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Shiga Toxin producing *E. coli* (STEC) in food – which serotypes are important?

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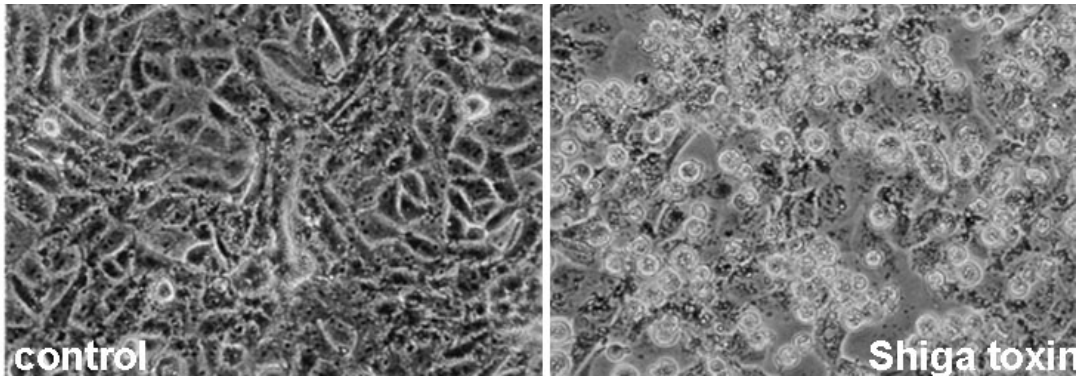
A short history of Shiga (Vero) Toxin producing *E. coli*

1977: Detection of Verocytotoxic activity in some *E. coli* strains by the Vero cell toxicity test Konowalchuk et al. IAI 18: 775: (1977)



1979/1980: First description of STEC as human pathogens

Wade et al. Lancet 8: 1235 (1979), Wilson & Bettelheim Lancet i 201 (1980)

1982: First description of foodborne outbreaks with STEC (O157:H7)

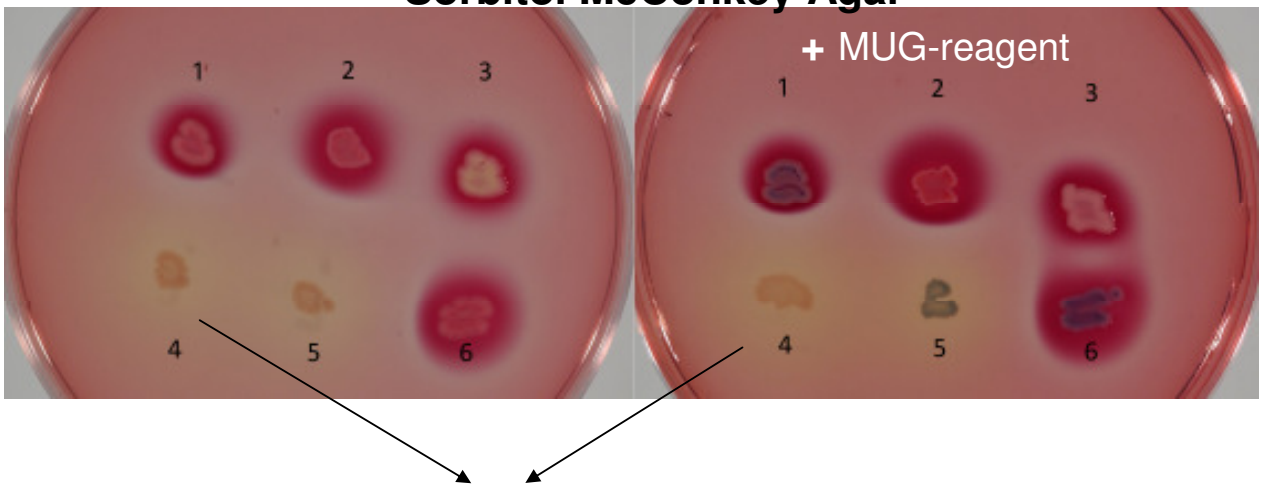
Riley et al. New Engl. J. Medicine 308: 681 (1983)

A specific case: EHEC O157:H7

STEC O157 infections in humans were found frequently associated with outbreaks and severe clinical illness: Haemorrhagic colitis (HC) and Haemolytic uraemic syndrome (HUS)

STEC O157 differs from other *E. coli* by absence of sorbitol fermentation and beta-glucuronidase activty. Sorbitol McConkey Agar was introduced for rapid selection of STEC O157:H7 strains

Sorbitol McConkey Agar



EHEC O157

As a consequence many countries concentrated on detection of STEC O157 only.

Non-O157 STEC: more than 400 serotypes from human patients (2005)

Scheutz & Strockbine. Genus: *Escherichia* Bergey's Manual Syst. Bacteriol (2005)

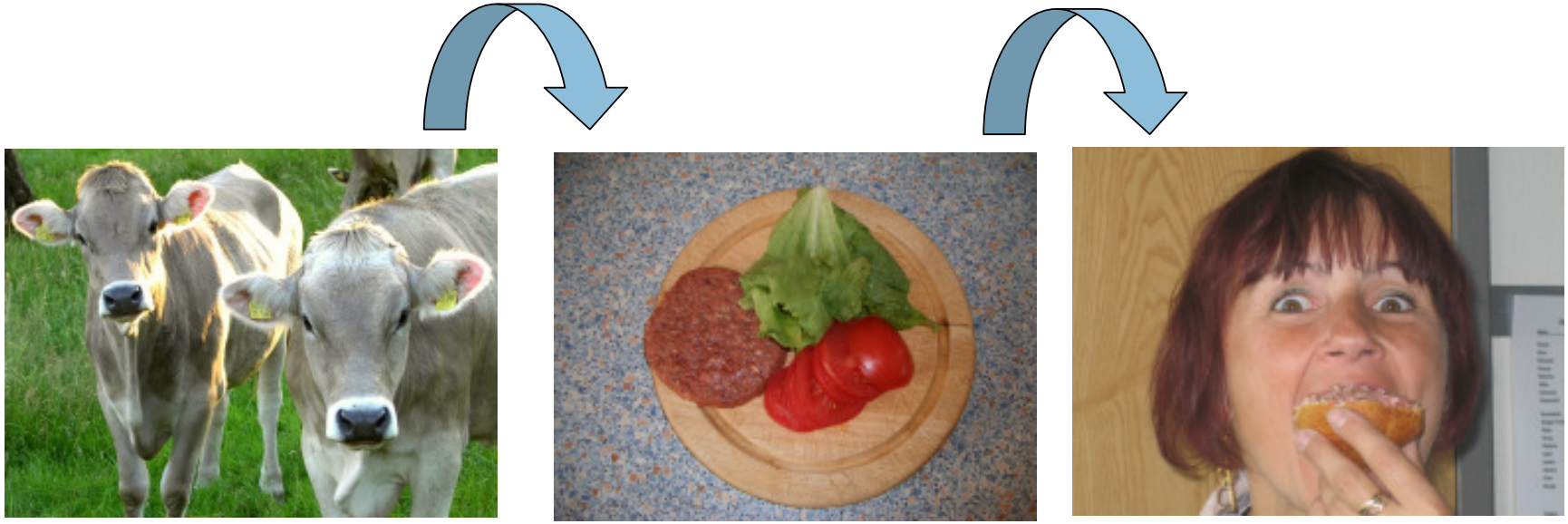
TABLE BXII.γ.205. Serotypes of non-O157 STEC/VTEC isolated from humans[a,b,c]

Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype
O1:H⁻	**O8:H21**	**O25:K2:H2**	O52:H23	**O83:H1**	O103:H18	O114:H4	O126:H20	O146:H11	O169:H⁻
O1:H1	O8:H25	O25:H14	O52:H25	**O84:H⁻**	O103:H21	O114:H48	O126:H21	O146:H14	**O171:H⁻**
O1:H2	**O9ab:H⁻**	**O26:H⁻**	O54:H21	O84:H2	O103:H25	**O114:H?**	**O126:H27**	O146:H21	O171:H2
O1:H7	O9:H7	O26:H2	**O55:H⁻**	O84:H20	O103:HNT	**O115:H10**	O127	O146:H28	**O172:H⁻**
O1:H20	O9:H21	O26:H8	**O55:H6**	**O85:H⁻**	**O104:H⁻**	O115:H18	**O128:H⁻**	O148:H28	**O172:H?**
O1:HNT	**O11:H⁻**	**O26:H11**	**O55:H7**	**O85:H10**	**O104:H2**	O116:H⁻	**O128ab:H2**	O150:H⁻	**O173:H2**
O2:H⁻	**O11:H2**	O26:H12	O55:H9	**O85:H23**	O104:H7	O116:H4	**O128:H7**	O150:H8	**O174:H⁻** [d]
O2:H1	O11:H8	O26:H32	**O55:H10**	**O86:H⁻**	O104:H16	O116:H10	O128:H8	O150:H10	**O174:H2**[d]
O2:K1:H2	O11:H49	O26:H46	O55:H19	O86:H10	**O104:H21**	O116:H19	O128:H10	O152:H4	O174:H8[d]
O2:H5	O12:H⁻	O27:H⁻	**O55:H?**	O86:H40	**O105ac:H18**	O117:H⁻	O128:H12	O153:H2	**O174:H21**[d]
O2:H6	**O14:H⁻**	O27:H30	O60:H⁻	O87:H16	O105:H19	**O117:H4**	**O128:H25**	O153:H11	O175:H16[e]
O2:H7	**O15:H⁻**	O28ab:H⁻	O64:H25	O88:H⁻	O105:H20	O117:H7	O128:H31	O153:H12	OX176:H⁻[f]
O2:H11	**O15:H2**	O28:H25	O65:H16	**O88:H25**	O106	O117:K1:H7	**O128:H45**	O153:H21	**OX177:H⁻** [f]
O2:H27	O15:H8	O28:H35	O68:H⁻	O89:H⁻	O107:H27	O117:H8	O129:H⁻	**O153:H25**	OX177:H11[f]
O2:H29	O15:H27	O30:H2	**O69:H⁻**	O90:H⁻	O109:H2	O117:H19	**O130:H11**	O153:H30	OX178:H7[f]
O2:H44	**O16:H⁻**	O30:H21	O69:H11	**O91:H⁻**	O109:H16	O117:H28	O131:H4	O153:H33	OX179:H8[f]
O3:H10	O16:H6	O30:H23	O70:H11	O91:H4	O110:H⁻	O118:H⁻	O132:H⁻	**O154:H⁻**	OX181:H15[f]
O4:H⁻	O16:H21	**O37:H41**	O71:H⁻	**O91:H10**	O110:H19	O118:H2	O133:H⁻	**O154:H4**	OX181:H49[c]
O4:H5	O17:H18	O38:H21	O73:H34	O91:H14	O110:H28	**O118:H12**	O133:H53	**O154:H19/20**	ONT:H⁻
O4:H10	O17:H41	O38:H26	O74	O91:H15	**O111:H⁻**	**O118:H16**	**O134:H25**	O156:H⁻	**ONT:H2**
O4:H40	**O18:H⁻**	O39:H4	**O75:H⁻**	**O91:H21**	**O111:H2**	**O118:H30**	**O137:H6**	O156:H4	ONT:H8
O5:H⁻	O18:H7	O39:H8	O75:H1	O91:H40	**O111:H7**	O119:H⁻	**O137:H41**	O156:H7	ONT:H18
O5:H16	O18:H12	O39:H28	**O75:H5**	O91:HNT	**O111:H8**	**O119:H5**	O138:H2	O156:H25	ONT:H19
O6:H⁻	O18:H15	O40:H2	O75:H8	O92:H3	O111:H11	**O119:H6**	O141:H⁻	O156:H27	ONT:H21
O6:H1	**O18:H?**	O40:H8	O76:H7	O92:H11	O111:H21	O119:H25	O141:H2	O156:HNT	**ONT:H25**
O6:H2	O20:H⁻	O41:H2	O76:H19	O95:H⁻	O111:H30	**O120:H19**	O141:H8	**O160:H?**	ONT:H41
O6:H4	O20:H7	O41:H26	**O77:H⁻**	O96:H10	O111:H34	**O121:H⁻**	O142	**O161:H⁻**	ONT:H47
O6:H12	**O20:H19**	O44	O77:H4	**O98:H⁻**	O111:H40	O121:H8	O143:H⁻	O162:H4	**ONT:K39:H48**
O6:H28	**O21:H5**	O45:H⁻	O77:H7	O98:H8	O111:H49	O121:H11	O144:H⁻	O163:H⁻	Orough:H⁻
O6:H29	**O21:H8**	O45:H2	O77:H18	**O100:H25**	**O111:H?**	**O121:H19**	**O145:H⁻**	**O163:H19**	Orough:H2
O6:H31	**O21:H?**	O45:H7	O77:H41	O100:H32	**O112ab:H2**	O123:H19	O145:H4	O163:H25	**Orough:H5**
O6:H34	O22:H⁻	O46:H2	O78:H⁻	**O101:H⁻**	O112:H19	O123:H49	**O145:H8**	**O165:H⁻**	**Orough:K1:H6**
O6:H49	**O22:H1**	**O46:H31**	**O79:H7**	O101:H9	O112:H21	O124:H⁻	O145:H16	O165:H10	Orough:K1:H7
O7:H4	**O22:H5**	O46:H38	O79:H14	O102:H6	O113:H2	O125:H⁻	**O145:H25**	**O165:H19**	**Orough:H11**
O7:H8	**O22:H8**	**O48:H21**	O79:H23	**O103:H⁻**	**O113:H4**	O125:H8	O145:H26	O165:H21	**Orough:H16**
O8:H⁻	O22:H16	**O49:H⁻**	O80:H⁻	**O103:H2**	O113:H5	**O125:H?**	**O145:H28**	**O165:H25**	Orough:H18
O8:H2	O22:H40	**O49:H10**	O81:H?	O103:H4	O113:H7	O126:H⁻	O145:H46	O166:H12	Orough:H20
O8:H9	O23:H7	O50:H⁻	O82:H⁻	O103:H6	**O113:H21**	O126:H2	O145:HNT	O166:H15	Orough:H21
O8:H11	O23:H16	**O50:H7**	O82:H5	O103:H7	O113:H32	O126:H8	O146:H⁻	O166:H28	Orough:H28
O8:H14	O23:H21	O51:H49	O82:H8	O103:H11	O113:H53	O126:H11	L	O168:H⁻	Orough:H46
O8:H19	O25:H⁻	O52:H19	O83:H⁻						

Most STEC are zoonotic agents

STEC form a phenotypically heterogeneous group

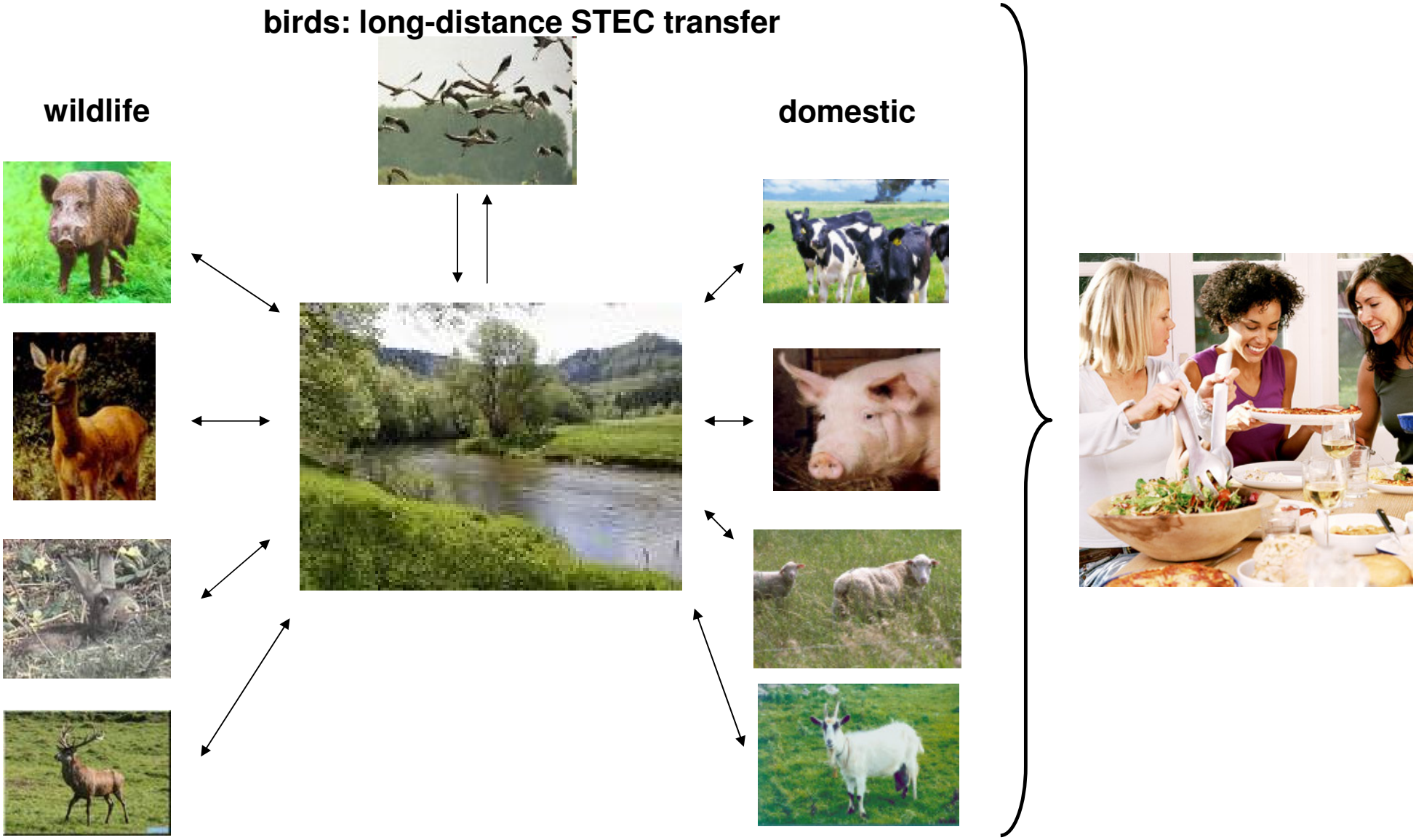
No common traits except for Stx-production / *stx*-gene(s)



CDC: 85% of STEC infections in humans are food borne

Mead et al. EID 5: 841 (1999)

There is a broad animal reservoir of STEC in nature and transmission to the environment, food and humans is important



Enteroaggregative enterohemorrhagic *E. coli* O104:H4: paradigm change, humans as primary source of food contamination

birds: long-distance STEC transfer

wildlife

domestic

Enteroaggregative STEC O104

Human as source of transmission to food

human, environment

Survival of STEC in food

- Survive well chilled and frozen temperatures
- Don‘t grow at temperatures <7°C
- Modified atmosphere packaging is not protective
- Resistant to dessication, may survive many drying and fermentation processes (survive in salami).
- Relatively acid tolerant (survive in apple juice)
- Not resistant to heat
- Stress conditions (high salt, low temperature) may result in VBNC state (not recovered by culture but still causing illness).

Persists as viable organism in soil and water over long periods (up to 20 months in soil) (shown for O157:H7)

Human STEC infections: EU and USA

USA: 1.9 /100,000 population for 1996-2010

EU: 0.83/100,000 population for 2010

The numbers of unreported infections is likely to vary between O157 and non-O157 and between different states, geographical locations.

EHEC O157: minimal infective dose: 10-100 organisms for humans

Transmission routes of outbreaks (USA: 1982-2002)

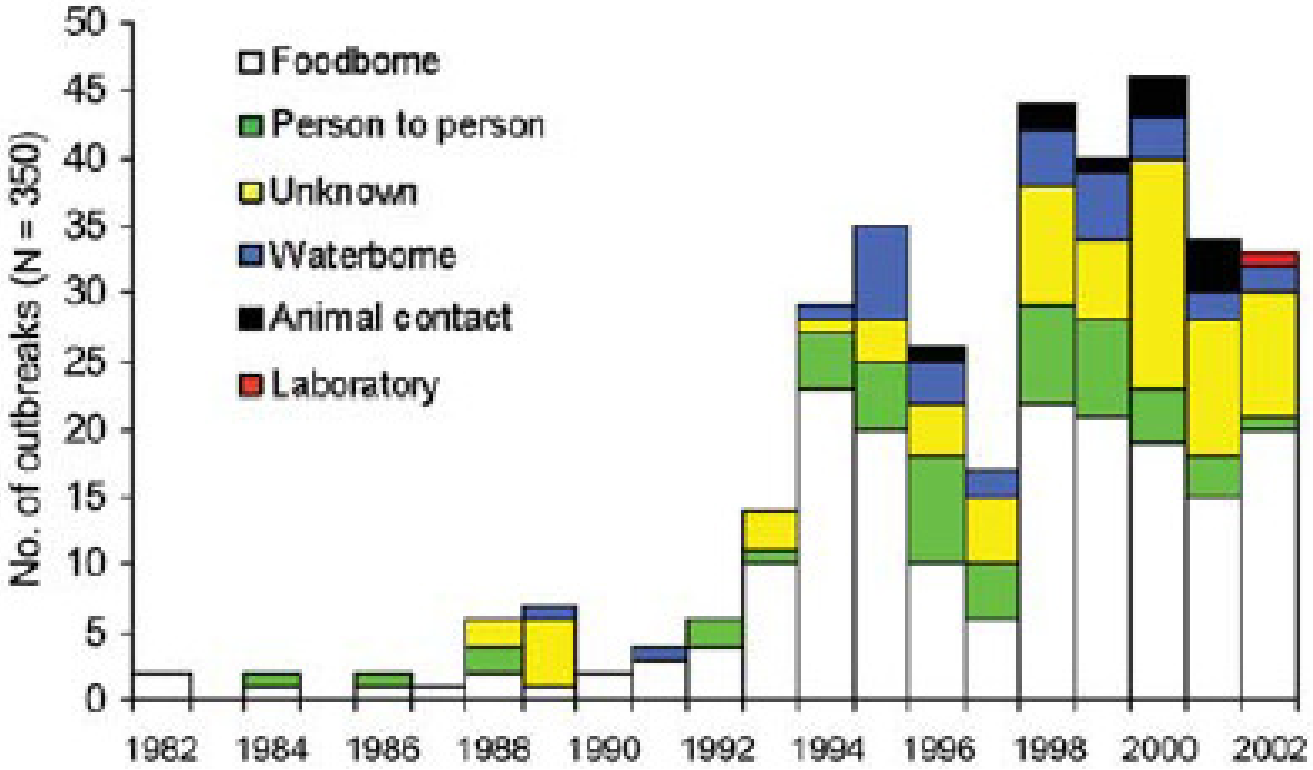


Figure 3. Transmission routes of *Escherichia coli* O157 outbreaks by year.

Rangel et al. Epidemiology of E. coli O157:H7 outbreaks, United States, 1982-2002. EID 2005 11:603-9.

Vehicles of foodborne outbreaks (USA: 1982-2002)

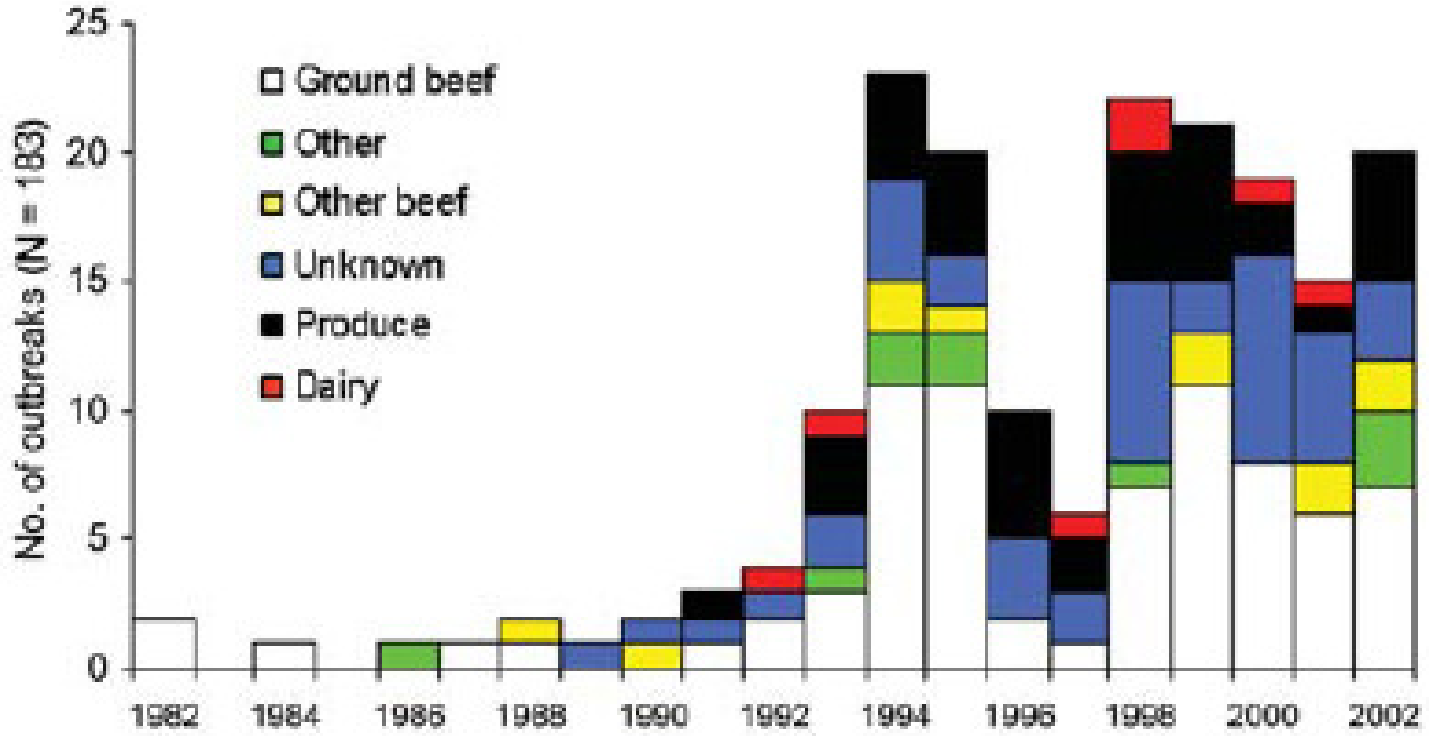


Figure 4. Vehicles of foodborne *Escherichia coli* O157 outbreaks by year.

Rangel et al. Epidemiology of E. coli O157:H7 outbreaks, United States, 1982-2002. EID 2005 11:603-9.

Not all STEC cause disease in humans and some STEC strains, called enterohemorrhagic *E. coli* (EHEC) are more virulent than others

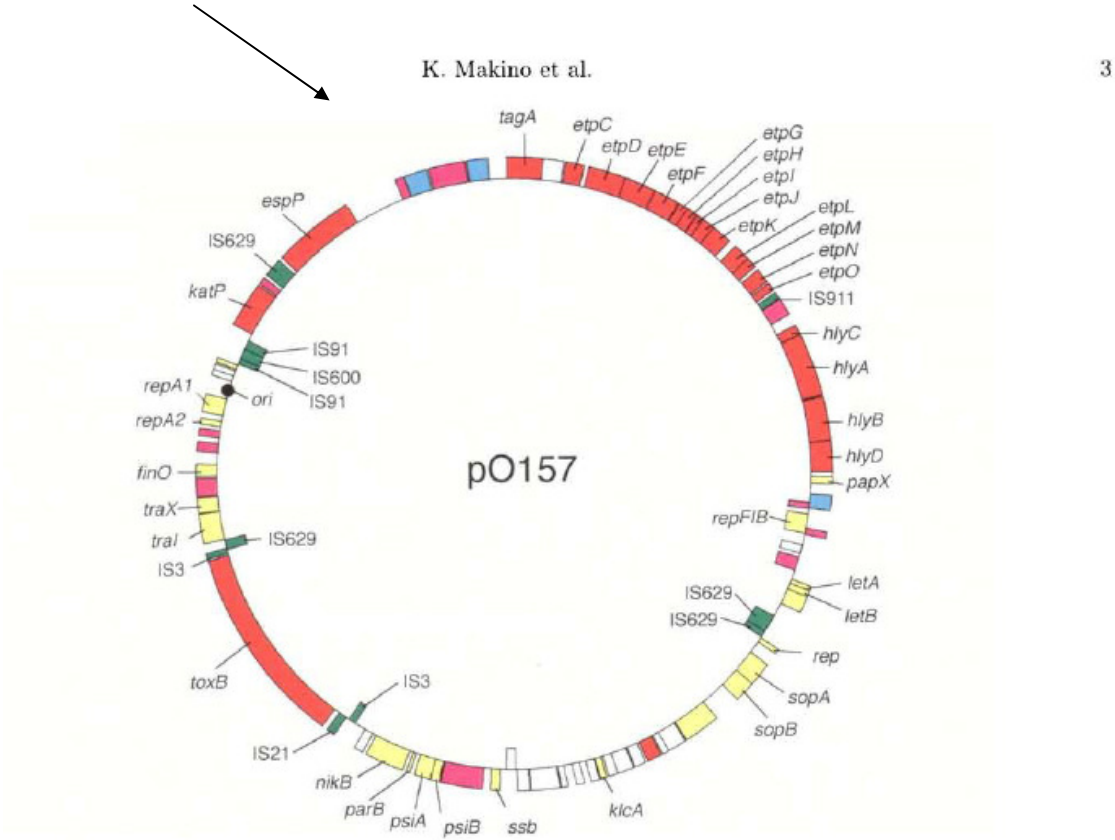
How to identify human virulent EHEC types?

Defining EHEC: clinical picture and EHEC plasmid-gene probe

1987

Levine MM. J Infect Dis. 1987 55:377-89.

Coining the term „EHEC“ for virulent STEC strains causing HC + HUS, a DNA probe for the EHEC-plasmid

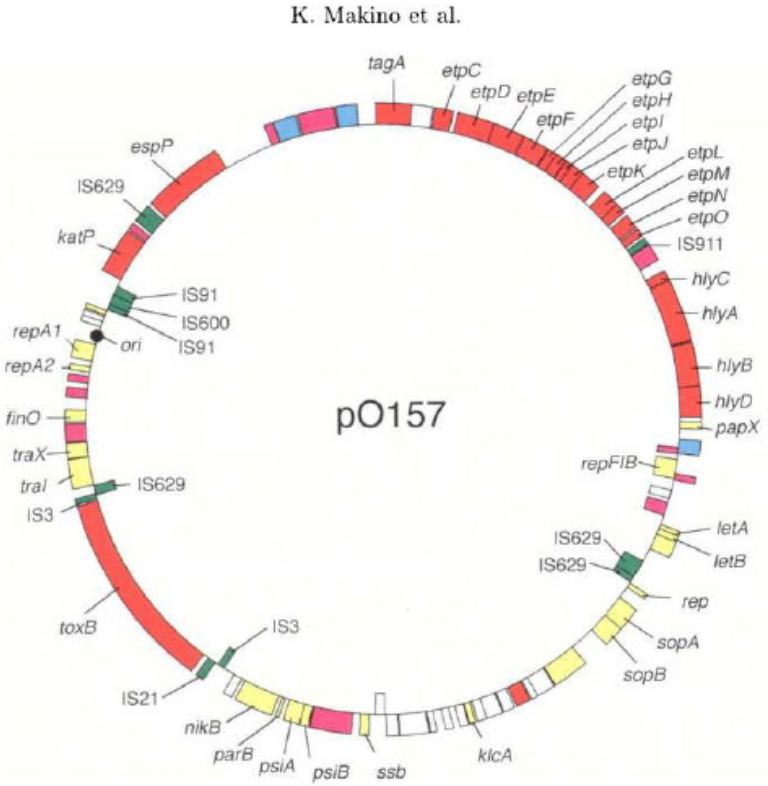


Defining EHEC: EHEC plasmid and enterohemolytic phenotype

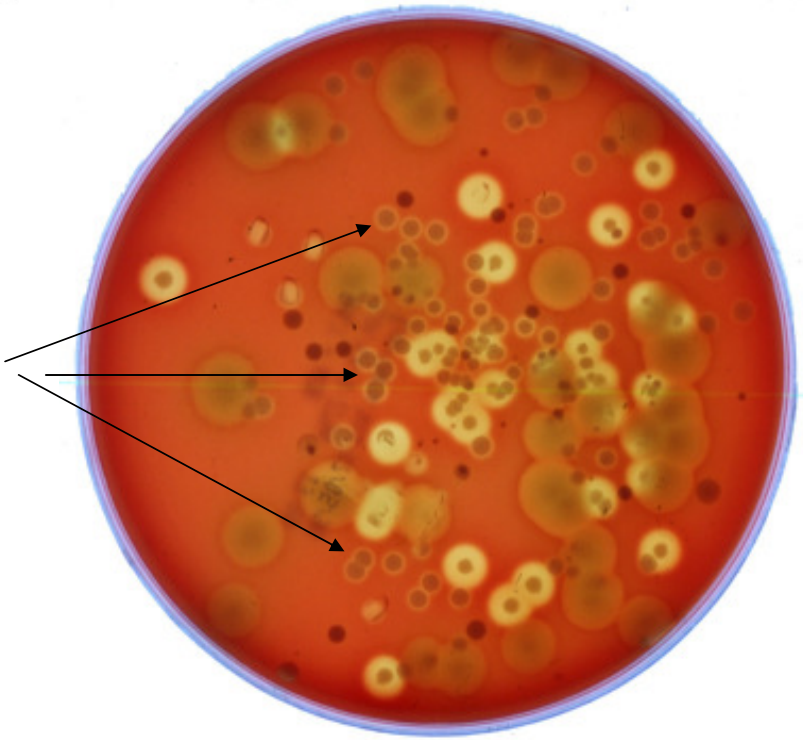
1989

Beutin et al. J Clin Microbiol 1987 55:377-89.

Presence of EHEC-plasmid by detection of enterohemolysin



E-hlyA



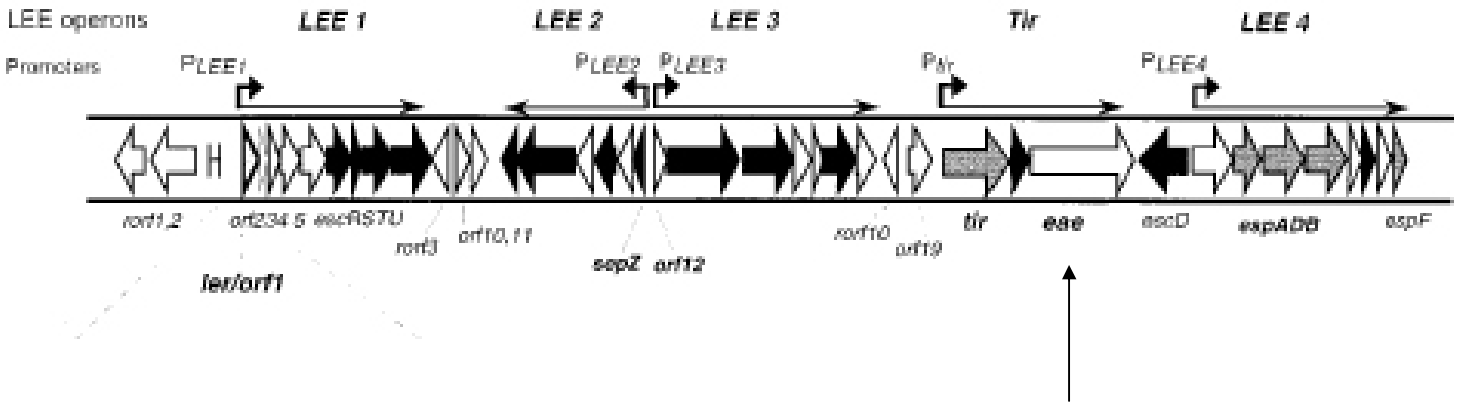
Defining EHEC, Locus of Enterocyte Effacement (LEE), Attaching and Effacing Mechanism (A/E)

1998

Sperandio V et al. 1998 FEMS Microbiol Lett. 164:133-9.

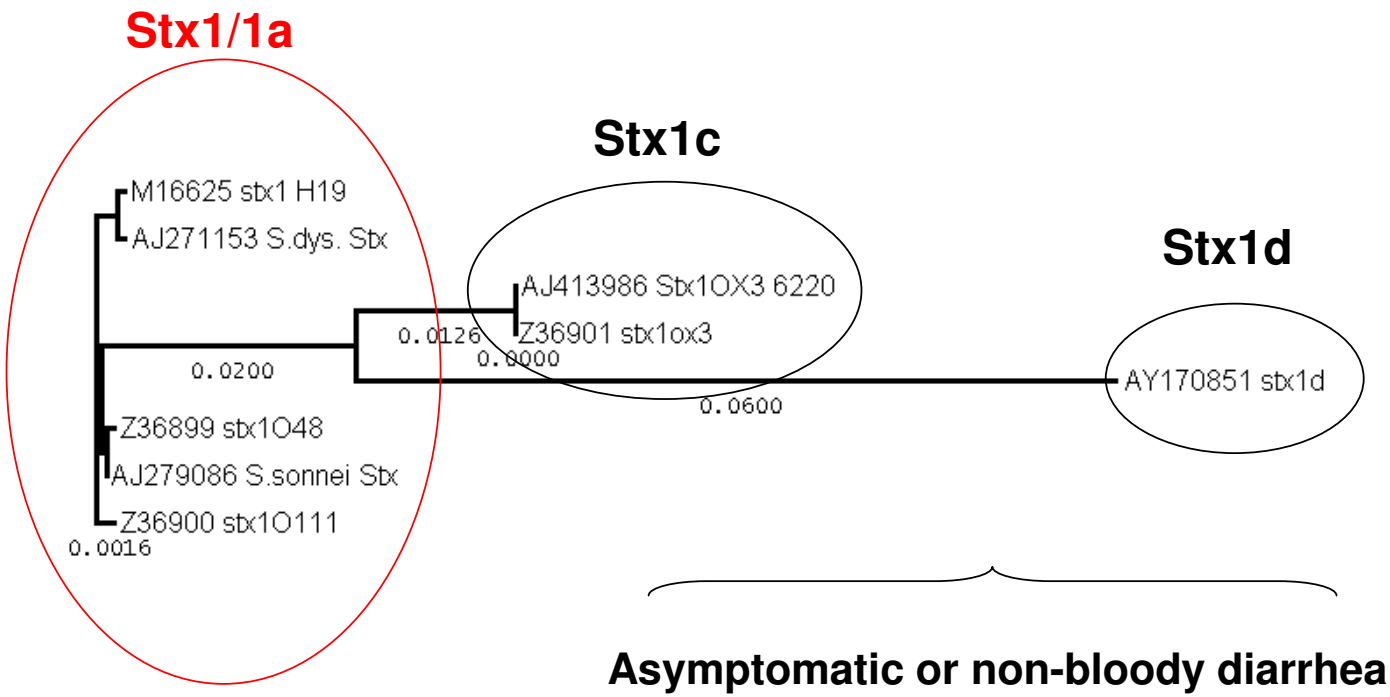
Role of the LEE locus in virulence of EHEC

Intimate adhesion, attaching and effacing (A/E) phenotype



eae as hallmark of EHEC strains

Defining EHEC: Role of Shiga Toxin 1 (Stx1) subtypes



Asymptomatic or non-bloody diarrhea

Friedrich AW, J Clin Microbiol. 2003: 2448-53.

Stx1a: frequent in EHEC

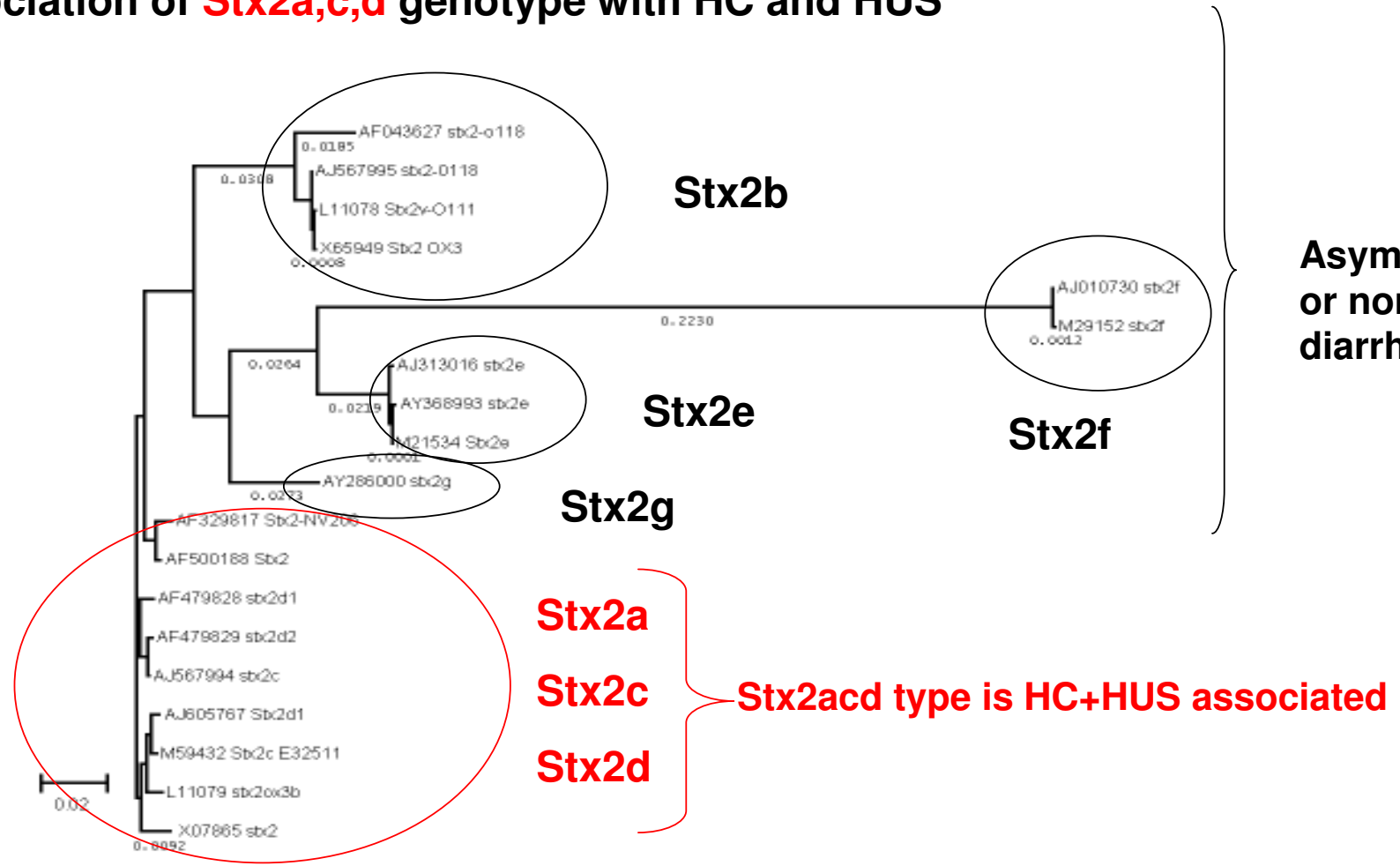
O26, O103, O111, O145 strains

Defining EHEC: role of Shiga Toxin 2 (Stx2)-subtype

2001

Friederich A et al. 2001 J Infect Dis 2002 185:74-84

Association of Stx2a,c,d genotype with HC and HUS

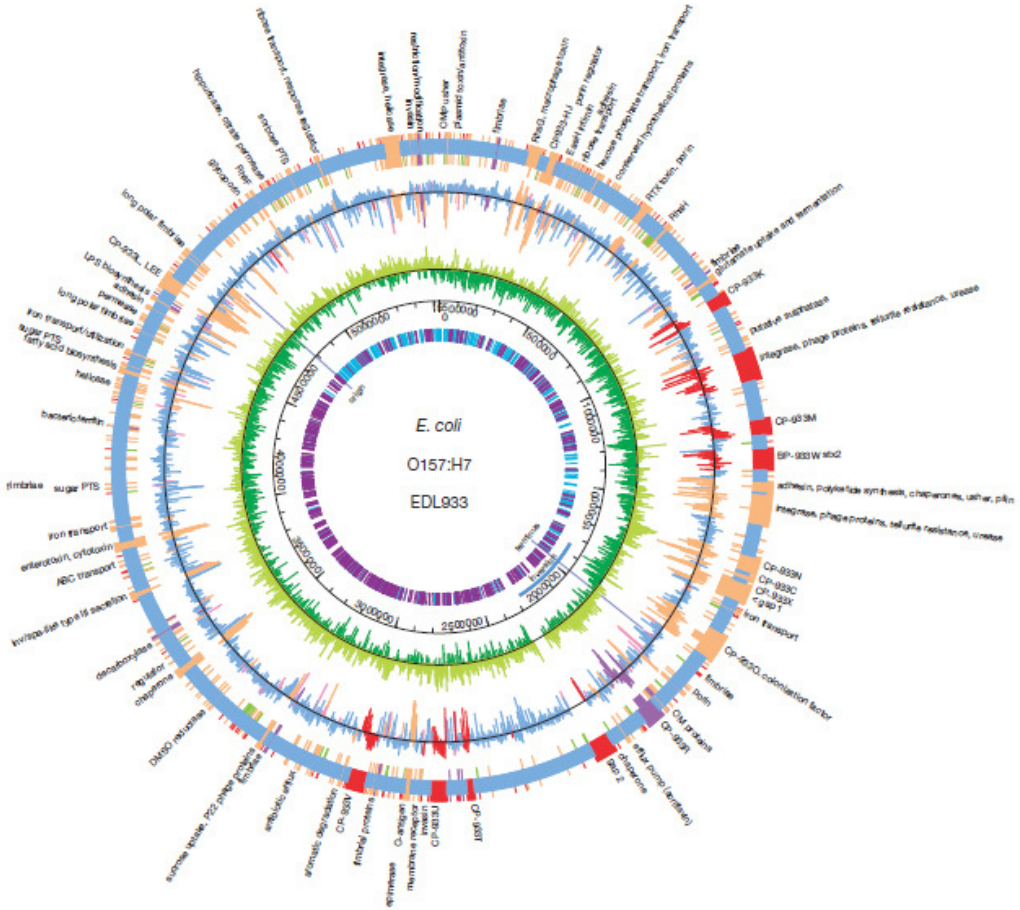


Defining EHEC, decoding of the EHEC O157:H7 genome

2001

Perna NT et al. Nature. 2001 Jan 25;409(6819):529-33.

Genome sequence of EHEC O157



177 O-islands >50kb

1387 genes (26%)

9 virulence associated O-islands

18 (pro)phages

E. coli basisgenome 4,1 Mba

O157 O-islands 1,34 Mba

Defining EHEC: seropathotype concept

2003

Karmali et al. *J Clin Microbiol* 2003, 41: 4930-4940.

O Island 122 of *E. coli* O157, Seropathotypes linked to Epidemic and/or Serious Disease.

TABLE 1. Classification of VTEC serotypes into seropathotypes

Seropathotype	Relative incidence	Frequency of involvement in outbreaks	Association with severe disease[a]	Serotypes
A	High	Common	Yes	O157:H7, O157:NM
B	Moderate	Uncommon	Yes	O26:H11, O103:H2, O111:NM, O121:H19, O145:NM
C	Low	Rare	Yes	O91:H21, O104:H21, O113:H21; others
D	Low	Rare	No	Multiple
E	Nonhuman only	NA[b]	NA	Multiple

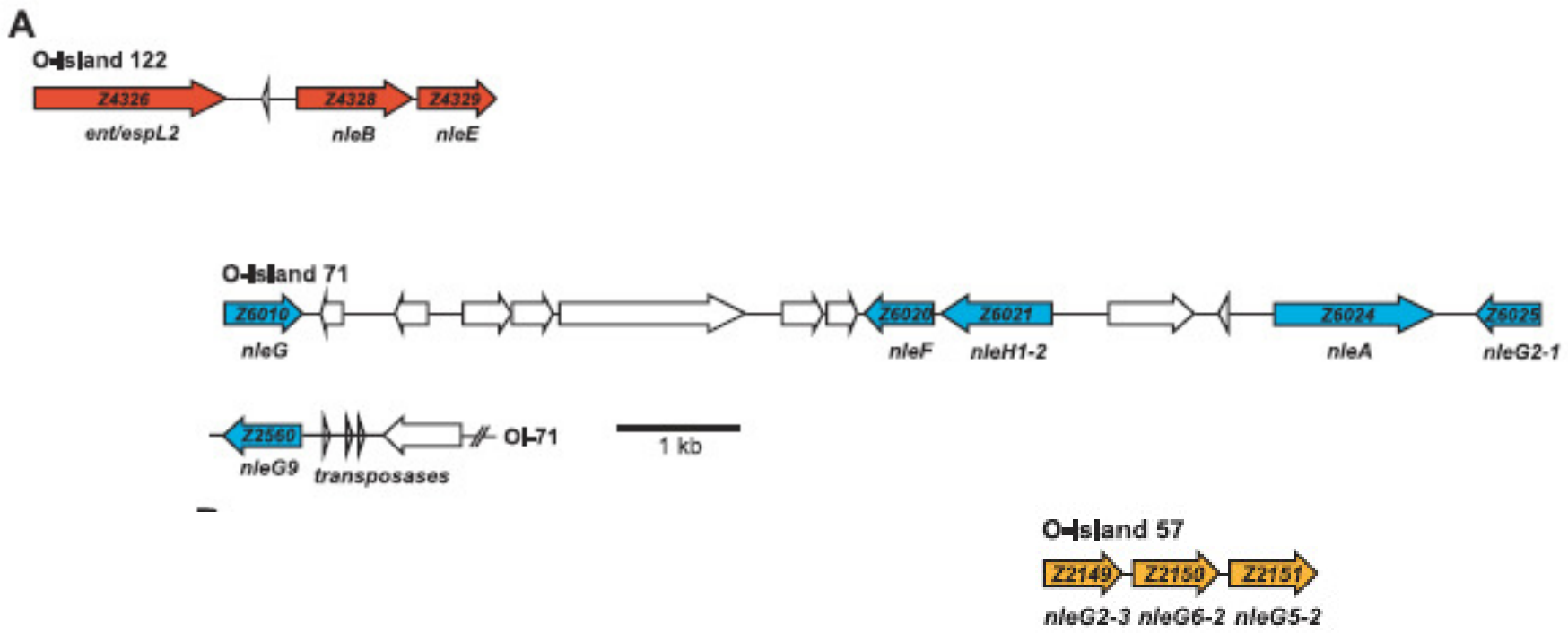
[a] HUS or hemorrhagic colitis.
[b] NA, not applicable.

Defining EHEC: concept of Molecular Risk Assessment (MRA)

2008

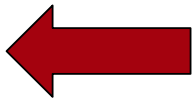
Coombes et al. *Appl Environ Microbiol* 2008, 74: 2153-2160

Nle genes on O-islands 122, 71, 57, MRA concept



Identification of new emerging EHEC types by MRA typing:

PATHOTYPE	MRA CLUSTER 1 EHEC	MRA CLUSTER 2 other	SEROPATHO-TYPE (Karmali 2003)	SEROTYPES
typical EHEC	24	1	A + B	O26:H11, O103:H2, O111:H8, O121:H19, O145:H25/H28, O157:H7
emerging EHEC	20	0	n.a.	O5:H-, O15:H2, O45:H2, O55:H7, O103:H11, O103:H25, O118:H16, O123, O165:H25, O172:H25, O177:H-
atypical EHEC	0	9	C	O91:H21, O104:H21, O113:H21
STEC	0	102	D + E	multiple
EPEC	8	13	n.a.	multiple
fecal *E. coli*	0	30	n.a.	multiple



cooperation ANSES (France) + NRL E.coli BfR (Berlin)

From: Bugarel M et al. Int J Food Microbiol.142:318-29

STEC minus EHEC: what about the rest?
Are these significant as food contaminants?

TABLE BXII.γ.205. Serotypes of non-O157 STEC/VTEC isolated from humans[a,b,c]

Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype
O1:H⁻	**O8:H21**	**O25:K2:H2**	O52:H23	**O83:H1**	O103:H18	O114:H4	O126:H20	O146:H11	O169:H⁻
O1:H1	O8:H25	O25:H14	O52:H25	**O84:H⁻**	O103:H21	O114:H48	O126:H21	O146:H14	**O171:H⁻**
O1:H2	**O9ab:H⁻**	[redacted]	O54:H21	O84:H2	O103:H25	**O114:H?**	**O126:H27**	O146:H21	O171:H2
O1:H7	O9:H7	O26:H2	**O55:H⁻**	O84:H20	O103:HNT	**O115:H10**	O127	O146:H28	**O172:H⁻**
O1:H20	O9:H21	O26:H8	**O55:H6**	**O85:H⁻**	**O104:H⁻**	O115:H18	**O128:H⁻**	O148:H28	**O172:H?**
O1:HNT	**O11:H⁻**	[redacted]	**O55:H7**	**O85:H10**	**O104:H2**	O116:H⁻	**O128ab:H2**	O150:H⁻	**O173:H2**
O2:H⁻	**O11:H2**	O26:H12	O55:H9	**O85:H23**	O104:H7	O116:H4	**O128:H7**	O150:H8	**O174:H⁻** [d]
O2:H1	O11:H8	O26:H32	**O55:H10**	**O86:H⁻**	O104:H16	O116:H10	O128:H8	O150:H10	**O174:H2**[d]
O2:K1:H2	O11:H49	O26:H46	O55:H19	O86:H10	**O104:H21**	O116:H19	O128:H10	O152:H4	O174:H8[d]
O2:H5	O12:H⁻	O27:H⁻	**O55:H?**	O86:H40	**O105ac:H18**	O117:H⁻	O128:H12	O153:H2	**O174:H21**[d]
O2:H6	**O14:H⁻**	O27:H30	O60:H⁻	O87:H16	O105:H19	**O117:H4**	**O128:H25**	O153:H11	O175:H16[e]
O2:H7	**O15:H⁻**	O28ab:H⁻	O64:H25	O88:H⁻	O105:H20	O117:H7	O128:H31	O153:H12	OX176:H⁻[f]
O2:H11	**O15:H2**	O28:H25	O65:H16	**O88:H25**	O106	O117:K1:H7	**O128:H45**	O153:H21	**OX177:H⁻** [f]
O2:H27	O15:H8	O28:H35	O68:H⁻	O89:H⁻	O107:H27	O117:H8	O12[redacted]	**O153:H25**	OX177:H11[f]
O2:H29	O15:H27	O30:H2	**O69:H⁻**	O90:H⁻	O109:H2	O117:H19	[redacted]	O153:H30	OX178:H7[f]
O2:H44	**O16:H⁻**	O30:H21	O69:H11	**O91:H⁻**	O109:[redacted]	[redacted]	[redacted]	O153:H33	OX179:H8[f]
O3:H10	O16:H6	O30:H23	O70:H11	O91:H4	O1[redacted]	[redacted]	[redacted]	**O154:H⁻**	OX181:H15[f]
O4:H⁻	O16:H21	O37:H41	O71:H⁻	[redacted]	[redacted]	[redacted]	[redacted]:H⁻	**O154:H4**	OX181:H49[e]
O4:H5	O17:H18	O38:H21	O73:H34	[redacted]	[redacted]	[redacted]	O133:H53	**O154:H19/20**	ONT:H⁻
O4:H10	O17:H41	O38:H26	O74	[redacted]	[redacted]	[redacted]	**O134:H25**	O156:H⁻	**ONT:H2**
O4:H40	**O18:H⁻**	O39:H4	**O75:H⁻**	[redacted]	[redacted]	**O118:H30**	**O137:H6**	O156:H4	ONT:H8
O5:H⁻	O18:H7	O39:H8	O75:H1	[redacted]	**O111:H7**	O119:H⁻	**O137:H41**	O156:H7	ONT:H18
O5:H16	O18:H12	O39:H28	**O75:H5**	[redacted]NT	[redacted]	**O119:H5**	O138:H2	O156:H25	ONT:H19
O6:H⁻	O18:H15	O40:H2	O75:H8	O92:H3	O111:H11	**O119:H6**	O141:H⁻	O156:H27	ONT:H21
O6:H1	**O18:H?**	O40:H8	O76:H7	O92:H11	O111:H21	O119:H25	O141:H2	O156:HNT	**ONT:H25**
O6:H2	O20:H⁻	O41:H2	O76:H19	O95:H⁻	O111:H30	**O120:H19**	O141:H8	**O160:H?**	ONT:H41
O6:H4	O20:H7	O41:H26	**O77:H⁻**	O96:H10	O111:H34	[redacted]	O142	**O161:H⁻**	ONT:H47
O6:H12	**O20:H19**	O44	O77:H4	**O98:H⁻**	O111:H40	O121:H8	O143:H⁻	O162:H4	**ONT:K39:H48**
O6:H28	**O21:H5**	[redacted]	O77:H7	O98:H8	O111:H49	O121:H11	O144:H⁻	O163:H⁻	Orough:H⁻
O6:H29	**O21:H8**	[redacted]	O77:H18	**O100:H25**	**O111:H?**	[redacted]	[redacted]	**O163:H19**	Orough:H2
O6:H31	**O21:H?**	O45:H7	O77:H41	O100:H32	**O112ab:H2**	O123:H19	O145:H4	O163:H25	**Orough:H5**
O6:H34	O22:H⁻	O46:H2	O78:H⁻	**O101:H⁻**	O112:H19	O123:H49	**O145:H8**	**O165:H⁻**	**Orough:K1:H6**
O6:H49	**O22:H1**	**O46:H31**	**O79:H7**	O101:H9	O112:H21	O124:H⁻	O145:H16	O165:H10	Orough:K1:H7
O7:H4	**O22:H5**	O46:H38	O79:H14	O102:H6	O113:H2	O125:H⁻	[redacted]	**O165:H19**	**Orough:H11**
O7:H8	**O22:H8**	**O48:H21**	O79:H23	[redacted]	**O113:H4**	O125:H8	O145:H26	O165:H21	**Orough:H16**
O8:H⁻	O22:H16	**O49:H⁻**	O80:H⁻	[redacted]	O113:H5	**O125:H?**	[redacted]	**O165:H25**	Orough:H18
O8:H2	O22:H40	**O49:H10**	O81:H?	O103:H4	O113:H7	O126:H⁻	O145:H46	O166:H12	Orough:H20
O8:H9	O23:H7	O50:H⁻	O82:H⁻	O103:H6	**O113:H21**	O126:H2	O145:HNT	O166:H15	Orough:H21
O8:H11	O23:H16	**O50:H7**	O82:H5	O103:H7	O113:H32	O126:H8	O146:H⁻	O166:H28	Orough:H28
O8:H14	O23:H21	O51:H49	O82:H8	O103:H11	O113:H53	O126:H11	**L**	O168:H⁻	Orough:H46
O8:H19	O25:H⁻	O52:H19	O83:H⁻						

yes, they are!!

Scheutz & Strockbine. Genus: *Escherichia* Bergey's Manual Syst. Bacteriol (2005)

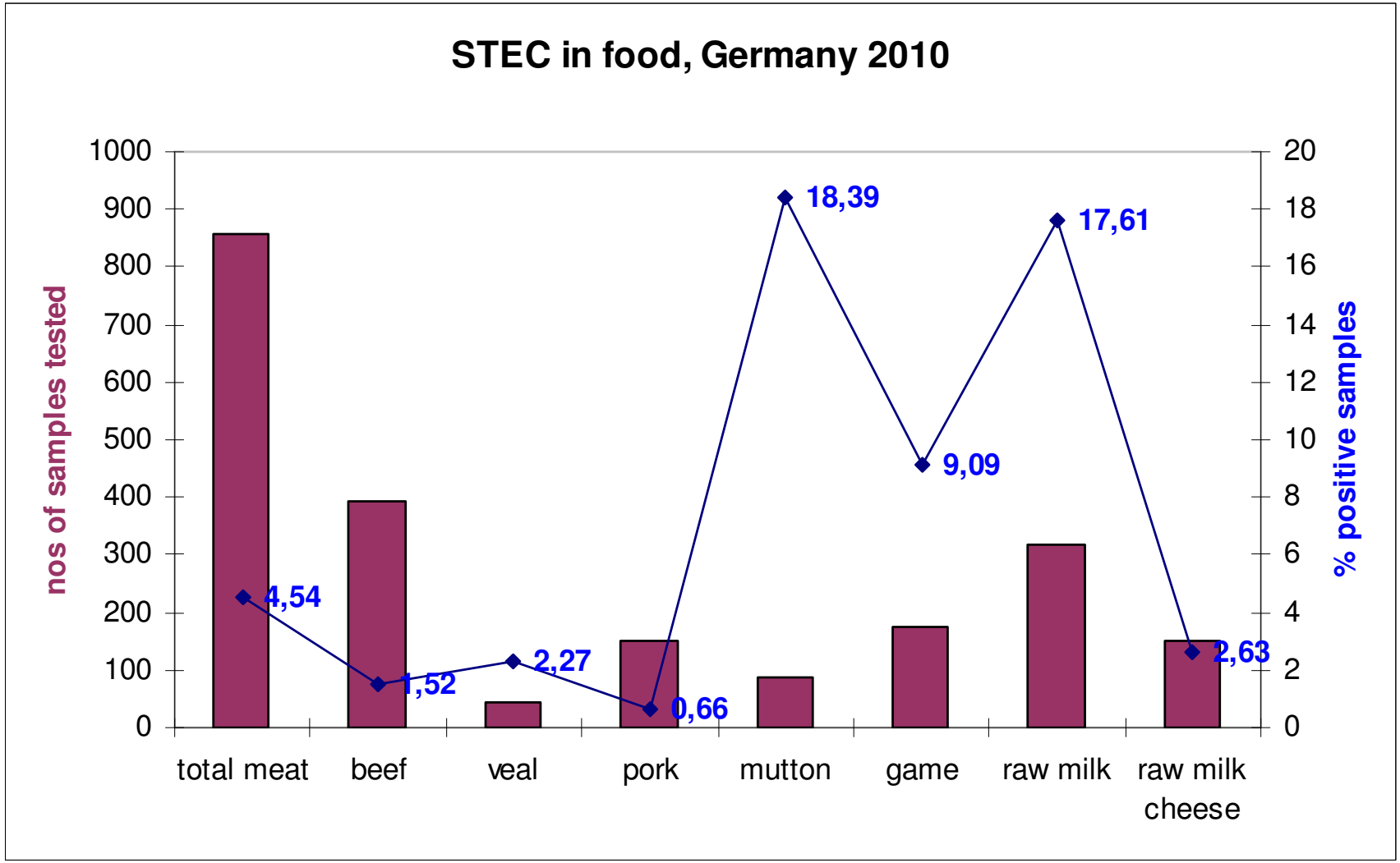
593 STEC from food isolated between 2005-2009

73 (19.5%) of 375 human pathogenic STEC serotypes are found in food

Martin A. & Beutin L.: Int. J. Food Microbiol. 146: 99 (2011)

Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype
O1:H-	O8:H21	O25:K2:H2	O52:H23	O83:H1	O103:H21	O114:H?	O127	O148:H28	O172:H-
O1:H1	O8:H25	O25:H14	O52:H25	O84:H-	O103:H25	O115:H10	O128:H-	O150:H-	O172:H?
O1:H2	O9ab:H-	O26:H-	O54:H21	O84:H2	O103:HNT	O115:H18	O128:H2	O150:H8	O173:H2
O1:H7	O9:H7	O26:H2	O55:H-	O84:H20	O104:H-	O116:H-	O128:H7	O150:H10	O174:H-
O1:H20	O9:H21	O26:H8	O55:H6	O85:H-	O104:H2	O116:H4	O128:H8	O152:H4	O174:H2
O1:HNT	O11:H-	O26:H11	O55:H7	O85:H10	O104:H7	O116:H10	O128:H10	O153:H2	O174:H8
O2:H-	O11:H2	O26:H12	O55:H9	O85:H23	O104:H16	O116:H19	O128:H12	O153:H11	O174:H21
O2:H1	O11:H8	O26:H32	O55:H10	O86:H-	O104:H21	O117:H-	O128:H25	O153:H12	O175:H16
O2:K1:H2	O11:H49	O26:H46	O55:H19	O86:H10	O105ac:H18	O117:H4	O128:H31	O153:H21	O176:H-
O2:H5	O12:H-	O27:H-	O55:H?	O86:H40	O105:H19	O117:H7	O128:H45	O153:H25	O177:H-
O2:H6	O14:H-	O27:H30	O60:H-	O87:H6	O105:H20	O117:K1:H7	O129:H-	O153:H30	O177:H11
O2:H7	O15:H-	O28ab:H-	O64:H25	O88:H-	O106	O117:H8	O130:H11	O153:H33	O178:H7
O2:H11	O15:H2	O28:H25	O65:H16	O88:H25	O107:H27	O117:H19	O131:H4	O154:H-	O179:H8
O2:H27	O15:H8	O28:H35	O68:H-	O89:H-	O109:H2	O117:H28	O132:H-	O154:H4	O181:H15
O2:H29	O15:H27	O30:H2	O69:H-	O90:H-	O109:H16	O118:H-	O133:H-	O154:H19/20	O181:H49
O2:H44	O16:H-	O30:H21	O69:H11	O91:H-	O110:H-	O118:H2	O133:H53	O156:H-	ONT:H-
O3:H10	O16:H6	O30:H41	O70:H11	O91:H4	O110:H19	O118:H12	O134:H25	O156:H4	ONT:H2
O4:H-	O16:H21	O37:H41	O71:H-	O91:H10	O110:H28	O118:H16	O137:H6	O156:H7	ONT:H8
O4:H5	O17:H18	O38:H21	O73:H34	O91:H14	O111:H-	O118:H30	O137:H41	O156:H25	ONT:H18
O4:H10	O17:H41	O38:H26	O74	O91:H15	O111:H2	O119:H-	O138:H2	O156:H27	ONT:H19
O4:H40	O18:H-	O39:H4	O75:H-	O91:H21	O111:H7	O119:H5	O141:H-	O156:HNT	ONT:H21
O5:H-	O18:H7	O39:H8	O75:H1	O91:H40	O111:H8	O119:H6	O141:H2	O157:H7	ONT:H25
O5:H16	O18:H12	O39:H28	O75:H5	O91:HNT	O111:H11	O119:H25	O141:H8	O157:NM	ONT:H41
O6:H-	O18:H15	O40:H2	O75:H8	O92:H3	O111:H21	O120:H19	O142	O160:H?	ONT:H47
O6:H1	O18:H?	O40:H8	O76:H7	O92:H11	O111:H30	O121:H-	O143:H-	O161:H-	ONT:K39:H48
O6:H2	O20:H-	O41:H2	O76:H19	O95:H-	O111:H34	O121:H8	O144:H-	O162:H4	Orough:H-
O6:H4	O20:H7	O41:H26	O77:H-	O96:H10	O111:H40	O121:H11	O145:H-	O163:H-	Orough:H2
O6:H12	O20:H19	O44	O77:H4	O98:H-	O111:H49	O121:H19	O145:H4	O163:H19	Orough:H5
O6:H28	O21:H5	O45:H-	O77:H7	O98:H8	O111:H?	O123:H19	O145:H8	O163:H25	Orough:K1:H6
O6:H29	O21:H8	O45:H2	O77:H18	O100:H25	O112ab:H2	O123:H49	O145:H16	O165:H-	Orough:K1:H7
O6:H31	O21:H?	O45:H7	O77:H41	O100:H32	O112:H19	O124:H-	O145:H25	O165:H10	Orough:H11
O6:H34	O22:H-	O46:H2	O78:H-	O101:H-	O112:H21	O125:H-	O145:H26	O165:H19	Orough:H16
O6:H49	O22:H1	O46:H31	O79:H7	O101:H9	O113:H2	O125:H8	O145:H28	O165:H21	Orough:H18
O7:H4	O22:H5	O46:H38	O79:H14	O102:H6	O113:H4	O125:H?	O145:H46	O165:H25	Orough:H20
O7:H8	O22:H8	O48:H21	O79:H23	O103:H-	O113:H5	O126:H-	O145:HNT	O166:H12	Orough:H21
O8:H-	O22:H16	O49:H-	O80:H-	O103:H2	O113:H7	O126:H2	O146:H-	O166:H15	Orough:H28
O8:H2	O22:H40	O49:H10	O81:H?	O103:H4	O113:H21	O126:H8	L	O160:H28	Orough:H46
O8:H9	O23:H7	O50:H-	O82:H-	O103:H6	O113:H32	O126:H11	O146:H11	O168:H-	
O8:H11	O23:H16	O50:H7	O82:H5	O103:H7	O113:H53	O126:H20	O146:H14	O169:H-	
O8:H14	O23:H21	O51:H49	O82:H8	O103:H11	O114:H4	O126:H21	O146:H21	O171:H-	
O8:H19	O25:H-	O52:H19	O83:H-	O103:H18	O114:H48	O126:H27	O146:H28	O171:H2	

STEC in different food matrices, Germany 2010



M. Hartung & A. Käsbohrer: Fleischwirtschaft 91 (12), S. 101 -108, (2011)

The EHEC virulence (HUS / HC) associated Stx2acd gene is present in more than 40% of foodborne STEC

TABLE 4. Frequencies and combinations of *stx* genotypes in STEC strains from food

stx gene(s)[a]	No. (%) of strains
stx_1	22 (10.0)
stx_{1c}	16 (7.3)
stx_{1d}	3 (1.4)
$stx_1 + stx_2/stx_{2d}$	37 (16.9)
$stx_1 + stx_{2\text{-}O118}$	2 (0.9)
$stx_{1c} + stx_{2\text{-}O118}$	8 (3.7)
stx_2/stx_{2d}	52 (23.7)
$stx_2/stx_{2d} + stx_{2\text{-}O118}$	3 (1.4)
$stx_2/stx_{2d} + stx_{2g}$	3 (1.4)
$stx_{2\text{-}O118}$	25 (11.4)
stx_{2e}	42 (19.2)
stx_{2g}	6 (2.7)

n= 219

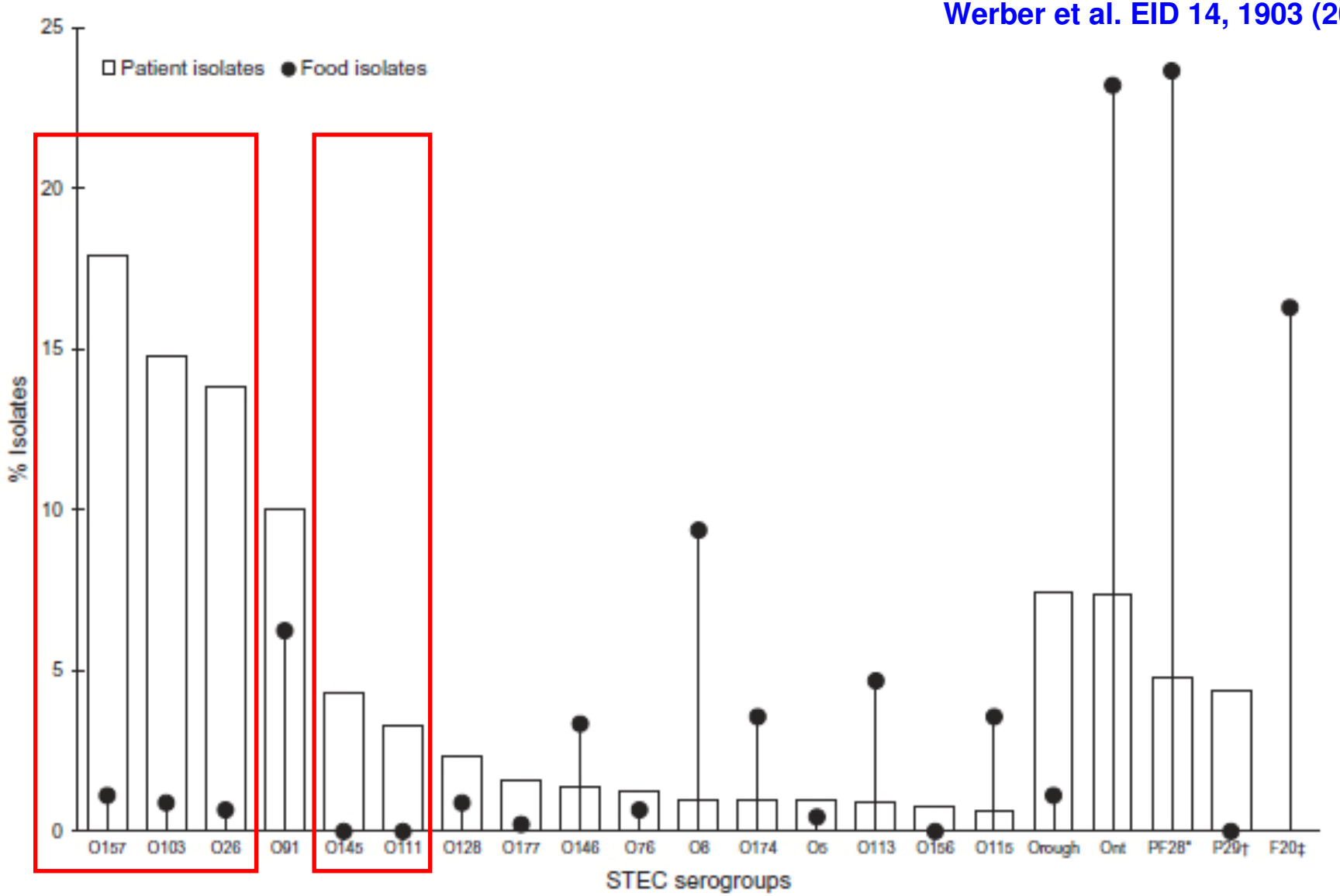
Beutin et al. Appl Environ Microbiol. 2007 73:4769-75.

Stx2a present in the most frequently isolated STEC serotypes from food but these are all negative for the A/E adherence mechanism

Martin A. & Beutin L.: Int J Food Microbiol 146: 99 (2011)

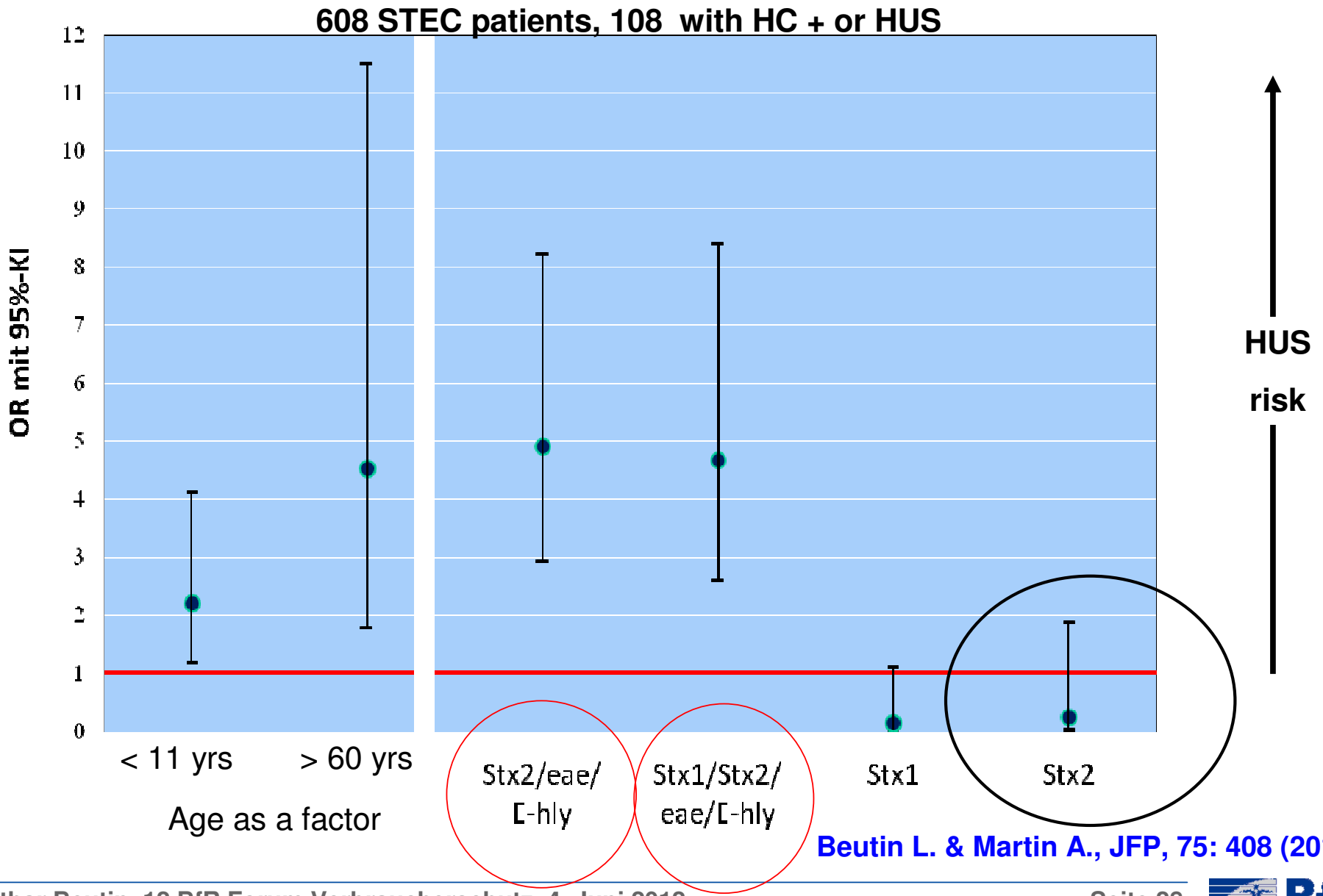
The top foodborne A/E negative STEC are rarely isolated from human patients and not involved in HUS and HC

Werber et al. EID 14, 1903 (2008)



classical EHEC types

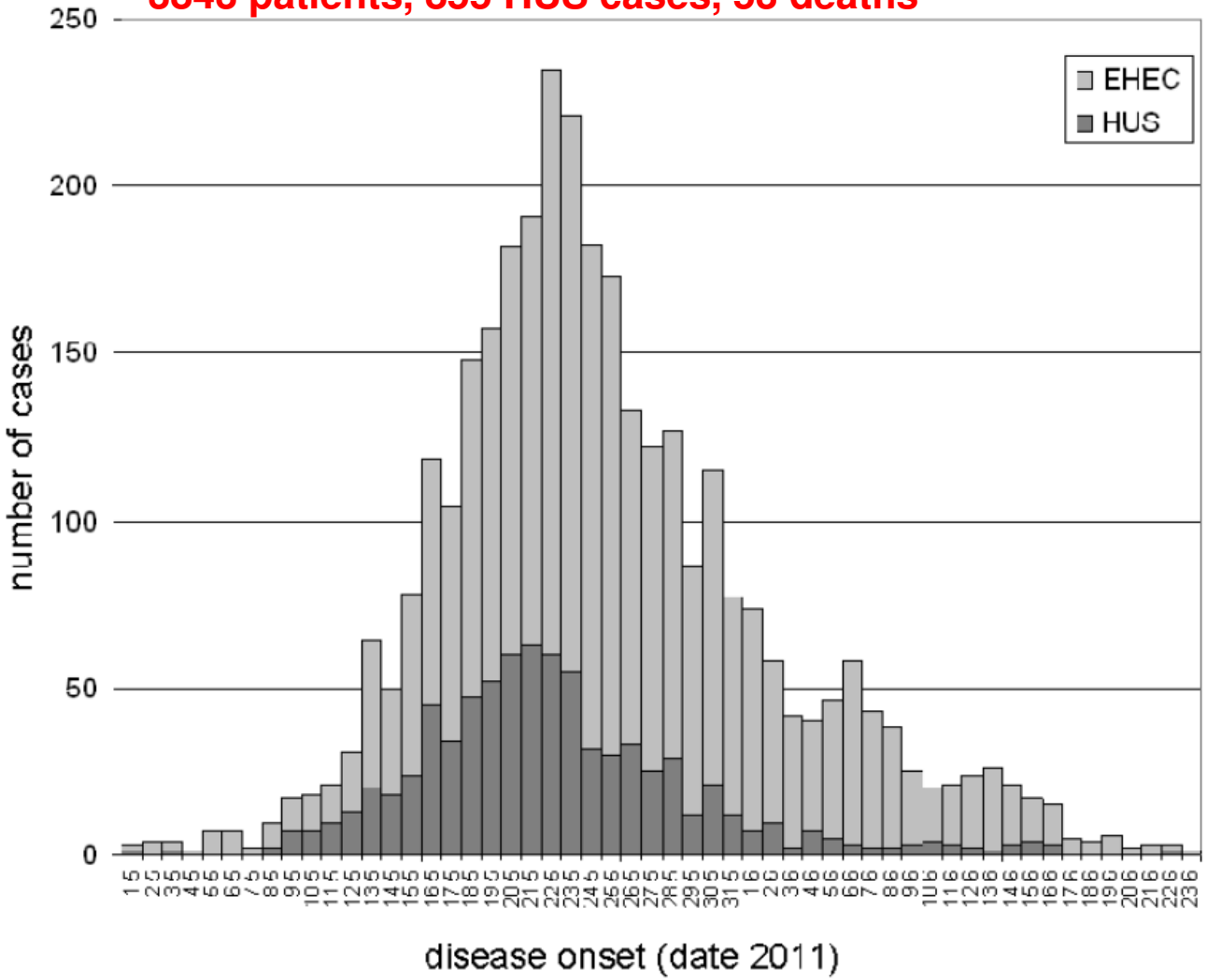
The combination of Stx2 and the A/E adherence increases the risk for HC and HUS in STEC patients



Beutin L. & Martin A., JFP, 75: 408 (2012)

Paradigm change: STEC O104:H4 outbreak in Germany 2011:
High virulence of an Stx2a positive, A/E (LEE) negative strain

3843 patients, 855 HUS cases, 53 deaths



From: Robert Koch Institute: Technical Report EHEC/HUS O104:H4 outbreak Germany, May/June 2001

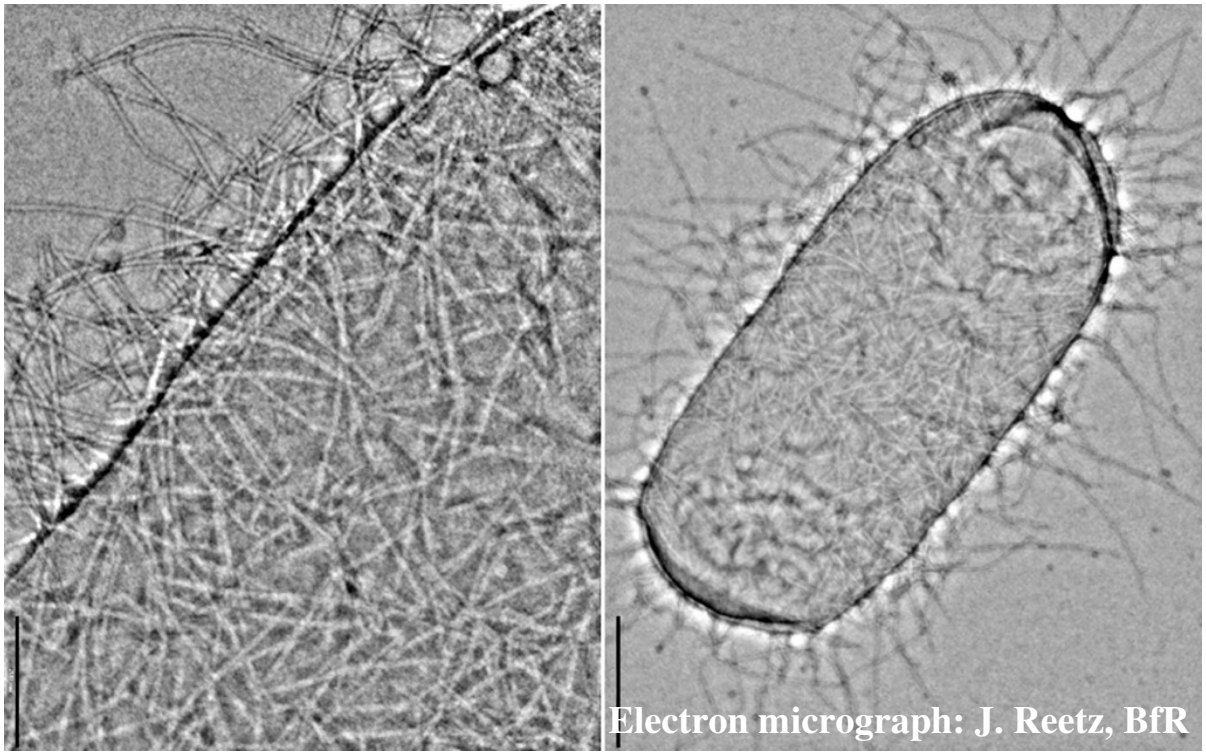
STEC O104:H4: same clinical picture as with classical EHEC

STEC O104:H4 resembles those STEC that are frequently found in food but rarely in human patients with severe disease

What makes STEC O104:H4 strains so different from many other LEE negative STEC strains?

STEC O104:H4 are derivatives of enteroaggregative *E. coli* (EAEC) carrying an Stx2a encoding bacteriophage

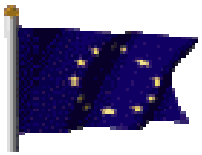
A new *E. coli* pathovar: Enteroaggregative hemorrhagic *E. coli* (EAHEC) O104:H4



Aggregative adherence (AAF/I) Pili on the surface of EAHEC O104:H4

Beutin L. & Martin A., JFP, 2012

STEC in food: situation in the EU



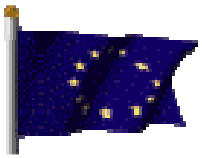
Not all EU member states search for all types of STEC types, results reported are diverging between different member states

The methods used for STEC detection vary between different member states

The food samples chosen for STEC monitoring vary between member states.

little is known on the frequency of non-O157 STEC in domestic and wildlife animals.

ISO TS 13136: restriction to five major EHEC types



Vote requested: ISO/TS 13136 (STEC) Microbiology of food and animal feed -- Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens -- Horizontal method for the detection of Shiga toxin-producing Escherichia coli (STEC) belonging to O157, O111, O26, O103 and O145 serogroups

limited to major EHEC strains

O26, O103, O111, O145, O157

The big five group!

In 2010, about half (46.6%) of human STEC isolates in the EU are not belonging to the „big five“ group!

Table VT3. Reported confirmed VTEC cases in humans by serogroup (top 10), 2009-2010

2010			EFSA Journal 2012;10(3):2597	2009	
Serogroup	**No. of cases**	**% total**	**Serogroup**	**No. of cases**	**% total**
O157	1,501	41.1	O157	1,848	51.7
NT[1]	1,230	33.7	NT1	1,008	28.2
O26	257	7.0	O26	192	5.4
O103	90	2.5	O103	82	2.3
O145	61	1.7	O91	48	1.3
O91	57	1.6	O145	47	1.3
O63	42	1.2	O146	31	0.9
O111	41	1.1	O128	26	0.7
O128	29	0.8	O111	25	0.7
O146	28	0.8	O113	22	0.6
Other[2]	315	8.6	Other [2]	244	6.8
Total	**3,651**		**Total**	**3,573**	

1. NT = untyped/untypeable.
2. Other included 8 (2010) and 12 (2009) confirmed cases where antigen O was reported as unknown.

Source: Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Luxembourg, Malta, Netherlands, Poland, Romania, Slovakia, Slovenia, Spain, Sweden and United Kingdom (N=3,651).

STEC in food: opinion of the FDA

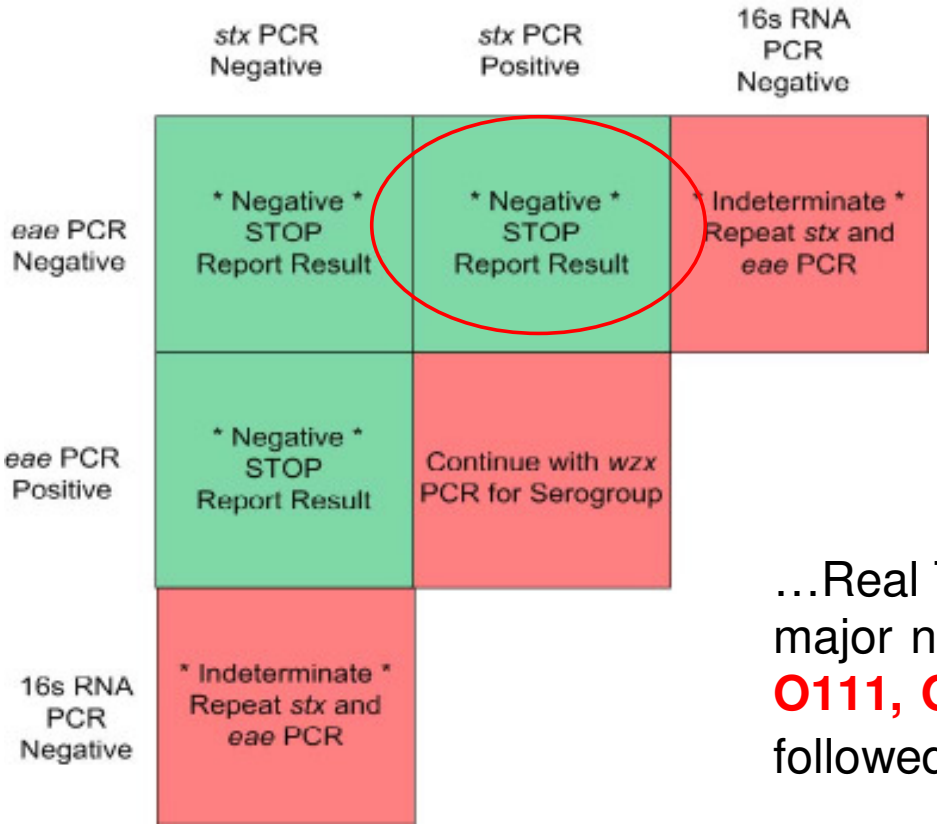
The ability of STEC to cause disease is dependent on combination of virulence genes and the ability to express them

Based on the current state of the science (high uncertainty) it is **unlikely that the simple detection of an isolate with a *stx* gene would be sufficient to take action against food**

Robert L. Buchanan HHS Food and Drug Administration Center for Food Safety and Applied Nutrition

Laboratory Guidebook MLG 5B.01
Looking only for the „Big Seven“ (for meat only!!)

USDA



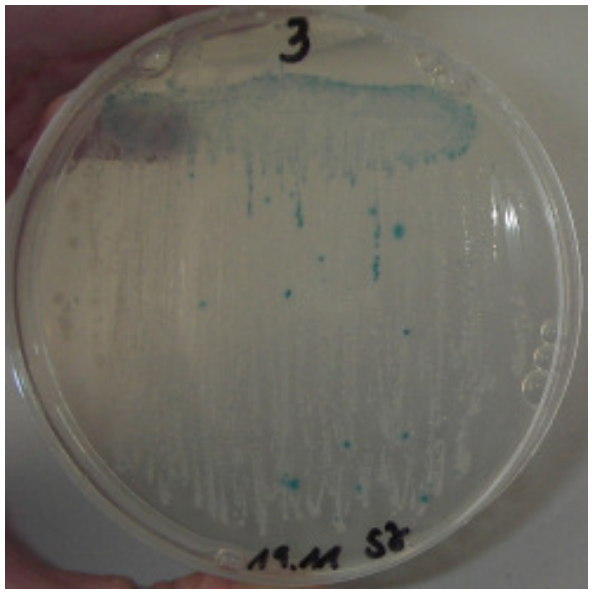
…CDC study showed that from 1983-2002 approx. 70% of non-O157 STEC infections were caused by strains from one of six major serogroups…

…Real Time-PCR method to detect and isolate the six major non-O157 STEC serogroups **(O26, O45, O103, O111, O121 and O145**) in ground beef and beef trim followed by cultural isolation.

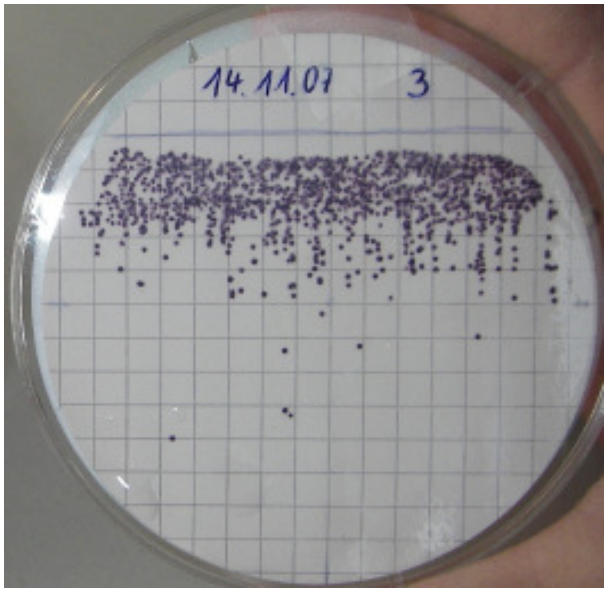
STEC in food: Austria + Germany: Looking for all!

STEC form a phenotypically heterogeneous group

No common traits except for Stx-production / *stx*-gene(s)



Bacteria from meat after 6h enrichment plated on Chromagar E. coli



Stx-specific colony immunoblot from the enrichment culture

Problems with testing for all types STEC in food

1. Detection and isolation of all STEC types in food is labourious, (colony immunoblot, colony hybridzation method).

2. Only very few EU member states are searching for all STEC types in food. Some EU members are only screening for *stx* and *eae* positive STEC in food. Legal problems?

3. Search for all STEC can be time consuming and costly (the procedure may exceed shelf life of food). As a consequence only a limited number of food samples can be tested.

4. STEC not considered as pathogenic for humans (for example Stx2e strains) are treated equally with highly pathogenic EHEC strains. Economical impact?

Future research needs for defining human virulent STEC:

The human virulence of a given STEC strain depends on the **production of Stx and on the effective colonization** of the human host.

EHEC possess both an colonization mechanism **(A/E) and Stx production**

EAHEC have substituted the A/E colonization mechnism by the AAF mechanism shown to be as well effective for producing severe disease in humans.

More research is needed to find other colonization systems in *eae* and AAF negative STEC which enable these to colonize humans efficiently.

BUNDESINSTITUT FÜR RISIKOBEWERTUNG

BfR

Risiken erkennen – Gesundheit schützen

Federal Institute for Risk Assessment

National Reference Laboratory for Escherichia coli

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