid-encoded genes and fimbriae in non-O157 virulence remains to be clarified.

Other bacterial factors

A number of other factors may be important in the pathogenesis of non-O157 STEC. Such factors may relate to the presence of acid resistance, which appears to be quite variable amongst non-O157 STEC isolates. Other factors may be important in determining the infectious dose of these organisms. Data from Australia suggest that an O111 outbreak strain had a low infectious dose that was comparable with some O157 outbreak strains. There are no data on the infectious dose of other non-O157 STEC. Non-O157 STEC have been documented to cause HUS following urinary tract infection. The pathogenic mechanisms involved with colonization of extra-intestinal sites are unknown.

Host factors

A variety of host factors may also be important in the pathogenesis of non-O157 STEC.

Age

The young and the elderly appear more susceptible to non-O157 STEC, but the mechanism for this is unclear. This may be related to immunity or other factors such as physiologic changes within the intestine.

Immune and other factors

As with dysentery associated with *Shigella dysenteriae* type 1, only a minority of patients with STEC infection develop an antibody response to STX. This is thought to be due to the high biological activity of the toxin. Thus the minute amount of toxin that is sufficient to induce disease may be insufficient to elicit a detectable antibody response. The absence of a primary antibody response may be associated with susceptibility to re-infection, while the presence of antibodies to Stx1 has been associated with

protection from disease. Circulating antibody to STX will also protect experimental animals from toxin challenge. Antibodies to lipopolysaccharides and several other bacterial proteins, including EspA, EspB, Tir and intimin, have been reported in human sera but it is not known whether these are protective. Breast milk may contain factors that protect against infection with O157 STEC, but this has not been examined in relation to non-O157 STEC. It is not known if there is genetic susceptibility to non-O157 STEC.

Role of antimicrobial agents

Certain antibiotics have been associated with increased Stx1 and Stx2 expression *in vitro* in both non-O157 and O157 STEC. It has been proposed that antibiotic use may be a risk factor for the development of HUS, but no definitive data are available.

Socioeconomic status and diet

There are suggestions that non-O157 STEC-associated disease is more common in developed countries than in poorer economic regions, but there is a lack of information to help clarify the issue. Diet is clearly a major factor in the acquisition of non-O157 STEC (*e.g.*, consumption of animal products) and there is a possibility that dietary factors may also play a role in the colonization of bacteria; diet does affect clinical expression of EPEC (LEE-positive bacteria) disease in pigs. Diet may even affect the expression of STX in infected individuals.

Non-specific disease mechanisms

The importance of host factors such as cytokine, chemokine and host white cells in disease pathogenesis remains speculative. There are enough data to suggest that these mechanisms are implicated in HUS but their precise role needs to be determined.

Conclusions and Gaps in Current Knowledge

Clinical manifestations

- Treatment of severe complications (HC, HUS, TTP) is insufficient and only supportive.
- No preventive measures nor interventions in the early stage of disease are presently available.
- Studies evaluating late sequelae are scarce, regional differences cannot be excluded and national trends are unknown.

Mechanisms of disease

Bacterial virulence factors

- What are the infectious doses of various non-O157 STEC?
- Is acid resistance correlated with infectious dose and pathogenicity?
- What is the influence of different Shiga toxins on disease pathogenesis?
- What factors control toxin regulation *in vivo*?
- What are the mechanisms and sites of toxin absorption in the intestine?
- Understanding of the ecology and mechanisms of transmission of STX phages is lacking.
- Where is the site of intestinal colonization?

- What are the mechanisms of LEE-negative colonization?
- Understanding of the variations in LEE is incomplete.
- What is the role of enterohemolysins and EspP (*i.e.*, putative virulence factors other than STX and LEE-mediated factors) in pathogenesis?
- How do virulence factors move between bacterial strains?
- Further characterization of virulence factors is required for non-O157 STEC lacking A/E ability and enterohemolysin production.

Host factors

- Are anti-toxin antibodies protective in humans?
- Are antibodies to other factors protective in animals (*e.g.*, LEE or plasmid encoded proteins)?
- Are there any protective antigens?
- What host differences (immune, physiologic, etc.) account for variations in age susceptibility?
- Does therapy with antibiotics increase either the risk of infection (*e.g.*, by removing competitive flora) or the likelihood of developing HUS?
- How is non-O157 infection related to socioeconomic status, occupation and diet?
- Is breast-feeding protective? If so, what is the mechanism?
- What is the role, if any, of diet and competitive exclusion in preventing colonization and infection?
- Are differences in mucus related to colonization?
- Are differences in receptor density and receptor type related to disease?
- What is the role of regulatory proteins in disease pathogenesis?
- What is the role of the inflammatory system in disease pathogenesis and contribution to tissue damage?

III. Surveillance and Frequency of Isolation

There is a general lack of information on the occurrence of specific non-O157 STEC serotypes in persons with HUS and diarrhoea, by population and in relation to other enteric pathogens. Surveillance for non-O157 STEC infections in humans and studies on the frequency of isolation of non-O157 STEC from humans are needed to determine overall disease trends, to determine trends by serotype, to estimate the burden of disease and to identify outbreaks.

Surveillance

Surveillance for isolates of non-O157 STEC and surveillance for cases of HUS provide complementary data. Surveillance for isolates depends on physicians requesting tests for STEC and laboratories examining stools for non-O157 STEC. Surveillance for HUS is easier and more complete because patients are hospitalized and the diagnosis of HUS does not depend on stool culture. However, HUS surveillance provides less information because $\leq$10% of persons with STEC infection develop HUS and because HUS develops about a week after the onset of diarrhoea, when isolation of the pathogen from stool is more difficult.

With regard to isolates of non-O157 STEC, a basic amount of laboratory information needs to be collected in order to maximize the isolates' usefulness for surveillance purposes. This information includes confirmation of STX production or presence of *STX* genes; O:H serotype; antimicrobial resistance pattern; characterization of other known virulence genes; biochemical confirmation as an *E. coli*; as well as any further typing information available.

Three types of data sources are used for surveillance for non-O157 STEC infections: notifiable systems in which infections are reportable by law, sentinel systems in which selected sites report the number of infections, and ad hoc sources such as short-term studies by medical centers. Most countries now rely on the latter.

Frequency of Isolation

Overall, in studies of persons with diarrhoea, non-O157 STEC are isolated more frequently than O157, with a median of a 4-fold higher isolation rate, but with wide variation among studies. The only area where non-O157 STEC have been isolated less frequently than O157 in recent studies of diarrhoea is North America, where they are isolated about half as frequently as O157. In any given location, however, non-O157 STEC may be either one of the most frequently isolated pathogens from diarrhoeal stools or one of the least frequently isolated.

Non-O157 STEC are an important cause of HUS. Studies from all parts of the developed world have examined the frequency of non-O157 STEC in patients with HUS. Overall, non-O157 STEC account for about 25% of STEC isolations in studies of persons with HUS. However, there is wide variation among studies, with the proportion of non-O157 varying from 7% to 90% of the STEC isolated from persons with HUS.

Among the over 200 non-O157 STEC serotypes, those in serogroups O26, O103, O111 and O145 are most commonly isolated from humans and are clearly recognized as human pathogens. Thus, at present, the most important non-O157 STEC serogroups, from an epidemiologic perspective, are O26, O103, O111 and O145. Table 1 shows the non-O157 STEC serotypes that have been isolated from humans.

Table 1. Serotypes of non-O157 STEC isolated from humans

O1:H-	O15:H27	**O49:H-**	O98:H8	O117:H19	O153:H11
O1:H1	**O16:H-**	**O49:H10**	O100:H25	O118:H12	O153:H12
O1:H2	O16:H6	O50:H-	O100:H32	**O118:H16**	**O153:H25**
O1:H7	O17:H18	**O50:H7**	**O101:H-**	O118:H30	**O154:H-**
O1:H20	**O18:H-**	O52:H23	O101:H9	O119:H-	**O154:H4**
O1:HNT	O18:H7	**O55:H-**	**O103:H-**	**O119:H5**	**O154:H19/20**
O2:H1	O18:H12	**O55:H6**	**O103:H2**	O119:H6	**O161:H-**
O2:H5	**O18:H?**	**O55:H7**	O103:H4	**O121:H-**	**O163:H19**
O2:H6	O20:H7	**O55:H10**	O103:H25	O121:H8	O165:H-
O2:K1:H2	**O21:H5**	**O55:H?**	O103:HNT	**O121:H19**	O165:H10
O2:H7	**O21:H?**	O60:H-	O104:H-	O123:H49	**O165:H19**
O2:H27	O22:H-	O65:H16	**O104:H2**	O124:H-	**O165:H25**
O2:H29	**O22:H1**	**O69:H-**	O104:H7	O125:H-	O166:H12
O2:H44	**O22:H8**	O70:H11	O104:H21	O125:H8	O166:H15
O4:H-	O22:H16	O73:H34	**O105ac:H18**	O126:H-	O166:H28
O4:H5	O22:H40	**O75:H-**	O105:H20	O126:H2	O168:H-
O4:H10	O23:H7	O75:H5	O109:H2	O126:H8	O169:H-
O4:H40	O23:H16	O76:H19	O110:H-	O126:H21	**O171:H-**
O5:H-	O25:H-	O77:H4	O110:H19	**O126:H27**	O171:H2
O5:H16	**O25:K2:H2**	O77:H18	**O111:H-**	O128:H-	O172:H-
O6:H-	**O26:H-**	**O79:H7**	**O111:H2**	**O128ab:H2**	ONT:H-
O6:H1	O26:H8	O80:H-	**O111:H8**	O128:H8	**ONT:H2**
O6:H2	**O26:H11**	O82:H-	O111:H30	O128:H12	ONT:H18
O6:H4	O26:H21	O82:H5	O111:H34	**O128:H25**	ONT:H21
O6:H28	O26:H32	O82:H8	O112:H21	O132:H-	**ONT:H25**
O6:H29	O27:H-	O83:H1	O113:H2	**O137:H41**	ONT:H47
O6:H31	O30:H2	O84:H2	O113:H4	O141:H-	O-rough:H-
O7:H4	O30:H21	**O85:H-**	O113:H7	O144:H-	**O-rough:H5**
O7:H8	O30:H23	**O85:H10**	**O113:H21**	**O145:H-**	**O-rough:H11**
O8:H-	O37:H41	**O85:H23**	O114:H4	O145:H16	**O-rough:H16**
O8:H14	O38:H21	O90:H-	**O115:H10**	**O145:H25**	O-rough:H20
O8:H21	O39:H4	O91:H-	O115:H18	**O145:H28**	O-rough:H21
O9ab:H-	O39:H8	**O91:H10**	O116:H19	O145:HNT	**OX3:H2**
O11:H49	O45:H-	O91:H14	O117:H-	O146:H21	**OX3:H21**
O14:H-	O45:H2	**O91:H21**	**O117:H4**	O146:H28	
O15:H-	O45:H7	O91:HNT	O117:H7	O150:H10	
O15:H2	**O48:H21**	O98:H-	O117:K1:H7	O153:H2	

Serotypes in **bold** represent strains isolated from patients with HUS.

An updated list of STEC, with literature references, is maintained by Dr K. Bettelheim (National *E. coli* Reference Laboratory, Melbourne, Australia) and can be found on the World Wide Web at:
http://www.microbionet.com.au/frames/feature/vtec/brief01.html

Conclusions

Although non-O157 STEC are an important cause of HUS and diarrhoea, infections are markedly under-recognized because clinicians do not request testing of stools for STEC and few laboratories screen stools for non-O157 STEC. Surveillance for non-O157 STEC is almost non-existent.

Variations in the frequency of isolation of non-O157 STEC compared with O157 and other pathogens in studies of diarrhoea are likely related to variations in the frequency of occurrence of these other enteric pathogens in those populations and to differences in the ecology and epidemiology of specific non-O157 STEC serotypes. Differences among studies may also be due to changes over time and differing methodologies.

The reason that overall a lower proportion of non-O157 is reported in STEC-associated HUS as compared to the proportion of non-O157 in STEC-associated diarrhoea, has not been determined. Some of the STEC isolated from diarrhoea may not be the cause of the diarrhoea and some may have a low potential to cause HUS. Similarly, some non-O157 STEC isolates can be found in healthy individuals and may not be pathogens. Further, there have been reports of multiple STEC serotypes isolated from a single patient; in such cases the contribution of each serotype to the pathogenesis of disease is difficult to determine.

Gaps in Current Knowledge

- The basic public health infrastructure for surveillance for non-O157 STEC is lacking in most countries.
  - Few countries have reporting systems for STEC with case definitions that clearly include non-O157.
  - Due to the lack of surveillance systems, variability in data sources and incompleteness of reporting, trends over time have not been determined, outbreaks are unlikely to be recognized, and data from various countries cannot easily be compared.
- Information is lacking on the incidence of HUS and on the frequency of isolation of non-O157 STEC in HUS, diarrhoea and healthy persons.
  - HUS:
    - It has not been documented whether the incidence of HUS has changed since it was first described in 1955.
    - Most countries lack surveillance systems for HUS.
    - Data are lacking on the serotypes of STEC isolated from persons with HUS in many countries and on temporal trends. These data are especially lacking from developing countries. Data are lacking on the relative importance of various serotypes by country.
    - Data are lacking on the proportion of persons with diarrhoea, caused by various STEC serotypes, who develop HUS.
  - Diarrhoea:
    - Data are lacking on temporal trends in the isolation rate of various STEC serotypes by country.
    - Data are lacking on the proportion of persons infected with various STEC serotypes who develop bloody diarrhoea.

- Healthy persons:
  - Additional data on the isolation of non-O157 STEC from healthy persons, by serotype and virulence factors, would be helpful in assessing the pathogenicity of various serotypes. There is a need to look at healthy persons in different environments - urban versus rural areas, for example - because of variation in exposure to animals that may be the major source of non-O157 STEC.

♦ Most countries have no recommendations for routine laboratory identification of non-O157 STEC.

IV. Sources and Modes of Transmission

Sources of STEC

Animals

STEC have been isolated from a variety of animals, particularly ruminants. Non-O157 STEC isolated from healthy animals belong to a variety of serotypes and an individual animal can carry more than one serotype, including both O157 and non-O157 serotypes. Table 2 shows serotypes of non-O157 STEC isolated from animals. Some of the STEC isolated from animals are of the same serotype as human isolates and many of these have been associated with HC or HUS in humans.

Studies of non-O157 STEC in food animals have not been done in all geographical areas and there have been only a limited number of studies in areas with a relatively higher incidence of human non-O157 illness. Results are difficult to compare due to varying study designs and detection methods, but excretion of non-O157 STEC has been detected in up to 21% of adult cattle and up to about 50% of calves. Sheep and goats have been found to have higher excretion rates, while pigs and chickens do not appear to be significant reservoirs of these bacteria. Bovine excretion of specific serotypes is intermittent and the causes of this are not clear. Due to intermittent excretion and to the difficulty of isolating non-O157 STEC, the true prevalence of STEC in cattle and other animals is probably higher than some studies suggest.

Table 2. Serotypes of non-O157 STEC isolated from animals

O2:H25	O26:H-	O76:H21	O113:H4	O145:H-	ONT:H8
O2:H29	O26:H11	O77:H4	O113:H21	O145:H28	ONT:H17
O2:H49	O35:H21	O82:H2	O116:H21	O146:H8	ONT:H18
O3:H-	O38:H16	O82:H8	O119:H8	O146:H21	ONT:H19
O4:H4	O39:H7	O82:H40	O119:H25	O147:H11	ONT:H21
O5:H-	O39:H21	O84:H28	O120:H-	O147:H29	ONT:H25
O8:H2	O39:H40	O87:H16	O120:H2	O153:H	ONT:H31
O8:H19	O39:H48	O87:H21	O120:H18	O153:H9	ONT:H34
O8:H25	O43:H2	O88:H25	O120:H42	O153:H19	ONT:H42
O9:H-	O46:H2	O90:H24	O123:H10	O153:H25	O-rough:H7
O10:H21	O46:H38	O91:H-	O126:H20	O156:H-	O-rough:H8
O15:H-	O65:H-	O91:H10	O126:H21	O156:H21	O-rough:H14
O15:H4	O65:H48	O91:H21	O128:H2	O156:H46	O-rough:H19
O15:H27	O68:H-	O91:H49	O130:H38	O168:H8	O-rough:H21
O17,77:H18	O69:H28	O100:H-	O131:H2	O169:HNT	O-rough:H34
O20:H19	O71:H12	O103:H2	O132:H-	O170:H8	O-rough:H38
O21:K5:H4	O73:HNT	O104:H21	O136:H12	O171:H2	O-rough:H42
O22:H8	O74:HNT	O105:H18	O136:H20	ONT:H-	O-rough:H47
O22:H16	O74:H29	O110:H2	O139:H8	ONT:H2	OX3:H8
O22:H21	O75:H8	O111:H-	O141:H4	ONT:H7	

An updated list of STEC, with literature references, is maintained by Dr K. Bettelheim (National E. coli Reference Laboratory, Melbourne, Australia) and can be found on the World Wide Web at: http://www.microbionet.com.au/frames/feature/vtec/brief01.html

Table 3. Serotypes of non-O157 STEC isolated from foods of animal origin

Serotype	Food	Serotype	Food
O2:H32	sausage	O84:H21	lamb
O4:H21	retail beef	O87:H16	beef
O6:H-	lamb	O91:H-	pork sausages, sausage
O6:H10	lamb, milk, cheese	O91:H21	milk, beef
O6:H34	milk	O96:H-	lamb
O7:H-	minced beef, lamb	O100:H-	pork sausages
O7:H16	minced beef	O100:H16	retail chicken
O8:H-	minced beef, lamb	O103:H2	milk, beef
O8:H9	beef	O103:H21	beef
O8:H16	beef	O104:H-	minced beef
O8:H25	pork sausages	O104:H12	minced beef
O8,60:H-	lamb	O107:H7	minced beef
O8,60:H51	lamb	O112ac:H-	lamb
O9:H-	pork	O112:H2	beef
O14:H-	minced beef	O113:H-	minced beef, beef
O17:H18	minced beef	O113:H4	milk, sausage, beef
O21:H21	milk	O113:H19	beef
O22:H-	minced beef	O113:H21	beef
O22:H4	minced beef	O114:H4	milk
O22:H5	minced beef	O115:H-	pork sausages
O22:H8	unpasteurized milk, milk,	O115:H10	pork sausages
	sausage, beef	O116:H-	cheese
O22:H16	beef	O117:H8	retail beef
O23:H-	minced beef	O125ab:H-	minced beef
O23:H15	minced beef	O128:H2	pork sausages
O26:H11	milk filters	O128:H30	minced meat
O26:H32	cheese	O138:H-	sausage
O28:H4	beef	O146:H8	pork sausages
O30:H-	minced beef	O146:H28	beef
O30:H8	milk	O148:H8	minced meat
O38:H30	minced beef	O149:H45	retail beef
O43:H2	milk filters	O153:H25	milk filters
O44:H-	milk filters	O156:H25	milk
O46:H-	minced beef	O166:H-	beef
O46:H8	minced beef	O171:H2	beef
O49:H-	pork	O171:H25	beef
O54:H21	retail chicken	ONT:H-	soft cheese, retail beef, lamb, milk,
O55:H9	beef		sausage, beef
O56:H56	beef	ONT:H2	milk, beef
O57:H-	beef	ONT:H5	minced beef
O60:H9	pork sausages	ONT:H7	milk, beef
O62:H-	minced beef	ONT:H8	pork sausages, milk filters, beef
O62:H8	minced beef	ONT:H9	sausage
O65:H-	milk filters, lamb	ONT:H10	pork
O71:H21	minced meat	ONT:H16	beef
O73:H-	minced beef	ONT:H18	sausage
O73:H16	minced beef	ONT:H19	milk filters, beef
O73:H31	minced beef	ONT:H21	sausage, beef
O74:H-	minced beef	ONT:H23	minced beef
O74:H37	minced beef	ONT:H28	beef
O74:H39	minced beef	ONT:H47	minced meat, beef
O75:H5	lamb	O-rough:H23	minced beef
O82:H8	beef	O-rough:H48	beef

An updated list of STEC, with literature references, is maintained by Dr K. Bettelheim (National *E. coli* Reference Laboratory, Melbourne, Australia) and can be found on the World Wide Web at: http://www.microbionet.com.au/frames/feature/vtec/brief01.html

Non-O157 STEC can also cause disease in animals, such as diarrhoea (or dysentery) in calves and oedema disease in pigs. Information is limited for other animal species. Non-O157 STEC associated with disease in animals belong to a limited number of serotypes, some of which have also been associated with human disease. For example, STEC causing disease in cattle are frequently serotypes O5:H-, O26:H11, O103:H2, O111:H- and O145:H-.

Foods

Foods are recognized as a major vehicle of transmission of O157 STEC and are likely to play the same role for non-O157 STEC, but there is limited information on the occurrence of non-O157 STEC in the food supply. Meat and meat products, dairy products and any foods cross-contaminated by animal products during preparation may be contaminated. Foods such as fruit and vegetables may be contaminated during growth, harvest and production.

As ruminants have been identified as an important reservoir of STEC, raw meat and milk have been most frequently studied due to their potential for contamination during production. Only a limited number of other foods have been studied. In studies of raw meat, non-O157 STEC has been isolated from up to 25% of samples; most studies have found around 10% of samples contaminated. About 1% of raw milk samples have been found contaminated with non-O157 STEC. As in animal surveys, the difficulty in isolating non-O157 STEC suggests that the true prevalence of non-O157 STEC in foods is probably higher than some studies have reported. Table 3 shows the serotypes of non-O157 STEC that have been isolated from foods of animal origin.

Modes of Transmission

Foods

As with O157 STEC, the intestinal tracts of bovines and other ruminants, as well as foods originating from these animals, are likely the major sources for human infection with non-O157 STEC. Data on the role of specific foods as sources of non-O157 STEC human illness are lacking. Documented non-O157 STEC outbreaks have been attributed to the consumption of fermented meat and contaminated milk.

Human-to-human

It is now well-established that human-to-human transmission of O157 STEC is facilitated by a low infectious dose. The O111:H- STEC implicated in an outbreak related to fermented meat was shown to be present in very low numbers, which supports the concept of a low infectious dose for non-O157 STEC. However, data on human-to-human transmission of infection due to other non-O157 STEC are lacking.

Animals and the environment

From on-farm studies, it appears that the ecology of the non-O157 STEC in animals and the environment is similar to O157 STEC. The spread of this bacterium in the farm environment and among animals may be facilitated by feed and water contaminated by animal waste, waste management

practices, animal-to-animal contact and possibly by other domestic or wild animals.

Attention to farm management and animal husbandry will contribute to reduction in animal carriage and excretion. Human infection through direct contact with non-O157 STEC contaminated water or animals needs to be better defined.

Risk factors for infection

The risk factors for human infection with STEC extend from the farm to the table. It is assumed that many of the risk factors for non-O157 are the same as O157 STEC, but there may be some differences, for example, related to animal reservoirs. Possible risk factors for human STEC infection include:

- Higher prevalence of STEC in animal populations;
- Contamination or cross contamination of food products with animal faeces;
- Cross contamination amongst cooked and non-cooked food products;
- Inadequate cooking of foods of animal origin;
- Contact with other STEC-infected persons;
- Contact with animals shedding STEC.

Conclusions and Gaps in Current Knowledge

Distribution in animals and food

- Animal reservoirs for non-O157 STEC need to be definitively determined; knowledge of the distribution of non-O157 STEC in animals, including bovines, is limited on a global scale.
- There is a particular need for more knowledge of the distribution in non-bovine animals of non-O157 STEC serotypes known to cause human disease.
- The pathogenic potential of the many non-O157 STEC in animals and foods is yet to be fully defined.
- Knowledge of the incidence of non-O157 STEC in foods throughout the entire food chain is limited, other than for raw animal products (*e.g.*, meat and milk).
- There is little information on quantification of non-O157 STEC in foods incriminated in human disease.

Modes of transmission

- Little is known about which foods might be potential vehicles of transmission of non-O157 STEC.
- Lack of detailed knowledge about outbreaks has limited our knowledge of the infectious dose of non-O157 STEC.
- Are there differences between non-O157 and O157 STEC in terms of specific risk factors for human acquisition?
- Is there significant human-to-human transmission of non-O157 STEC infection?
- What is the role of water in the transmission of non-O157 STEC to humans?
- Direct animal-to-human transmission of non-O157 STEC has not yet been demonstrated.
- The factors that influence the carriage rate and levels of shedding of non-O157 STEC by food animals are not fully understood.
- Strategies for reducing the carriage rate and levels of shedding of non-O157 STEC by food animals have not been identified.

V. Laboratory Methodologies

The principles for the routine diagnosis of human STEC infections, including those associated with non-O157 STEC, are the demonstration of STX in faecal filtrates and the isolation of STEC from primary stool cultures. The use of serological tests for the detection of antibodies to the lipopolysaccharides of specific non-O157 STEC serogroups can also be helpful in providing evidence of STEC infection.

A method which is suitable for screening and isolation of non-O157 STEC strains should be based on the detection of STX or *STX* genes. Commercially available diagnostic kits for screening large numbers of samples are suitable for use in diagnostic laboratories. Differences between methods for O157 STEC and STEC of all other serogroups will be considered throughout this section.

Detection

There are three types of methods that can be used for STEC screening. They are cell culture cytotoxcity assays and immunological tests for STX, and DNA-based methods for *STX* genes. The method of choice will depend on the type of sample (clinical, veterinary, food, environment), for example, direct cell culture is not appropriate for foods.

Cell culture cytotoxicity assays

Cell culture cytotoxicity assays should be performed with Vero cells which are the most sensitive to different types of STX. These tests can be used for screening of clinical samples. Food samples need to be processed before testing because the physical nature of foods precludes direct testing. STEC are usually present in low numbers in foods and preformed toxin may not be detectable. The cell assay is very sensitive but is not as specific as DNA-based methods. Therefore a positive cytotoxicity assay has to be confirmed by a STX-specific method. For example, neutralization with STX-specific monoclonal or polyclonal antibodies should be performed if possible.

Immunological tests

Enzyme immunoassays (EIAs) are available for the detection of STX in stool, stool cultures, food cultures and isolates. There are differences in sensitivity with respect to detection of the various toxin types. The commercially available kits are suitable for non-specialized laboratories. These EIAs are generally not as sensitive as cell culture and false positive reactions have been reported, particularly where stool samples are used. For testing of food samples enrichment is essential to ensure a high concentration of toxin.

DNA-based methods

An assay based on polymerase chain reaction (PCR) amplification can be used for screening of different types of STEC and should detect all variants of *STX* genes. Currently published primer sequences need evaluation for their ability to detect all members of the STX family. Samples of all types

may contain inhibitors of the PCR reaction. Thus, processing of samples before amplification is required. This may be DNA preparation, or culture in selective or non-selective liquid or solid media prior to DNA preparation.

Other approaches

Screening for the enterohaemolytic phenotype can be helpful for identification of STEC from samples, particularly clinical specimens. This is performed on agar containing washed sheep erythrocytes. STX production by suspected colonies has to be confirmed. STEC of known specific serogroups can be detected using slide agglutination with appropriate antisera. This also requires testing of suspected colonies for STX production.

Serodiagnosis

An alternative approach to provide evidence of non-O157 STEC infection is the detection of STEC-related antibodies in patients' sera. Serodiagnosis is of importance when screening and isolation tests for non-O157 STEC are negative, particularly in cases of HUS and in outbreak investigations. Except for *E. coli* O157:H7, little is known about the immune response to different STEC-related antigens in humans. However, screening for STX-antibodies is not recommended, as such antibodies are not consistently produced in infected humans following a single infection.

Isolation

Isolation should be considered the definitive diagnostic procedure for identifying STEC strains in clinical, food and other samples. The detection of STX production or *STX*-related genes without attempting the subsequent isolation of STEC is incomplete and should be considered to be only a presumptive result.

Methods of isolation

Colony immunoblot and colony DNA hybridization assays are very valuable for the isolation of STX-positive strains from samples shown to have been positive by one or more of the screening methods. Every other currently available method requires testing of individual colonies for STX production or *STX* genes. Frequently, STEC are present in low numbers and isolation may involve testing large numbers of colonies.

Enrichment methods

Screening of food and environmental samples for STEC requires enrichment because low numbers of STEC are generally present. Numbers of STEC in clinical samples can be quite variable and may be low in cases of HUS. The enrichment procedures developed for *E. coli* O157:H7 need to be evaluated for non-O157 STEC. At present, specific procedures for non-O157 STEC cannot be recommended.

Identification

Identification is done by reference or specialized laboratories. The criteria for referral to such laboratories are isolation of a single colony which is positive for STX or *STX* genes, or positive for

commonly occurring human pathogenic STEC "O" serogroups (O26, O103, O111, O145, O157). Positive isolates must be confirmed as STEC.

If the primary laboratory is not able to produce an isolate, the stool specimen or the primary plate culture may be referred to a specialized laboratory based on the following criteria:

- Hospitalized patient with diarrhoea or HUS;
- Suspected STEC infection, for example, bloody diarrhoea;
- Suspected link with consumption of food;
- Part of an outbreak investigation.

Confirmation of isolates as STEC

The methods currently used are as follows:

- STX production:
  - Vero cell assay neutralization for Stx1 and Stx2
  - EIA kit(s)
  - STEC-reversed passive latex agglutination (RPLA)
- Presence of *STX* genes:
  - PCR
  - DNA probe hybridization using oligonucleotides or polynucleotides

In all cases, isolates require biochemical confirmation as *E. coli* or other STX-positive organisms.

Characterization of STEC

Several methods have been used for the characterization of STEC. These include both typing and fingerprinting techniques. Bacterial typing systems can be defined as those capable of identifying strains accurately and reproducibly, at different times and in different laboratories. Fingerprinting methods are those suitable only for grouping or excluding isolates in small scale or local studies of bacterial transmission, such as outbreak investigations. For outbreak investigations first line typing or screening methods are needed to provide rapid results at the level of field investigation. Confirmation by secondary methods may be obtained when control measures may be required.

Methods can be broadly categorized as phenotypic, based on expressed properties such as somatic and flagellar antigens, susceptibility to bacteriophages (phage typing), enzyme activity; and genotypic, based on specific molecular characteristics of the chromosomal, plasmid or phage DNA. The following methods will be considered:

- Serotyping
- Resistance to antimicrobial agents and heavy metals
- Subtyping *STX* genes
- Plasmid DNA analysis
- Genomic DNA analysis
  - restriction fragment length polymorphism (RFLP)
  - ribotyping
  - pulsed field gel electrophoresis (PFGE)

- PCR-based techniques, including amplified fragment length polymorphism (AFLP)

♦ Virulence properties other than STX production
- A/E ability
- enterohaemolytic phenotype

Serotyping

Serotyping is based on the identification of the somatic O antigens and flagellar H antigens. The schemes at present identify O antigens from 1 to 173 and H antigens from 1 to 56. In most laboratories, STEC of serogroup O157 can be identified readily, but few laboratories have the capacity for serotyping non-O157 STEC. Results of serotyping have shown that non-O157 STEC associated with human disease belong to a large number of different serotypes. The development of O:H serotyping has led to the identification of new STEC serotypes and this allows the study of their epidemiology. The other application of serotyping information is the development of specific tests. For example, magnetic beads were coated with *E. coli* O111 antiserum for detection in a mettwurst-associated outbreak in Australia in 1995.

Resistance to antimicrobial agents and heavy metals

Resistance to antimicrobial drugs can be a useful epidemiological marker for non-O157 STEC. However, at present, data are very limited and need to be collected on a standardized basis. Resistance to tellurite has been described for O157 STEC, but little information is available for non-O157 STEC. Testing resistance to several heavy metals may provide useful markers, as well as being applicable for isolation methods, as shown in the case of *E. coli* O157:H7.

Subtyping STX genes

Variants of Stx1 and Stx2 have now been reported, with most variation seen in the stx_2 gene. These variants can be detected by PCR or use of oligonucleotide probes. For non-O157 STEC a wide range of *STX* genes has been identified. Further analysis of stx_2 genes can be performed using restriction digestion of the PCR products or by DNA sequencing.

Plasmid DNA analysis

This method is easy to perform and many non-O157 STEC strains possess plasmids of different sizes. Plasmid profiles and RFLP analysis can be useful in epidemiological studies.

Genomic DNA analysis

The analysis of genomic DNA can be performed in several ways and the most commonly applied methods are summarized below.

RFLP analysis using STX gene probes

Hybridization of genomic DNA digested with restriction enzymes with probes for *STX* genes allows identification of the fragments carrying these genes. This technique has been applied for non-O157 STEC. The use of a single enzyme alone does not allow significant discrimination for epidemiological

studies, but the combination of two enzymes has been useful in such investigations.

RFLP analysis using phage DNA as a probe

In many non-O157 STEC the *STX* genes are located on the genome of lambdoid phages which are integrated in the host chromosome. Genomic DNA of non-O157 STEC strains can be hybridised with phage DNA and this yields different RFLP patterns, even within a serotype.

Ribotyping

Ribotyping systems have been developed for *E. coli*, including non-O157 STEC. These include computer identification of *E. coli* rRNA gene restriction patterns and have been applied to different non-O157 STEC serogroups. This approach has been recommended in combination with other methods such as "O" serogrouping.

PFGE

PFGE analysis of non-O157 STEC provides a highly discriminatory and reproducible method for some serotypes, but its suitability for many others is not known. The Centers for Disease Control and Prevention in Atlanta have set up a national network for the subtyping of O157 STEC. A standardized protocol for performing PFGE and interpreting the patterns has been developed. Such an approach has allowed a national database for O157 STEC to be developed. This approach has not been applied in a systematic manner for non-O157 STEC.

PCR-based techniques

Several PCR based methods have been evaluated as faster alternatives to methods such as PFGE. Some of the many variations on amplification based methods are arbitrarily primed PCR, random amplified polymorphic DNA and the use of repetitive sequences. These techniques have significant limitations for typing but may be very useful in local settings, for example, investigation of institutional outbreaks. A high degree of standardization is required in order to achieve inter-laboratory comparison of data.

AFLP is another PCR-based technique that has recently been investigated for non-O157 STEC. Preliminary results suggest AFLP has the potential to provide a high degree of discrimination. Another typing approach that has been proposed recently is that of multilocus sequence typing (MLST) involving "housekeeping" genes. The advantage of MLST is that sequence data are portable between laboratories permitting one expanding global database that could be put on the World Wide Web, enabling the exchange of molecular typing data. This approach has not yet been reported for STEC.

Virulence properties other than STX production

A/E ability

The ability of certain non-O157 STEC to adhere intimately to intestinal epithelium, resulting in the effacement of the microvillus surface, is considered to be an important virulence property of these organisms and is encoded by LEE. STEC can be tested for the presence of *eae* genes, which encode intimin, and other genes in LEE. The *eae* genes can be subdivided further using specific primers.

Enterohaemolytic phenotype

Another property associated with some non-O157 STEC is that of enterohemolysin production. Strains can be examined for the property on agar plates containing washed sheep erythrocytes and the genes encoding this property can be detected with DNA probes or PCR.

The application of typing and other characterization methods for non-O157 STEC in epidemiological investigations has been very limited compared to studies involving O157 STEC. Serotyping provides an internationally recognized and standardized scheme that has been demonstrated to be very helpful in the characterization of non-O157 STEC. However, it is limited in application because the full scheme is only employed in certain specialized laboratories. A variety of other methods can be applied to non-O157 STEC and several studies have now shown the value of tests for virulence factors such as A/E ability and the production of enterohemolysin. At present, a combination of several methods appears to provide the most informative approach to the characterization of non-O157 STEC, particularly together with clinical and epidemiological information on infected patients. For example, the use of serotyping, *STX* gene analysis and tests for virulence factors provides a very useful combination. For more detailed analysis these tests can be complemented by other techniques such as PFGE.

Criteria for Reporting Characteristics of Non-O157 Isolates from Clinical Samples

- For clinical purposes, reporting the confirmation of STX production or the presence of *STX* genes should be the priority.
- For epidemiological and surveillance purposes, reporting should include the following characteristics:
  - biochemical confirmation as *E. coli* (or other STX-positive organism);
  - O:H serotype;
  - antimicrobial resistance pattern;
  - characterization of other known virulence genes;
  - further typing information where available.

Conclusions and Gaps in Current Knowledge

- The only reliable marker for non-O157 STEC is STX production or possession of *STX* genes.
- Several satisfactory screening methods for the detection of non-O157 STEC have been reported and these are becoming increasingly available as commercial kits.
- Standardized procedures including enrichment are essential for detection and isolation of non-O157 STEC from foods. Such methods require optimisation because methods developed for O157 STEC may not be appropriate.
- There are many methods for the typing and characterization of non-O157 STEC. However, apart from serotyping, there is little standardized comparison and exchange of data on non-O157 STEC.

VI. Recommendations

Clinical Manifestations

Risk groups

Studies comparing incidence and aetiological relevance of STEC in normal individuals, patients with intestinal disease and patients with complications, are necessary on a regional, national and international level. It is important that these studies be performed in different age groups (infants, children, women after pregnancy, elderly) to identify risk groups for the development of non-O157 STEC-associated disease. There is a need for further information on non-O157 STEC as cause of severe colitis, HUS and TTP in patients treated with antibiotics and/or immunosuppressive agents (oncology, haematology, nephrology, etc.).

Differences related to serotype

Clinical manifestations and risk factors for developing disease should be evaluated and defined with regard to STEC serotype. In addition, the pathogenic potential of the many non-O157 STEC serotypes in animals and foods should be determined.

Detection, intervention and prevention

Because treatment for disease caused by non-O157 STEC is non-specific and generally insufficient, programmes supporting early detection, early intervention and preventive measures are needed.

Mechanisms of Disease

Animal model

The development of an animal model that reflects human disease should be a high priority. Such a model is needed to investigate various issues such as acid resistance, infectious dose, role of immune factors as it relates to the specific model, etc.

Shiga toxin

As STX is the primary cause of disease resulting from STEC infection, a research priority should be to determine how toxin is regulated *in vivo* and how and where it is absorbed. It is also important to elucidate the pathogenic significance of each of the different toxin types. The ecology and biology of toxin phages must also be determined.

Colonization

The mechanisms and sites involved in LEE-negative colonization need to be defined, as well as determining the role of additional LEE genes found on strains with larger LEE. The effects of different types of mucus on colonization of STEC should also be determined.

Other bacterial factors

In addition to determining the frequency and mechanisms by which putative virulence factors move between bacterial strains, a high priority should be placed on defining the role of other virulence factors, if any, in the disease process or in the ecology of STEC organisms. Specific virulence characteristics also need to be defined by serotype.

Susceptibility and immunity to disease

A priority should be placed on determining if specific antimicrobials increase the risk of disease and, if so, what is the mechanism (increased intestinal toxin production?). Determination is also needed on the role of diet in susceptibility to non-O157 STEC and whether breast feeding is protective. If it is, what are the mechanisms involved?

A toxin-based immunotherapy, either serum-based or colostrum-based, should be a development priority. Its efficacy in preventing disease must then be determined. If toxin immunotherapy is found not to be the answer, it should be determined which other virulence factors might have a role in immunotherapy.

The role of cytokines in preventing or enhancing toxin-mediated tissue damage needs to be better understood. This should be done through determining cytokine response over time in patients infected with non-O157 STEC and in experimental models.

The nature of immunity to non-O157 STEC should be determined through population-based studies in both the developed and developing world. Disease susceptibility needs to be correlated with age, socioeconomic and genetic determinants.

Surveillance and Frequency of Isolation

Reporting of STEC

All STEC infections should be reportable. To have an optimal reporting system, the following are helpful:

- indications for physicians to submit specimens for STEC culture;
- indications for laboratories as to which specimens to culture for STEC;
- simple, inexpensive screening or diagnostic methods for non-O157;
- reference laboratories to assist with serotyping, toxin testing, and strain characterization;
- clear case definitions for STEC that include non-O157 STEC and that account for different disease presentations and asymptomatic excretion;

- a mechanism for reporting by physicians and laboratories;
- resources for compiling data and providing feedback.

Surveillance for HUS

Surveillance should be conducted for HUS. In most locations, surveillance for HUS is the logical first step in surveillance to monitor the incidence of non-O157 STEC infections over time, to determine which serotypes are predominant and whether prevention measures are effective. Components of HUS surveillance, in order of priority should include:

- determination of the population-based incidence over time;
- isolation and characterization of strains isolated from stools and other clinical specimens, with determination of their population-based incidence over time;
- testing of serum specimens for antibody to LPS of major serogroups.

Surveillance for STEC in diarrhoeal disease

Sentinel surveillance systems for diarrhoea due to STEC, including non-O157 STEC, should be developed. Such studies are needed to determine the incidence of non-O157 STEC infections over time, the serotypes isolated, and the proportion of persons who develop HUS by serotype. These studies can be used as a platform for case-control studies to determine food vehicles and other risk factors for infection, and to compare the isolation rate of various serotypes from persons with diarrhoea to healthy controls from the same population. Studies of non-O157 STEC diarrhoea by other groups, *e.g.*, medical centers, should also be encouraged because they provide complementary data. If resources do not permit testing of all specimens, those with certain characteristics or from particular populations could be selected. The following are examples of selection criteria, listed in order of priority/low expense:

- history of bloody diarrhoea;
- bloody stool received by laboratory;
- children <5 years old +/- persons >60 years old;
- persons hospitalized for diarrhoea;
- other populations, *e.g.*, travellers, all diarrhoeal specimens during warm weather, all diarrhoeal specimens (perhaps excluding nosocomial diarrhoea).

Laboratories

Clinical laboratories should have the capacity to examine stools for non-O157 STEC. Stools from all patients with HUS should be examined for non-O157 STEC. Examination of specimens from persons with diarrhoea is also encouraged; if resources do not permit testing of all specimens from persons with diarrhoea, the above selection criteria should be used to determine specimens for culture.

Public health networks

Public health officials should develop networks to compare trends in isolation rates of non-O157 STEC; to exchange knowledge about incidence, risk factors, clinical manifestations, and complications; and to improve detection of international outbreaks. Further, early warning systems, both at the national and international levels, should be established for non-O157 STEC.

Sources and Modes of Transmission

Distribution in animals and food

The distribution of non-O157 STEC in bovines and other animals should be studied in broader geographic areas. The ecology of non-O157 STEC in foods throughout the food chain should also be determined. Although they may be similar to other STEC, the reservoirs of non-O157 STEC need to be definitively determined.

On-farm factors

Factors that influence the ecology of non-O157 STEC in farm animals should be defined, as should management practices that might influence those factors. Most importantly, this should be followed up by developing on-farm intervention mechanisms whenever there is good epidemiological evidence indicating that they are important (*e.g.*, water trough contamination). If there are additional factors that influence the carriage rate and levels of shedding of non-O157 STEC by food animals they should be identified and strategies to reduce such carriage and shedding should be developed.

Transmission

Careful epidemiological investigations of outbreaks and case-control studies of sporadic cases are needed to determine vehicles of infection. The potential of foods other than animal-derived products to serve as vehicles of non-O157 STEC transmission should be assessed. Additionally, the role of human-to-human, waterborne and animal-to-human transmission of non-O157 STEC infection should be established.

The minimum infectious dose of an organism is an important factor in determining whether transmission will lead to infection. Thus it is important to further investigate and determine the minimum infectious dose of various non-O157 STEC.

The WHO recommendations previously made for the prevention of STEC transmission are relevant and are endorsed by this working group (*Prevention and Control of Enterohaemorrhagic* Escherichia coli *[EHEC] Infections, Report of a WHO Consultation, Geneva, Switzerland, 28 April-1 May, 1997; WHO/FSF/FOS/97.6*).

Laboratory Methodologies

Detection

Serological diagnostics should be established and improved; new useful serological markers need to be found in order to determine the aetiology of an infection independently from the isolation of organisms from stool cultures.

Isolation

Standardization of rapid, cheap and widely available methods for the isolation of non-O157 STEC is required. These should be suitable for clinical, veterinary, food and environmental samples. Optimized enrichment procedures need developing, particularly for testing of foods.

Characterization

Optimal typing and characterization methods and quality assessment for screening, isolation and typing methods for non-O157 STEC should be implemented. The most suitable screening methods for detection of non-O157 STEC require further application. At present there are EIAs for STX production and PCR for *STX* genes.

Primary microbiological laboratories

It is important that more primary microbiological laboratories have the capability to identify non-O157 STEC. This requires the improvement of current diagnostic measures for non-O157 STEC and the urgent development of new methods that are cost effective in order to establish a rapid, accurate and inexpensive diagnosis.

National reference laboratories

National reference laboratories for all STEC must be recognized and established worldwide. Mandatory reporting of clinical isolates of non-O157 STEC to national reference laboratories should be implemented.

International collaboration

A pilot global database on non-O157 STEC should be established. The information should be comprehensive and include details of sources, clinical information, typing and characterization of isolates.

A large, representative, available strain collection should be established for consensus between reference laboratories and for international collaborative studies. Studies to identify and follow the spread of clinical groups of non-O157 STEC on a worldwide basis should be implemented.

International workshops to standardize data collection, typing methods and reporting systems should be organized. Protocols should be harmonized and data compared on a worldwide basis, including characterization of antimicrobial resistance according to a standardized protocol. The global spread of mobile virulence determinants and antimicrobial resistance should be monitored.

List of Abbreviations

A/E	-	Attaching and effacing
AFLP	-	Amplified fragment length polymorphism
eae	-	*E. coli* attaching and effacing gene
EHEC	-	Enterohaemorrhagic *E. coli*
EIA	-	Enzyme immunoassay
EPEC	-	Enteropathogenic *E. coli*
EspA, B, D, E	-	*E. coli* secreted proteins A, B, D or E
HC	-	Haemorrhagic colitis
HUS	-	Haemolytic uraemic syndrome
LEE	-	Locus of enterocyte effacement
MLST	-	Multilocus sequence typing
NM	-	Non-motile, in reference to H antigen type (*i.e.*, no flagellar antigen)
NT	-	Not typable, in reference to O or H antigen type
PCR	-	Polymerase chain reaction
PFGE	-	Pulsed field gel electrophoresis
RFLP	-	Restriction fragment length polymorphism
RPLA	-	Reversed passive latex agglutination
STEC	-	Shiga toxin-producing *E. coli*
STX	-	Any member of the Shiga toxin family, including Shiga-like toxin
STX	-	Any Shiga toxin gene, including genes that code for Shiga-like toxin
Stx1, 2, etc.	-	Shiga-like toxin 1, 2, etc.
stx_1, stx_2, etc.	-	Gene for Shiga-like toxin 1, 2, etc.
Tir	-	Translocated intimin receptor
TTP	-	Thrombotic thrombocytopaenic purpura

List of Participants and Observers

Participants

Dr D. Acheson
Division of Geographic Medicine
and Infectious Diseases
New England Medical Center
Hospital
750 Washington Street
Boston, MA 02111
USA
Tel: 1 617 636 7002
Fax: 1 617 636 5292
E-mail:
david.acheson@es.nemc.org

Dr Andrea Ammon *
Robert Koch-Institut
Fachbereich 4
Stresemannstrasse 90-102
D-10963 Berlin
Germany
Tel: 49 30 4547 3481
Fax: 49 30 4547 3533
E-mail: ammona@rki.de

Prof. Dr G. Baljer **
Institut für Hygiene und
Infektionskrankheiten der Tiere
Frankfurter Str. 89-91
D-35392 Giessen
Germany
Tel: 49 641 993 300
Fax: 49 641 993 309
E-mail:
georg.baljer@vetmed.uni-giessen.de

Dr K. Bettelheim
National *E. coli* Reference
Laboratory
Victorian Infectious Diseases
Reference Laboratory
Private Bag 815, Carlton South
Melbourne, Victoria
Australia
Tel: 61 3 9349 4391
Fax: 61 3 9342 9830
E-mail: kabsm@jolt.mpx.com.au

Dr L. Beutin
(**Vice-Chairperson**)
Robert Koch-Institut
Fachbereich 2
Nordufer 20
D-13353 Berlin
Germany
Tel: 49 30 4547 2484
Fax: 49 30 4547 2673
E-mail: beutinl@rki.de

Dr Martina Bielaszewska *
Institut für Hygiene und
Mikrobiologie Universität
Würzburg
Josef-Schneider Strasse 2
D-97080 Würzburg
Germany
Tel: 49 931 201 5162
Fax: 49 931 201 5166 or 3445

Dr R. Clarke **
Health Canada
Health Protection Branch
HPB Building 0701B1, Tunney's
Pasture
Ottawa, K1A 0L2
Canada
Tel: 1 613 952 2558
Fax: 1 613 941 6242
E-mail: robert_clarke@hc-sc.gc.ca

Dr Patricia Desmarchelier
(**Rapporteur**)
Food Science Australia
P.O.Box 3312
Tingalpa D.C.
Queensland 4173
Australia
Tel: 61 7 3214 2032
Fax: 61 7 3214 2062
E-mail: Patricia.Desmarchelier@
foodscience.afisc.csiro.au

Dr B. Gericke *
Robert Koch-Institut
Fachbereich 2
Burgstrasse 37
D-38855 Wernigerode
Germany
Tel: 49 3943 679 257
Fax: 49 3943 679 207
E-mail: gerickeb@rki.de

Dr Patricia Griffin
National Center for Infectious Diseases
Centers for Disease Control and Prevention
MS A38
1600 Clifton Road
Atlanta, GA 30333
USA
Tel: 1 404 639 3384
Fax: 1 404 639 2205
E-mail: pmg1@cdc.gov

Dr Francine Grimont **
Institut Pasteur
28, rue Dr. Roux
Paris 75724
France
Tel: 33 1 4568 8344
Fax: 33 1 4568 8837
E-mail: fgrimont@pasteur.fr

Dr Beatriz Guth
Universidade Federal de Sao Paolo
Escola Paulista de Medicina
Rua Botucato, 862-3 andar
Sao Paulo 04023-062
Brazil
Tel: 55 11 5084 3213
Fax: 55 11 571 6504
E-mail: becguth.dmip@epm.br

Dr Maria Herpay
"B. Johan" National Institute of Public Health
Gyali ut 2-6
Budapest 1097
Hungary
Tel: 36 1 215 2250
Fax: 36 1 215 2250

Dr B. Kaijser **
University Goteborg Sweden
Department of Clinical Bacteriology
Guldhedsgatan 10
Goteborg 41346
Sweden
Tel: 46 31 604 908
Fax: 46 31 410 024
E-mail: bertil.kaijser@mic.gu.se

Prof. Dr H. Karch
Institut für Hygiene und Mikrobiologie Universität Würzburg
Josef-Schneider Strasse 2
D-97080 Würzburg
Germany
Tel: 49 931 201 5162
Fax: 49 931 201 3444
E-mail: hkarch@hygiene.uni-wuerzburg.de

Dr M. Karmali
(**Chairperson**)
Division of Microbiology
The Hospital for Sick Children
555 University Avenue
Toronto, Ontario M5G 1X8
Canada
Tel: 1 416 813 5994
Fax: 1 416 813 6257
E-mail: mohamed.karmali@mailhub.sickkids.on.ca

Dr E. Lopez
Infectious Diseases Division
Hospital de Niños "Dr. Ricardo Gutierrez"
(1452) Guido 2676 - Piso 10
Buenos Aires
Argentina
Tel: 54 1 963 8705
Fax: 54 1 961 4671
E-mail: elopez@pccp.com.ar

Dr L. Monnens **
University Hospital Nijmegen
Department of Pediatric Nephrology
P.O. Box 9101
Nijmegen 6500
The Netherlands
Tel: 31 24 361 6862
Fax: 31 24 361 6428

Dr H. Moon
Veterinary Medical Research Institute
Iowa State University
Ames, Iowa 50011
USA
Tel: 1 515 294 9327
Fax: 1 515 294 1401
E-mail: hwmoon@iastate.edu

Dr L. Petersen *
Robert Koch-Institut
Fachbereich 4
Stresemannstrasse 90-102
D-10963 Berlin
Germany
Tel: 49 30 45 47 3404
Fax: 49 30 45 47 3533
E-mail: petersenl@rki.de

Dr D. Piérard
Akademisch Ziekenhuis Vrije Universiteit Brussel (AZVUB)
Laarbeeklaan 101
B-1090 Brussels
Belgium
Tel: 32 2477 5002
Fax: 32 2477 5015
E-mail: labomicro@az.vub.ac.be

Dr H. Richter
Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (BgVV)
Fachbereich 5, Jahnstrasse 8
D-06846 Dessau
Germany
Tel: 49 340 6400 0120
Fax: 49 340 6400 0281
E-mail: h.richter@bgvv.de

Dr Marta Rivas
Instituto Nacional de Enfermedades Infecciosas Anlis – "Dr. Carlos Malbran"
Servicio Fisiopatogenia
Av. Diaz Velez Sarsfield 563
Buenos Aires 1281
Argentina
Tel: 54 1 303 1801
Fax: 54 1 303 1801
E-mail: isturiz@mail.retina.ar

Dr G. Rizzoni **
Children's Research Hospital Bambino Gesu
Piazza S. Onofrio 4
Rome 00165
Italy
Tel: 39 6 6859 2126
Fax: 39 6 6859 2602
E-mail: rizzoni@itcaspur.caspur.it

Dr F. Scheutz
The International *Escherichia* Centre (WHO)
Statens Serum Institut

Artillerivej 5
Copenhagen 2300
Denmark
Tel: 45 3268 3334
Fax: 45 3268 3036
E-mail: fsc@ssi.dk

Dr Anja Siitonen
Laboratory of Enteric Pathogens
National Public Health Institute
Mannerheimintie 166
FIN-00300 Helsinki
Finland
Tel: 35 8 947 448 245
Fax: 35 8 947 448 238
E-mail: anja.siitonen@ktl.fi

Dr H. Smith
Laboratory of Enteric Pathogens
Central Public Health Laboratory
London NW 9
United Kingdom
Tel: 44 181 200 4400 Ext. 3146
Fax: 44 181 905 9929
E-mail: hsmith@phls.co.uk

Dr J. Spika **
Health Canada
Center for Disease Control
P.I. 0603E1, Tunney's Pasture
Ottawa K1A 0L2
Canada
Tel: 1 613 957 4243
Fax: 1 613 941 7708
E-mail: john_spika@hc-sc.gc.ca

Dr Y. Takeda **
Research Institute
International Medical Center of Japan
1-21-1 Toyama, Shinjuku-ku
Tokyo 162
Japan
Tel: 81 3 3202 7181
Fax: 81 3 3202 7364
E-mail: resedr@imcj.go.jp

Dr A. Tozzi **
Istituto Superiore di Sanita
Laboratory of Epidemiology and Biostatistics
Viale Regina Elena 299
Rome 00161
Italy
Tel. 39 6 4990 2744
Fax: 39 6 4938 7292
E-mail: tozzi@iss.it

Dr L. Wieler *
Institut für Hygiene und Infektionskrankheiten der Tiere
Fachbereich Veterinärmedizin der Justus-Liebig-Universität Giessen
Frankfurter Strasse 85-89
D-35392 Giessen
Germany
Tel: 49 641 99 38 300
Fax. 49 641 99 38 309
E-mail: lothar.h.wieler@vetmed.uni-giessen.de

Observers

Dr. Edda Bartelt
BgVV
Fachbereich 3
Diedersdorfer Weg 1
D-12277 Berlin
Germany
Tel: 49 30 8412 2101
Fax: 49 30 8412 2951

Dr F. Gunzer
Medizinische Hochschule
Hannover
Carl-Neuberg-Strasse 1
D-30625 Hannover
Germany
Tel: 49 511 532 4532
Fax: 49 511 532 4366
E-mail: fgunzer@
mikbio.h.shuttle.de

Dr Susanne Heckötter
Institut für Tierärztliche
Nahrungsmittelkunde
Fachvereich Veterinärmedizin der
Justus-Liebig-Universität Giessen
Frankfurter Strasse 92
D-35392 Giessen
Germany
Tel: 49 641 99 38 250
Fax: 49 641 99 38 259

Dr Sonja Kiessling
Robert Koch-Institut
Fachbereich 4
Stresemannstrasse 90-102
D-10963 Berlin
Germany
Tel: 49 30 4547 3451
Fax: 49 30 4547 3514
E-mail: kiesslings@rki.de

Prof. Dr R. Kurth
Robert Koch-Institut
Nordufer 20
D-13353 Berlin
Germany
Tel: 49 30 4547 2261
Fax: 49 30 4547 2610

Dr Kerstin Ludwig
Universitätskinderklinik und
-poliklinik
Martinistrasse 52
D-20251 Hamburg
Germany
Tel: 49 40 4717 2702
Fax: 49 40 4717 5053
E-mail: kludwig@uke.uni-
hamburg.de

Dr D. Protz
BgVV
Fachbereich 5
Diedersdorfer Weg 1
D-12277 Berlin
Germany
Tel: 49 30 8412 2264
Fax: 49 30 8412 2952
E-mail: d.protz@bgvv.de

Prof. Dr L. Zimmerhackl
Universitäts-Kinderklinik der
Albert Ludwigs Universität
Mathildenstrasse 1
D-79106 Freiburg i. Br.
Germany
Tel: 49 6761 270 4309
Fax: 49 6761 270 4407
E-mail:
hackl@kkl200.ukl.uni-freiburg.de

Dr Christel Zimmermann
Robert Koch-Institut
Fachbereich 4
Stresemannstrasse 90-102
D-10963 Berlin
Germany
Tel: 49 30 4547 3139
Fax: 49 30 4547 3133
E-mail: zimmermannc@rki.de

WHO Secretariat

Dr R. Crom
(**Secretary**)
Zoonotic Diseases
Division of Emerging and other
Communicable Diseases
Surveillance and Control (EMC)
World Health Organization
CH-1211 Geneva 27
Switzerland
Tel: 41 22 791 2842
Fax: 41 22 791 4893
E-mail: cromr@who.ch

Mr A. Reilly
Food Safety Unit
Programme of Food Safety and
Food Aid
World Health Organization
CH-1211 Geneva 27
Switzerland
Tel: 41 22 791 3462
Fax: 41 22 791 4807
E-mail: reillya@who.ch

Dr K. Stöhr
Zoonotic Diseases
EMC
World Health Organization
CH-1211 Geneva 27
Switzerland
Tel: 41 22 791 2529
Fax: 41 22 791 4893
E-mail: stohrk@who.ch

* Participant nominated
by Robert Koch-Institut

** Unable to attend

Agenda and Timetable

Tuesday, 23 June 1998

TIME	SUBJECT	SPEAKER
13:00	Opening and Introductions	Reinhard KURTH, RKI
13:20	Objectives	Klaus STÖHR / Randy CROM, WHO
Overview of Non-O157 STEC - Detection of infections		
13:30	Isolation and identification methods	Lothar BEUTIN, Germany
14:05	Additional information on isolation and identification	Heinz RICHTER, Germany, Flemming SCHEUTZ, Denmark
14:25	Discussion	
15:00	Break	
15:15	Issues related to typing and subtyping	Henry SMITH, United Kingdom
16:00	Discussion	
16:30	Break	
16:45	Reports on surveillance of non-O157 STEC in humans	David ACHESON, USA, Maria HERPAY, Hungary Flemming SCHEUTZ, Denmark Anja SIITONEN, Finland Alberto TOZZI, Italy
17:30	Discussion	
18:00	Adjournment	

Wednesday, 24 June 1998

8:30	Surveillance for non-O157 STEC in humans (Europe)	Andrea AMMON, Germany
8:50	Discussion	
Overview of Non-O157 STEC - Epidemiology		
9:15	Risk factors for human infection	Eduardo LOPEZ, Argentina
10:00	Discussion	
10:30	Break	
11:00	Sources of non-O157 STEC infection: animal reservoirs	Patricia DESMARCHELIER, Australia
11:45	Discussion	
12:15	Discussion of new non-O157 STEC types	All
13:00	Lunch	

Wednesday, 24 June 1998 (continued)

Overview of Non-O157 STEC - Pathogenesis

14:00	Intestinal colonization mechanisms and host resistance	David ACHESON, USA
14:45	Discussion	
15:15	Break	
15:30	Challenges of non-O157 STEC	Mohamed KARMALI, Canada
16:15	Discussion	
16:45	Charge to the Working Groups:Conclusions and recommendations on agenda areas	
17:15	Adjournment for the day	

Thursday, 25 June 1998

Working in Groups

9:00	Conclusions and recommendations on agenda areas
10:30	Break
11:00	Conclusions and recommendations on agenda areas (continued)
12:30	Lunch
13:30	Conclusions and recommendations on agenda areas (continued)
15:00	Break
15:30	Conclusions and recommendations on agenda areas (continued)
17:00	Adjournment for the day

Friday, 26 June 1998

Final Plenary Session

8:30	Finalize conclusions and recommendations from the working group reports
10:00	Break
10:30	Finalise conclusions and recommendations from the Working Group reports (continued)
12:00	Concluding Remarks / Adjournment