Zoonosis from the ground

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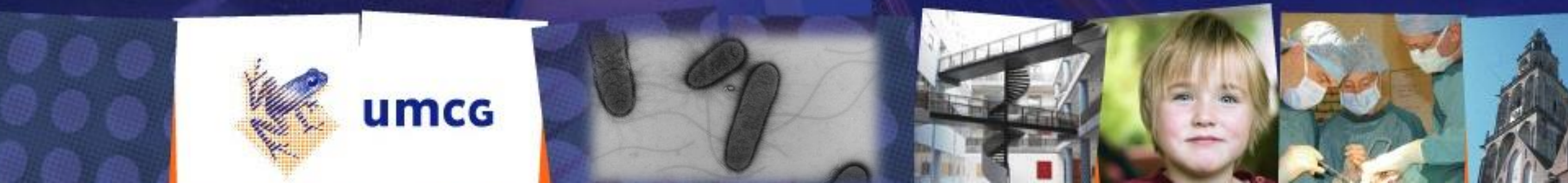
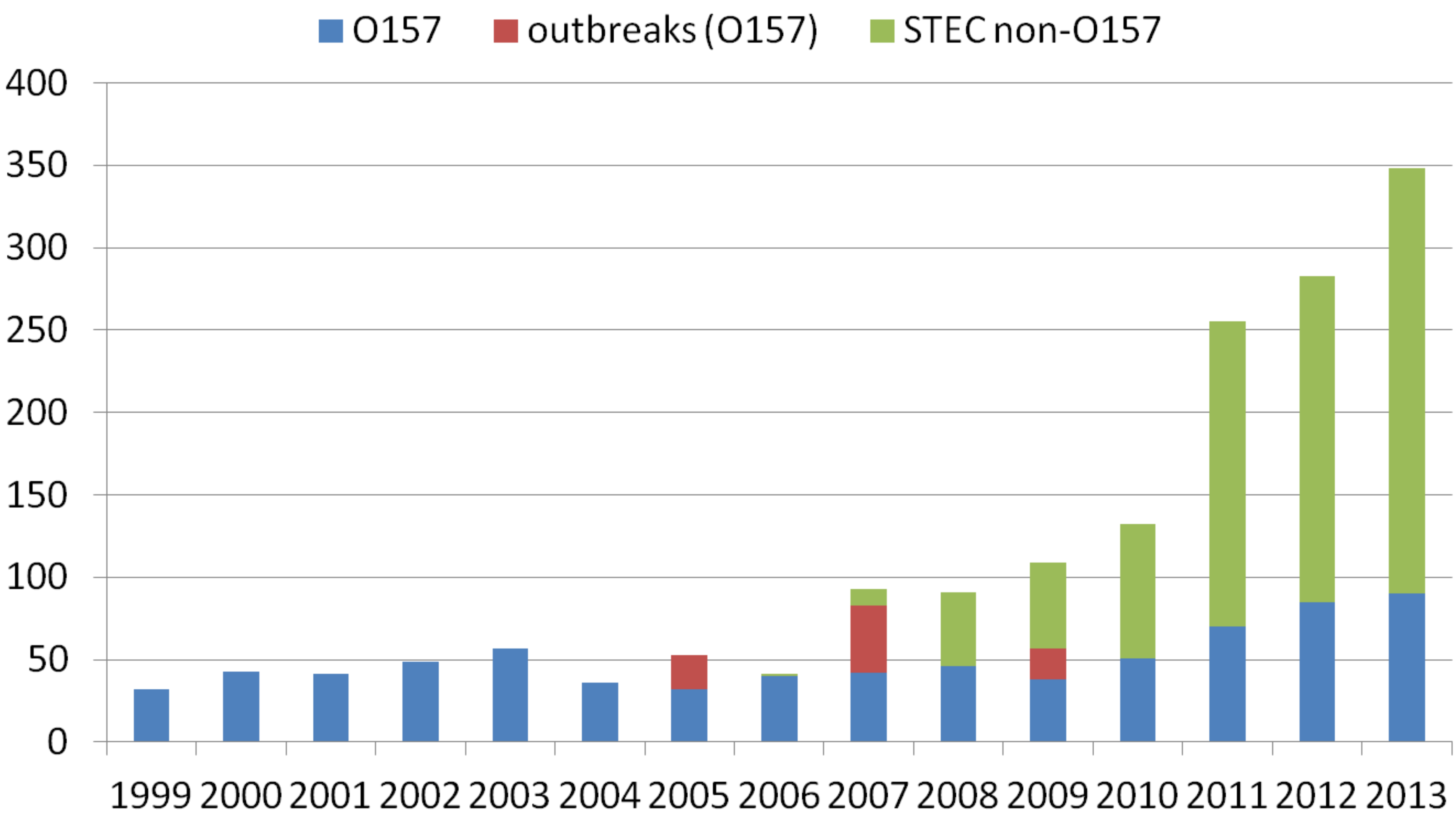


Reported hospitalisation and case-fatality rates due to zoonoses in confirmed human cases in the EU, 2011

Zoonotic Diseases	Causative agent	Confirmed human cases	Reported hospitalised cases	Hospitalisation rate (%)	Reported deaths	Case fatality rate (%)
Campylobacteriosis	Campylobacter spp.	220,209	8,137	47.9	43	0.04
Salmonellosis	Salmonella spp.	95,548	4,557	45.7	56	0.12
STEC infections	Shiga toxin-producing *E. coli*	9,485	721	33.8	56	0.75
Yersiniosis	Yersinia spp.	7,017	427	55.2	1	0.02
Listeriosis	L. monocytogenes	1,476	604	93.6	134	12.7
Echinococcosis	Echinococcus spp.	781	96	67.6	2	0.90
Brucellosis	Brucella spp.	330	118	66.3	1	0.74
Trichinellosis	Trichinella spp.	268	153	74.3	1	0.49

EFSA Journal 2013;11(4):3129

STEC reports in the Netherlands



Data: Ingrid Friesema, RIVM

Infections due to *E. coli*

Urinary tract infections

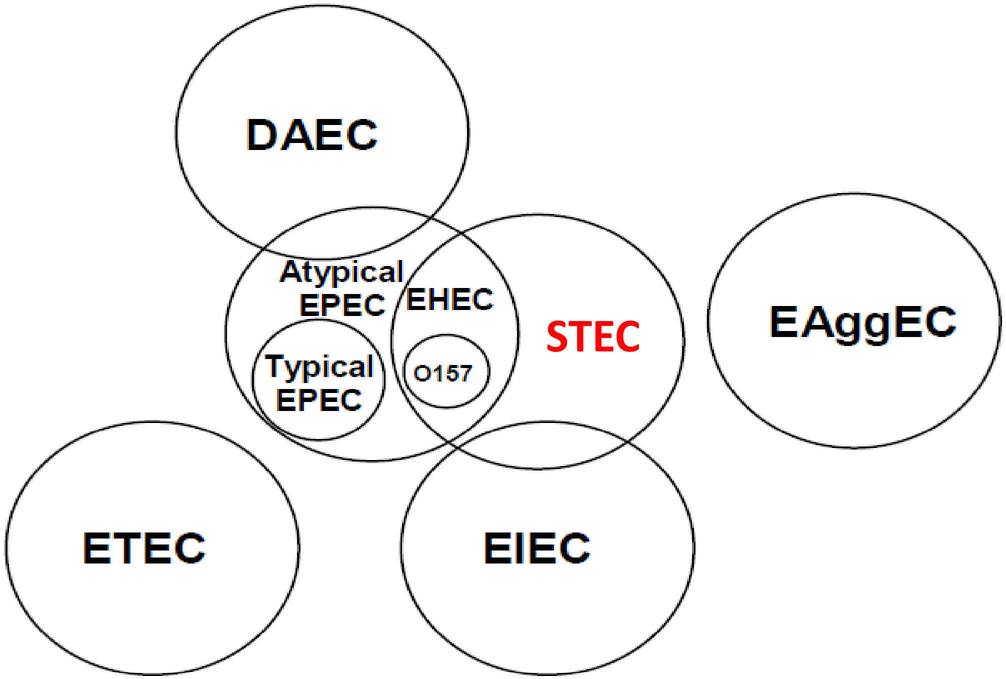
Neonatal encephalitis

Enteropathogenic *E. coli*

Other opportunistic infections

Enteropathogenic *E. coli*

- Enterotoxigenic *E. coli* (ETEC)
- Enteropathogenic *E. coli* (EPEC)
- Shiga toxin-producing (STEC) /Enterohaemorrhagic *E. coli* (EHEC)/ VTEC
- Enteroaggregative *E. coli* (EAEC)
  - children in 3rd world, in HIV patients, chron. Diarrhea. Malnutrition, highly antibiotic resistant
- Enteroinvasive *E. coli* (EIEC)



http://www.antimicrobialresistance.dk/data/images/protocols/e%20coli%20methods.pdf

EHEC O157:H7 outbreak in Canada

7 died, 2000 with diarrhea
cause: contaminated water

Clinical importance and danger to public health

- HUS (most frequent cause for acute renal failure)

Table 7 Reported food- and waterborne *E. coli* outbreaks to EFSA in accordance with Directive 2003/99/EC in 2004–2009

STEC/VTEC/EHEC outbreaks	2009	2008	2007	2004–2006
Food-borne outbreaks	75	75	61	195
Waterborne outbreaks	5	4	4	5
Human cases in food-borne outbreaks	595	339	479 (includes only verified outbreaks)	2 345 (data missing from some outbreaks)
Human cases in waterborne outbreaks	12	22	62	26

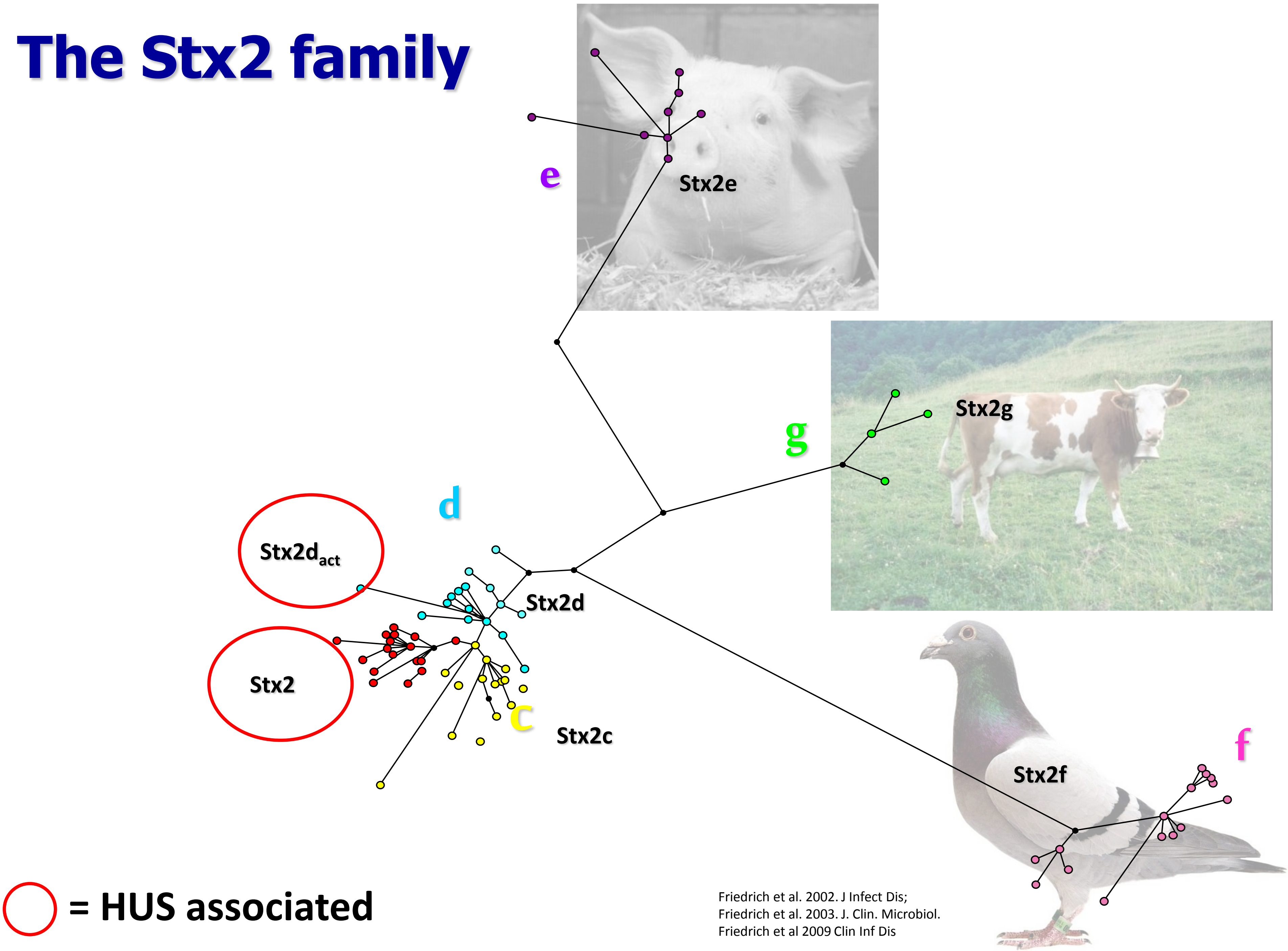
- No specific therapy
- No specific prevention (e.g. vaccination)
- Complex diagnostics

(Zoonotic) STEC Infection sources

HUS-association of molecular markers in EHEC diagnostics

PCR results				Percentage of pos. PCR on pat with HUS	*E. coli* isolates
sfpA	*rfbO157*	*eae*	*stx*		
+	+	+	+	81%	SF O157:HNM
-	+	+	+	60%	NSF O157:H7
-	-	+	+	25%	non O157 EHEC
-	-	-	+	13%	STEC
-	-	+	-	0%	EPEC

The Stx2 family



Friedrich et al. 2002. J Infect Dis;
Friedrich et al. 2003. J. Clin. Microbiol.
Friedrich et al 2009 Clin Inf Dis

EHEC: major concern of food born outbreak

✓ Common O-serogroups reported to cause foodborne illness are

O157:H7/HNM
O26,
O45
O103,
O104:H4
O111,
O121 and
O145

All eae +, except …

- **Asscociation with raw fruits & vegetables?**

EHEC on fruits and veg

1997	*E. coli* O157:H7	Sprouted seeds (alfalfa)	Sivaplasingham *et al.* 2004
1997	*E. coli* O157:H7	Salad	Anon (2005a)
1998	*E. coli* O157:H7	Salad	Anon (2001a,b)
1998	*E. coli* O157:H7	Fruit salad	Anon (2001a,b)
1998	*E. coli* O157:H7	Coleslaw	Anon (2001a,b)
1998	*E. coli* O157:H7	Sprouted seeds (clover/alfalfa)	Taormina *et al.* 1999
1998	*E. coli* O157:H7	Unpasteurized apple juice	Anon (2001a,b)
1998	*E. coli* O157:H7	Parsley	Sivaplasingham *et al.* 2004
1999	*E. coli* O157:H7	Coriander (cilantro)	Campbell *et al.* 2001
1999	*E. coli* O157:H7	Unpasteurized apple juice	Anon (2001a,b)
2003	*E. coli* O157:H7	Cucumber	Duffell *et al.* (2003)
2003	*E. coli* O157:H7	Lettuce	Anon (2005a)
2005	*E. coli* O157:H7	Lettuce	Söderström *et al.* (2005)
2006	*E. coli* O157:H7	Spinach	CDC (2006b)
2006	*E. coli* O157:H7	Lettuce	CDC (2006c)

Heaton and Jones, J Appl Microbiol 2007

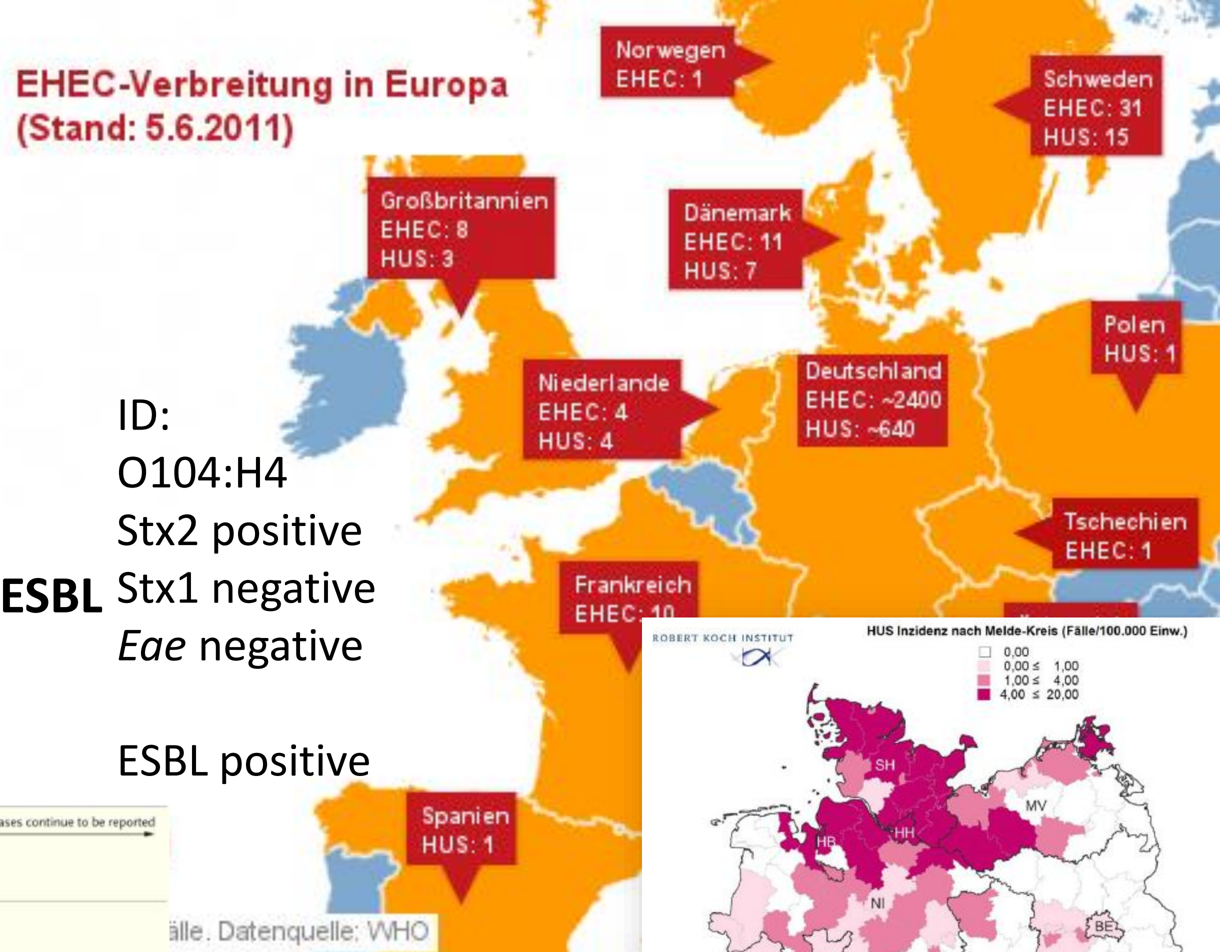
EHEC O104:H4 outbreak
In Germany

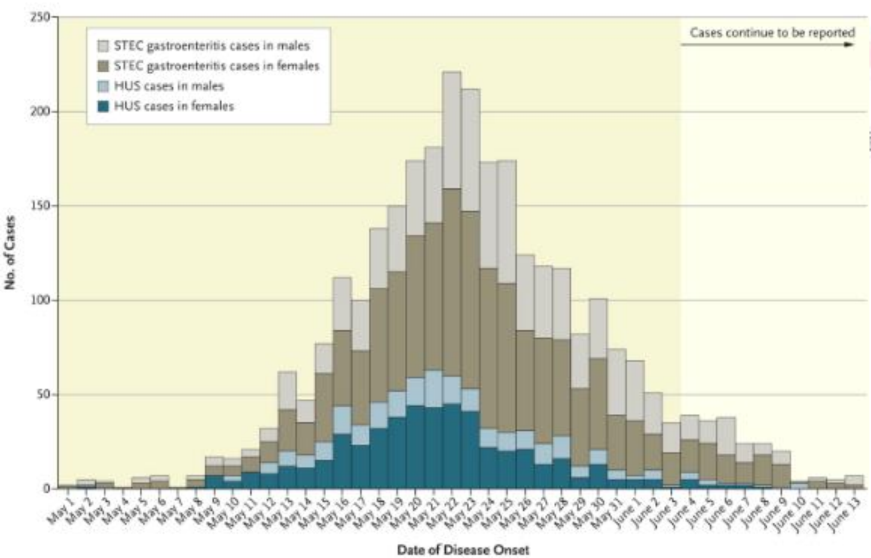
- **>5922 cases**
- **>850 HUS**
- **53 patients died**

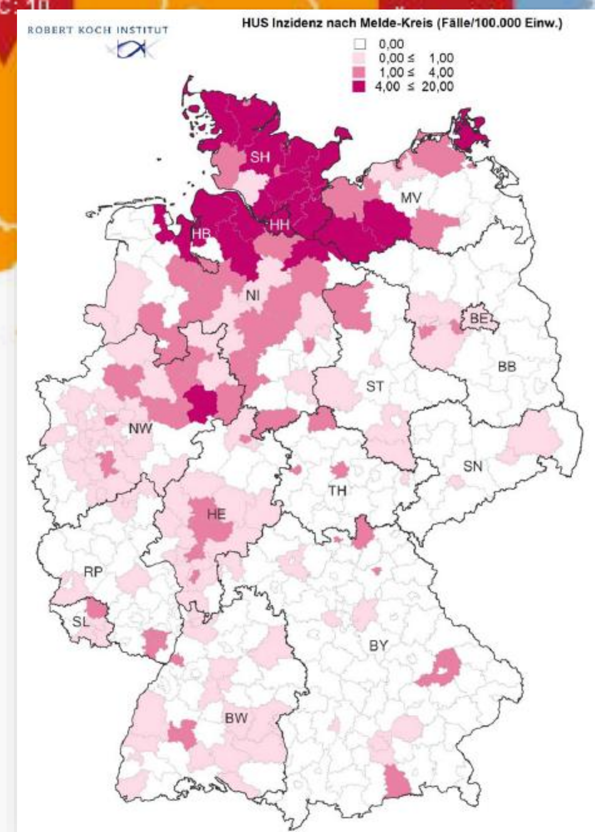
- **E. coli O104:H4 HUSEC41/ESBL**

ID:
O104:H4
Stx2 positive
Stx1 negative
Eae negative

ESBL positive







Frank et al. 2011 N Engl J Med

De Telegraaf **BUITENLAND**

Doelwit Libië
Atoomangst Japan
Video's Buitenland

Buitenland RSS

21:23 President Obama in Joplin
19:41 Jonathan beëdigd
19:25 Plattegrond Titanic geveild
18:56 Aardappelveld vernield
18:43 Duitse klinieken overvol
18:42 Betoging tegen arrestatie
17:44 Opnieuw bloedig optreden Syrische troepen
17:30 Crisisoverleg EHEC in Berlijn
16:26 EHEC in Scandinavië
16:04 Minister weg om seksaffaire

zo 29 mei 2011, 18:43 | 86 reacties

Duitse klinieken overbelast door darmbacterie

HAMBURG - Duitsland krijgt de gevaarlijke darmbacterie EHEC maar niet onder controle. Het aantal patiënten loopt gestaag op. Veel van hen zijn er erg slecht aan toe. Ziekenhuizen zijn overvol en het bloed voor transfusies raakt op.

Foto: EPA

EHEC brak enkele weken geleden uit en verspreidde zich snel over Duitsland. De bacterie kan darmbloedingen, hevige diarree, misselijkheid, koorts, buikkrampen, bloedarmoede en nierfalen veroorzaken. Bijkomend probleem is dat de Duitse bacterie agressiever dan gebruikelijk is en bestand blijkt tegen veel gangbare antibiotica.

Inmiddels zijn er al meer dan duizend gevallen bekend, terwijl de bacterie normaal gesproken ongeveer negenhonderd mensen in een heel jaar treft. Gedurende het weekeinde zijn vier patiënten overleden: twee vrouwen in Kiel (38 en 84), een 86-jarige dame in Lübeck en een 87-jarige vrouw in Hamburg. Daarme... de Duitse autoriteiten gaan ervan uit dat het daar ...

Ziekenhuizen kunnen de toestroom patiënten nau... academisch ziekenhuis van Hamburg tachtig men... op is berekend. Bij dertig patiënten werken de niere... mensen verliezen. We moeten er rekening mee hou... bezwijken", geeft ziekenhuisdirecteur Jörg Debatin ...

UMCG klaar voor EHEC-patiënten

ACHTERGROND
EHEC IN NEDERLAND

- RIVM: Geen zorgen over EHEC-bacterie in Nederland
- UMCG bereid Duitse patiënten op te nemen

Besmette Nederlanders liepen darminfectie op in Duitsland

▲ De consument laat komkommers links liggen. Foto: ANP/Koen Suyk

'Roken en autorijden veel gevaarlijker'

- Paniek rond komkommer volgens groentetelers onnodig

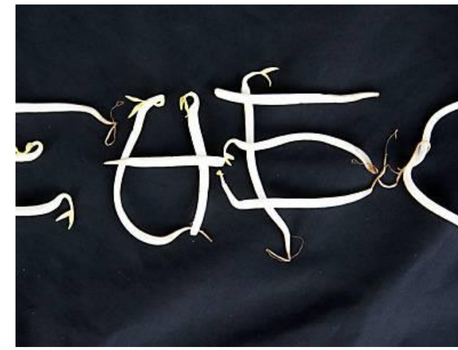
Waar komen de komkommers vandaan?

Mission uncompleted…

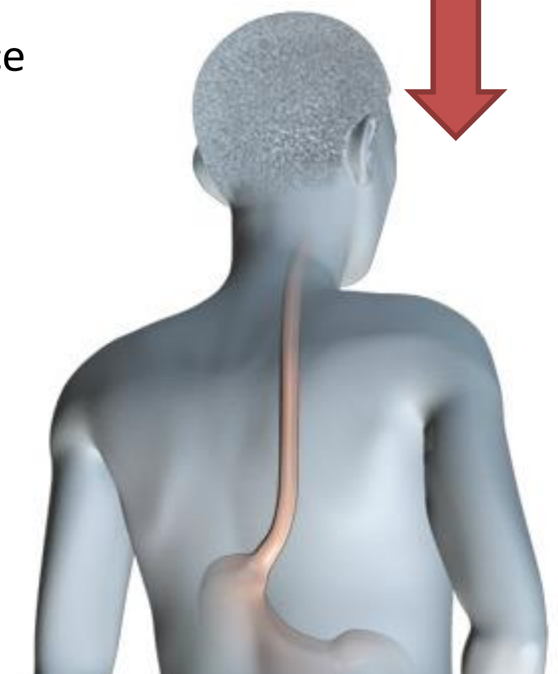
Reservoir
???

Primary source
„fennugreek“ et al.

outbreak source



2nd source



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Why Sprouts ??

- Seeds and beans need warm and humid conditions to sprout and grow
- These sprouting conditions are also ideal for the growth of bacteria such as *E. coli*

http://www.efsa.europa.eu/en/faqs/faqecolishigatoxin.htm

Tenacity of EHEC

Pathogen	Environment	Survival (day)	References
Escherichia coli O157:H7	Soil + animal manure	30	Nicholson *et al.* (2005)
E. coli O157:H7	Soil + animal manure	99	Nicholson *et al.* (2005)
E. coli O157:H7	Animal manure	60	Avery *et al.* (2005)
E. coli O157:H7	Slurries	60	Avery *et al.* (2005)
E. coli O157:H7	Abattoir waste	60	Avery et al. (2005)
E. coli O157:H7	Sewage sludge	60	Avery et al. (2005)
E. coli O157:H7	Nonaerated ovine manure	>365	Kudva *et al.* (1998)
E. coli O157:H7	Aerated ovine manure	120	Kudva *et al.* (1998)
E. coli O157:H7	Nonaerated slurry	600	Kudva *et al.* (1998)
E. coli O157:H7	Aerated slurry	30	Kudva *et al.* (1998)
E. coli	Slurry + dirty water	90	Nicholson *et al.* (2005)

Heaton and Jones, J Appl Microbiol 2007

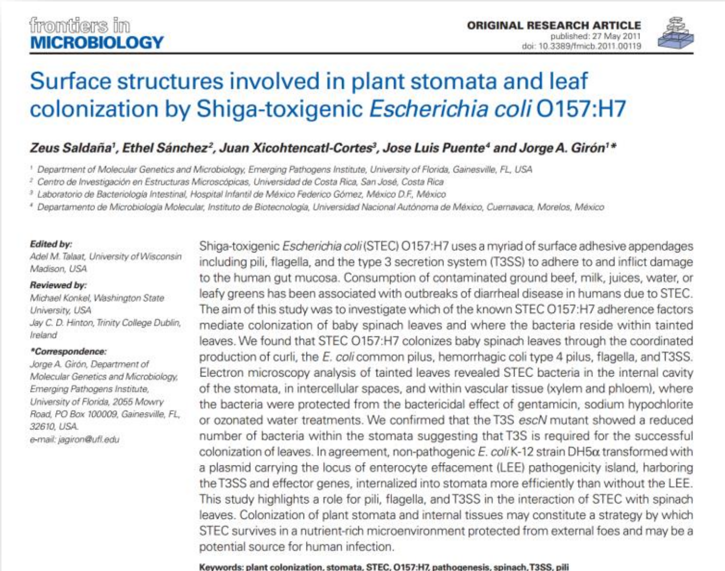
Prevention of Gastrointestinal Zoonotic diseases

Keep cleaning

Food safety

Animal Contact

In and not on plants



frontiers in MICROBIOLOGY

ORIGINAL RESEARCH ARTICLE
published: 27 May 2011
doi: 10.3389/fmicb.2011.00119

Surface structures involved in plant stomata and leaf colonization by Shiga-toxigenic *Escherichia coli* O157:H7

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Shiga-toxigenic *Escherichia coli* (STEC) O157:H7 uses a myriad of surface adhesive appendages including pili, flagella, and the type 3 secretion system (T3SS) to adhere to and inflict damage to the human gut mucosa. Consumption of contaminated ground beef, milk, juices, water, or leafy greens has been associated with outbreaks of diarrheal disease in humans due to STEC. The aim of this study was to investigate which of the known STEC O157:H7 adherence factors mediate colonization of baby spinach leaves and where the bacteria reside within tainted leaves. We found that STEC O157:H7 colonizes baby spinach leaves through the coordinated production of curli, the *E. coli* common pilus, hemorrhagic coli type 4 pilus, flagella, and T3SS. Electron microscopy analysis of tainted leaves revealed STEC bacteria in the internal cavity of the stomata, in intercellular spaces, and within vascular tissue (xylem and phloem), where the bacteria were protected from the bactericidal effect of gentamicin, sodium hypochlorite or ozonated water treatments. We confirmed that the T3S *escN* mutant showed a reduced number of bacteria within the stomata suggesting that T3S is required for the successful colonization of leaves. In agreement, non-pathogenic *E. coli* K-12 strain DH5α transformed with a plasmid carrying the locus of enterocyte effacement (LEE) pathogenicity island, harboring the T3SS and effector genes, internalized into stomata more efficiently than without the LEE. This study highlights a role for pili, flagella, and T3SS in the interaction of STEC with spinach leaves. Colonization of plant stomata and internal tissues may constitute a strategy by which STEC survives in a nutrient-rich microenvironment protected from external foes and may be a potential source for human infection.

Keywords: plant colonization, stomata, STEC, O157:H7, pathogenesis, spinach, T3SS, pili

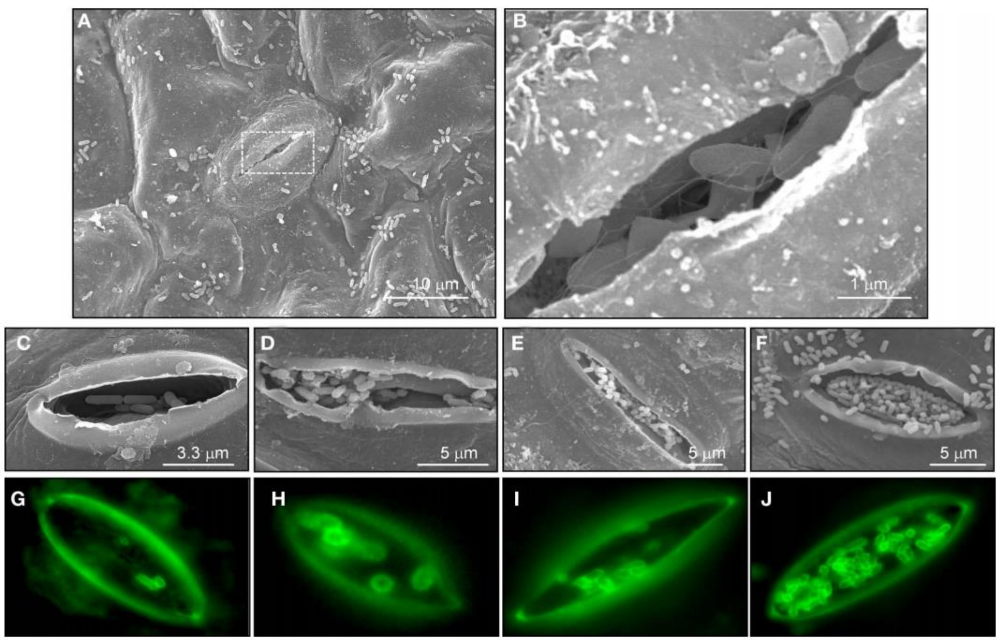


FIGURE 4 | Evidence of STEC in stomata. (A) Scanning electron micrograph showing bacteria on leaf epidermis at 6 h of infection. **(B)** High magnification of boxed area in **(A)** showing flagellate bacteria internalized in the stomata. **(C–F)** Micrographs (60X) of time-course EDL933 infection experiments between 3, 6, 12, and 24 h showing progressive association of bacteria with stomata. **(G–J)** Same experiment as before employing IFM and anti-0157 antibodies to stain bacteria (green).

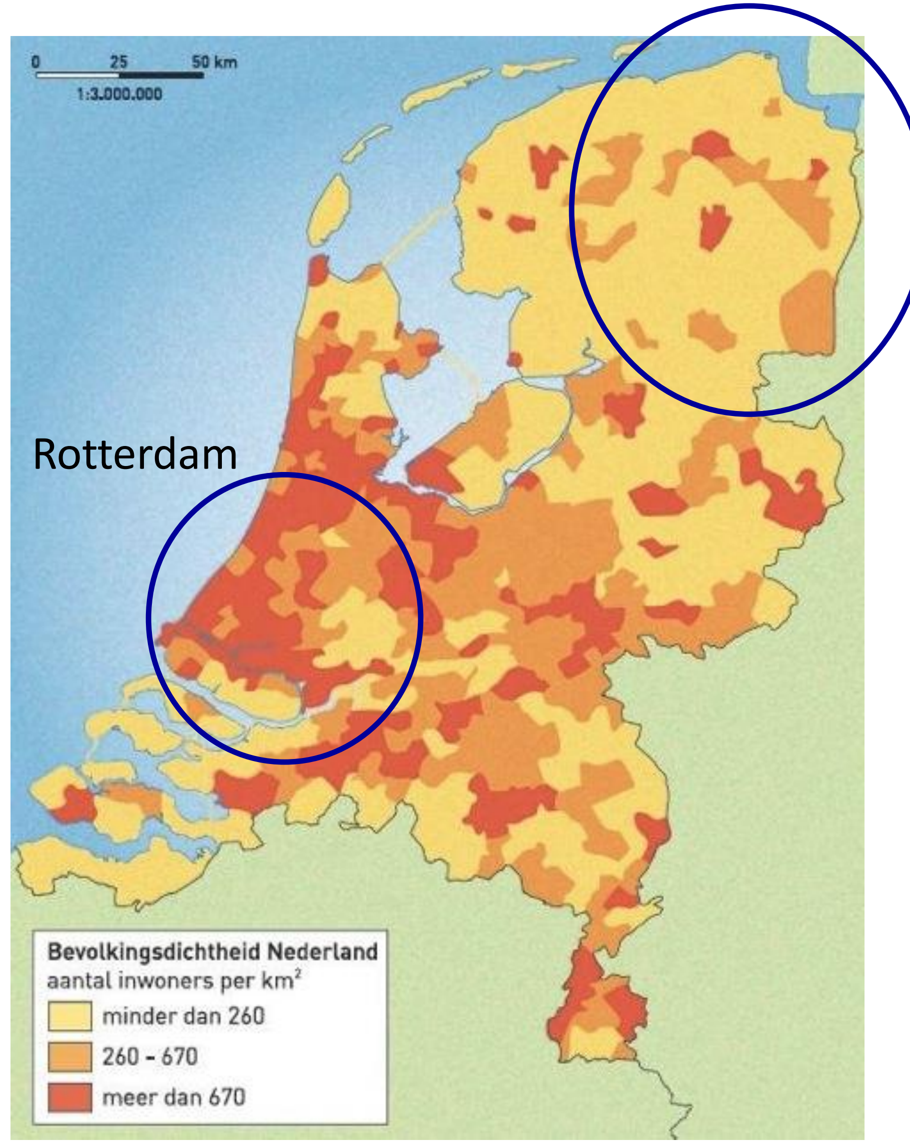
Stx-negative variants of EHEC O157

Outbreak no.	Year	Disease	No. of isolates		
			***stx*-positive**	***stx*-negative**	**Total**
1	2003	HUS	27	2	29
2	1995/96	HUS	12	1	13
3	2002	HUS	10	1	11
4	1993	D	1	6	7
5	1994	D	0	4	4
6	1994	D	0	3	3

Clin. Infect. Dis. 2007

STEC-ID-net

- Running multicenter study in NL
- 25.000 patients from GP with Diarrhea/BD/HUS
- Identify prevalence of STEC-EHEC/HUSEC
- Capacity building of STEC-diagnostics
- Create an expert network
- Microbiological risk assessment for Public Health
- Create Dutch HUSEC collection



John Rossen, UMCG

Mirjam Kooistra, CERTE

CERTE Medische Diagnostiek & Advies, Zeker voor zorg

umcg

rijksuniversiteit groningen

Star-Medisch Diagnostisch Centrum

Rijksinstituut voor Volksgezondheid en Milieu, Ministerie van Volksgezondheid, Welzijn en Sport

GGD Rotterdam-Rijnmond

GGD Groningen

GGd Drenthe

STEC-ID net: direct PCR-detection

April 2013 t/m Augustus 2013
Included patients: n= 9582 (Groningen n=6358; Rotterdam n=3224)

Target(s)	Groningen		Rotterdam	
	n	%	n	%
stx1	23	0.4	15	0.5
stx1,eae	30	0.5	22	0.7
stx2	23	0.4	12	0.4
stx2,eae	30	0.5	16	0.5
stx1,stx2	6	0.1	4	0.1
stx1,stx2,eae	13	0.2	7	0.2
eae	638	10.0	410	12.7
TOTAAL	763	12.1	468	15.1

Shiga toxin 2-encoding bacteriophages in human fecal samples from healthy individuals.

Alexandre Martinez-Castillo, Pablo Quirós, Ferran Navarro, Elisenda Miró, Maite Muniesa

Department of Microbiology, University of Barcelona, Diagonal 643, Annex, Floor 0, 08028 Barcelona, Spain.

Applied and environmental microbiology (Impact Factor: 3.69). 06/2013; DOI:10.1128/AEM.01158-13
Source: PubMed

ABSTRACT Shiga toxin-converting bacteriophages (Stx phages) carry the stx gene and convert non-pathogenic bacterial strains into Shiga toxin-producing bacteria. Previous studies have shown that high densities of free and infectious Stx phages are found in environments polluted with feces and also in food samples. Taken together, these two

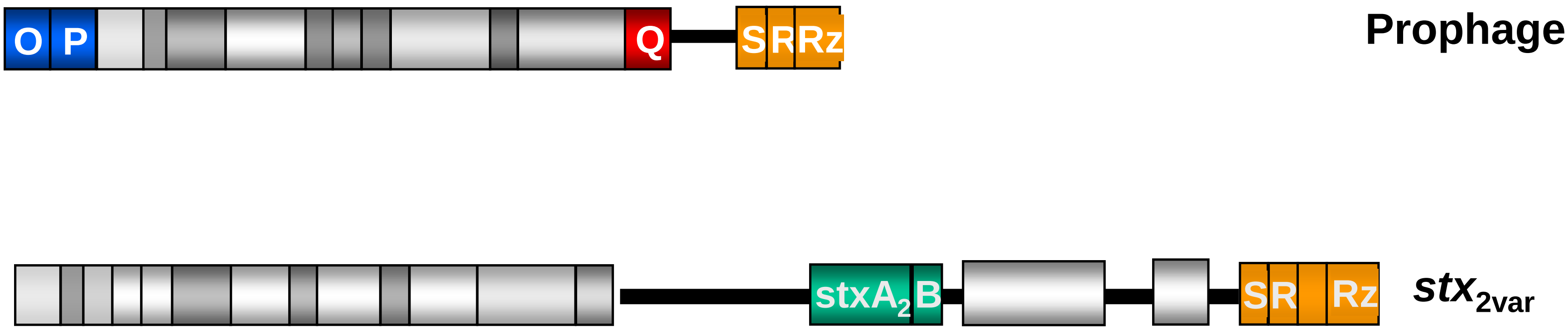
	Groningen	Rotterdam	Totaal
STEC-prevalence	0,8%	0,4%	0,7%
EHEC-prevalence	1,1%	1,4	1,2%

	Groningen n	Groningen %	Rotterdam n	Rotterdam %	Totaal n	Totaal %
Direct-PCR *stx1/stx2/eae*	**763**	**100%**	**486**	**100%**	**1249**	**100%**
after Pre-enrichment:	**718**	**94.1%**	**457**	**94.0%**	**1175**	**94.1%**

?

Data analyzed: Mirjam Kooistra, UMCG/Certe

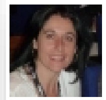
Structure analysis of *stx*-Gene



Antibiotics in pre-HUS-phase increase risk for HUS

2011 outbreak: Antibiotics after HUS improved clinical outcome

stx-genes: common in nature



Maite Muniesa 35.90
University of Barcelona

Article

Persistence of infectious Stx bacteriophages after disinfection treatments.

Anna Allué-Guardia, Alexandre Martínez-Castillo, Maite Muniesa

Applied and environmental microbiology (Impact Factor: 3.69). 01/2014;
DOI:10.1128/AEM.04006-13
Source: PubMed

ABSTRACT In Shiga toxin-producing Escherichia coli (STEC), induction of Stx phages causes the release of free phages that can later be found in the environment. The ability of Stx phages to survive different inactivation conditions will determine their prevalence in the environment, the risk of stx transduction and the generation of new STEC. We evaluated the infectivity and genomes of two Stx phages (Φ534 and Φ557) in different conditions. Infectious Stx phages were stable at 4, 22 and 37°C and at pH 7 and 9 after one month of storage, but were completely inactivated at pH 3. Infective Stx phages decreased moderately when treated with UV (2.2 log10 reduction for an estimated UV dose of 178.2 mJ/cm(2)) or after treatment at 60 and 68°C for 60 min (2.2 and 2.5 log10 reduction respectively) and were highly inactivated (3 log10) by 10 ppm of chlorine in one min. Assays in a mesocosm showed lower inactivation of all microroganisms in winter than in summer. The number of Stx phage genomes did not decrease significantly in most cases, and STEC inactivation was higher than phage inactivation in all conditions. Moreover, Stx phages retained the ability to lysogenize E. coli after some of the treatments [less]

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Appl Environ Microbiol. 2009 Mar;75(6):1764-8. Epub 2009 Jan 23.

Phage-mediated Shiga toxin 2 gene transfer in food and water.

Imamovic L, Jofre J, Schmidt H, Serra-Moreno R, Muniesa M.
Department of Microbiology, Faculty of Biology, University of Barcelona, Diagonal 645, E-08028 Barcelona, Spain.

Abstract
Shiga toxin (stx) transduction in various food matrices has been evaluated with lysogens of Stx phages. stx transduction events were observed for many phages under appropriate conditions. Transduction did not occur at low pH and low temperatures. A total of 10(3) to 10(4) CFU ml(-1) was the minimal amount of donor and recipient strains necessary to generate transductants.

PMID: 19168651 [PubMed - indexed for MEDLINE] PMCID: PMC2655461 **Free PMC Article**

Are we prepared?

- STEC of important zoonotic impact also via vegetables
- Stx-genes not enough to determine STEC/EHEC
- Risk analysis of STEC/EHEC needed for public health, infection control and food safety
- Need for national research network on STEC/EHEC for outbreak preparation

INVITATION

ONE HEALTH SYMPOSIUM FOCUS ON STEC - HUSEC

National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

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