ERKNet
The European
Rare Kidney Disease
Reference Network

european
society for
paediatric
nephrology

ESPN/ERKNet

Educational Webinars on Pediatric Nephrology & Rare Kidney Diseases

Date: **20 April 2021**

Topic: **STEC associated HUS**

Speaker: **Nicole van de Kar**

Moderator: **Francesco Emma**

LINK Shiga-toxin producing Escherichia coli and hemolytic uremic syndrome

Preliminary Communication

SPORADIC CASES OF HAEMOLYTIC-URAEMIC SYNDROME ASSOCIATED WITH FAECAL CYTOTOXIN AND CYTOTOXIN-PRODUCING ESCHERICHIA COLI IN STOOLS

MOHAMED A. KARMALI BRIAN T. STEELE*
MARTIN PETRIC CORAZON LIM

Departments of Bacteriology and Virology; Research Institute; and Division of Nephrology, Department of Pediatrics, Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8 and Department of Microbiology, University of Toronto

Summary A cytotoxin active on Vero cells, less active on hela cells, and inactive on WI38 cells Vero toxin [VT]) was detected in stool isolates of *Escherichia coli* from 8 of 15 sporadic cases of haemolytic uraemic syndrome (HUS). Stools from 5 of these 8 patients were examined for faecal VT activity, and all were positive. Of the 7 of 15 patients who did not have VT$^+$ *E. coli*, 2 were positive for faecal VT, and a third (patient K) had strong serological evidence of VT$^+$ *E. coli* infection. 2 HUS patients, including

EVIDENCE OF INFECTION BY VT$^+$ E. COLI IN 15 CASES OF SPORADIC HUS (A–O) AND 2 PATIENTS WITH UNCOMPLICATED DIARRHOEA WHO WERE SIBLINGS OF HUS PATIENTS

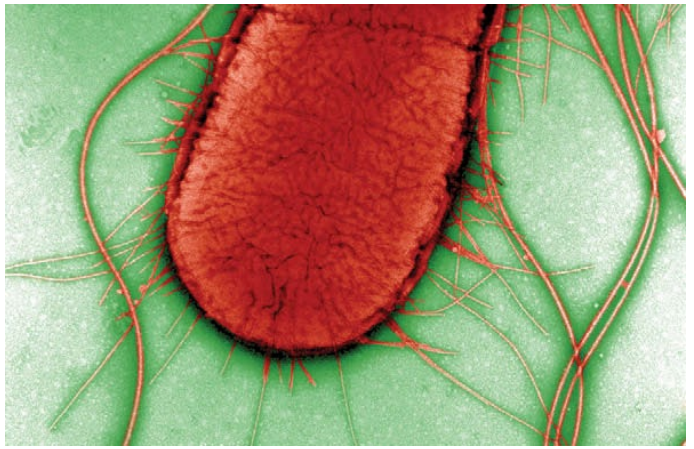
Patient	Age	Sex	VT$^+$ *E. coli* (serotype) in stools	Faecal VT titre	VT antibody titre
A	17 mo	M	Yes (O26:K60)	NA	NT
B	18 mo	M	Yes (O113:K75:H21)	NA	NT
C	12 yr	M	Yes (O111:K57:H8)	NA	NT
D	14 mo	F	Yes (O111:K58)NM	1:5120	NT
E	11 mo	M	Yes (O157:H7)	1:320	NT
F	16 mo	F	Yes (O157:H7)	1:320	1:80 (day 9) 1:1280 (day 43)
G	45 mo	F	Yes (UT)	1:320	NT
H	44 mo	F	Yes (0113:K57:H21)	1:40	NT
I	26 mo	F	No	1:80	NT
J	13 mo	F	No	1:80	NT
K	38 mo	F	No	-ve	1:80 (day 17) 1:320 (day 28)
L	32 mo	F	No	-ve	NT
M	18 mo	F	No	-ve	NT
N	32 mo	F	No	-ve	NT
O	1 mo	F	No	-ve	NT
Sib of F	66 mo	M	Yes (O157:H7)	1:2560	1:160 (day 30)
Sib of K	8 mo	M	Yes (O157:H7)	1:320	1:20 (day 4) 1:80 (day 35)

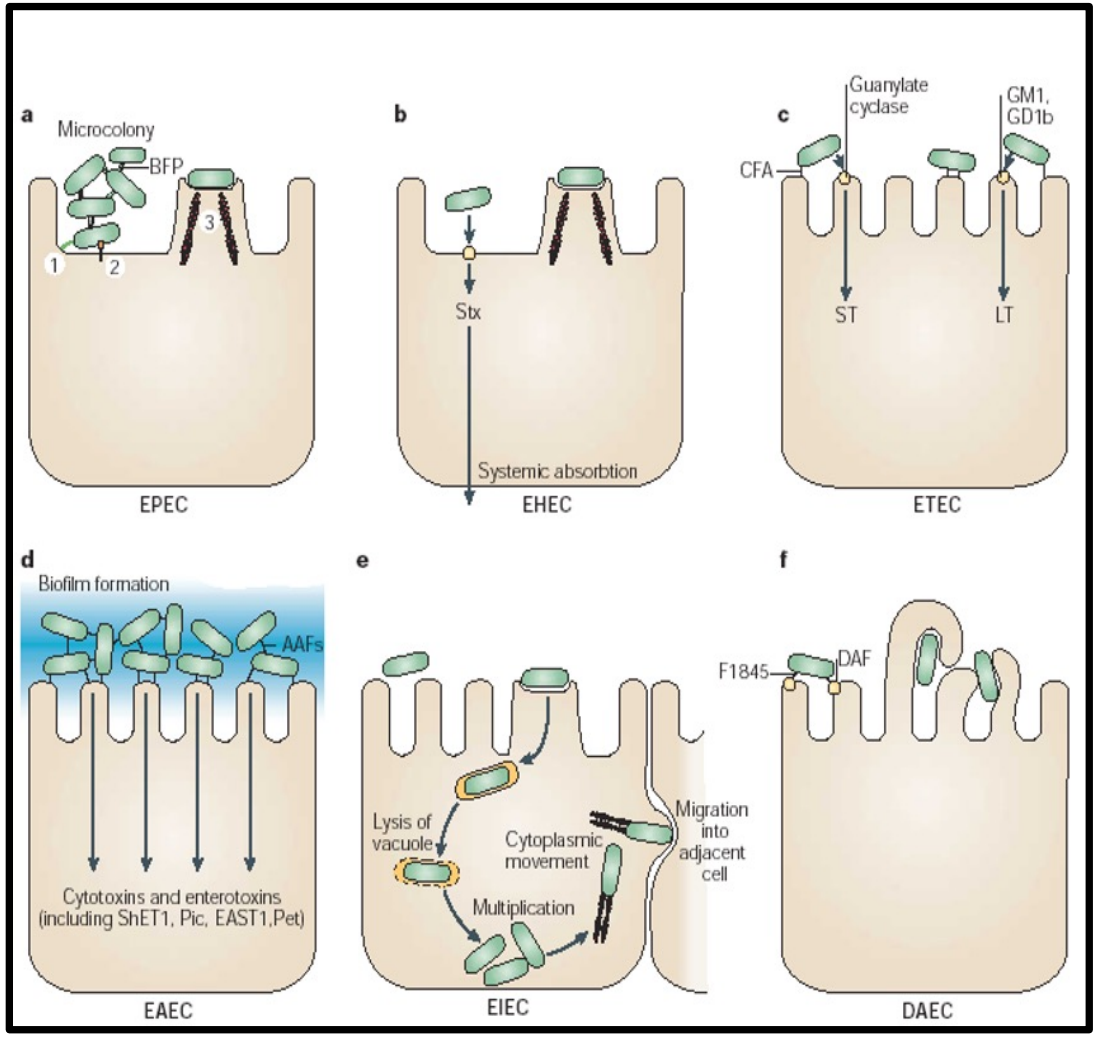
UT = untypable so far. NT = not tested so far. NA = stool not available for testing. NM = non-motile.

Karmali MA et al, Lancet 1983

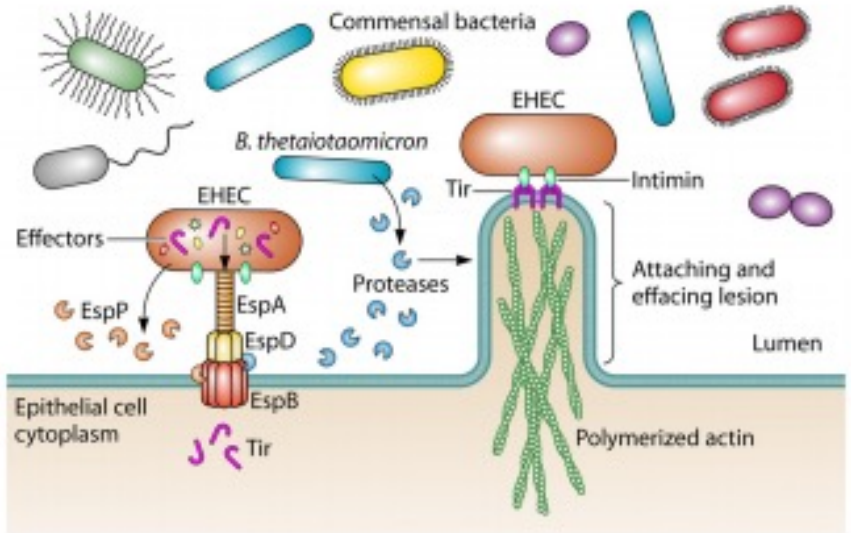
Nomenclature

- VTEC — Verocytoxin-producing Escherichia coli
  - Verocytoxin-1 (VT1), verocytotoxin-2 (VT2)
- STEC — Shiga toxin-producing Escherichia coli
  - Shiga toxin-1 (Stx1), shiga toxin-2 (Stx2)
- EHEC — Enterohemorrhagic Escherichia coli
  - STEC causing disease in humans (bloody diarrhea)
- ~~Typical HUS~~ — ~~HUS with bloody diarrhea~~
- **STEC-HUS** — **HUS caused by STEC-infection**





EPEC	Entero-pathogenic *E.coli*
EHEC	Entero-hemorrhagic *E.coli*
ETEC	Entero-toxigenic *E.coli*
EAEC	Entero-aggregative *E.coli*
EIEC	Entero-invasive *E.coli*
DAEC	Diffuse adherent *E.coli*



Epidemiology of STEC

Q1 : How many STEC-HUS patients have you treated (10y) ?

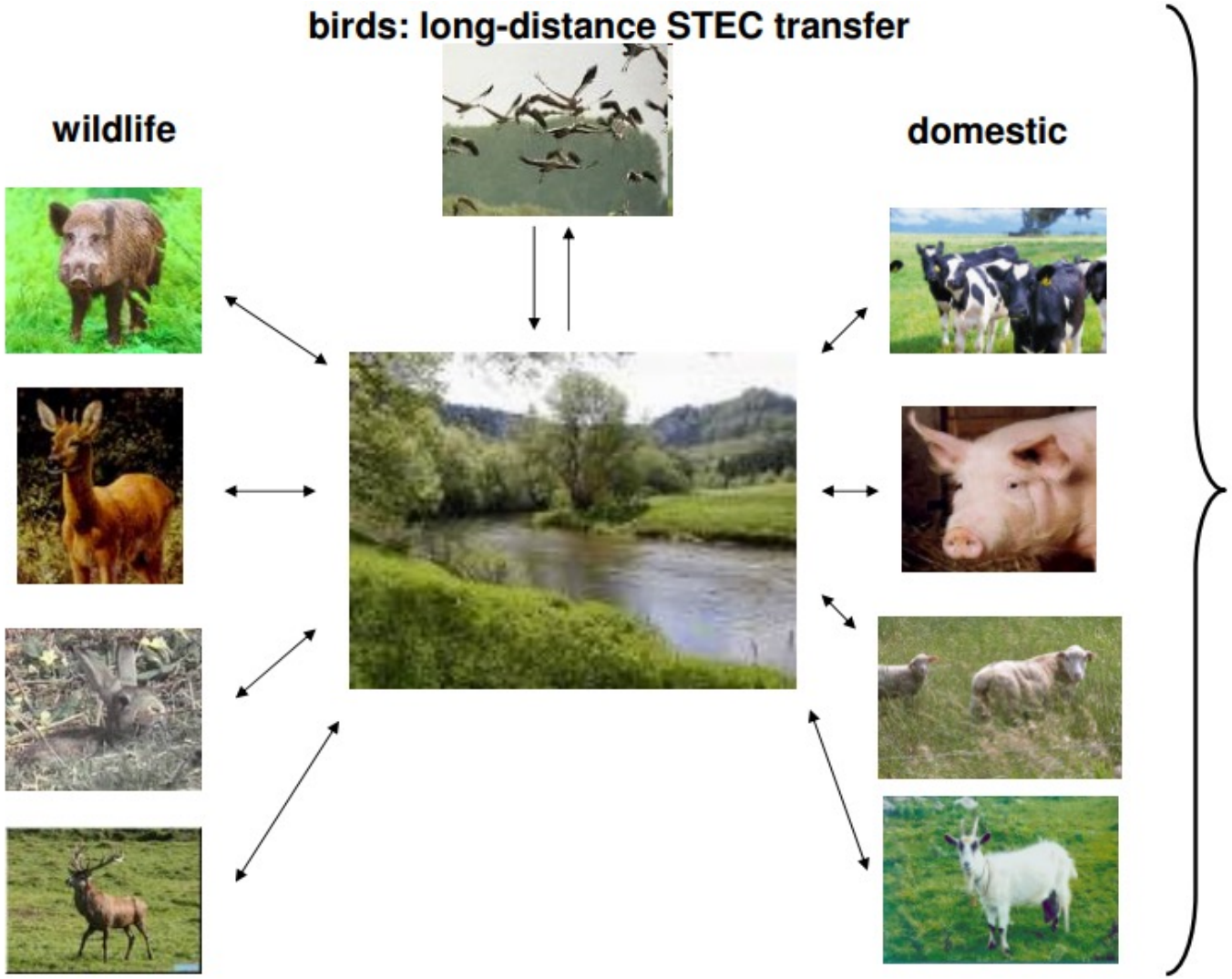
A < 5

B < 10

C < 20

D > 20

Broad animal reservoir of STEC



Human to human

Beutin L, BfR Forum Verbraucherschutz, 2012

85% of STEC infections in humans are food borne

Photo illustration

USDA says E. coli O157:H7 outbreak likely linked to ground beef

By Coral Beach on March 30, 2021

UPDATED: Comments from CDC added

Federal officials have concluded investigation of a previously unrevealed multi-state foodborne illness outbreak. Ground beef was identified as the likely source of the E. Coli O157:H7 behind the infections.

Photo illustration

E coli O157:H7 outbreak likely caused by leafy greens of unknown origin is over

By News Desk on December 22, 2020

Forty confirmed cases of E. coli O157:H7 sent 20 people to hospitals in 19 states in an outbreak linked to leafy greens. But the specific type or brand remains unknown and the outbreak is over, reports the federal Centers for Disease Control and Prevention.

Lake Nokomis in Fall

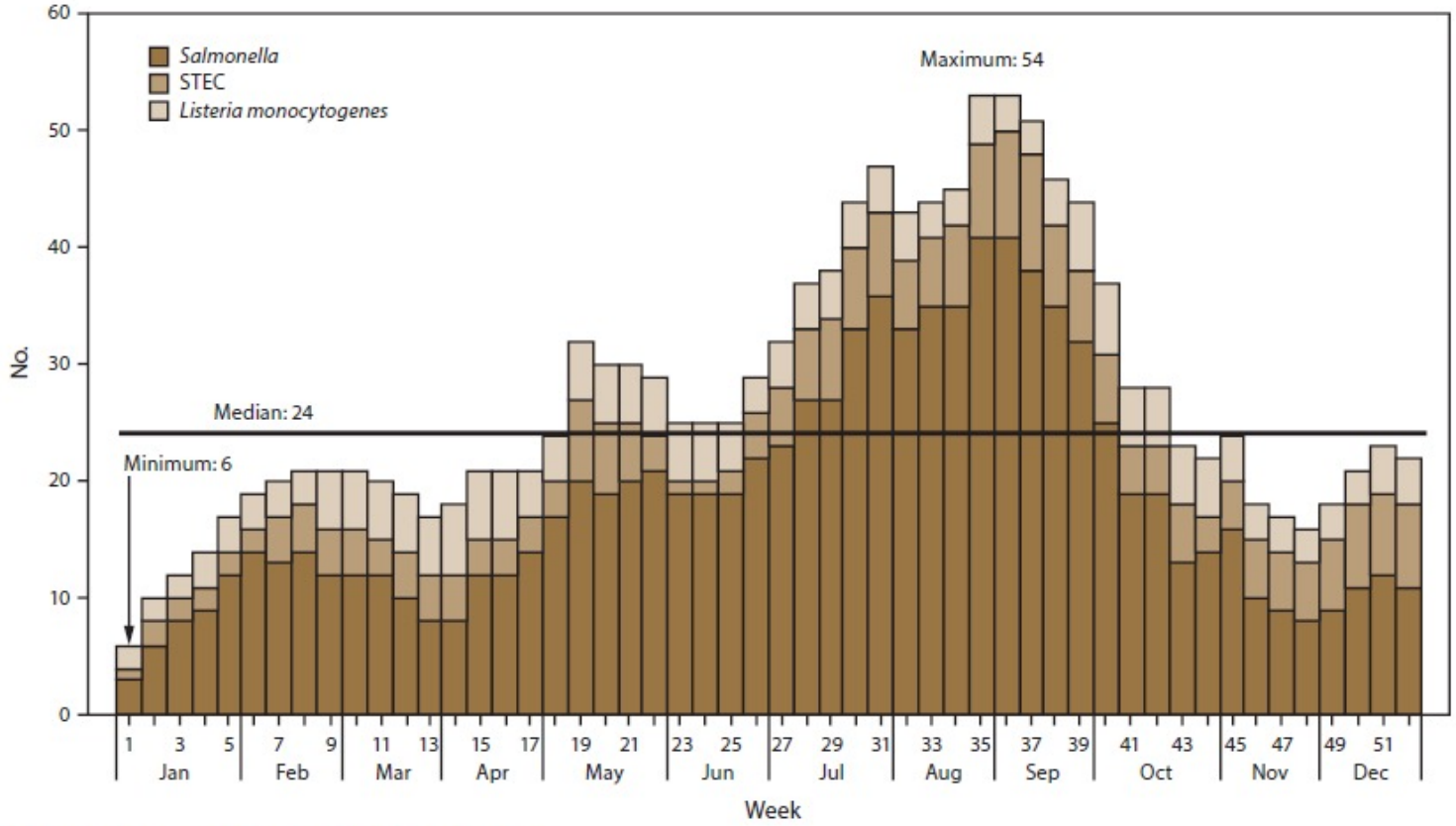
Up to 69 Shiga toxin-producing illnesses acquired from popular Minneapolis swimming beach

By News Desk on August 22, 2019

FSN | Food Safety News

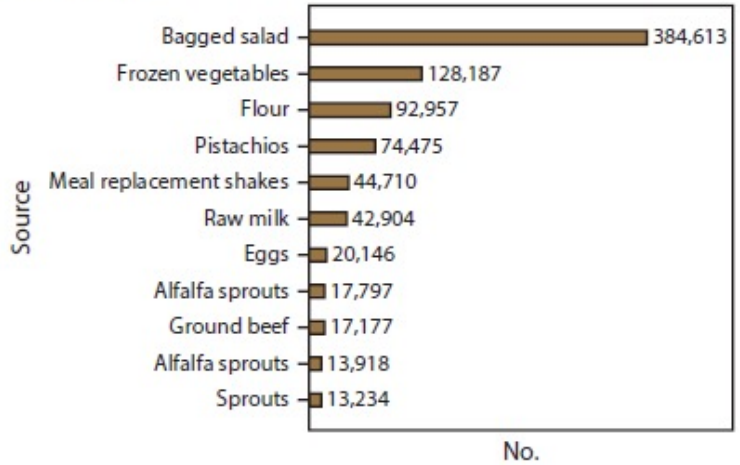
Breaking news for everyone's consumption

FIGURE 1. Number of ongoing possible multistate outbreak investigations,* by pathogen and week — United States, 2016

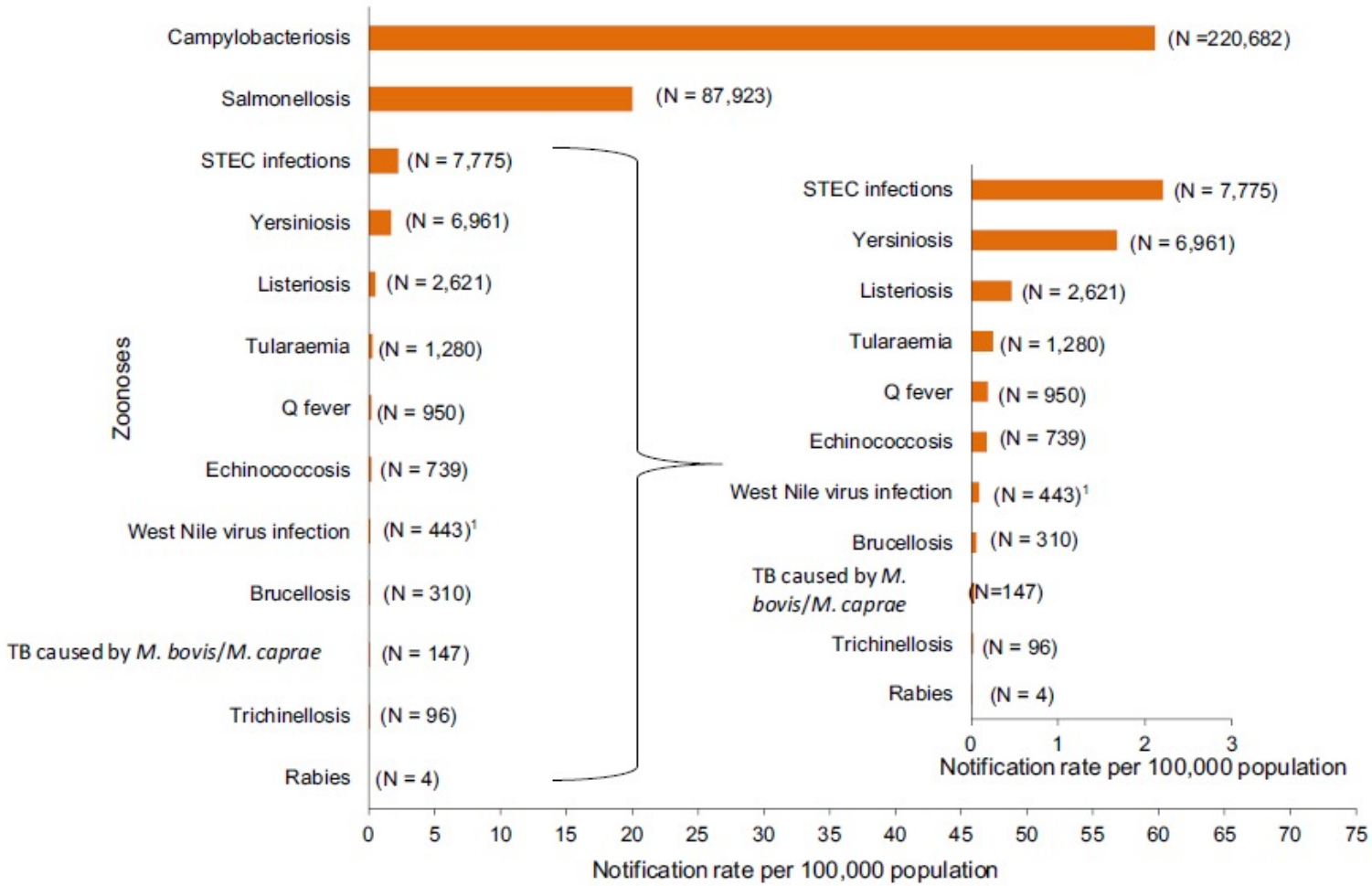


Abbreviation: STEC = Shiga toxin–producing *Escherichia coli*.
* n = 174.

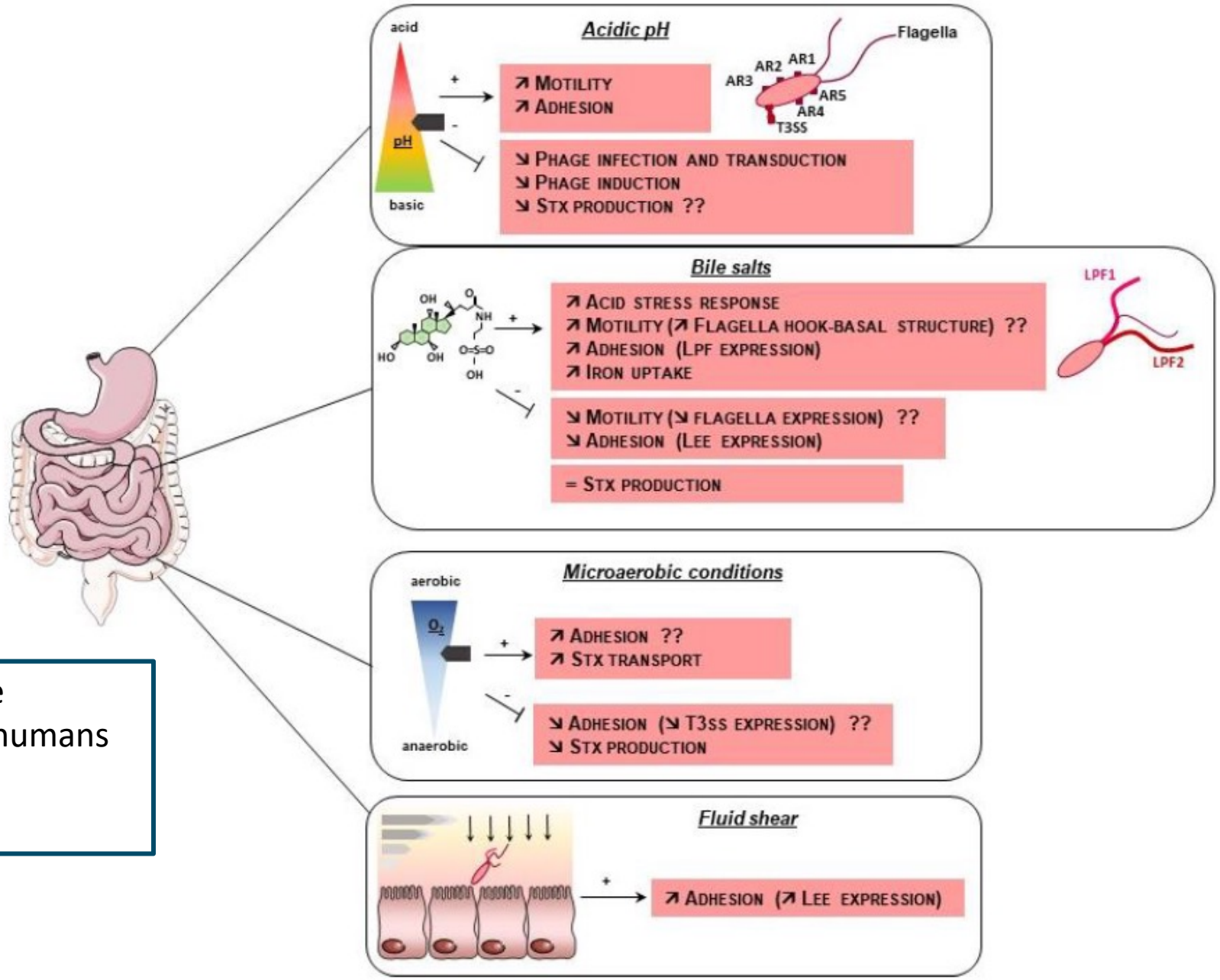
FIGURE 2. Number of webpage views for CDC announcements of multistate foodborne outbreaks,* by outbreak source — United States, 2016



* n = 11.



Note: The total number of confirmed cases is indicated between parentheses at the end of each bar.
[1] Exception: West Nile virus infection for which the total number of cases was used.



Minimal infective dose
10-100 organisms for humans

STEC is non-invasive

Jubelin G et al, Microorganisms 2018

STEC causing HUS

Serotypes - Shiga toxin 1 and / or 2

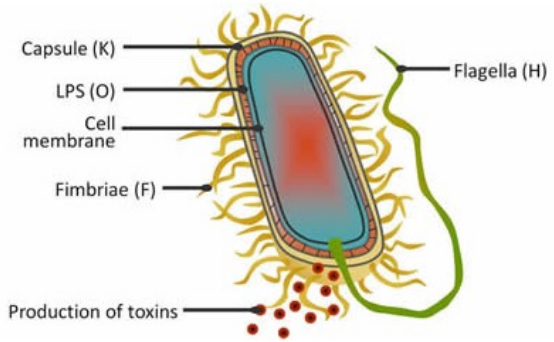


Table 1 Serotypes, *stx* genotypes, and additional virulence factors identified in 280 patients with STEC-HUS

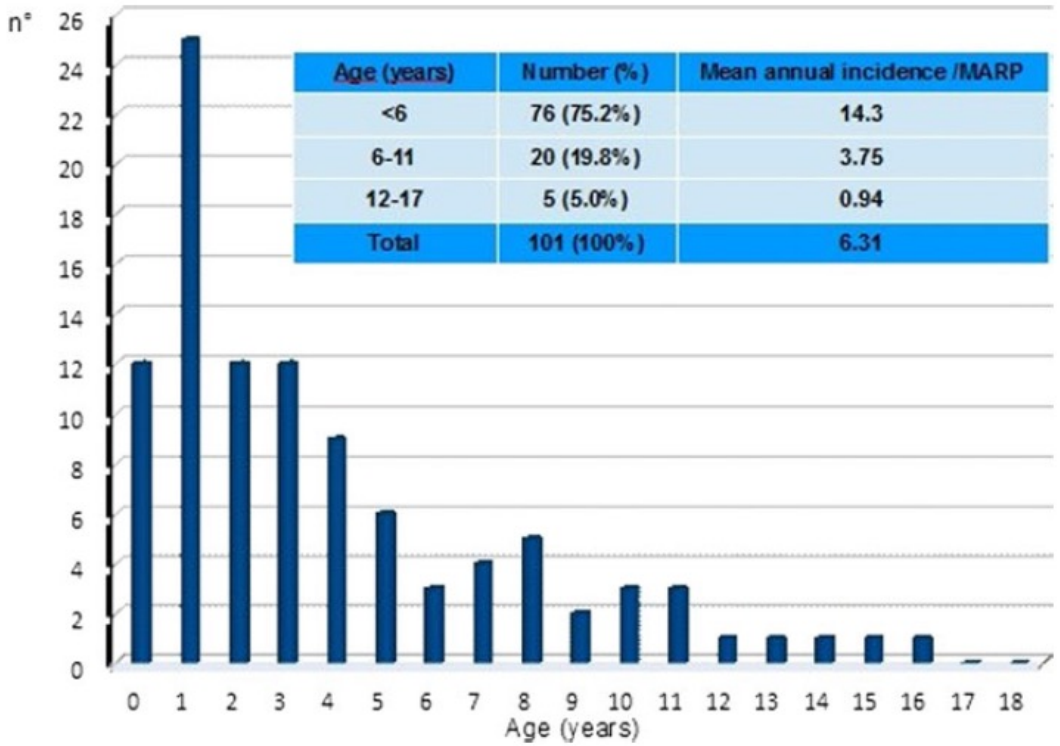
Serogroup (*n*=280)	Virulence genes					
	stx_{2a}/stx_{2c} (*n*=179)	stx_{2a} (*n*=94)	stx_{1a} (*n*=2)	stx_{1a}/stx_{2a} (*n*=5)	*eae* (*n*=274)	*ehx*A (*n*=277)
O157:H7 (206, 73.6%)	179	27	-		206	206
O145:NM (47, 16.8%)	-	47	-		47	47
O121:H19 (15, 5,4%)	-	15	-		15	15
O26:H11 (2, 0.7%)	-	1	1		2	2
O103:H2 (2, 0.7%)	-	-	-	2	2	2
O113:H21 (1, 0.35%)	-	1	-		-	1
O174:NM (1, 0.35%)	-	1	-		-	-
O59:H19 (1, 0.35%)	-	1	-		-	-
O8:H16 (1, 0.35%)	-	-	-	1	-	1
O91:NM (1, 0.35%)	-	-	-	1	-	-
ONT:HNM (2, 0.7%)	-	1	1		1	2
ONT:HNT (1, 0.35%)	-	-	-	1	1	1

ONT O non-typeable

Big 5

Alconcher LF et al, Pediatr Nephrol 2020

The patient with HUS – North Italian HUS Network



Age (years)	Number (%)	Mean annual incidence /MARP
<6	76 (75.2%)	14.3
6-11	20 (19.8%)	3.75
12-17	5 (5.0%)	0.94
Total	101 (100%)	6.31

TABLE 1. Number of Families With Positive Households in Relation to Those Investigated for Each Variable Considered: Case Patient Disease, *STX* and Serogroup of the Involved STEC

Variable	Type	N. Index Cases	N. Families With Positive Households	Prevalence %	95% CI	*P* Value
Condition	HUS	23	9	39.1	(22.2–59.2)	Ns
	BD	16	5	31.3	(14.2–55.6)	

Siblings > Mothers > Fathers > Grandparents

10y Follow-up : STEC-HUS 89 cases (median age 4.3y) and aHUS 12 cases (median age 3,7y)

Ardissino G et al, Eur J Pediatr 2016, Vittorio Luini M et al, Pediatr Infect Dis J 2021

STEC O104:H4 outbreak in Germany 2011 - adults

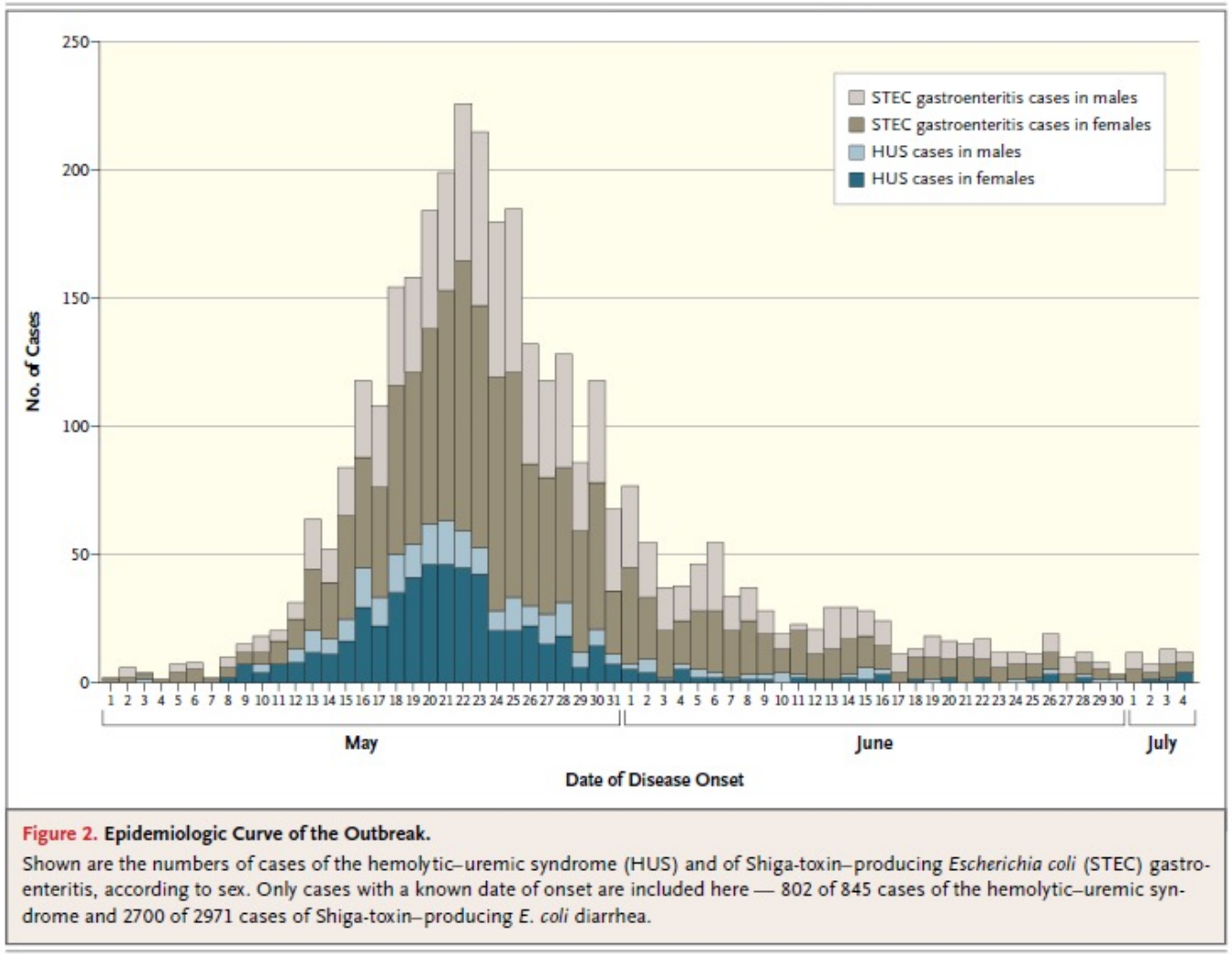
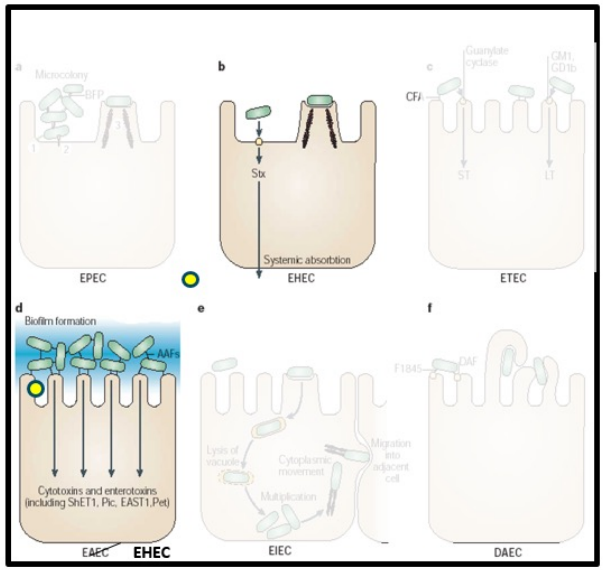


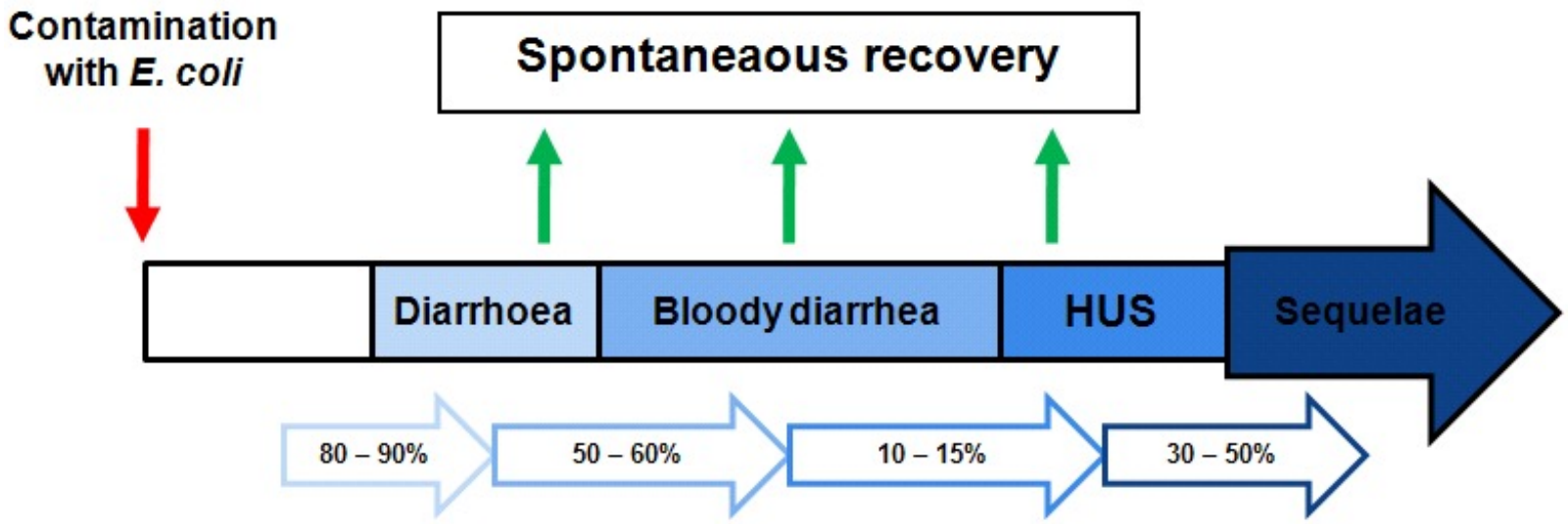
Figure 2. Epidemiologic Curve of the Outbreak.

Shown are the numbers of cases of the hemolytic–uremic syndrome (HUS) and of Shiga-toxin–producing *Escherichia coli* (STEC) gastroenteritis, according to sex. Only cases with a known date of onset are included here — 802 of 845 cases of the hemolytic–uremic syndrome and 2700 of 2971 cases of Shiga-toxin–producing *E. coli* diarrhea.



Frank C et al, NEJM 2011

STEC infection ⟶ STEC-HUS in children



Annual Incidence STEC-HUS children < 5 y varies per country: 10.4/100.000 in Argentina; 1.5-1.8/100.000 in Italy / France / Germany

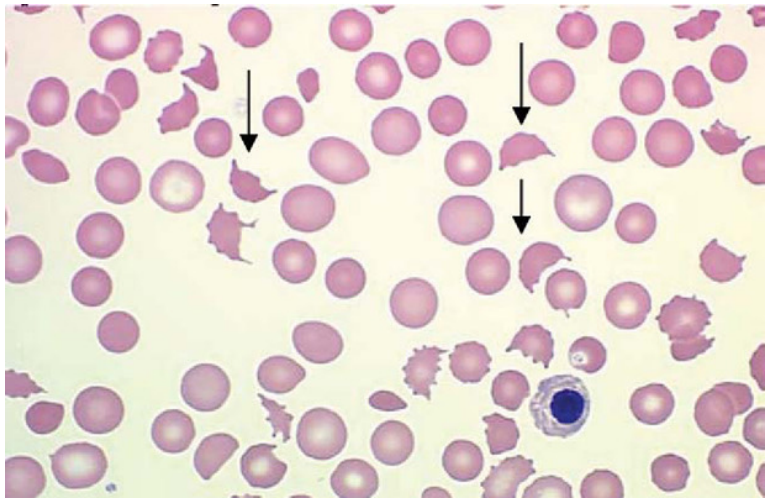
Symptoms STEC-HUS patients at presentation

- Previously healthy , age < 5y, seasonal variation, m=f

- Vomiting, nausea
- **Diarrhea (watery, often bloody) , however in 10% of STEC-HUS patients NO diarrhea at presentation**
- Pale,
- Jaundice in some
- **Fever** absent in most cases
- Petechiae , ecchymosis
- Restless, irritable, somnolence, convulsions
- Oliguria, anuria

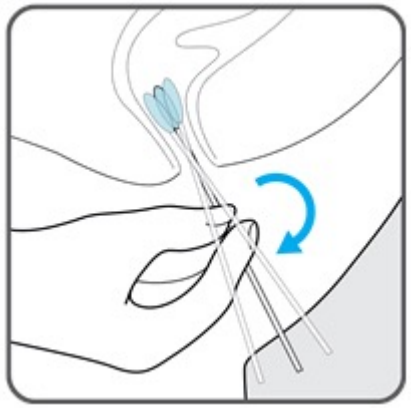
- …Pancreatic involvement
- …Cardiomyopathy
- ..

schizocytes →



Detection of STEC

Use rectal swab when no stool is immediate available



Enteropathogen detection in children with diarrhoea, or vomiting, or both, comparing rectal flocked swabs with stool specimens: an outpatient cohort study

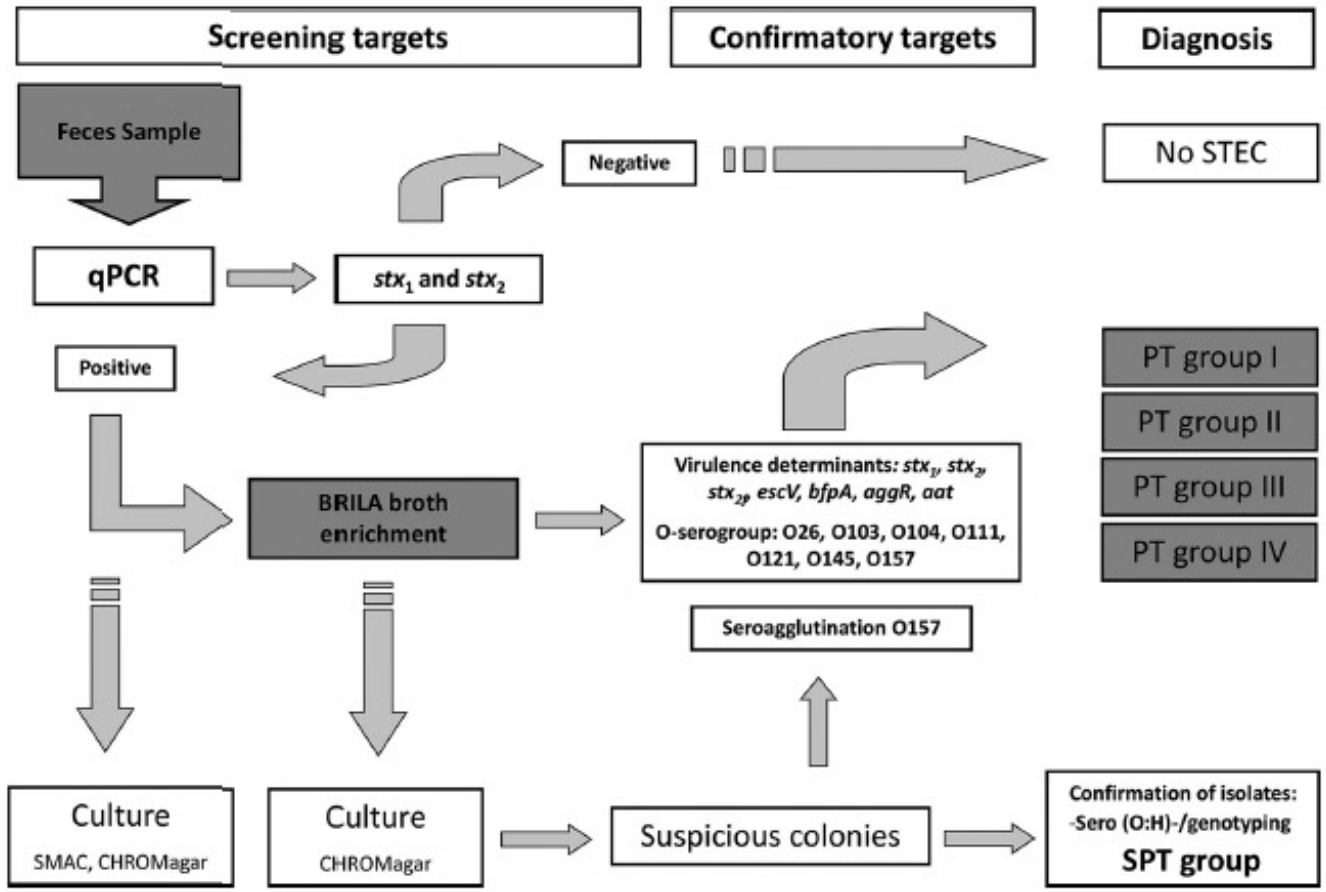
*Stephen B Freedman, Jianling Xie, Alberto Nettel-Aguirre, Bonita Lee, Linda Chui, Xiao-Li Pang, Ran Zhuo, Brendon Parsons, James A Dickinson, Otto G Vanderkooi, Samina Ali, Lara Osterreicher, Karen Lowerison, Phillip I Tarr, on behalf of the Alberta Provincial Pediatric EnTeric Infection TEam (APPETITE)**

N=1519

Interpretation Rectal swabs should be done when enteropathogen identification and rapid detection are needed, appropriate molecular diagnostic technology is available, and a stool specimen is not immediately available. In view of their high yield, we urge that the recommendation against the use of rectal swabs as diagnostic specimens be reconsidered.

Freeman S et al, Lancet Gastroenterol Hepatol 2017

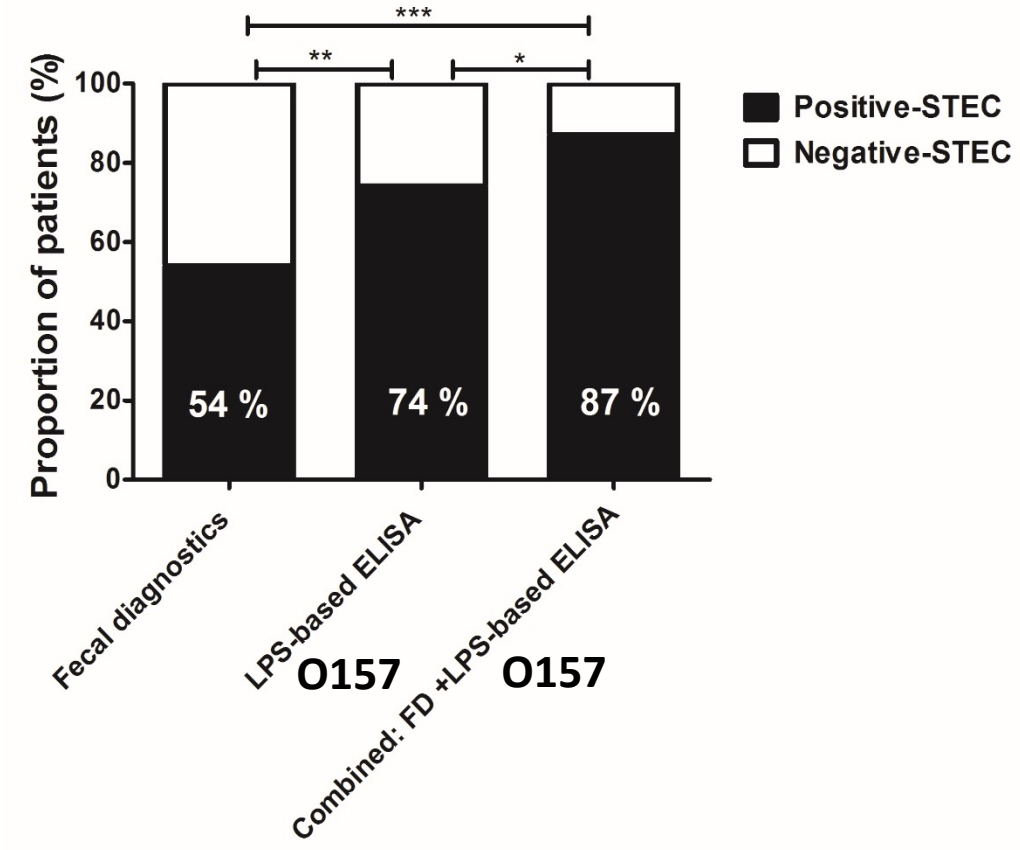
Fecal STEC diagnostic algorithm

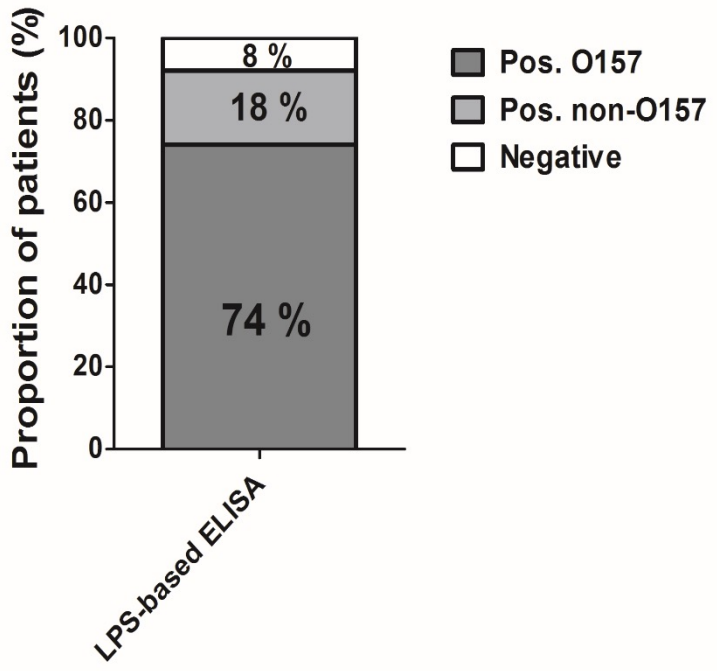


De Boer R et al, J Clin Microbiol 2015

Additional value of serology antibodies against LPS antigen (ELISA)

N=65



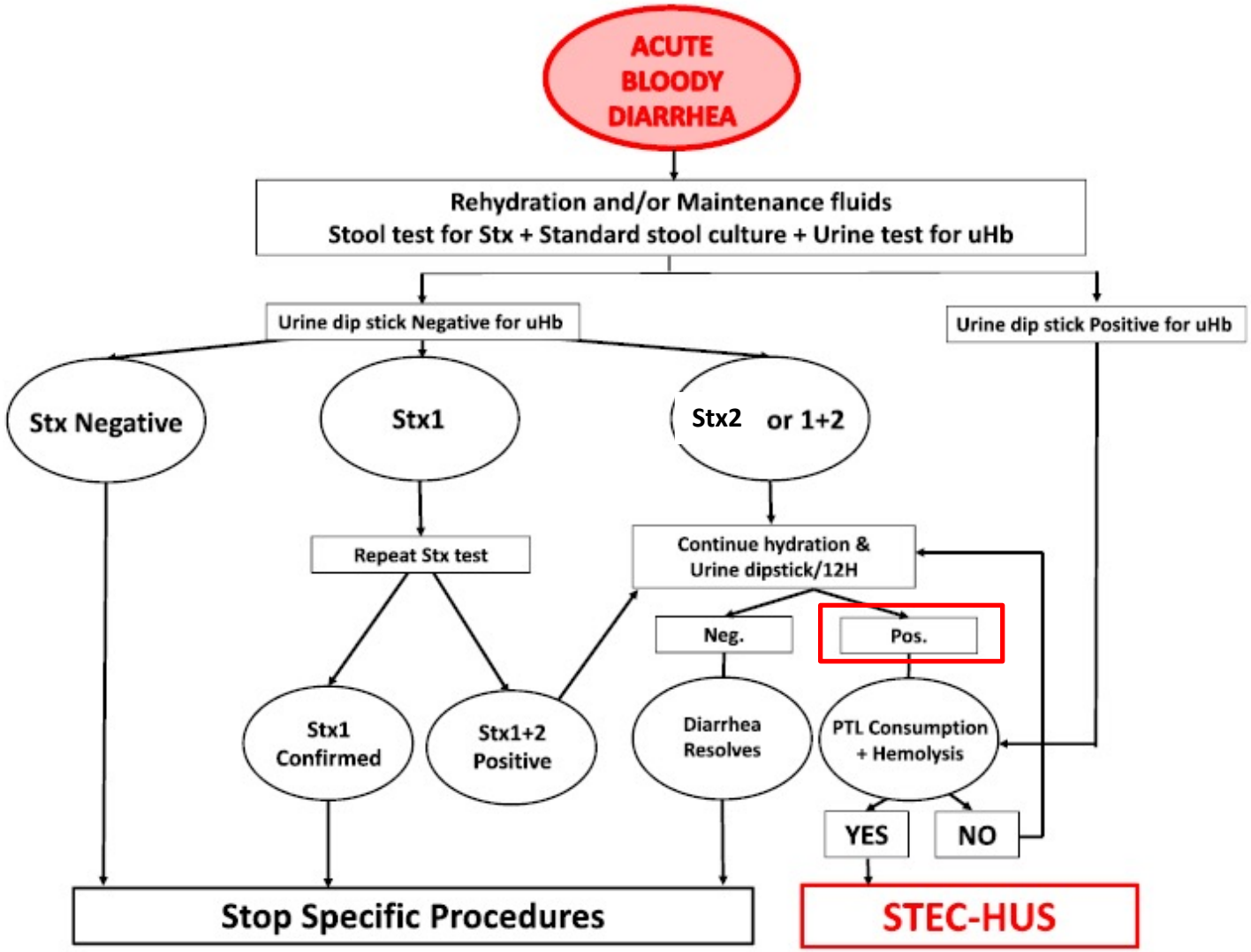


Wijnsma K et al, Pediatric Nephrol, 2016

Monitor urine when child with bloody diarrhea is STEC positive

N=100 Stx+ (10y)
Stx + BD n=63
22 ery+ **15/22 HUS**
41 ery- **no HUS**

Stx+ HUS n=37
37 ery+



Capone V et al, ItalKid-HUS Network, Eur J Pediatr 2021

Case presentation

- 16 month-old boy
- Previously healthy
- Physical examination
  - Fluctuating levels of alertness
  - Petechiae at lower limbs

Q2: Case

A Most likely STEC-HUS

B Most likely aHUS

C It can be STEC-HUS, it can be aHUS

D Most likely TTP

Q3: Eculizumab?

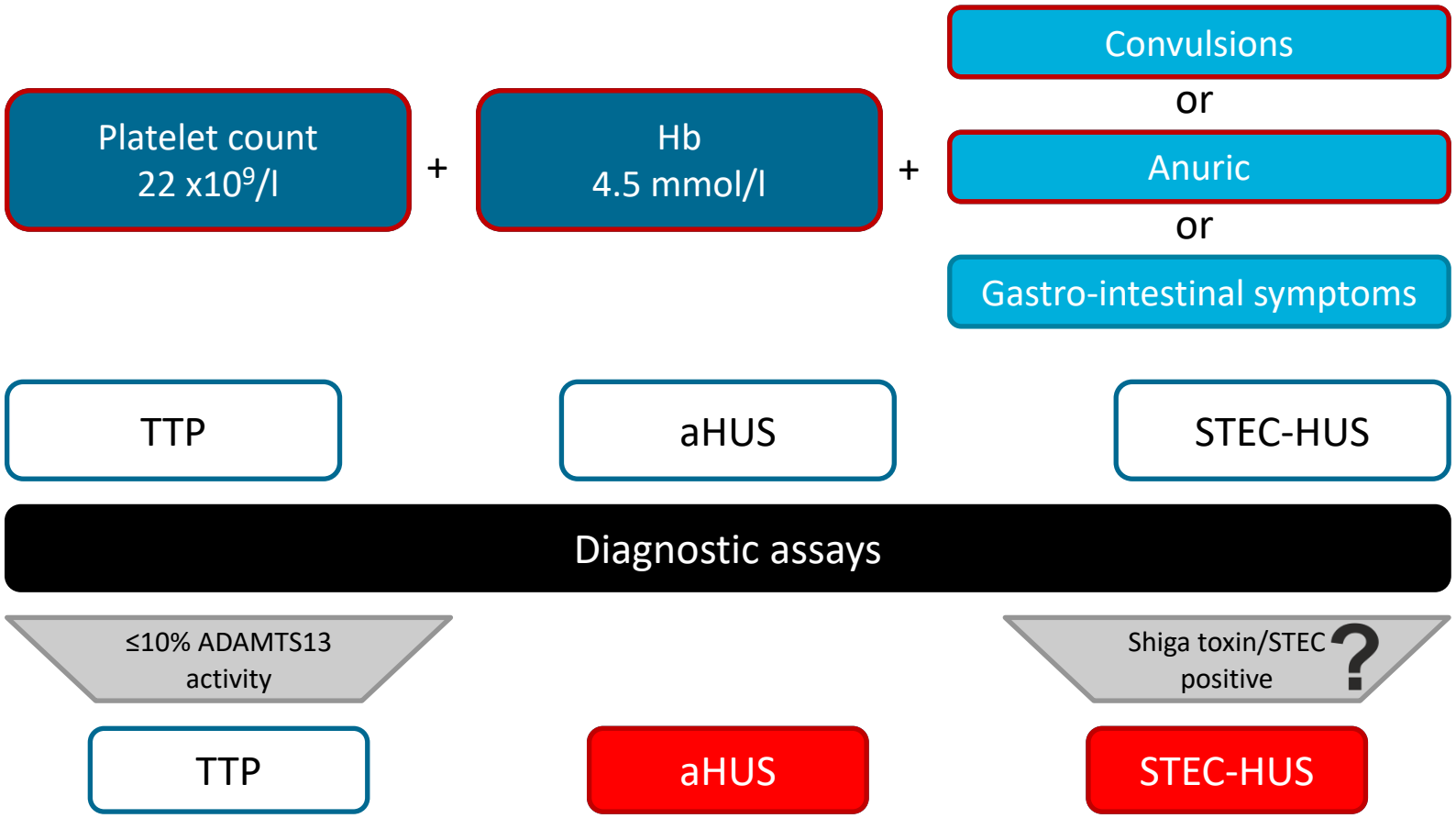
A Yes, it is aHUS

B Yes, but further diagnostics needed

C No, it is STEC

D No, Plasma therapy, ECU not available

Thrombotic microangiopathy



Diagnostics

Atypical HUS

- Complement
  - C3
  - C4
- Autoantibodies against factor H
- Complement DNA analysis

STEC – HUS

- PCR feces
  - *Stx 1 gene*
  - ***Stx 2 gene***
  - ***Eae gene***
- Serology
  - O157
  - O26
- Molecular serotyping
  - **O80:H2**

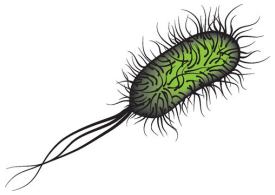
CERTE
MEDISCHE DIAGNOSTIEK & ADVIES
ZEKER VOOR ZORG

O80 serotype

Stx 2d gene

eae ξ

plasmid



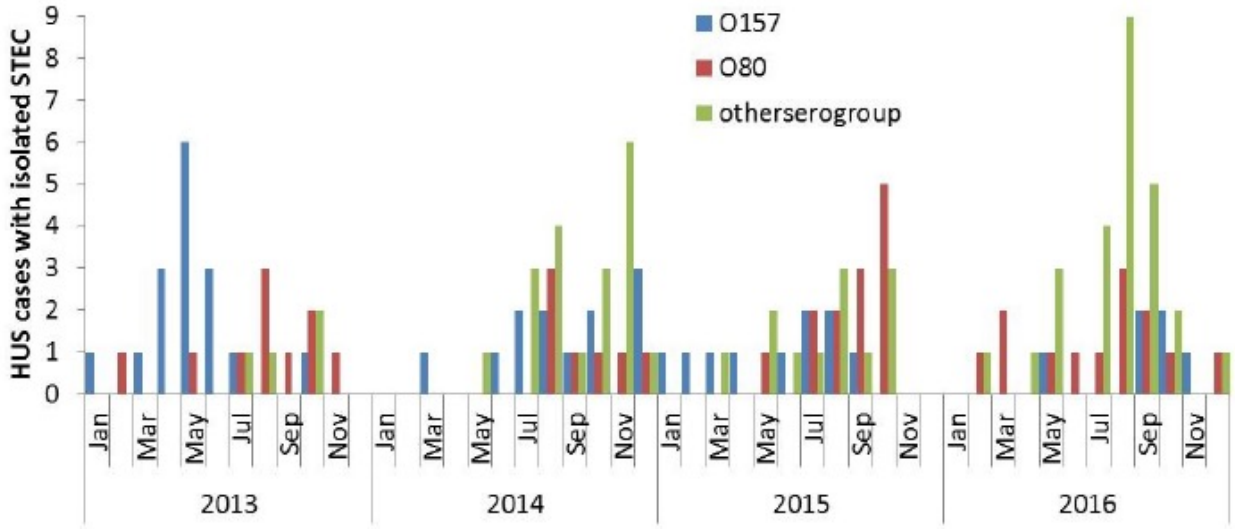
RESEARCH ARTICLE

Emerging Shiga-toxin-producing *Escherichia coli* serogroup O80 associated hemolytic and uremic syndrome in France, 2013-2016: Differences with other serogroups

Brecht Ingelbeen[1,2¤*], Mathias Bruyand[1], Patricia Mariani-Kurkjian[3,4], Simon Le Hello[5], Kostas Danis[1,2], Cécile Sommen[1], Stéphane Bonacorsi[3,4], Henriette de Valk[1]

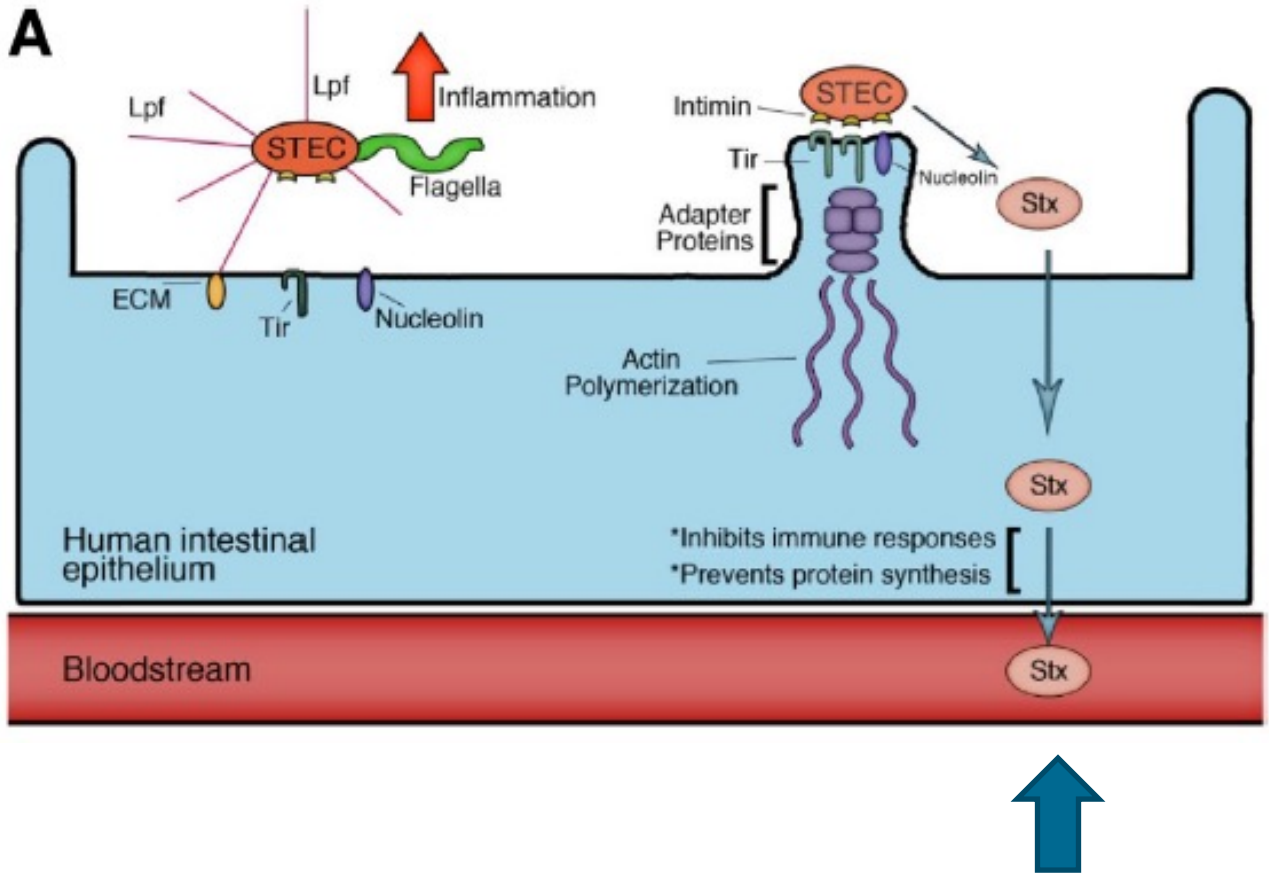
France 2013-2016 , STEC isolations 153 / 521 HUS

45 / 153 O80	**median age 1.1y**
46 / 153 O157	median age 4.0y
62 / 153 other	median age 1.8y

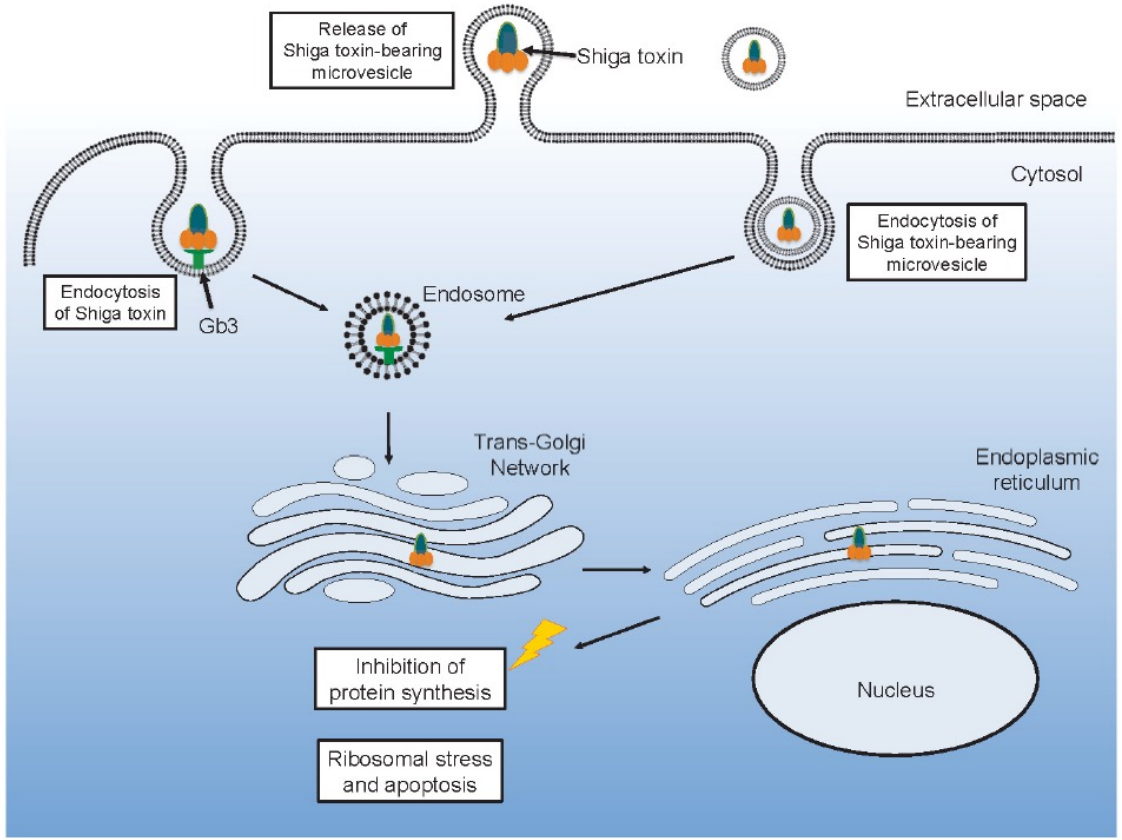


Ingelbeen et al, Plos One 2018

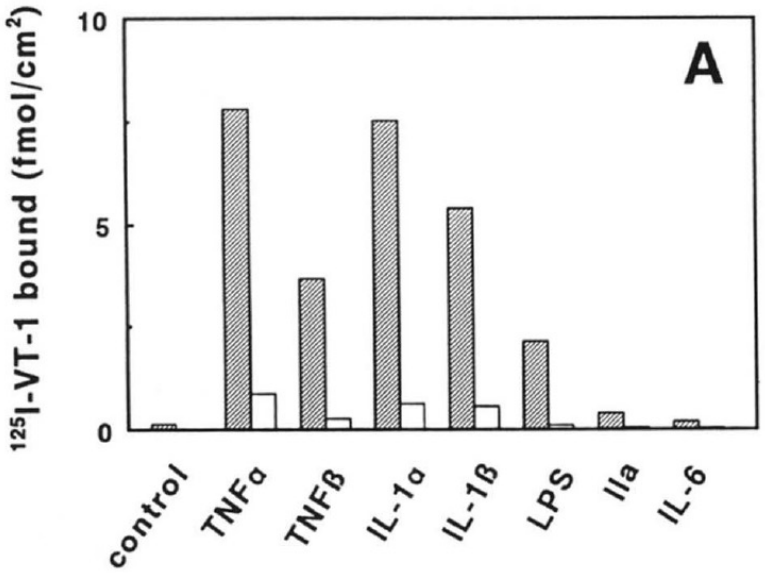
Pathogenesis STEC -HUS



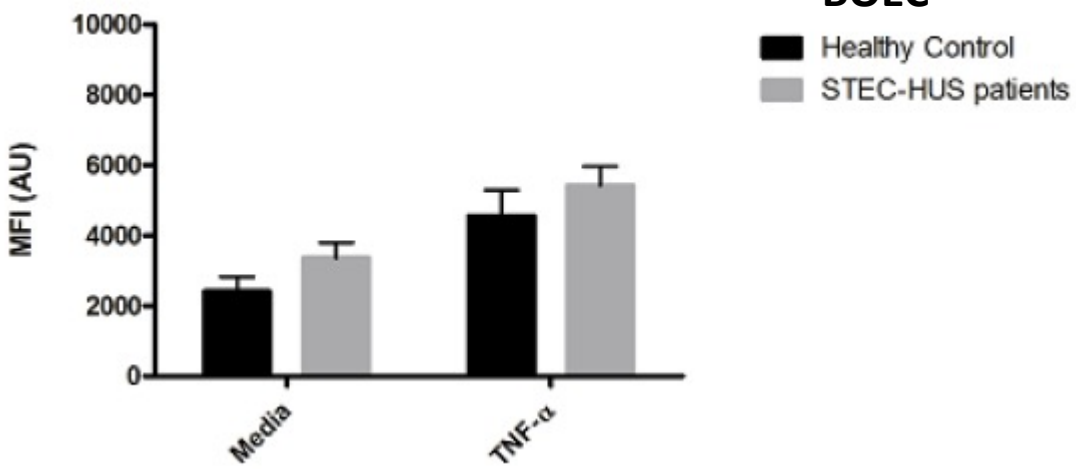
Farfan & Torres, Infection & Immunity 2011



HUVEC

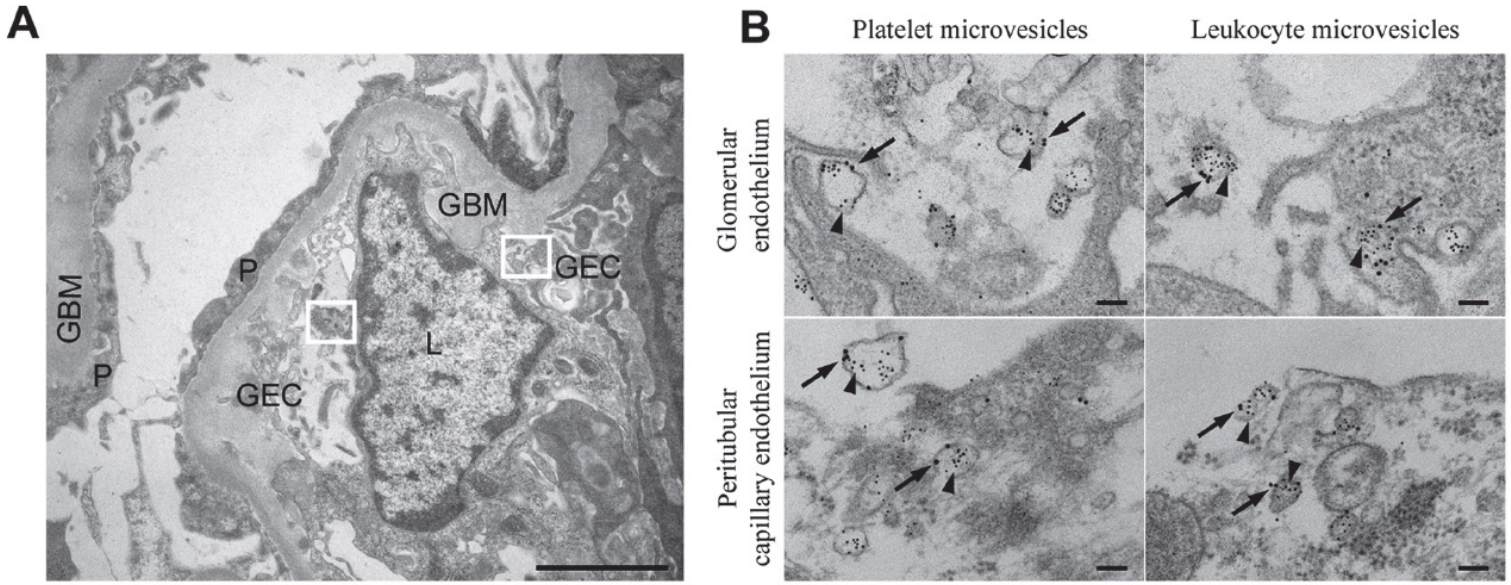


BOEC

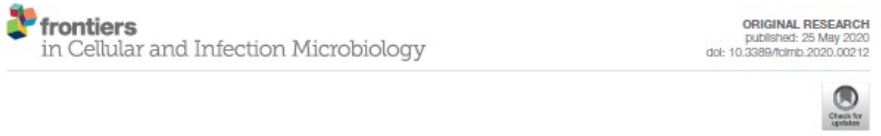


Villysson A et al, review, Toxins 2017, van de Kar et al, Blood 1991, van Setten et al Kidney Int 1996, Feitz et al, Toxins 2020

Shiga toxin and its receptor Gb3 needed for developing STEC-HUS



Stx2 containing blood cell-derived microvesicles in renal cortex of a HUS patient


frontiers in Cellular and Infection Microbiology

ORIGINAL RESEARCH
published: 25 May 2020
doi: 10.3389/fcimb.2020.00212

Shiga Toxin-Bearing Microvesicles Exert a Cytotoxic Effect on Recipient Cells Only When the Cells Express the Toxin Receptor

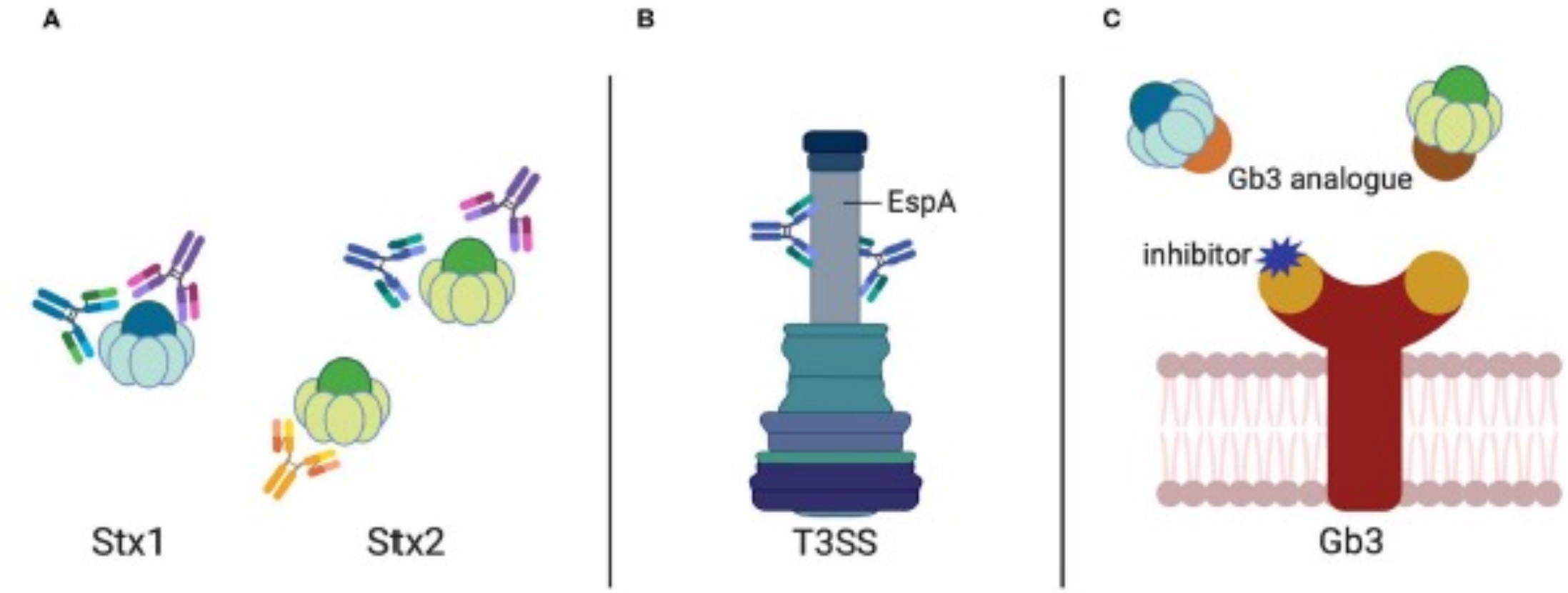
*Karl Johansson[1], Annie Willysson[1], Ann-Charlotte Kristoffersson[1], Ashmita Tontanahal[1], Daniel Gillet[2], Anne-lie Ståhl[1] and Diana Karpman[1]**

[1] Department of Pediatrics, Clinical Sciences Lund, Lund University, Lund, Sweden, [2] Université Paris-Saclay, CEA, INRAE, Médicaments et Technologies pour la Santé, (MTS), SIMoS, Gif-sur-Yvette, France

Stx has more biological effects:
-platelets, monocytes, neutrophils,..
-release cytokines/chemokines
-suppresses thrombomodulin expression
-etc etc etc

Stahl A et al, Plos Pathogens 2015, Johannes K et al, Front Cell Infect Microbiol 2020

Treatment options



Mühlen & Dersch, Front Cell Infect Microbiol 2020

Treatment STEC-HUS

Acute renal failure in infancy and childhood

Clinical course and treatment of 41 patients

Experience with 41 infants and children in acute renal failure is recorded; 28 of them had the "hemolytic-uremic" syndrome. The mortality attributed to renal failure was 37 per cent. Exchange transfusion was performed in 9 children, peritoneal dialysis in 6, and extracorporeal dialysis in only 2. The regimen for conservative treatment of acute renal failure is discussed, and the efficacy of exchange resins in the management of hyperkalemia is emphasized.

Carlos A. Gianantonio, M.D.,* Margarita Vitacco, M.D.; Javier Mendilaharzu, M.D., Fernando Mendilaharzu, M.D., and Arnaldo Rutty, M.D.

BUENOS AIRES, ARGENTINA

Table V. Peritoneal dialysis, results and biochemical data

Case No.	*Solution*		*Time (hours)*	*Water balance (ml.)*	*Urea*		*BUN (mg.%)*	
	mOsm./L.	*ml./Kg./hr.*			*Clearance (ml./min./Kg.)*	*Extraction (Gm.)*	*Pre*	*Post*
26	380	66	6	+400	1.04	17.5	450	360
23	450	65	7	–240	2.11	20.6	580	330
22	368	64	12	+120	1.54	26.4	630	240
	450	64	2	+ 40	1.49	5.9	560	450
	450	64	4	+100	1.15	7.5	480	350
	450	64	5	– 50	1.54	11.7	540	270
30	450	70	7	–145	0.84	5.8	305	171
	450	78	2	+ 60	0.79	2.3	347	300
4	540	72	5	–550	1.10	28.2	425	340
	450	70	8	–890	1.19	29.5	430	250
36	440	62	8	–150	1.56	9.7	260	180

*Gianantonio CA et al J Pediatr **1962***

Role for eculizumab in treatment of STEC-HUS?

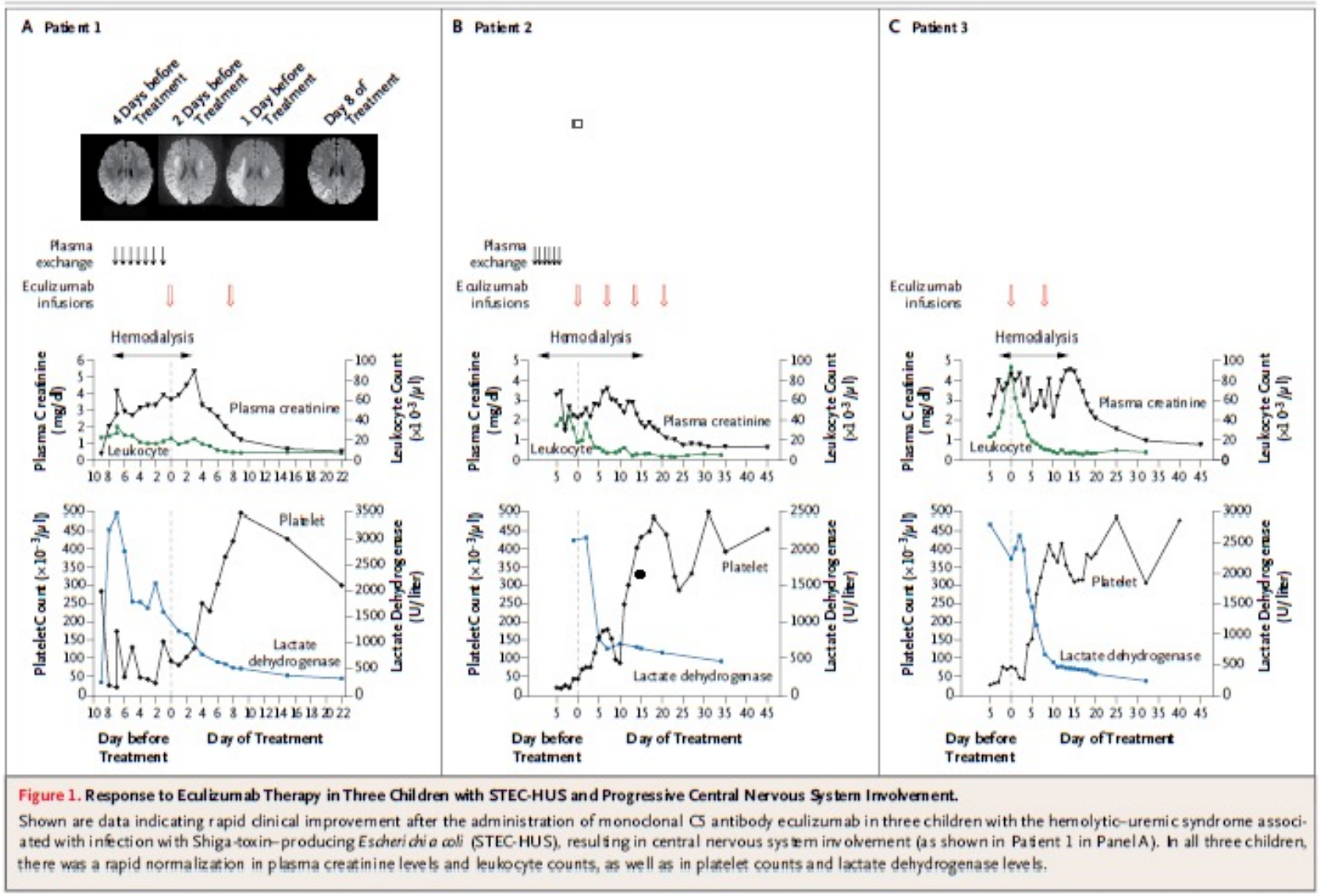


Figure 1. Response to Eculizumab Therapy in Three Children with STEC-HUS and Progressive Central Nervous System Involvement.

Shown are data indicating rapid clinical improvement after the administration of monoclonal C5 antibody eculizumab in three children with the hemolytic–uremic syndrome associated with infection with Shiga-toxin–producing *Escherichia coli* (STEC-HUS), resulting in central nervous system involvement (as shown in Patient 1 in Panel A). In all three children, there was a rapid normalization in plasma creatinine levels and leukocyte counts, as well as in platelet counts and lactate dehydrogenase levels.

The NEW ENGLAND JOURNAL of MEDICINE

Lapeyraque AL et al, NEJM 2011

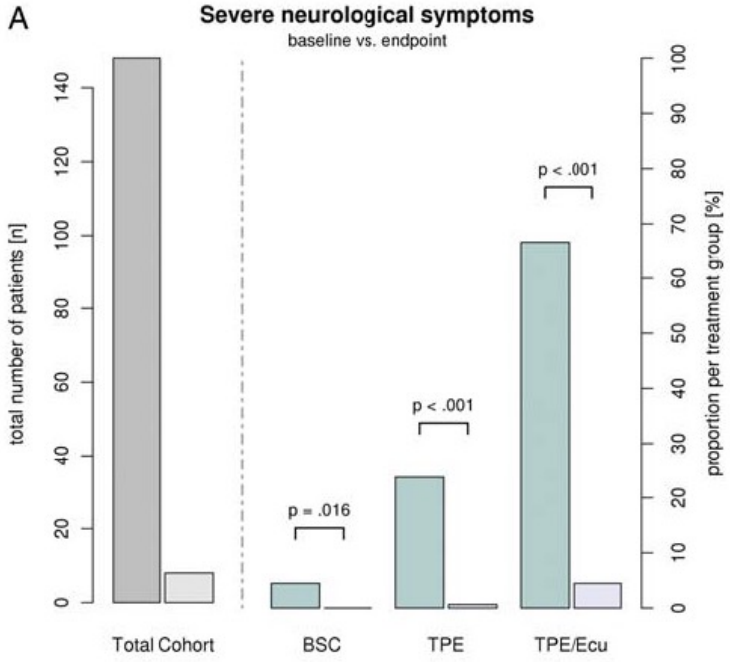
Is complement system activated in STEC-HUS ?? ➡ YES

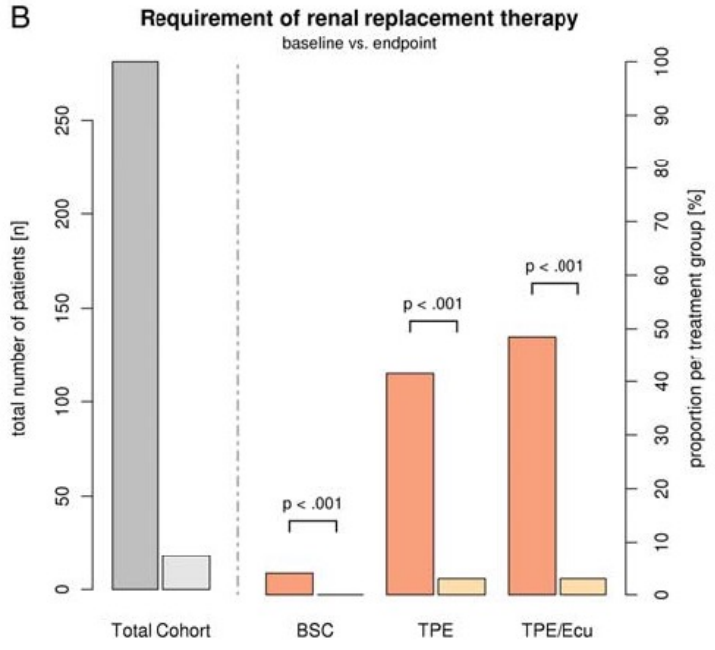
- Kim Y et al. Breakdown products of C3 and factor B in hemolytic uremic syndrome. J Lab Clin Med: 1977;89:845-850
- Monnens L et al. The complement system in hemolytic uremic syndrome in childhood. Clin Nephrol 1980;13:168-171
- Thurman JM et al. Alternative pathway of complement in children with diarrhea-associated hemolytic uremic syndrome. Clin J Am Soc Nephrol 2009
- Ahlenstiel-Grunow T et al. Systemic complement activation and complement gene analysis in enterohaemorrhagic Escherichia coli-associated pediatric haemolytic uraemic syndrome. Nephrol Dial Transplant 2016
- Westra D et al. Serological and genetic complement alterations in infection-induced and complement mediatied hemolytic uremic syndrome. Pediatr Nephrol 2016

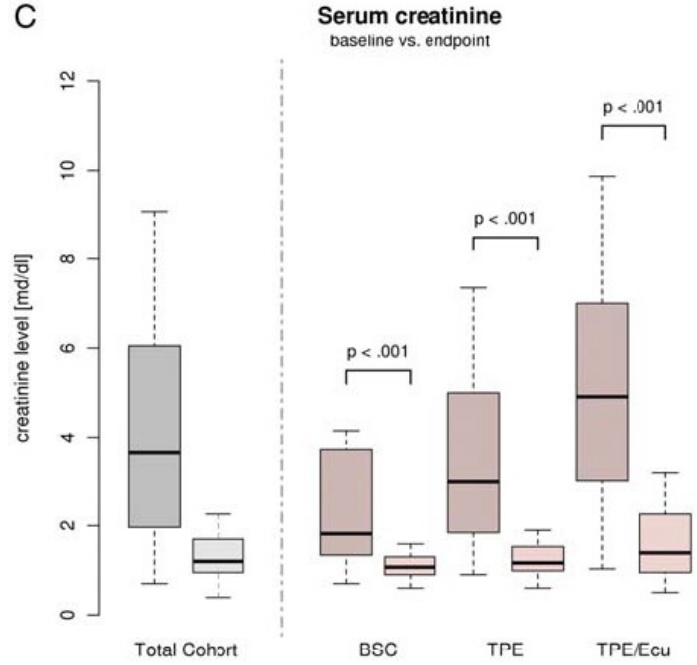
Best supportive care, plasma exchange, plasma exchange+eculizumab German Outbreak - STEC-HUS O104:H4 , 2011

No evidence for use plasmapheresis / eculizumab in STEC-HUS , n=491

Longterm?



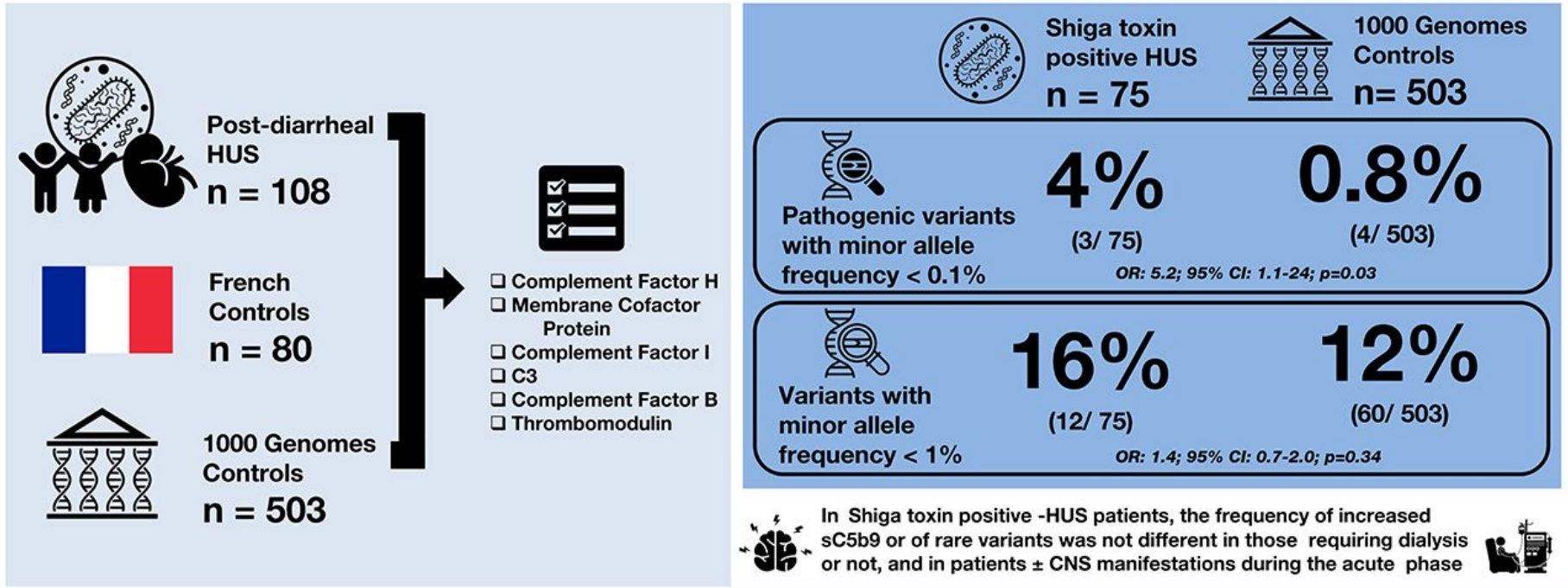




Kielstein et al , Nephrol Dial Transplant 2012

Complement gene variants and Shiga toxin producing *E. coli* associated hemolytic uremic syndrome

CJASN
Clinical Journal of American Society of Nephrology



Conclusions Rare variants and complement activation biomarkers were not associated with severity of Shiga toxin associated-HUS. Only pathogenic variants with minor allele frequency <0.1 % are more frequent in Shiga toxin positive-HUS patients than in controls.

Véronique Frémeaux-Bacchi, Anne-Laure Sellier-Leclerc, Paula Vieira-Martins, Sophie Limou, et al. ***Complement Gene Variants and Shiga Toxin Producing E. Coli Associated Hemolytic Uremic Syndrome.*** CJASN doi: 10.2215/CJN.05830518.
Visual Abstract by Edgar Lerma, MD, FACP, FASN

Véronique Frémeaux-Bacchi et al. CJASN 2019;14:364-377

CJASN

©2019 by American Society of Nephrology

Pediatric Nephrology
https://doi.org/10.1007/s00467-018-3972-9

EDITORIAL COMMENTARY

Eculizumab in STEC-HUS: need for a proper randomized controlled trial

Sebastian Loos[1] · Jun Oh[1] · Markus J. Kemper[2]

Randomized, double blinded, placebo controlled trial in UK, Dr S Johnson

Traitement précoce de patients pédiatriques présentant un syndrome hémolytique et urémique typique par l'anticorps monoclonal anti-C5 eculizumab: essai thérapeutique prospectif randomisé contrôlé contre placebo de phase III

Etude ECULISHU

Promoteur : CHU de Toulouse
Investigateur principal : Dr Arnaud GARNIER, garnier.a@chu-toulouse.fr
ARC coordinatrice: Isabelle KIEFFER, kieffer.i@chu-toulouse.fr

Randomized, single blinded, placebo-controlled

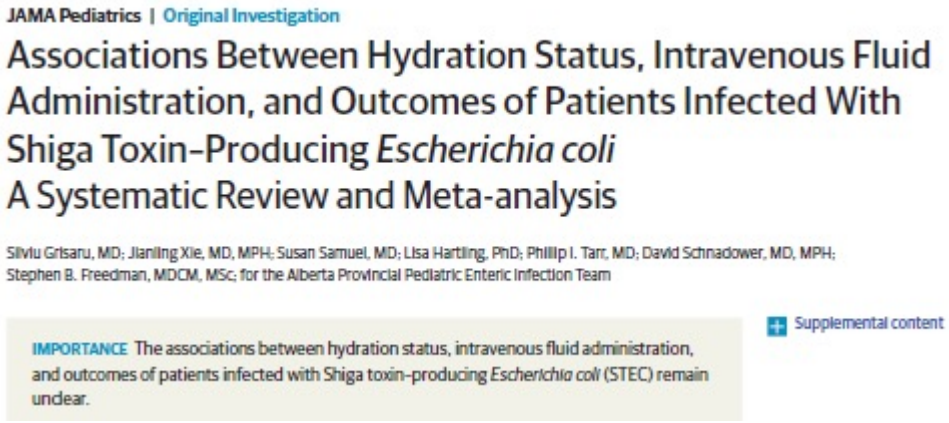
Treatment of STEC-HUS

Only symptomatic treatment

- **Timely rehydration intravenously!!**
- Renal replacement therapy
- Blood transfusions

- No antibiotics!

- Mortality: 1-4%
- 20-30% long term sequelae



JAMA Pediatrics | Original Investigation

Associations Between Hydration Status, Intravenous Fluid Administration, and Outcomes of Patients Infected With Shiga Toxin–Producing *Escherichia coli*
A Systematic Review and Meta-analysis

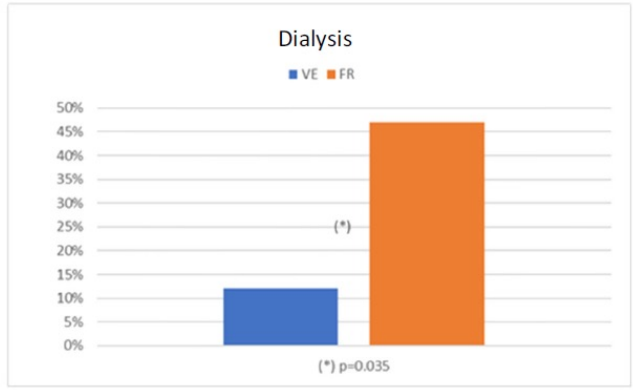
Silviu Grisaru, MD; Jianling Xie, MD, MPH; Susan Samuel, MD; Lisa Hartling, PhD; Phillip I. Tarr, MD; David Schnadower, MD, MPH; Stephen B. Freedman, MDCM, MSc; for the Alberta Provincial Pediatric Enteric Infection Team

Supplemental content

IMPORTANCE The associations between hydration status, intravenous fluid administration, and outcomes of patients infected with Shiga toxin–producing *Escherichia coli* (STEC) remain unclear.

For every 3 patients treated with volume-expansion , it is possible to prevent 1 dialysis

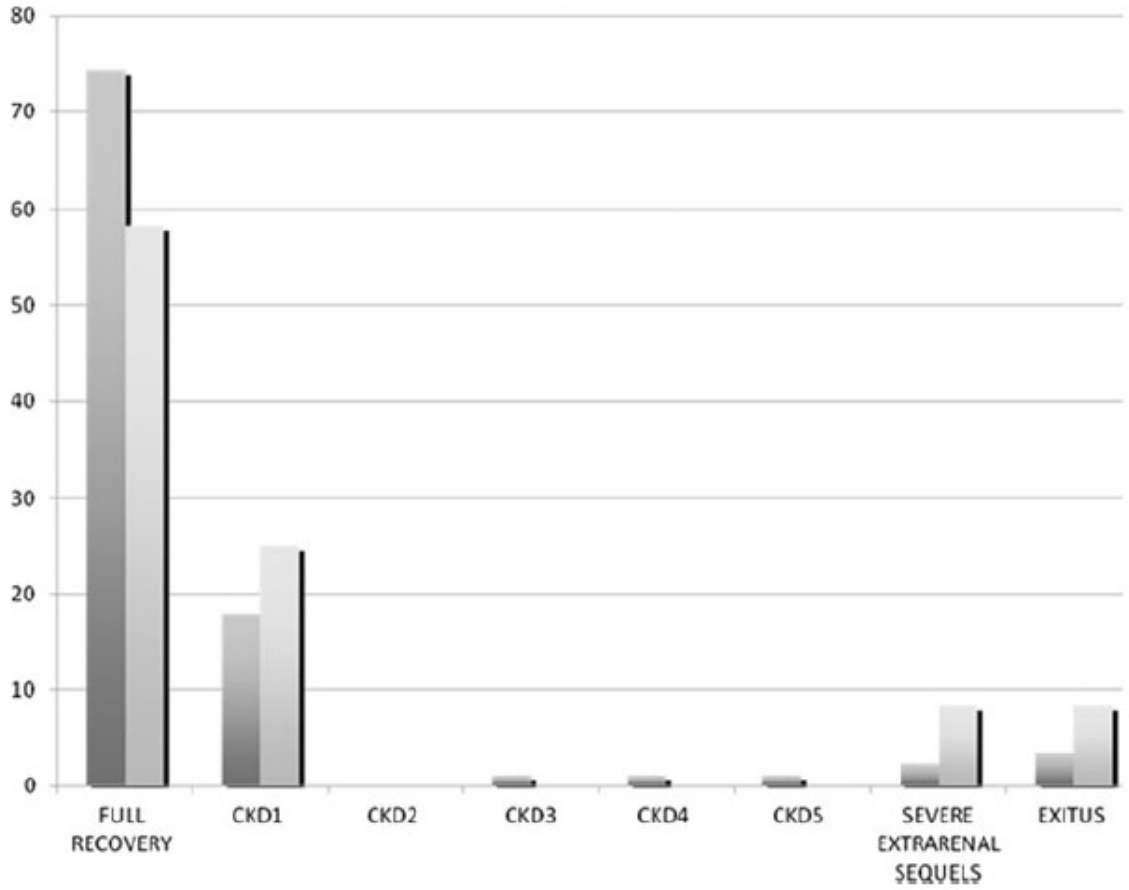
Bonany P et al, Pediatr Nephrol 2020



Ake J et al Pediatrics 2005, Hickey CA et al, Arch Pediatr Adolesc Med 2011, Ardissino G et al, Pediatrics 2016, Grisaru S et al JAMA Pediatr 2017, Bonany P et al Pediatr Nephrol 2020, McKee RS et al, Clin Infect Dis 2020

Outcome for majority of STEC-HUS patients is good

North Italian HUS Network
N=101 HUS / 10y (2003-2012)
STEC-HUS (dark grey)
aHUS (light grey)

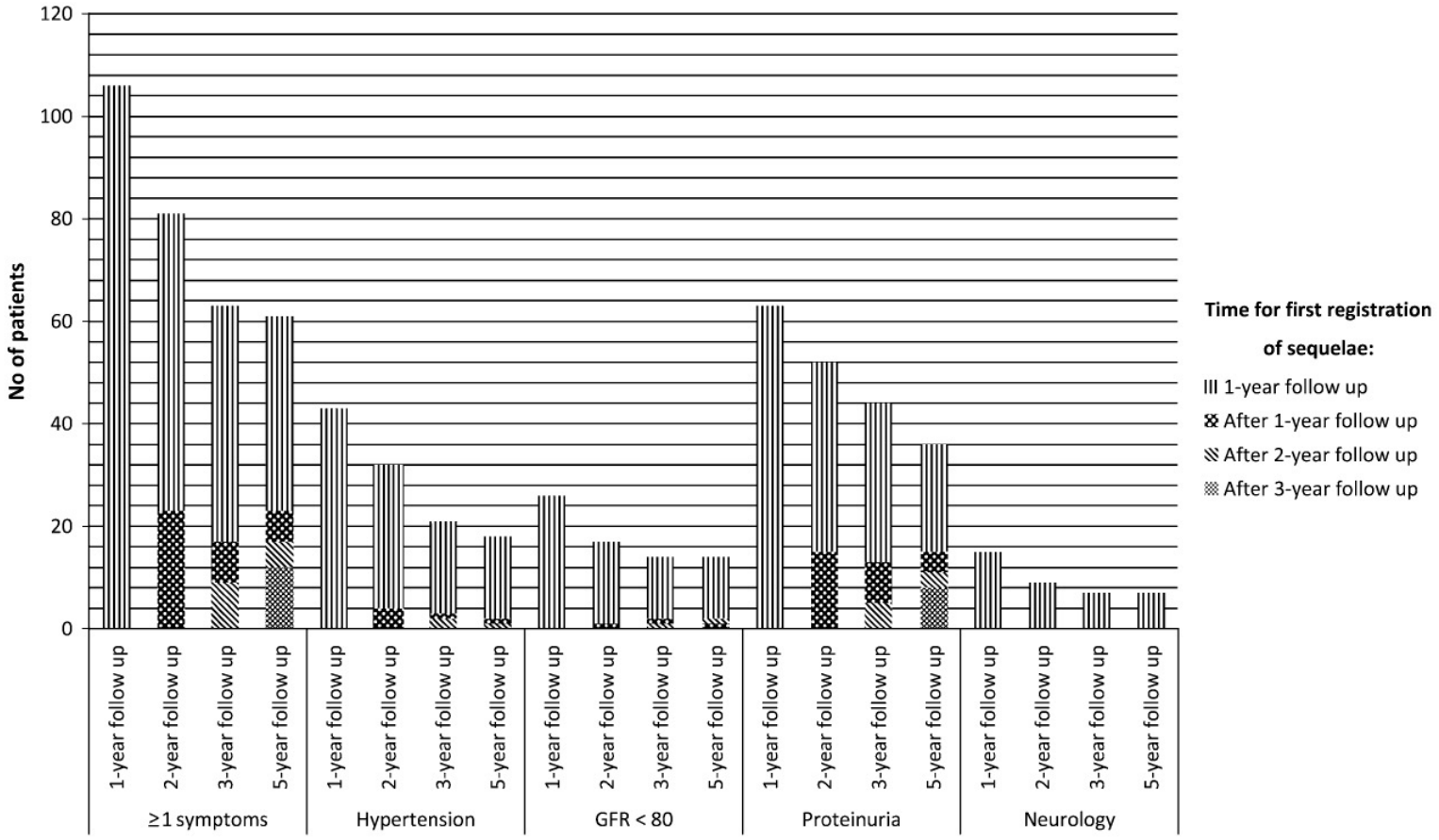


Ardissino GL et al, Eur J Pediatr 2016

Follow up STEC-HUS patients – Austria-Germany cohort

6y FOLLOW-UP
N=274

5y
- ≥1: 22% 60/274

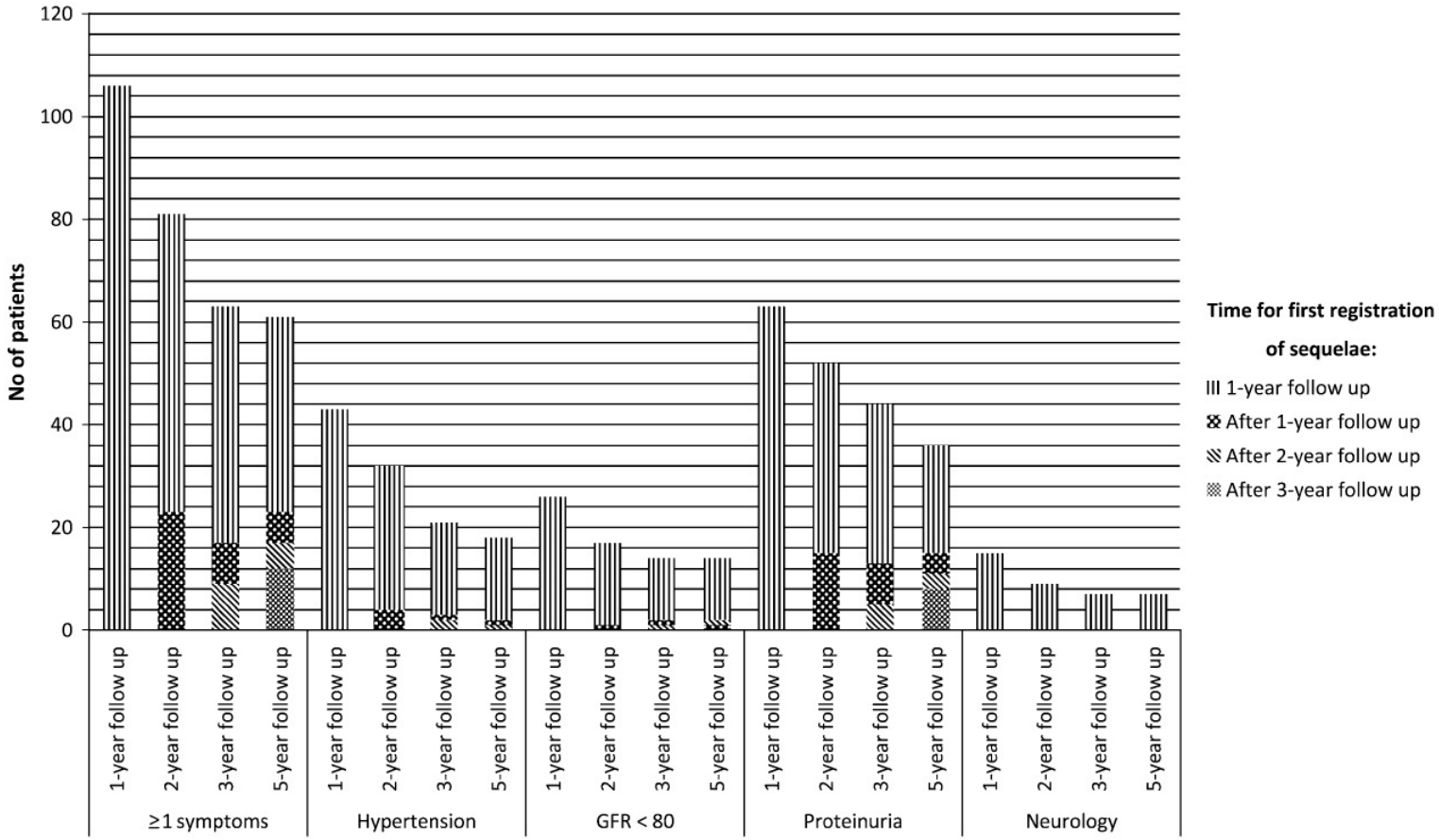


Rosales A et al, Clin Infect Dis 2012

Follow up STEC-HUS patients – Austria-Germany cohort

6y FOLLOW-UP
N=274

5y
- ≥1: 22% 60/274



Recommendation: Follow-up investigations yearly : Bloodpressure, protein/creatinin ratio

Rosales A et al, Clin Infect Dis 2012

Take home message

- Acute bloody diarrhea: think STEC!
  - Stool and Serology

- Use rectal swab if no immediate stool available (stool 3x)
  - If Stx2/Stx1+2 positive:
    - Dipstick urine – if hemoglobinuria – blood examination
    - Start rehydration!
    - Examination stool of family members!

- In 10% of STEC-HUS no diarrhea as presenting symptom
  - Emerging serotypes such as O80

- STEC activates the complement system, but complement is not the main driver of disease
  - No evidence for eculizumab as treatment of STEC-HUS

IMPORTANT UPDATE to our VTEC/STEC community: VTEC 2021 rescheduled to VTEC 2023

Online meetings to be held in May 2021 and 2022

Register for this year's online meeting here

May 11, 2021

VTEC2021 a 2,5h online meeting!
Free registration!
Registration & Program on www.VTEC.org

Next Webinars

era edta WGIKD working group
Working Group on Inherited Kidney Disorders

ERKNet
The European Rare Kidney Disease Reference Network

european society for paediatric nephrology

IPNA International Pediatric Nephrology Association
GREAT CARE FOR LITTLE KIDNEYS. EVERYWHERE

ERKNet/ERA-EDTA Advanced Webinars on Rare Kidney Disorders

Date: **04 May 2021**

Speaker: **Michael Somers**

Topic**: Acute post-streptoccocal GN**

ERKNet/ERA-EDTA Advanced Webinars on Rare Kidney Disorders

Date: **11 May 2021**

Speaker: **Savino Sciascia**

Topic**: TMA in Anti-phospholipid syndrome**

ESPN/ERKNet Educational Webinars on Pediatric Nephrology & Rare Kidney Diseases

Date: **01 June 2021**

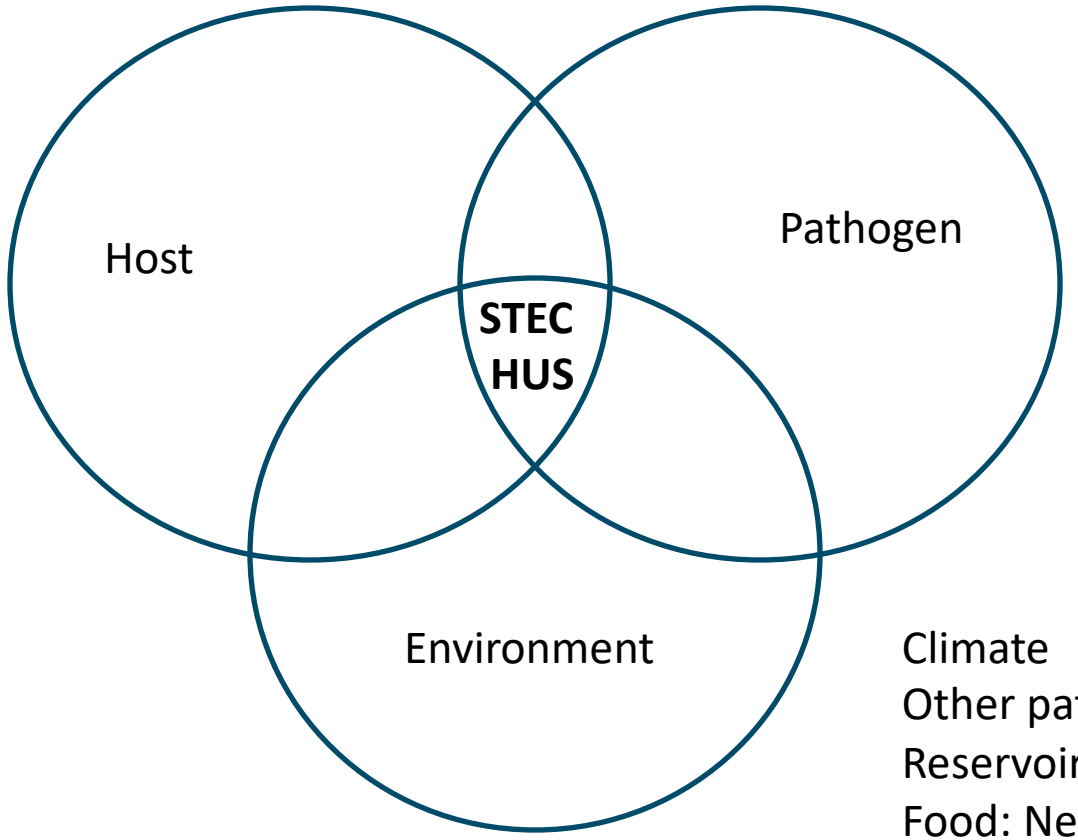
Speaker: **Marina Noris**

Topic: **Atypical Hemolytic Uremic Syndrome**

Subscribe the ERKNet and IPNA Newsletter and don‘t miss Webinars!

Determinants of health and diseases in STEC -HUS

Genetic profile
Innate immunity:
Complement
Coagulation
Cytokine
Development
Mucus

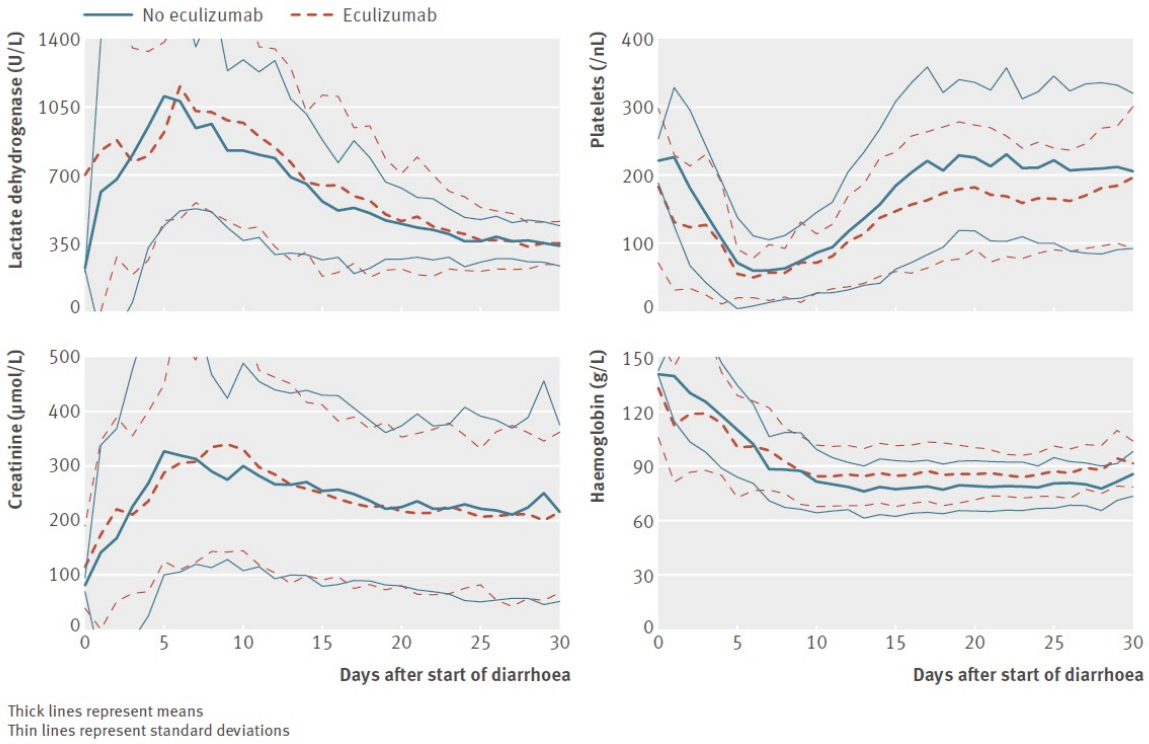


Serotype
Stx,SubAB,CLT
Pathogenecity islands
Plasmids
Esp subtypes, Ehem

Climate
Other pathogens
Reservoir
Food: Neu5Gc

Complement modulation in STEC-HUS patients

- Plasma therapy ‘80-’90
- German outbreak serotype O104:H4 2011



67 HUS with ECU
65 HUS matched without ECU

Longterm?

Menne J et al BMJ 2012