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Management of Shiga toxin-producing *Escherichia coli* (STEC) in cattle in the European Union

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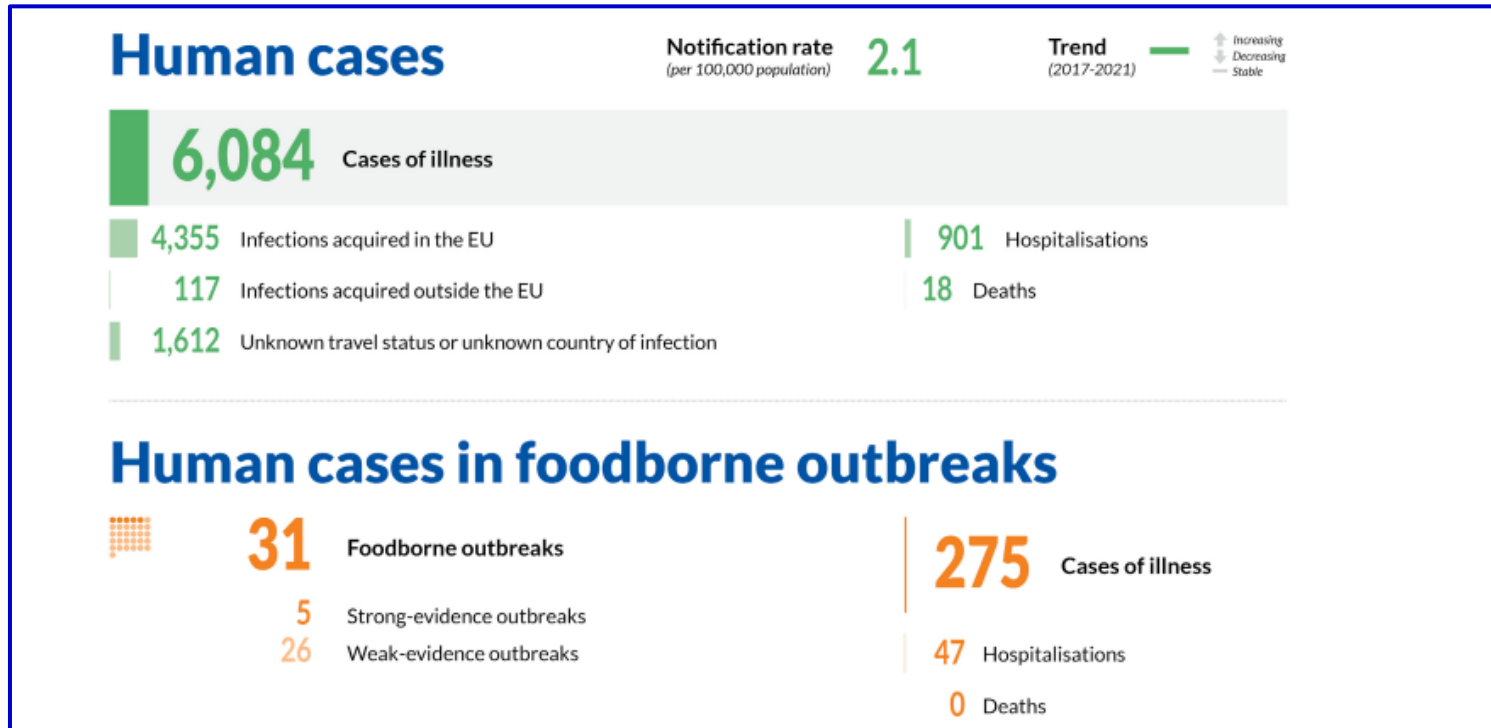
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** presenting person*



RIBMINS

Shiga toxin-producing *Escherichia coli* (STEC) in the EU, 2021



Foodborne outbreaks

Food vehicles causing strong-evidence outbreaks



Bovine meat and products thereof
2 Outbreaks



Milk
1 Outbreak



Vegetables and juices and other products thereof
1 Outbreak



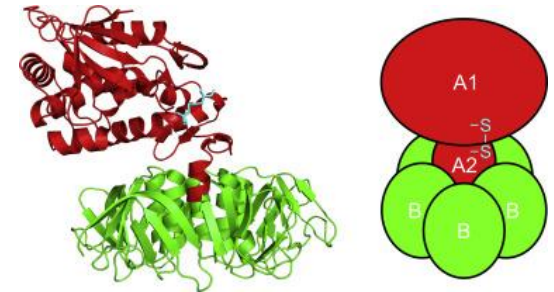
Meat and meat products, unspecified
1 Outbreak

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

- STEC belong to diarrhoeagenic *E. coli*
- Shiga toxins (Stxs) are **the major virulence factor** of STEC

- Two main groups
 - ➔ **Stx1** four subtypes (a,c,d and e)
 - ➔ **Stx2** 12 subtypes (a to l)

- Stxs bind to Gb3 receptors on the endothelial cells of target organs (kidneys, brain)



(Gardette et al., 2019; EFSA, 2020)

STEC virulence



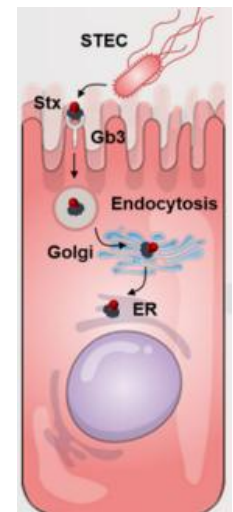
- Low infectious dose (< 100 CFU)
- Attachment to the large intestine enterocytes

LEE-STEC: expression of the LEE genes (*eae*, *tir*, *esp*)
formation of **attaching and effacing (A/E) lesions**

non-LEE STEC: alternative mechanisms

- Once attached STEC must multiply
- At this stage, STEC may produce Stxs

(Lee et al., 2021)



Clinical outcomes in humans

- Asymptomatic carriage
- Non-bloody diarrhoea
- Haemorrhagic colitis
- Haemolytic uraemic syndrome (HUS) especially in children < 5 y
- HUS is the leading cause of pediatric acute renal failure in the EU



«all blood and no stool»



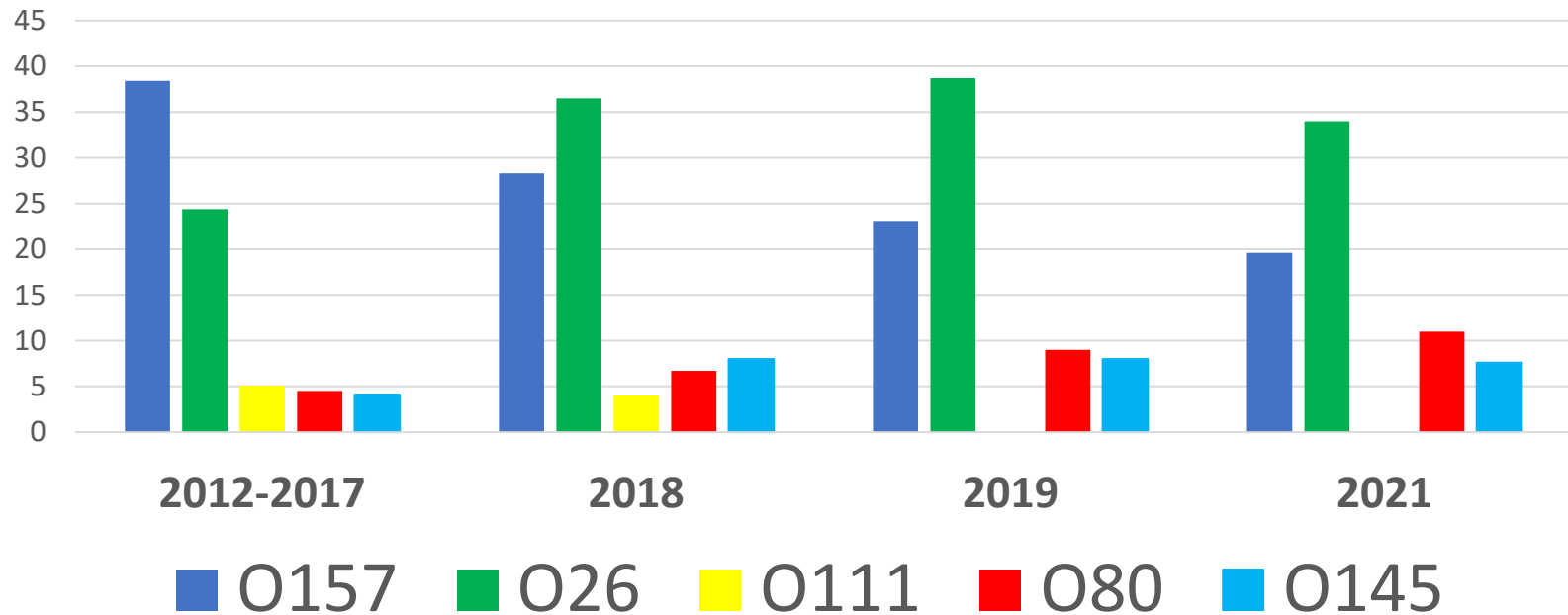
The top serogroups

- STEC belong to hundreds of serotypes
- Top five serogroups tested in food and animal samples: **O157, O26, O103, O111, O145** (plus O104:H4 for sprouts)
- ISO/TS 13136:2012
- In 2017, they **accounted only for 10.7%** of the STEC isolated from food in 23 MSs

(EFSA, 2020)



STEC serogroups in HUS cases in the EU



(EFSA and ECDC, 2022)

STEC, but not only

STEC	Shiga toxin-producing EC
EPEC	Enteropathogenic EC
ETEC	Enterotoxigenic EC
EAEC	Enteroaggregative EC
EIEC	Enteroinvasive EC
DAEC	Diffusely Adherent EC
AIEC	Adherent Invasive EC

Cross-pathotype strains

Strains harbouring pathogenicity genes associated with more than one pathotype

EAEC – STEC O104:H4

Once an *E. coli* carries the *stx* gene it may be considered a STEC

(EFSA, 2020)

The cross-pathotype O80:H2

- Highly virulent pathotype (HUS + bacteriemia)
- **ExPEC-STE C O80:H2**
- Stx2 genes (*stx2a*, *stx2c*, *stx2d*, or *stx2f*)
- intimin gene *eae-ξ*
- extraintestinal virulence genes (*sitA*, *cia*, *hylF*, *ompTP*, *iss*, *iroN*)
- Detected in **calves** in Belgium (Thiry et al., 2017)
- Not reported from food in the EU (2017-2021)



Other cross-pathotype serotypes

- **ETEC-STE**

- *stx1* and/or *stx2* genes

- ETEC heat-stable (ST) enterotoxin **Ia gene** (*estIa*)

1% (human STEC)

14% (animal and environmental STEC)

- Many serotypes found in **cattle** (**O2:H27, O15:H16, O101:H-, O128:H8, O141:H8**) are associated with human disease

(Nyholm et al., 2015)

Cow raw milk
cheese in Italy
(2017).
HUS in a 4y old
child

The animal reservoirs

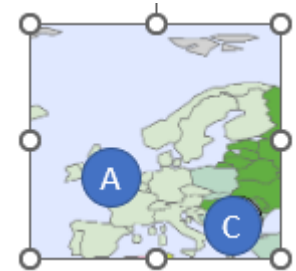
- Cattle are the main reservoirs of STEC worldwide
- STEC colonize the gut mucosa and the recto-anal junction (RAJ)
- Prolonged shedding
- Asymptomatic infections
- Gb3 expressed by peripheral and intestinal lymphocytes
- **Stxs act as immunosuppressive virulence factors in cattle, explaining both the lack of clinical symptoms and the persistent character of the infection**



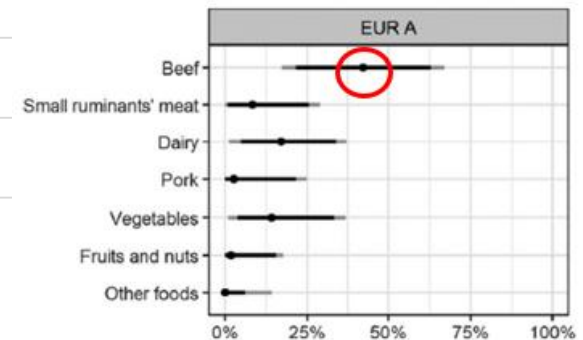
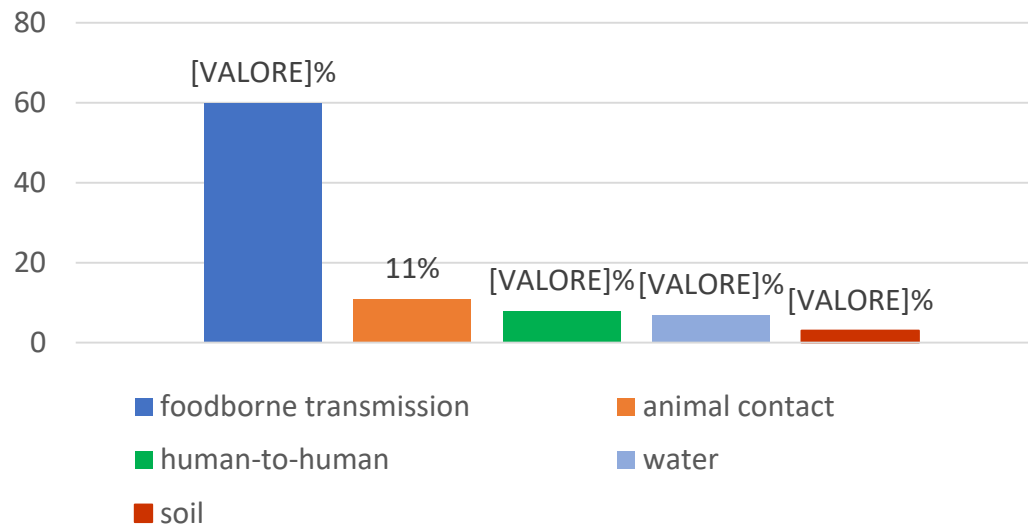
(Menge, 2020)

Source attribution studies

- European subregion-A (very low child and adult mortality)



STEC contamination



(Hoffmann et al. 2017)

STEC food safety criterion (Regulation EC 2073/2005)

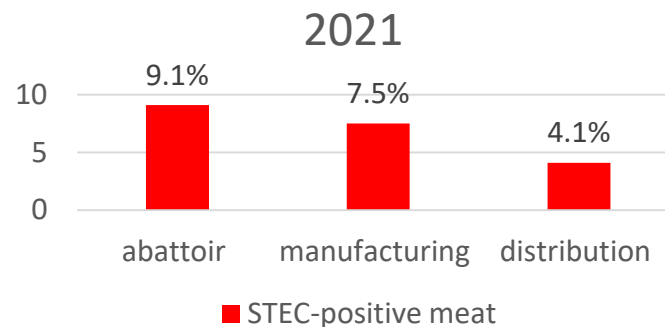
Food category	Micro-organisms/their toxins, metabolites	Sampling plan ⁽¹⁾		Limits ⁽²⁾		Analytical reference method ⁽³⁾	Stage where the criterion applies
		n	c	m	M		
1.29 Sprouts ⁽²³⁾	Shiga toxin producing <i>E. coli</i> (STEC) O157, O26, O111, O103, O145 and O104:H4	5	0	► M9 Not detected ◀ in 25 grams		CEN/ISO TS 13136 ⁽²²⁾	Products placed on the market during their shelf-life

Occurrence of STEC in sprouts in the EU		
	2021	2017-2020
No sampling units	617	1,730
No positive units (%)	1 (0.16)	0

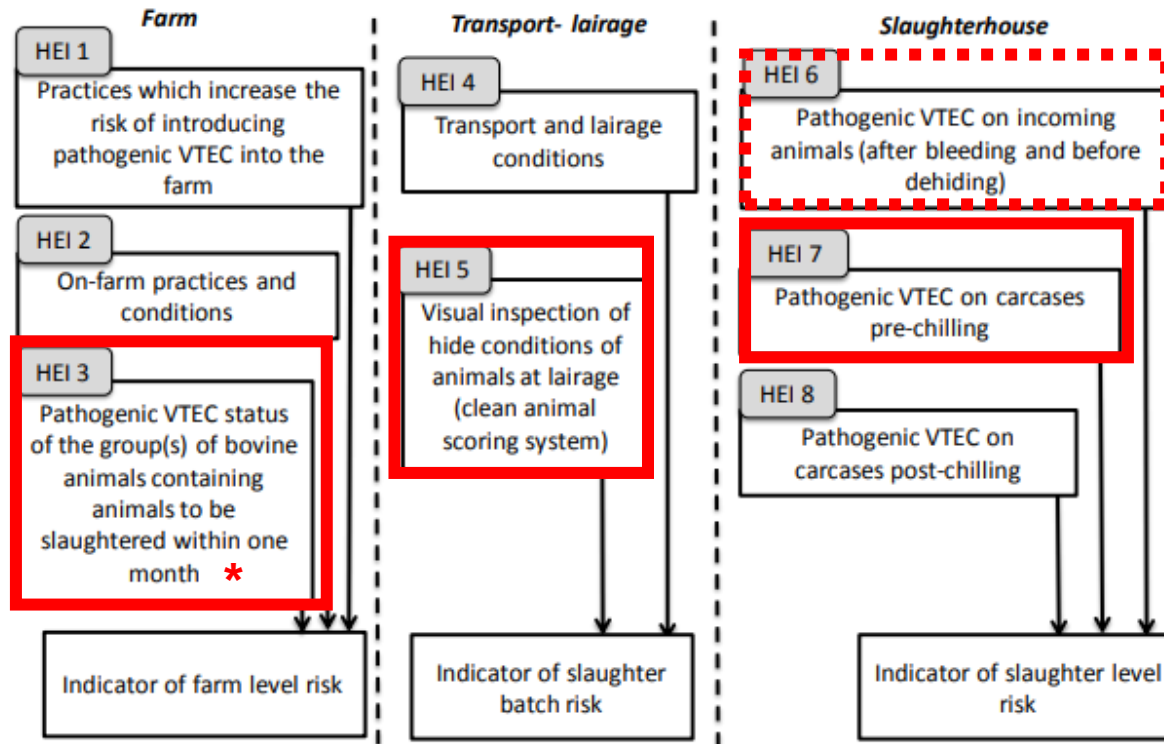
Monitoring on STEC in other foods

- Reporting obligations of MSs under **Directive 2003/99/EC**, which indicates that the presence of STEC should be investigated at the most appropriate stage of the food chain

Occurrence of STEC in fresh bovine meat in the EU		
	2021	2017-2020
No sampling units	5,095	21,017
No positive units (%)	288 (5.7)	514 (2.4)



HEIs for STEC in bovine animals (EFSA, 2013)



**HEI 5
+
HEI 7**

**should be
preferred
to HEI 3**

* Results must be included in FCI

Processing control strategies for STEC in beef

Processing stages mainly include:

1. Animals receiving and lairage
2. Slaughter
3. Carcass pre-chilling

(FAO and WHO, 2021)

Interventions at lairage

- Proper lairage pen and working facilities hygiene
- Reducing time spent at lairage
- Hide decontamination, if needed



Categorization and management of cattle prior to slaughter

1 – 2: **clean** animals

No actions at the abattoir



3: **moderately dirty** animals

GHP at the abattoir

No actions on farm



Categorization and management of cattle prior to slaughter

4: **dirty** animals

Interventions and GHP
at the abattoir *
Actions on farm



5: **dirty and mould** animals

Interventions and GHP
at the abattoir *
Actions on farm



- * - Holding cattle for a period of time on straw in lairage
 - Hide decontamination
 - Changing logistics in slaughter
 - Clipping hide after kill (before hide removal)
 - Slowing the slaughter line speed

Hide decontamination

Before stunning

- **Live animal hide washes**
 - ✓ water
 - ✓ ozonated or electrolyzed water
 - ✓ water with the addition of chemicals

(Bosilevac et al., 2005)

- **Bacteriophage** treatment specific for STEC O157:H7

(Arthur et al., 2017)



(Bosilevac, 2019)

Hide decontamination

After stunning

- **Washing**

- ✓ pressure hoses for 3'
- ✓ ozonated water
- ✓ chlorinated water
- ✓ electrolyzed-oxidized (EO) water
- ✓ sodium hydroxide
- ✓ lactic acid



- **Hide clipping, hide coating, chemical dehairing**

(Antic et al., 2021)



Slaughter and dressing

- Reducing processing speed
- Increasing distance between carcasses
- Good practices in hide removal
- Sealing off the rectum and the oesophagus
- Removing of faecal material from carcasses
 - ✓ Trimming
 - ✓ Steam vacuuming (82-95°C)



Carcass pre-chilling

- Hot water wash * ($\geq 85^{\circ}\text{C}$ for at least 10'')
- Steam pasteurization * ($\geq 85^{\circ}\text{C}$ for at least 13'')
- (Bosilevac, 2019)
- Lactic acid * - Regulation (EU) No 101/2013
- ✓ spraying 3 % LA solution in potable water at 55°C
- (Signorini et al., 2018)



* may result in carcass discolouration,
which generally disappears after 24 h of chilling

Degree of support for the *specific* control of STEC in raw beef (FAO and WHO, 2022)

- Proper lairage pen hygiene
 - Minimizing time spent in lairage
 - Animal cleanliness
 - Hide decontamination
 - Hide removal
 - Reducing speed of processing
 - Faecal material removal by washing
 - Lactic acid (pre-chilling)
 - Sealing off the rectum and the esophagus
 - Trimming of carcasses
 - Steam vacuuming
 - Hot water wash (pre-chilling)
 - Steam pasteurization
- LOW
- MEDIUM
- MEDIUM TO HIGH
- HIGH

STEC control programmes in the EU

- Only a few countries have implemented national control programmes for STEC in the beef chain
- In Sweden and Finland, they are based on two components:
 - 1) traceback from human cases
 - 2) monitoring at abattoirs (FBOp own-checks)

STEC control in Sweden



PASSIVE

Traceback from human cases

- Association between a human case of STEC infection and animal farms



trace back investigation on farm

(faeces, environmental swabs)

ACTIVE

Monitoring at abattoirs

- Survey in cattle:
 - ✓ annually between 1997-2002
 - ✓ then every third year
- The last survey: 2020–2021
- Prevalence in faecal samples:
 - STEC O157 : 2.8%**
 - STEC O26 : 0.7%**
 - STEC O103: 0.3%**



STEC control in Finland



PASSIVE

Traceback from human cases

- Association between a human case of STEC infection and animal farms



trace back investigation on farm

(faeces, environmental swabs)



STEC control in Finland



ACTIVE – Monitoring at abattoirs

- 2004-2021
- EHEC control programme
- STEC O157 and non-O157 that cause human illness
- **Faecal samples**
- ISO 16654 and NMKL 164



Positive samples

Official sampling on farm

Logistic slaughtering

Heat treatment of carcasses

- 2021-
- STEC monitoring programme
- **Carcass swabs**
- At least 500 swabs/year (>100 heads y)
- ISO/TS 13136



Positive samples

Improvement of slaughter hygiene

Review of process control

Official controls

- STEC in **faecal samples** from cattle
- At least every 5 years



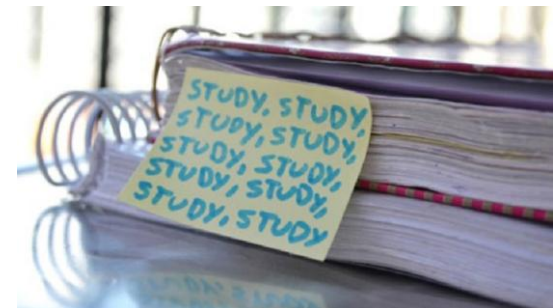
How to go further?

- Amending Regulation (EC) No 2073/2005
- ✓ **Process Hygiene Criterium** for STEC in bovine (and other ruminants') carcasses
- ✓ **Top five serogroups**: not sufficient, but better than nothing



Conclusions

- Complexity of STEC
- Plasticity of genome
- Cattle as asymptomatic reservoirs
- No food safety criteria in food at risk
- No control programmes in most EU countries
- STEC-risk only to be managed by FBOp at abattoirs
-and mandatory training for vets!





Thank you for your attention

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