Overview of Stx phages diversity and their role in virulence and evolution of *Escherichia coli*

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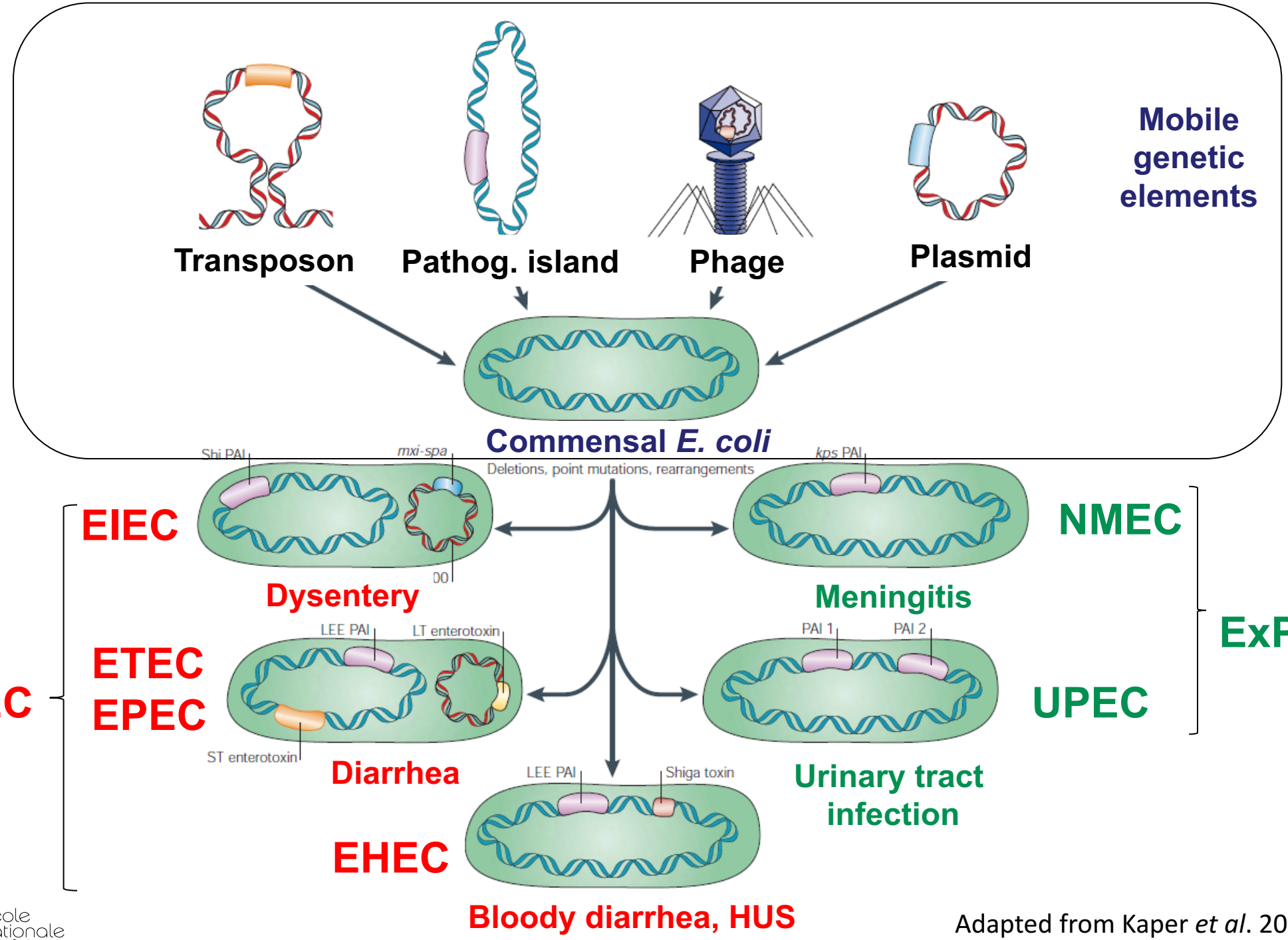
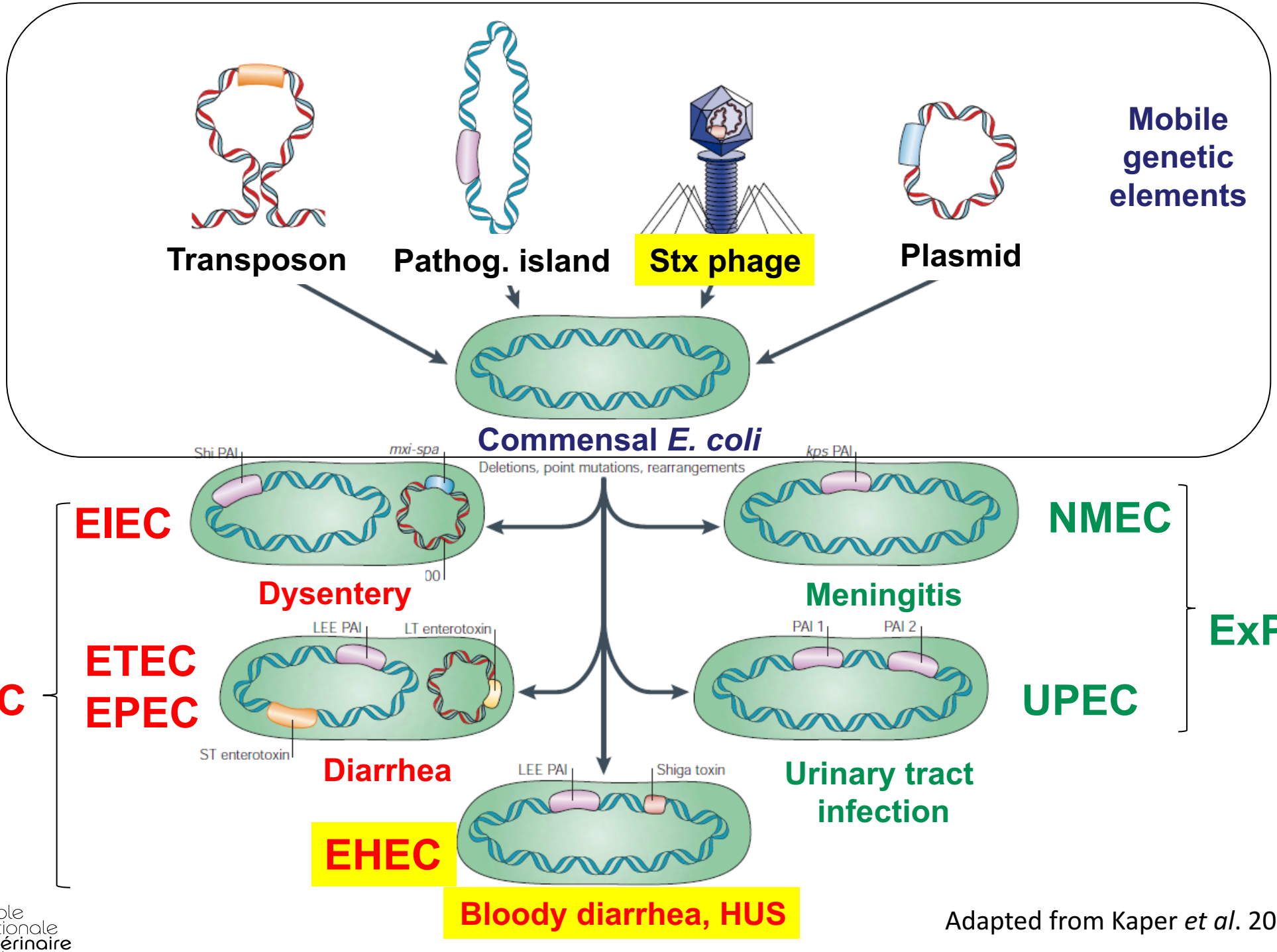
From commensal to pathogenic *E. coli*



Adapted from Kaper *et al*. 2004

From commensal to pathogenic *E. coli*



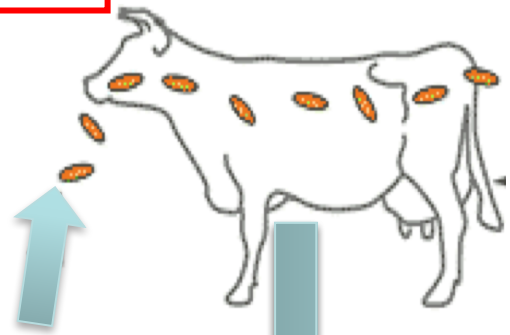
Adapted from Kaper *et al*. 2004

Reservoirs of EHEC

Main reservoir : ruminants (cattle)

STEC infections = life-threatening zoonosis

Asymptomatic carriage

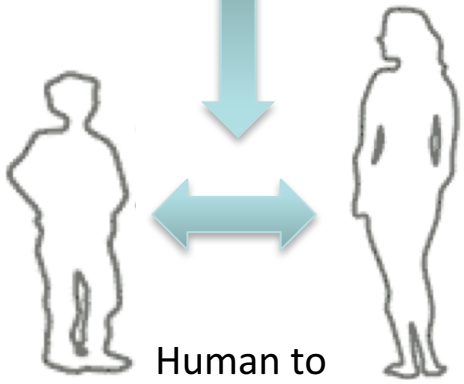


Fecal shedding
Contamination of the environment

Contamination of food & water

Ingestion of EHEC

Transmission through direct contact



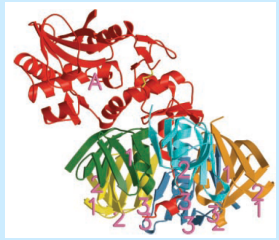
Human to human transmission

Ingestion of EHEC

Infection

Virulence factors of EHEC

Stx phage

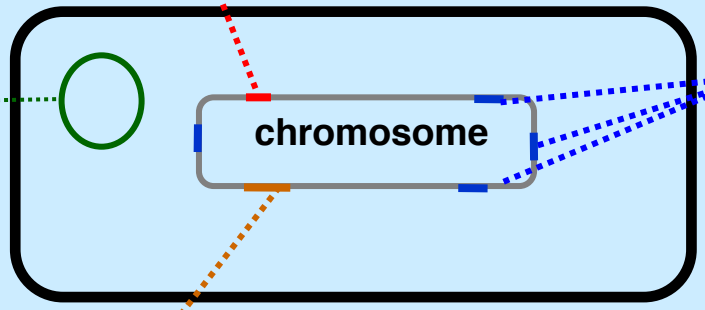


Stx

Damage to vascular endotelial cells (colon, kidney, brain)

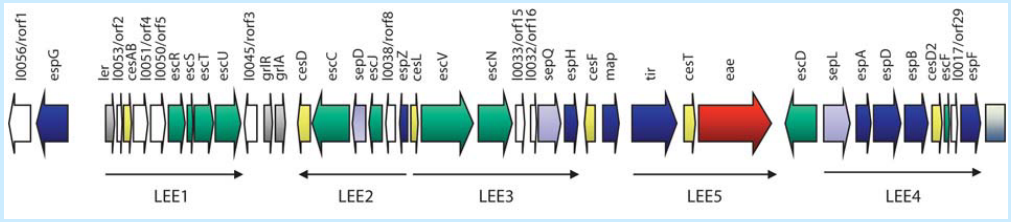
BD, HUS, encephalopathy

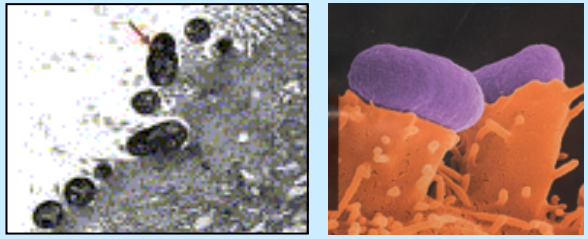
Plasmid pEHEC (pO157) ***(ehxA, katP…)***



O Islands (OI-122, OI-71, OI-57…) **Non-LEE encoded Type III effectors** (n > 32)
(ex: TccP, NleB, NleE, EspL…)

Locus of Enterocyte Effacement (LEE) *



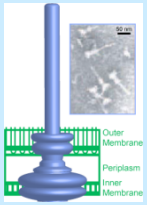


A/E Lesions (attachment/effacement)

Intimin (*eae*) and its receptor (Tir)

Type III secretion system

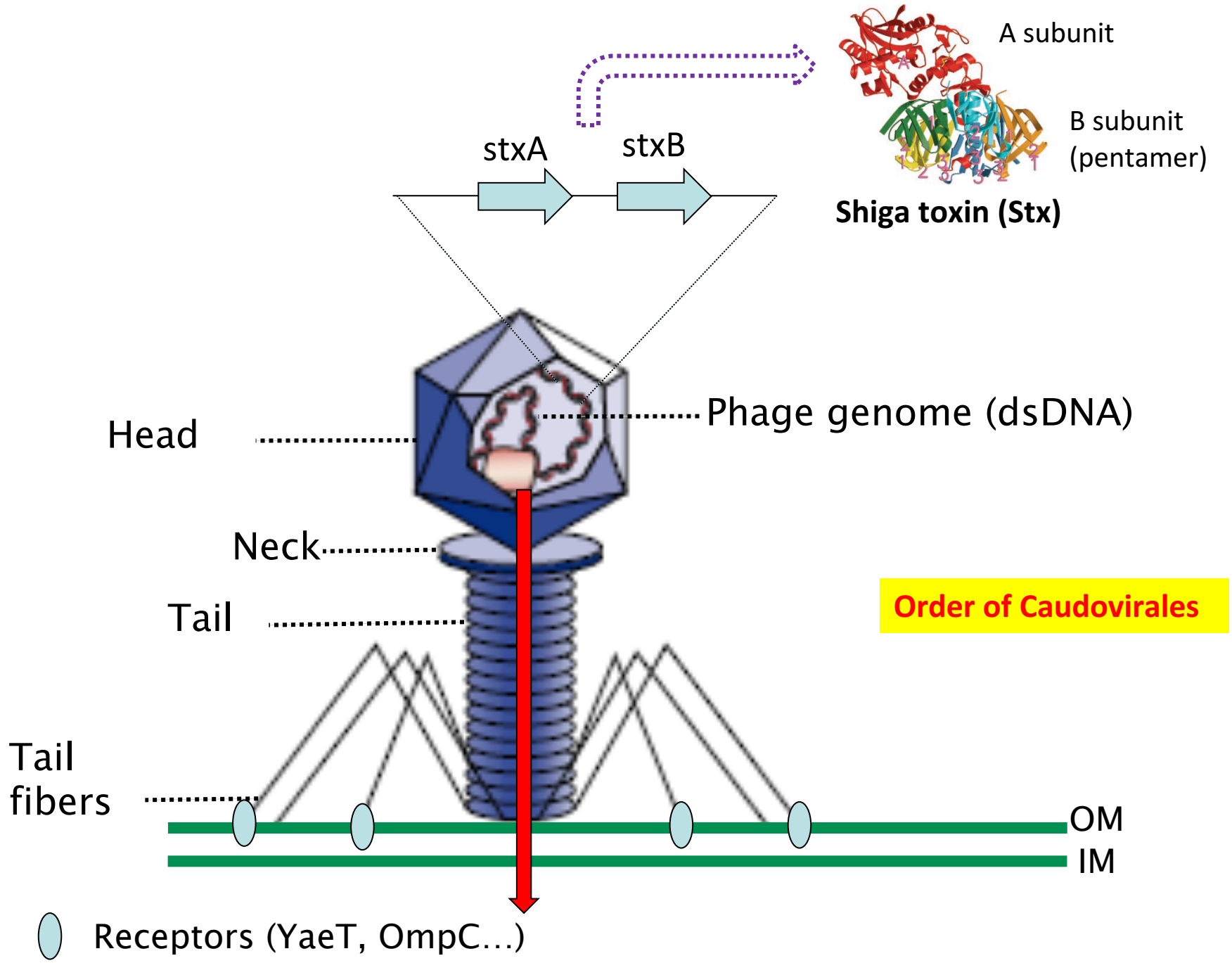
Type III effectors



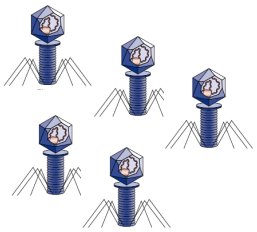
Adhesion and colonisation

* LEE also carried by EPEC

Shiga toxin-encoding phages (Stx phages)



Lytic & lysogenic cycles of Stx phages



Free Stx phages

(temperate phages, λ-like)

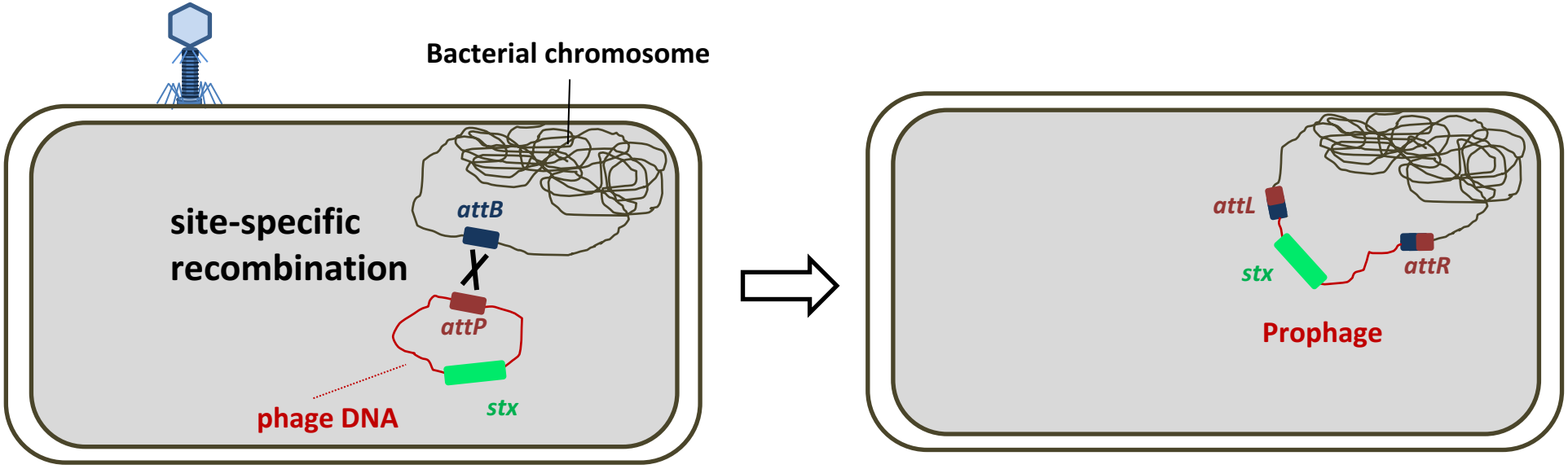
2 pathways

Lytic cycle ⇧⇩ **Lysogenic cycle (lysogenization)**

STEC (Shiga toxin-producing *E. coli*)

Lysogenic state

Chromosomal insertion of Stx phage DNA



E. coli

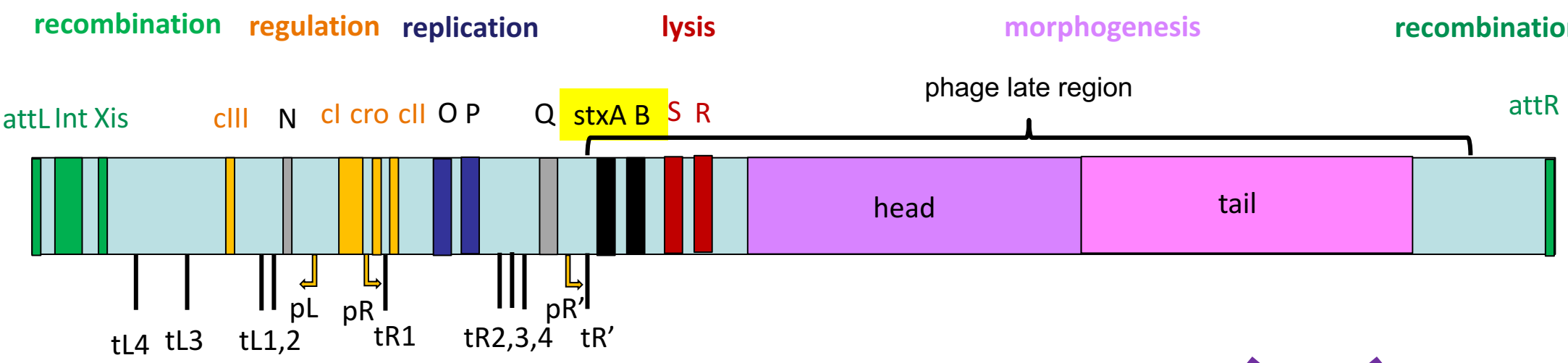
STEC

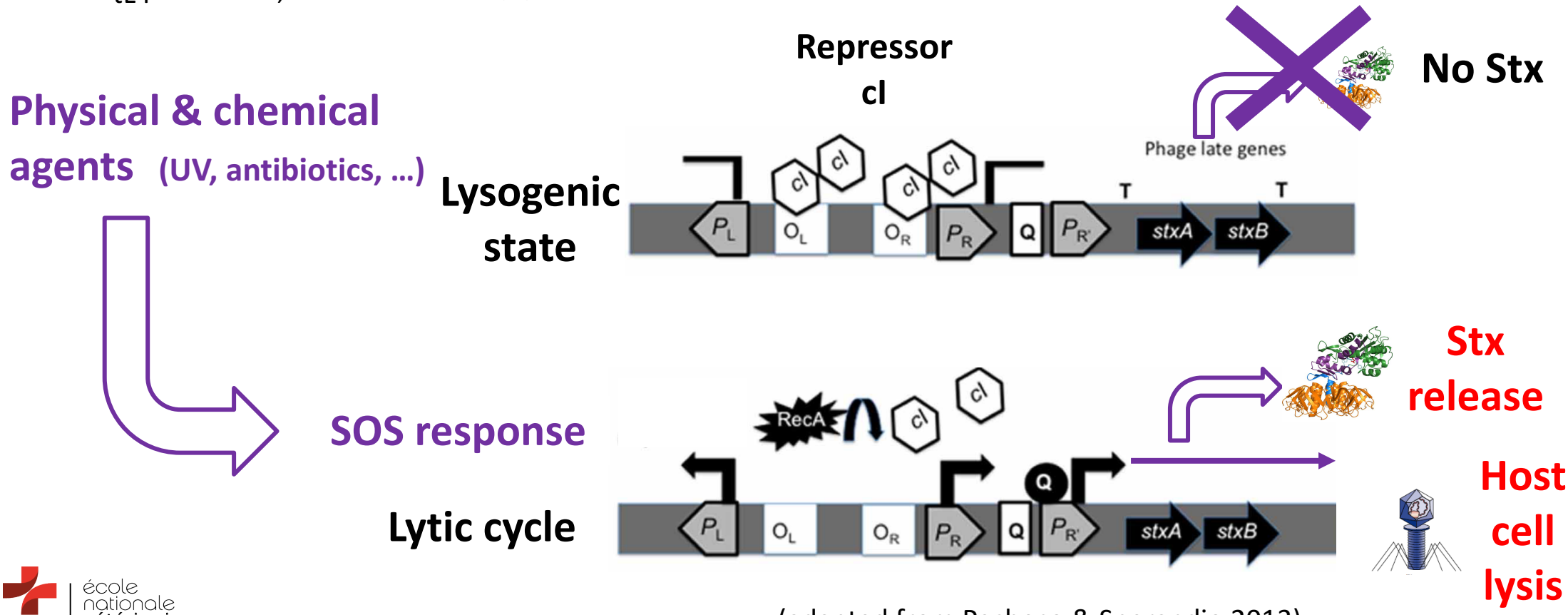
STEC (Shiga toxin-producing *E. coli*)

> 400 STEC serotypes

Broad host range of Stx phages

Genetic organization of Stx phage DNA





(adapted from Pacheco & Sperandio 2013)

Phages: vehicles for very diverse shiga toxin genes

- 2 antigenically distinct Stx types

  Stx1 & **Stx2** (Stx2 : 1000 x more cytotoxic to human renal endothelial cells)

- At least 3 *stx1* subtypes (a,c,d) and 8 *stx2* subtypes (a-h)

- Certain subtypes are circulating in particular reservoirs

stx2e

stx2f

stx2h

Oedema disease

- STEC strains may carry >1 Stx phage (=> carriage of multiple *stx* variants)

- Certain *stx* subtypes (*stx2a* , *stx2c* , *stx2d*) are carried by STEC more frequently associated with bloody diarrhea or HUS

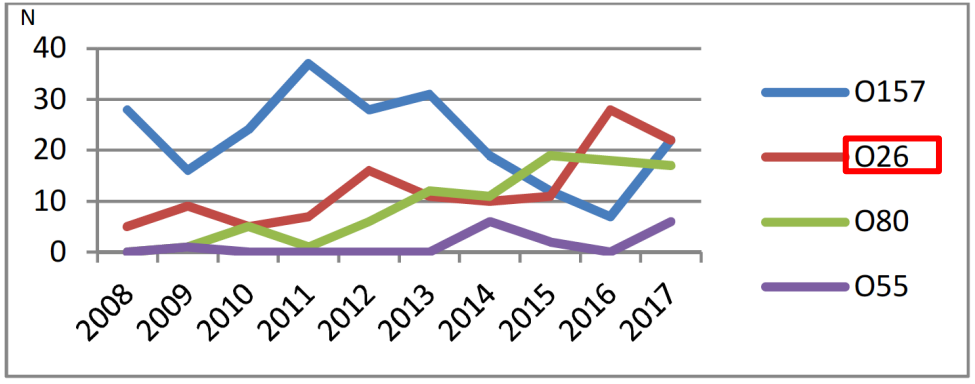
Characterization of Stx phages from EHEC O26:H11

STEC serogoups from confirmed human cases, 2015-2017 (EU/EEA)

Serogroup	2017			2016			2015		
	Cases	MS	%	Cases	MS	%	Cases	MS	%
O157	1,304	24	31.9	1,552	22	38.6	1,510	21	42.1
O26	582	18	14.3	671	19	16.7	537	16	15.0
NT[(a)]	493	11	12.1	335	12	8.3	397	10	11.1
O103	245	14	6.0	218	18	5.4	172	14	4.8
O91	179	14	4.4	149	11	4.0	114	12	3.2
O145	150	14	3.7	121	12	3.7	95	12	2.6
O146	140	10	3.4	158	11	3.0	75	10	2.1
O111	94	18	2.3	57	14	1.6	42	11	1.2
O113	56	8	1.4	60	11	1.5	25	7	0.7
NON-O157	48	4	1.2	25	5	1.4	29	3	0.8
O128	46	12	1.1	65	13	1.0	49	12	1.4
O80	42	7	1.0	42	8	0.8	24	4	0.7
O-rough[(b)]	37	3	0.9	26	4	0.7	44	8	1.2
O128ab	33	2	0.8	9	1	0.7	2	6	0.1
O76	31	7	0.8	20	6	0.6	31	9	0.9
O121	30	7	0.7	24	5	0.6	17	4	0.5
O55	30	9	0.7	34	10	0.6	28	8	0.8
O63	30	6	0.7	24	4	0.6	8	4	0.2
O117	29	4	0.7	28	7	0.6	23	7	0.6
O8	28	7	0.7	25	10	0.5	20	9	0.6
Other	455	–	11.1	369	–	7.8	348	–	9.7
Total	**4,082**	**25**	100.0	**4,012**	**25**	100.0	**3,590**	**21**	**100.0**

EFSA Journal 2018; 16(12): 5500.

STEC serogroups from pediatric HUS patients, 2008-2017 (France)



Characterization of Stx phages from STEC O26:H11

74 STEC O26:H11 strains : dairy products (31) ; human cases (31) ; cattle (12)

53 *stx1*-positive; 18 *stx2*-positive ; 3 *stx1*/*stx2*-positive

Stx subtypes :

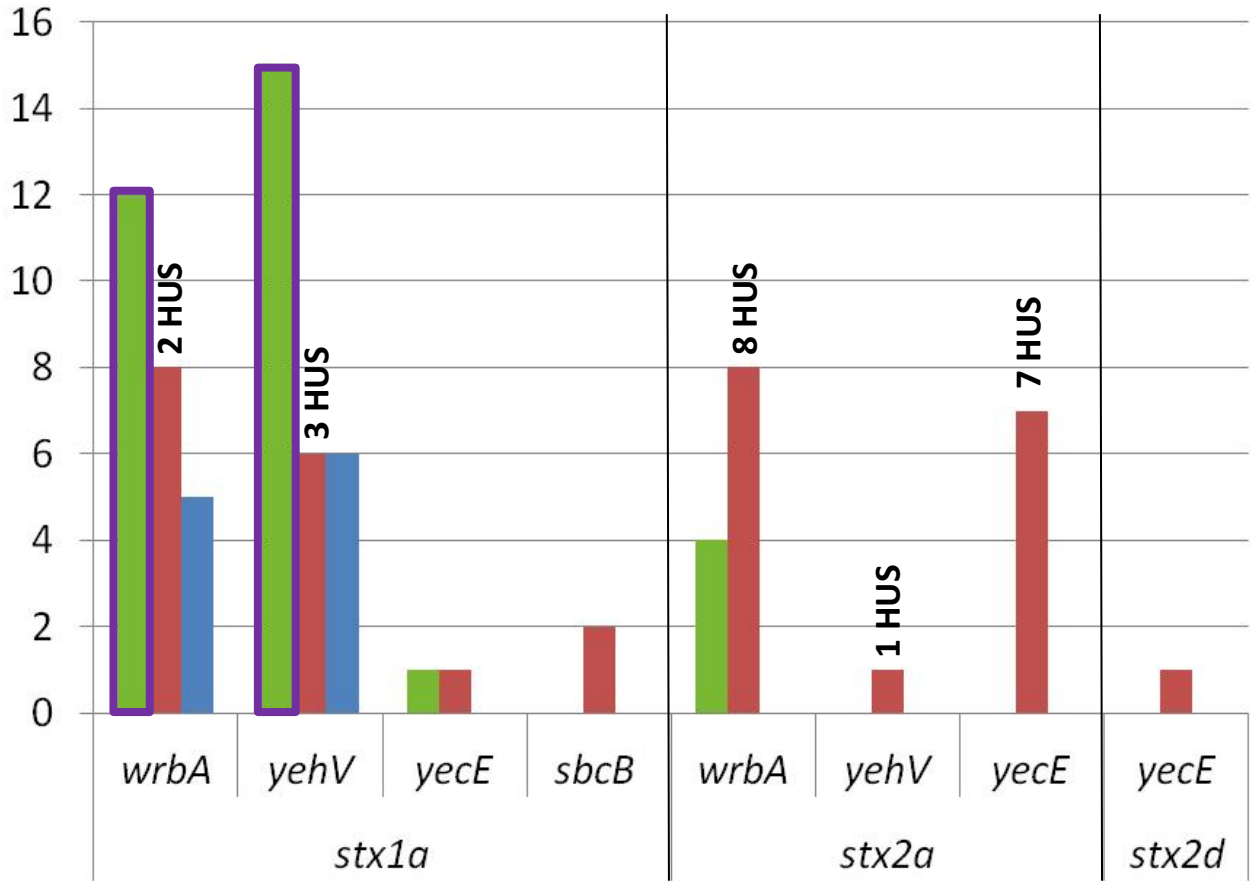
stx1a (56)
stx2a (20)
stx2d (1)

Insertion sites :

wrbA (37)
yehV (28)
yecE (10)
sbcB (2)

Z2577
argW
prfC
torST
ssrA

No inserted phage Stx

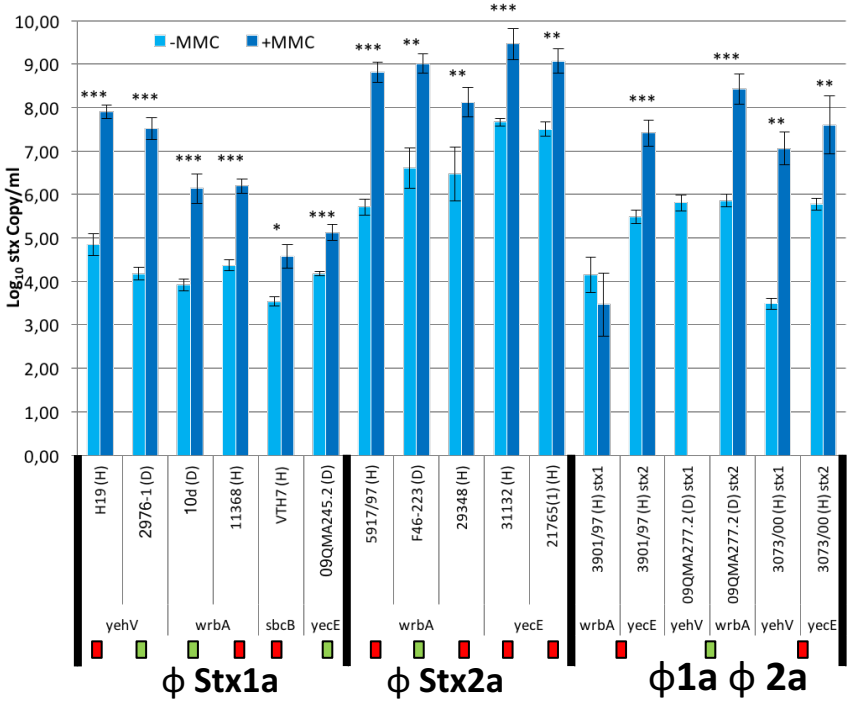


Bonanno *et al.*, AEM 2015

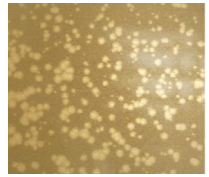
Induction of STEC O26:H11 Stx phages by MMC

14 STEC O26:H11 strains Human Dairy product

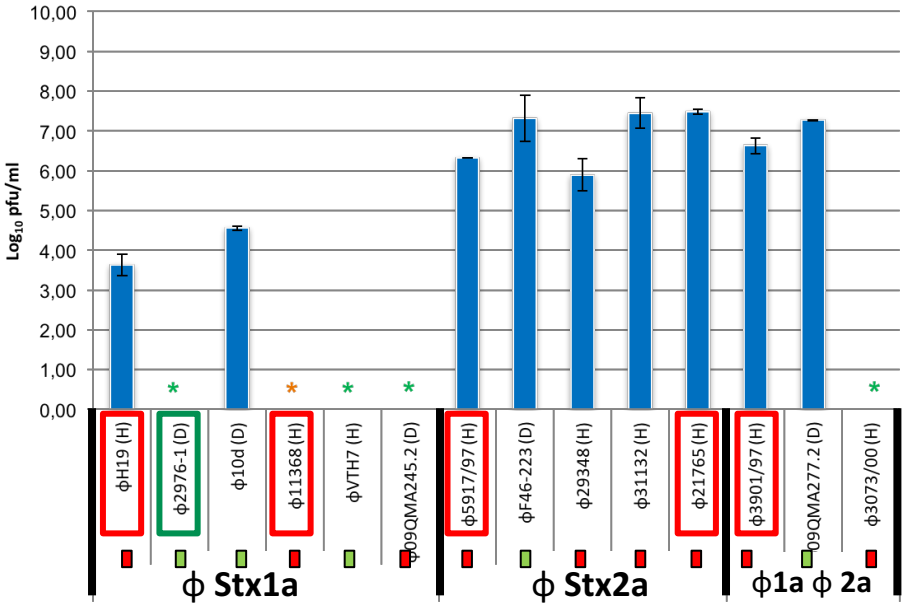
qPCR



Plaque assay



Recipient : DH5α



* No lysis * Confluent lysis

TEM

=> -MMC : spontaneous production
=> +MMC : ↗ 2 $\log_{10}$ [ϕ Stx]
=> ϕStx2 > ϕ Stx1 (2-3 $\log_{10}$)

=>[ϕ Stx2] > [ϕ Stx1] → 2 $\log_{10}$
=> presence of non-infectious ϕ Stx (pfu < qPCR)

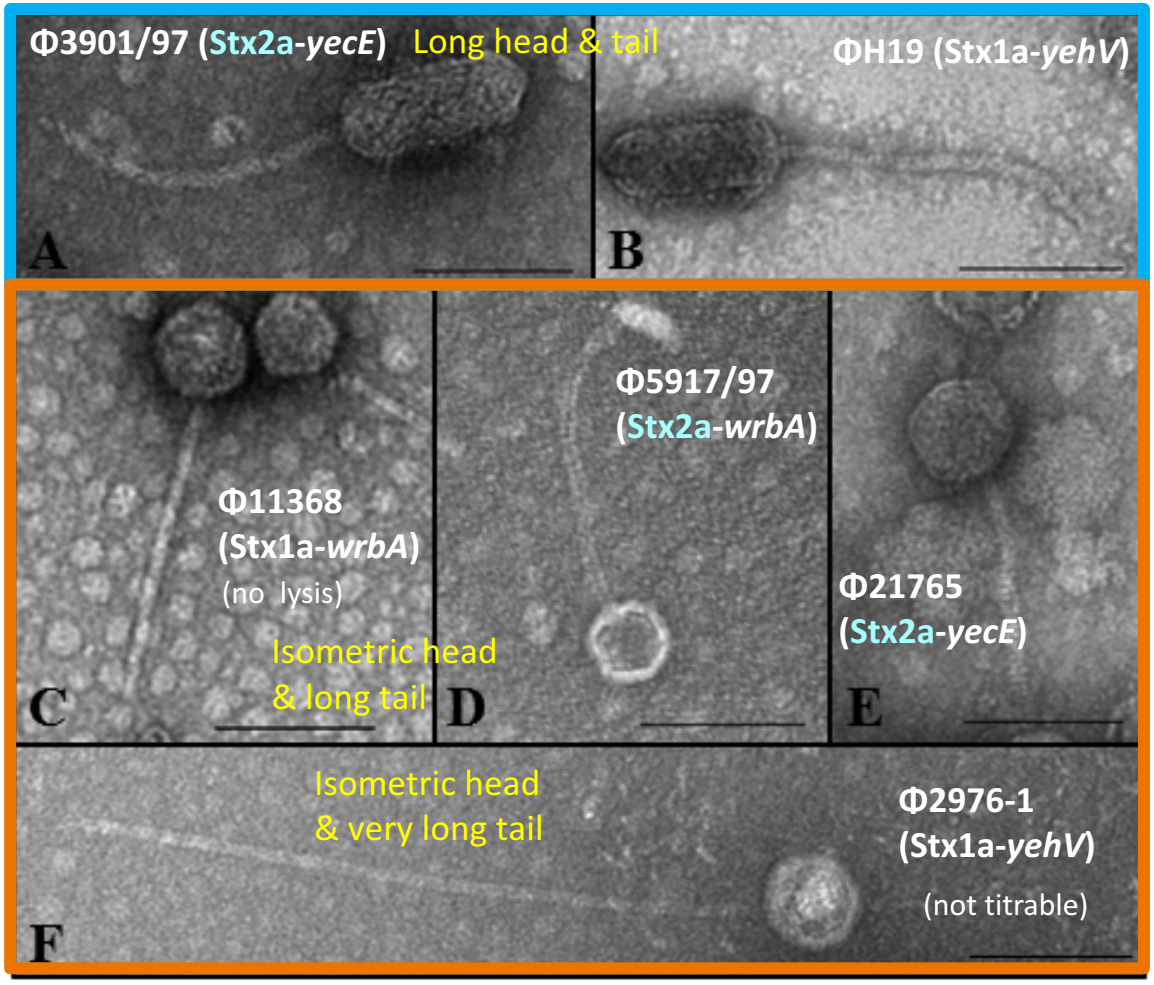
Bonanno *et al*. AEM 2016

Characterization of Stx phages from STEC O26:H11

Stx1a & Stx2a phages

Order of caudovirales (Siphoviridae, long & non-contractile tail)

Heterogeneity (not correlated to Stx type)



Bars = 100nm

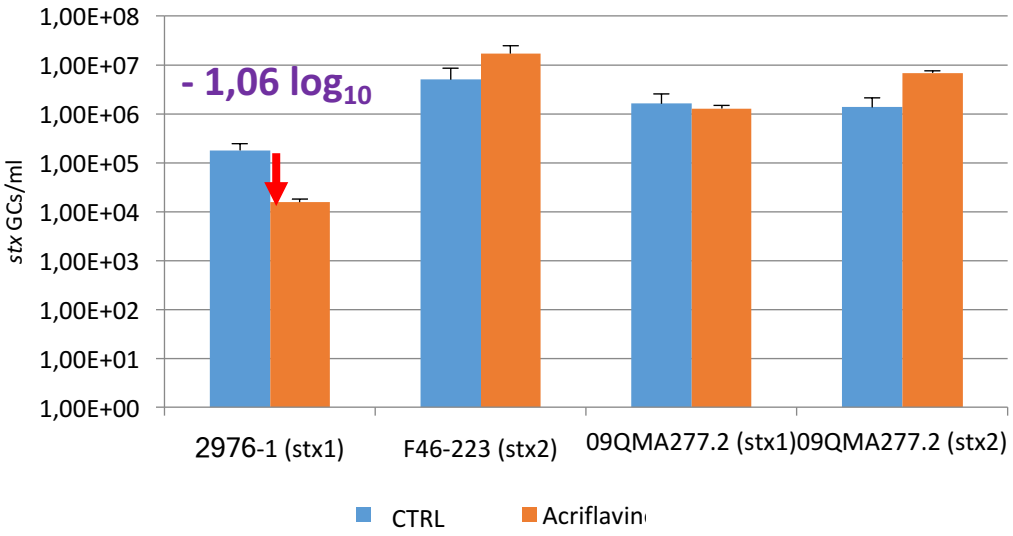
Bonanno *et al*. AEM 2016

Induction of Stx phages & diagnostics

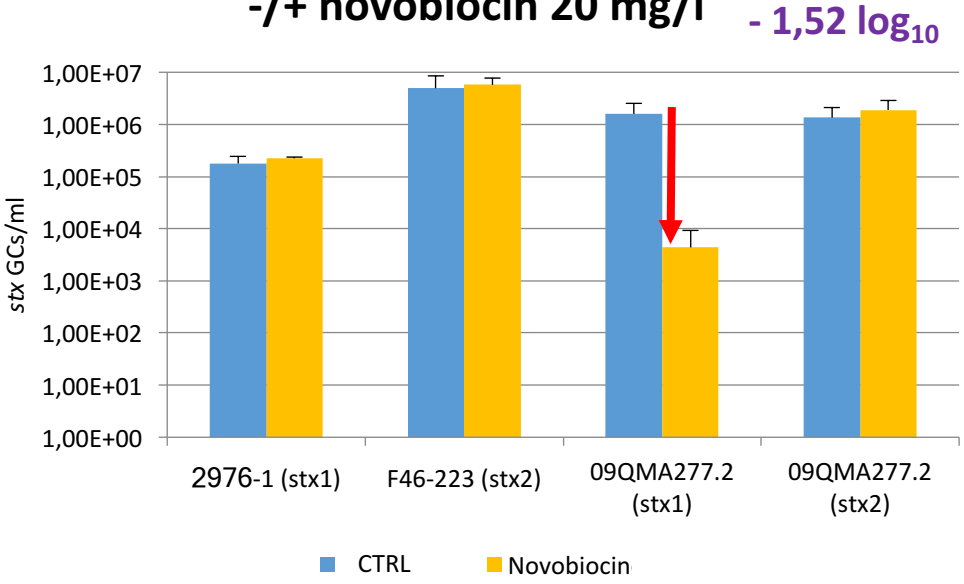
❑ qPCR-based quantification of Stx phages

3 STEC O26:H11 strains tested *in vitro* (+/- selective agent)

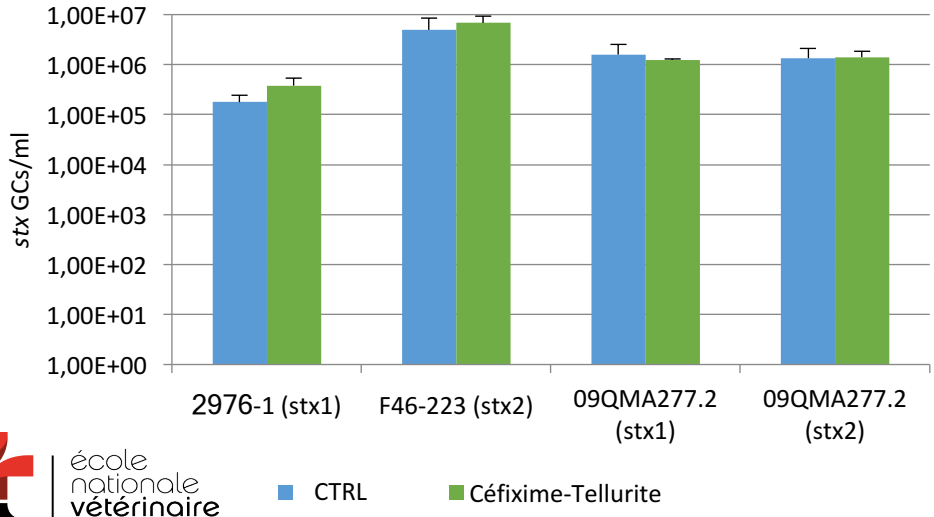
-/+ acriflavin 12 mg/l



-/+ novobiocin 20 mg/l



-/+ cefixime (0.05 mg/l) tellurite (2.5 mg/l)



Acriflavin, novobiocin, CT
=> no induction observed.

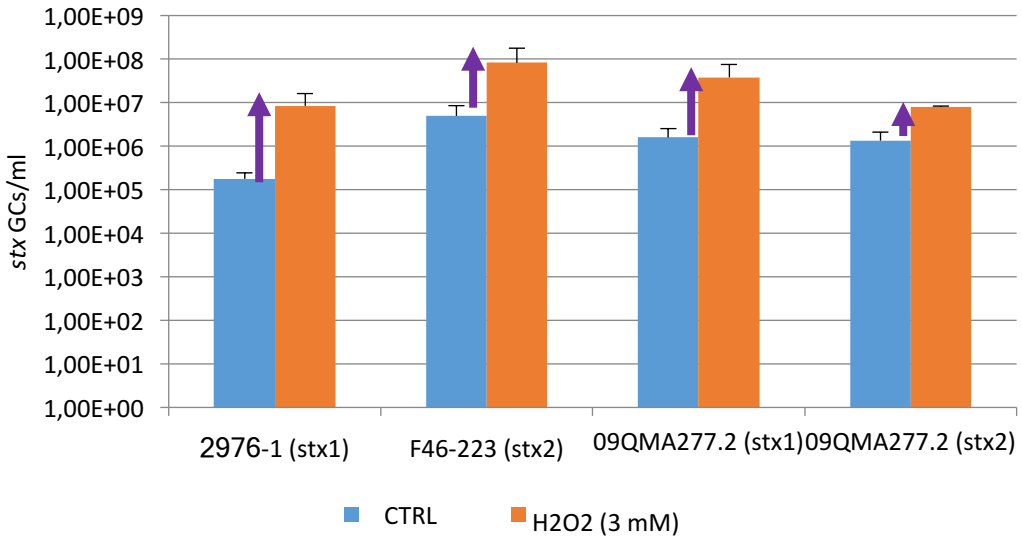
Bonanno *et al*. Front. Mic. 2017

Induction of Stx phages in cheeses

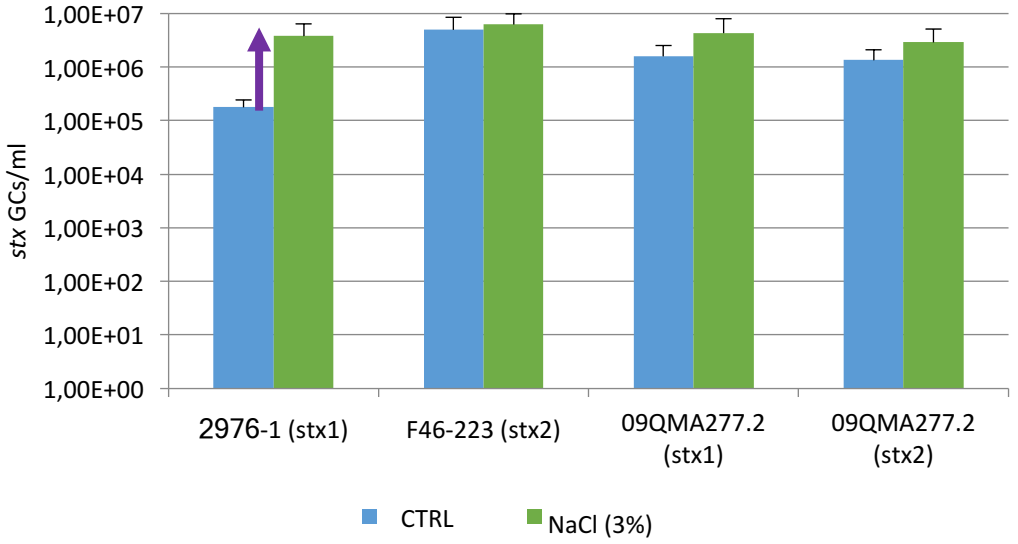
❑ qPCR-based quantification of Stx phages

3 STEC O26:H11 strains tested *in vitro* (LB medium +/- selective agent)

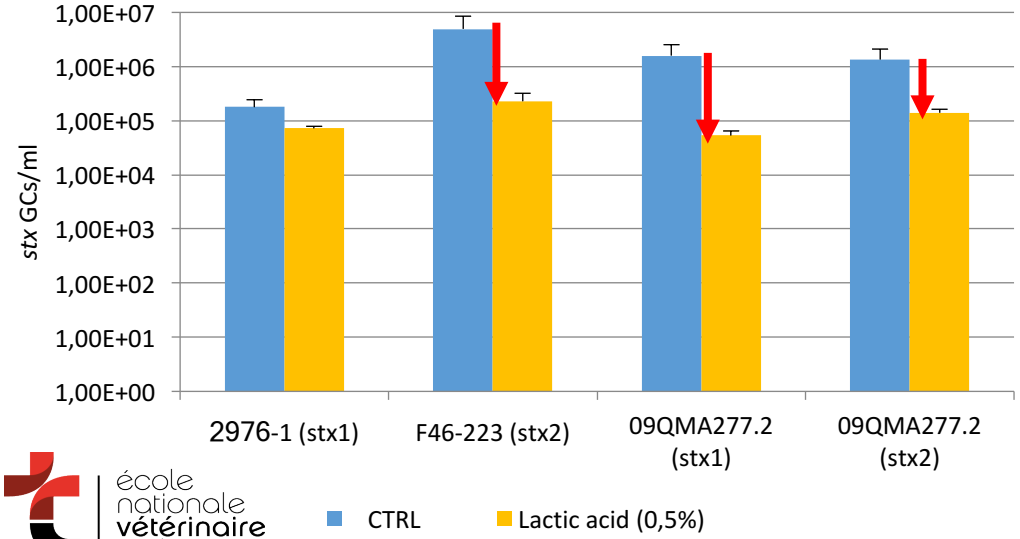
H_2O_2 3 mM **(mean: + 1.2 log_{10})**



(+ 1.33 log_{10}) **NaCl 3%**



Lactic acid 0.5% **(mean: - 1.0 log_{10})**



=> Stx phage production induced by H_2O_2

=> Stx phage production reduced by lactic acid

Bonanno *et al*. Front. Mic. 2017

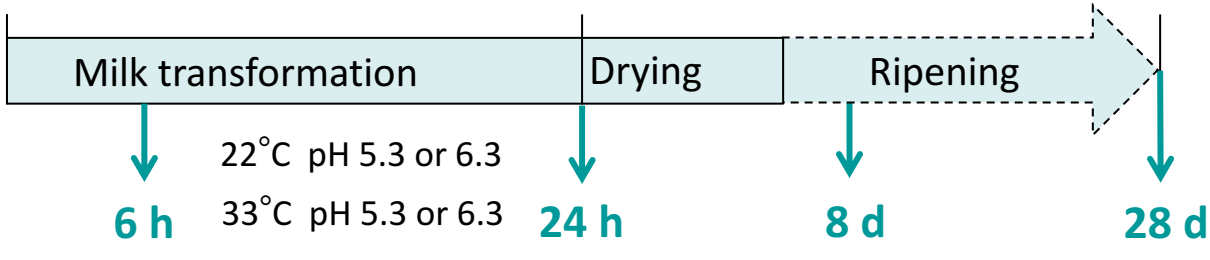
école nationale vétérinaire toulouse

Induction of Stx phages in cheeses

❑ Production of uncooked pressed cheese

STEC O26:H11 (100 CFU/mL)
2 strains (1 *stx1*+ ; 1 *stx2*+)

Pasteurized milk
(Lactose 51 g/L ; Fat 40 g/L)



Each production performed in triplicate
48 samples collected & analyzed / strain

Strain	stx1	stx2	Detected Stx phages	Concentration of Stx phage
21765	-	+	7/48 (14,6%)	$<10^4$ to 10^8 copies/ml
F15-338 A	+	-	3/48 (6,25%)	

➔ Stx phages could be induced during cheese making
- variation between replicates

Detection of free Stx phages in food & environnement

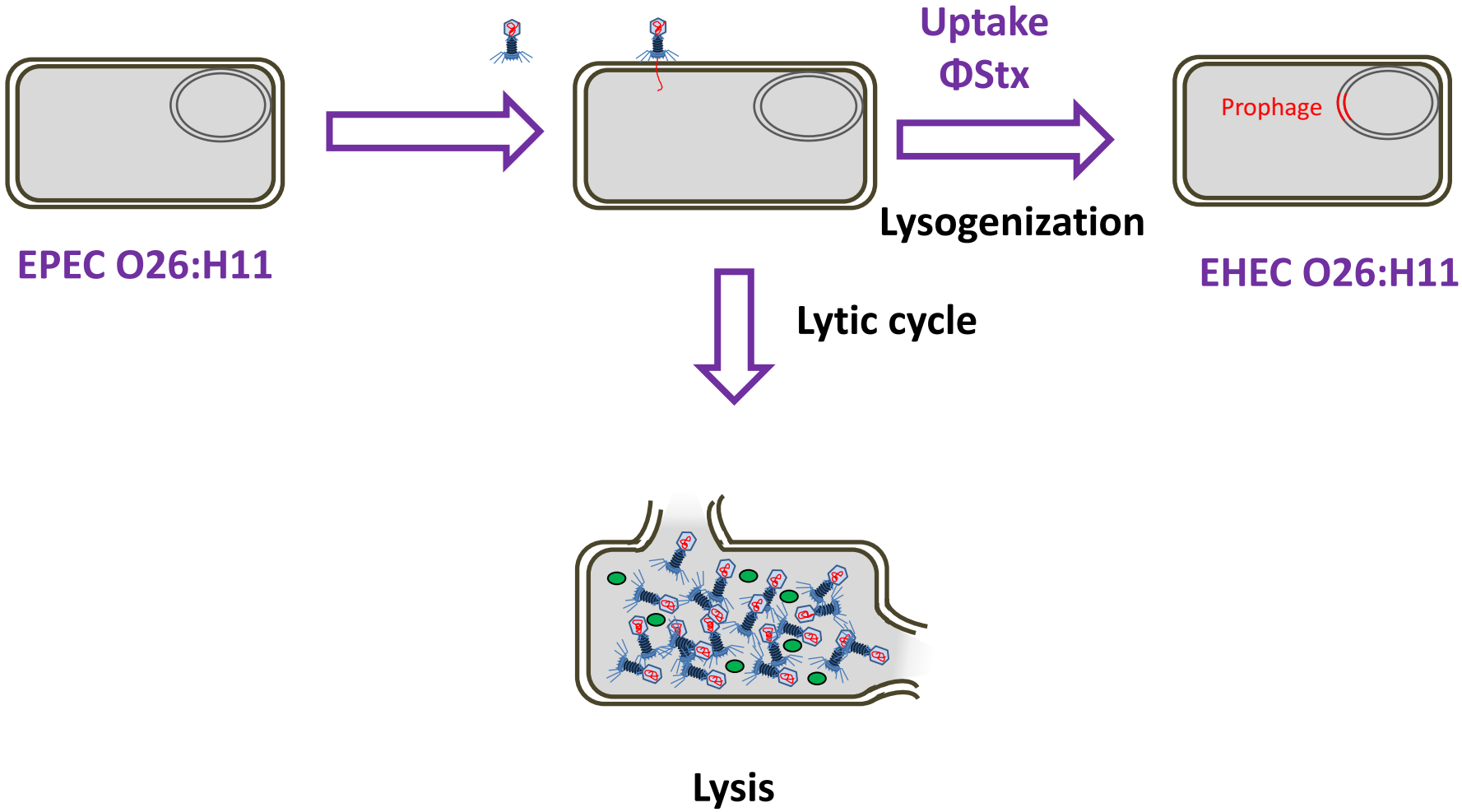
❑ Free Stx phages in foods (Imamovic & Muniesa, AEM 2011)

Samples	Nb. analysed	% positive samples (Stx phages)	% positive samples (infectious particles)
 Meat	36	100%	52-56%
 Salads	32	68,7%	39-65%

❑ Free Stx phages in the environnement & animal feces (Imamovic *et al.*, AEM 2010)

Samples	Nb analyzed samples.	% positive samples
 Urban sewage	50	70%
Slaughterhouse wastewater	36	94%
 Cattle feces	34	89%
   Other feces	22	31,8%

Lytic infection & lysogenization of EPEC O26:H11

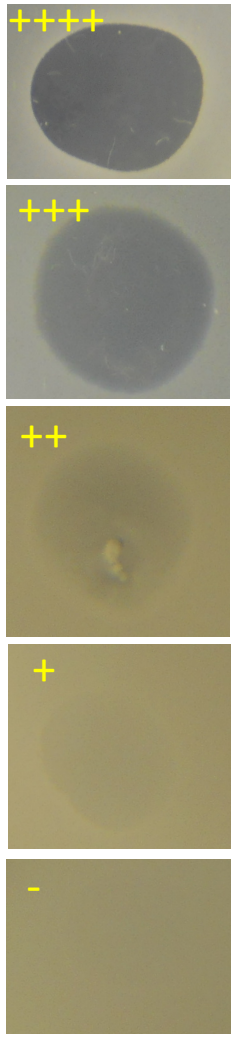


Lytic infection & lysogenization of EPEC O26:H11

6 Stx phages from STEC O26:H11

102 interactions tested

	Origin	Strain	ΦH19 (ϕStx1)	Φ5917 (ϕStx2)	Φ3901m (ϕStx1,Stx2)	ΦF46-223 (ϕStx2)	Φ277.2 (ϕStx2)	Φ21765 (ϕStx2)	Total
17 EPEC O26:H11	Dairy product	09QMA04.2	-	-	+	-	-	-	1
	Dairy product	09QMA315.2	+	++	+	-	+	-	4
	Dairy product	09QMA306.D	-	+	-	-	+	-	2
	Dairy product	FR14.18	-	+	+	-	-	-	2
	Dairy product	4198.1	-	-	+	++	++	+	4
	Dairy product	191.1	+	+	+	+	+	+	6
	Dairy product	64.36	-	-	-	-	-	-	0
	Dairy product	09QMA355.2	-	+	-	-	-	-	1
	Dairy product	F61-523	-	+	-	-	++	-	2
	Human (HUS)	5021/97	+	+	+	+	+	+	6
	Human (HUS)	5080/97	+	+	+	+	+	++	6
	Human (HUS)	318/98	-	-	+	+	++	-	3
	Human (HUS)	21474	-	+	++++	+++	++	+++	5
	Human (HUS)	21766	-	+	+	-	-	+	3
	Human (NK)	MB04	-	+	+	+	-	+	4
	Human (NK)	MB01	-	+	-	+	+	+	4
	Human (HUS)	29690	-	+	++	-	-	-	2
Lab.	K12	DH5α	++++	++++	++++	++++	++++	+++	6
	Total	18	5	14	13	9	11	9	



➔ **Variation in the infectivity of Stx phages against EPEC O26:H11**

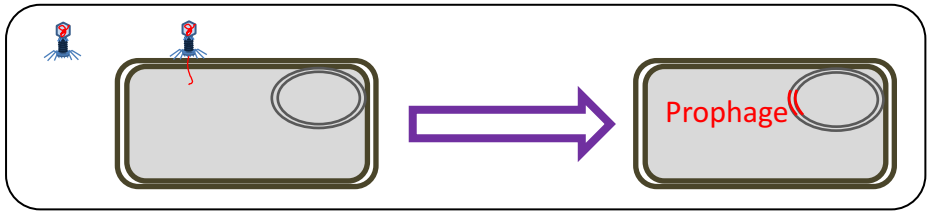
➔ **Variation in the sensitivity of EPEC O26:H11 to Stx phages**

Bonanno *et al.* AEM 2016

➔ **No EPEC lysogenized (only *E.coli* K12); Stable lysogenization of EPEC O26:H11 : rare.**

Lysogenization of EPEC O26:H11 : 10x < compared to a lab strain (C600) (Bielaszewska *et al.* AEM 2007)

Acquisition of Stx phages by *E. coli*



In water & food




☑ Favorable (experimental) conditions

But: 10e3 - 10e4 CFU *E. coli* recipient /mL required

☒ no transfer at 4°C

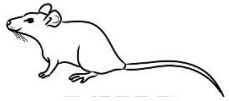
(Imamovic *et al*. AEM 2009; Imamovic & Muniesa AEM 2011; Picozzi *et al*. IJFM 2012)

☒ Unfavorable conditions (acidic pH)

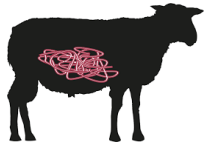
(Imamovic et al. AEM 2009)

In vivo

Gastro-intestinal tract

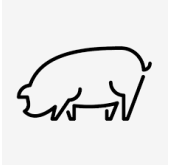


☑ (Acheson *et al*. Inf. Imm. 1998)



☑ (Sekse *et al*. JAM 2008; Cornick, Helgerson et al. 2006)

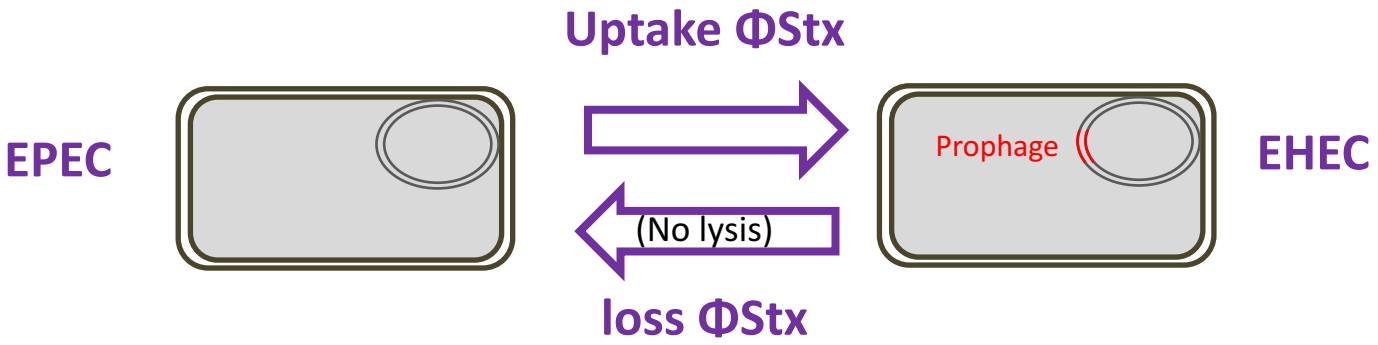
Ligated ileal loop



☑ (Toth *et al*. AEM 2003)

in vivo conditions more effective for Stx2 phage transduction (failures *in vitro*).

Interconversion of EHEC & EPEC



Loss of Stx phage

In human patients

For 5,5% of 787 patients with HUS (*infected with EHEC*):
the only isolated bacteria = EPEC O26:H11, O103:H2, O145:H28 and O157:H7 (90,7%)
(Bielaszewska, Kock *et al*. 2007)

Culture broth

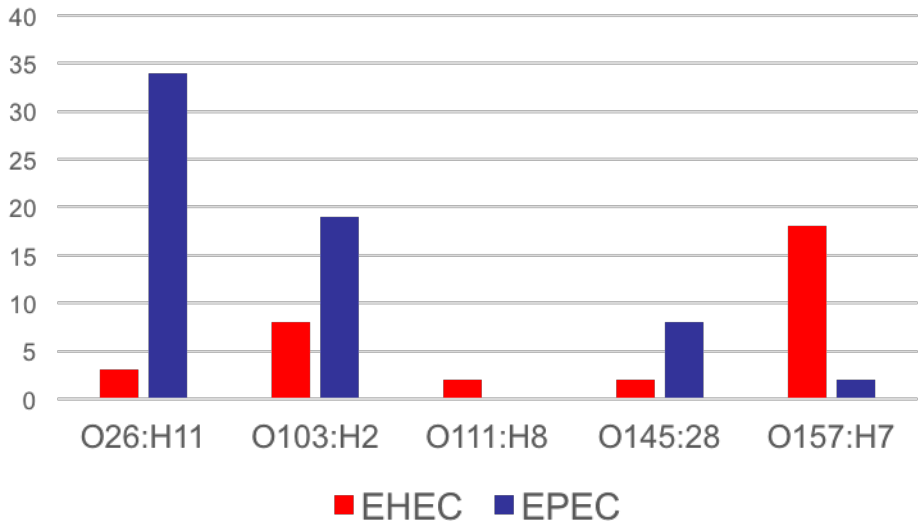
Upon subcultivation 15/45 clinical EHEC : loss of Stx phages => EPEC (Karch *et al.* 2002).

14% colonies *stx*-negative (EPEC) after plating EHEC O26:H11 (Bielaszewska, Prager *et al*. 2007).

Co-existence of EHEC & EPEC in cattle

EPEC: progenitors of EHEC ? Or derivatives of EHEC ?

Healthy cattle
N = 1318
(2010-2011)



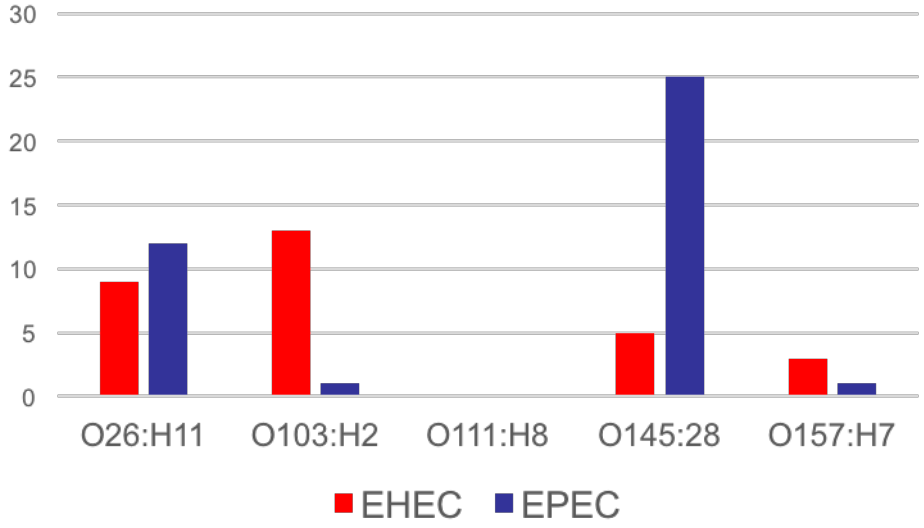
(2 animals carrying EHEC & EPEC of the same serotype)

33 EHEC strains isolated (top7 EHEC : 1.8%)

63 EPEC strains isolated

(Bibbal *et al*. AEM 2015)

Healthy veal calves
N = 500
(2017)



(4 animals carrying EHEC & EPEC of the same serotype)

30 EHEC strains isolated (top7 : EHEC 5.6%)

39 EPEC strains isolated

Gain/loss of Stx phages in wild boars ?

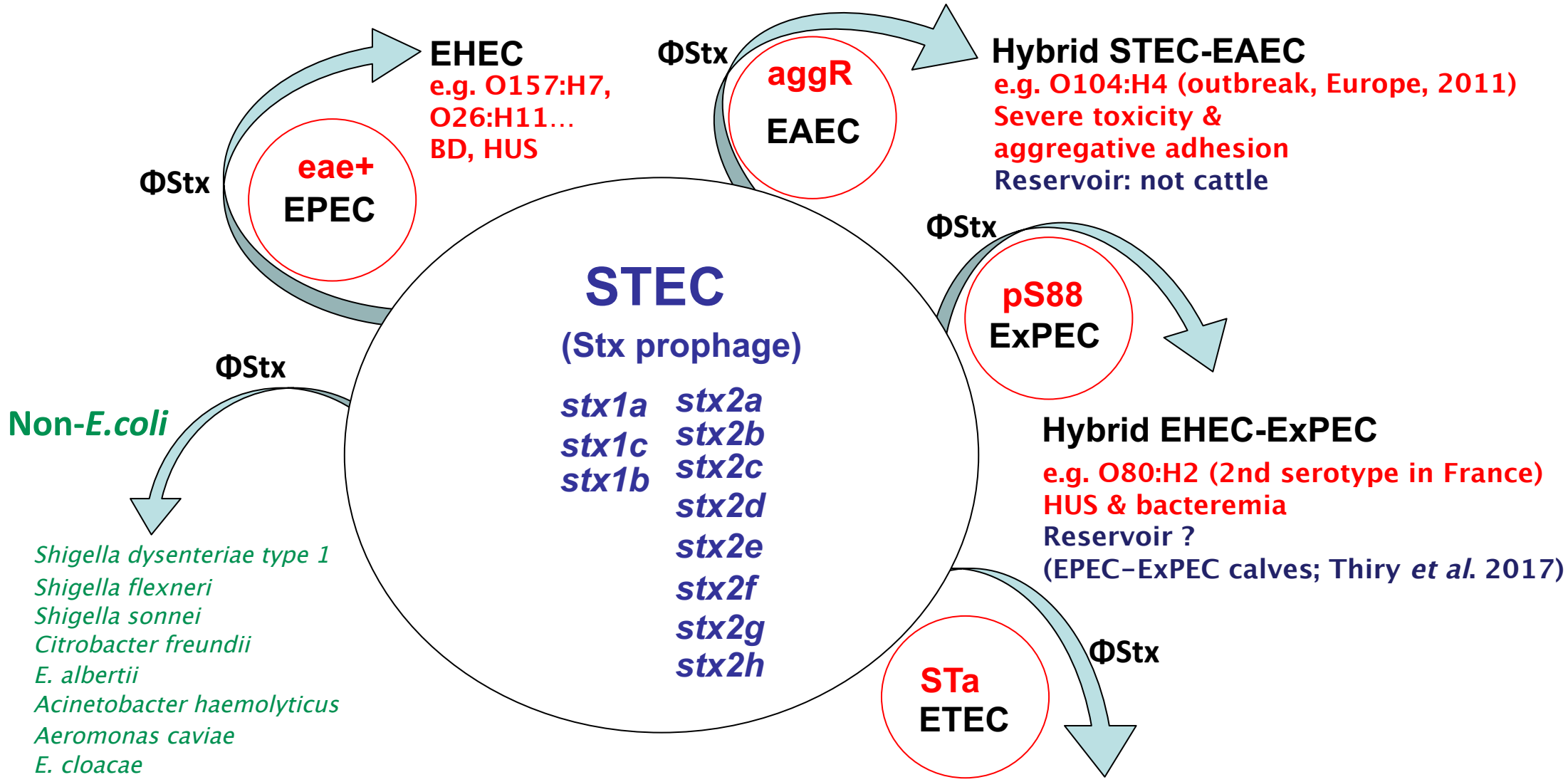
WGS analysis of 21 pig strains & 21 wild-boar strains (oedema disease).

2 *stx*-negative strains from wild boars with edema disease

Loss of Stx phage during infection or isolation ?

Spread of Stx phages to other *E. coli* pathovars

Hybrid pathovars : an ever-growing list…



Role of phage-encoded Stx in cattle colonization

☑ **Role of Stx in pathology in humans**

? **Role of Stx in cattle ? Selective advantage for *E. coli* ?**

Killing of protozoa

Killing of grazing protozoa, predators of *E. coli* in the rumen (controversial).

(Steinberg & Levin, PBS 2007; Schmidt *et al*. PLOS 2016)

Adherence

Cell culture (human, porcine); Mice : increase of the expression of intimin host receptors, other than Tir (i.e. nucleolin and integrin), facilitating gut colonisation.

(Robinson *et al*. PNAS 2006; Liu *et al*. AEM 2010)

Immune modulation

Suppression of host innate and adaptive immune responses during colonisation.

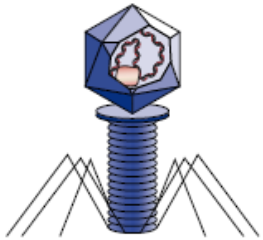
(Menge *et a*l. Inf Imm 2004; Hoffman *et al*. CVI 2006; Gobert *et al*. J immunol 2007.)

Super-shedding

Epidemiological association of Stx2a subtype with increased STEC fecal excretion levels (>10^e3 cfu/g feces) of *E. coli* O157 from cattle.

(Matthews et al. PNAS 2013)

Conclusions



Stx phages

A heterogeneous group of lambdoid temperate phages

Diverse morphology, host range, chromosomal location, *stx* variants (=> impact on pathogenesis; e.g. *stx2a*)

Role in Stx expression (=> impact on pathogenesis)

Production of Stx phages occurs either spontaneously or upon induction

Presence in food products & environment

High mobility (uptake and loss) => role in STEC genome diversification & evolution of new *E. coli* pathotypes (e.g. hybrid pathovars)

Selective advantage of Stx phages (& Stx) for STEC in cattle ? (role in cattle colonization ?)

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