Survey of shiga toxin-producing *E. coli* (STEC) on beef and leafy vegetables in Sweden

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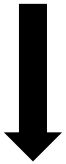
Aim of the study

Prevalence of STEC on beef and leafy vegetables in Sweden

Data used for:

Assessing the public health risk of STEC in food



Developing risk management strategies

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Analytical method

In-house method
(similar to ISO/TS 13136:2012)

Enrichment

Trypton Soy Broth (TSB)

Detection

1. Real-time PCR *stx*-genes
2. If positive for any stx-gene:
   Real-time PCR *eae* and the serogroups O157, O26, O103, O111 and O145

Isolation

If positive for any of the five serogroups → Immunomagnetic separation (IMS)

If negative for the five serogroups → Immunoblot

Presumptive colonies were verified by real-time PCR

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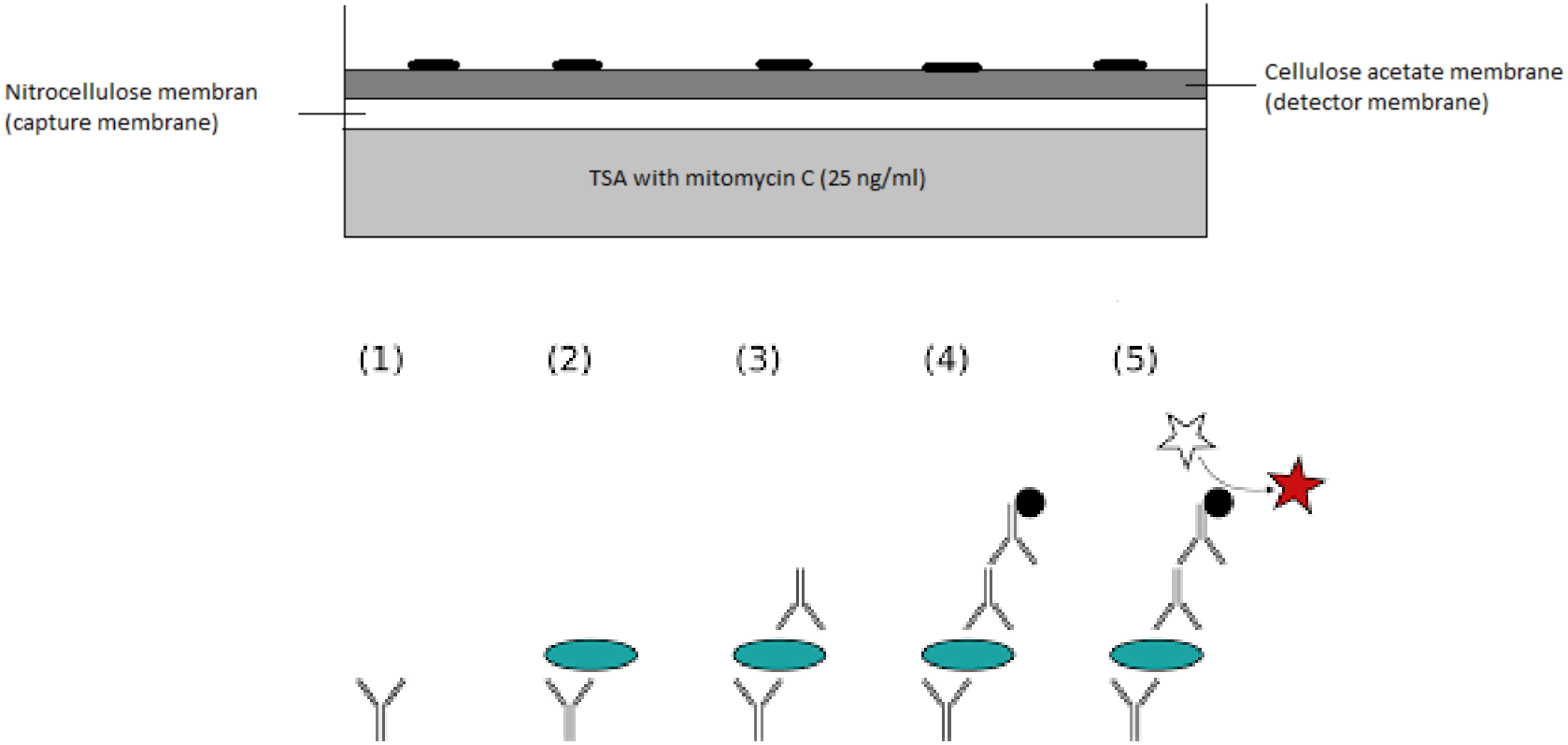
Membrane immunblot procedure



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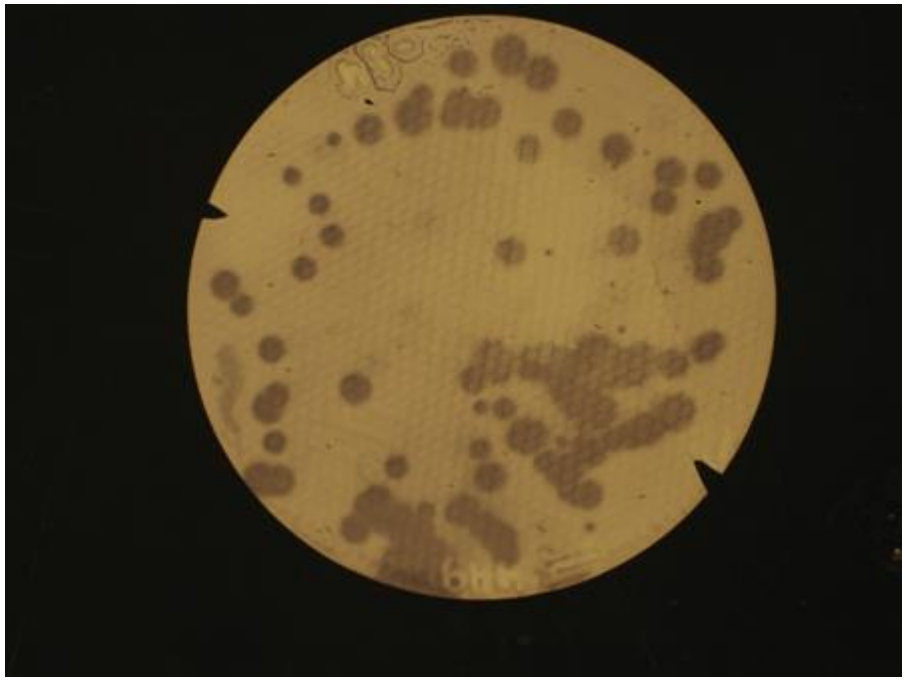
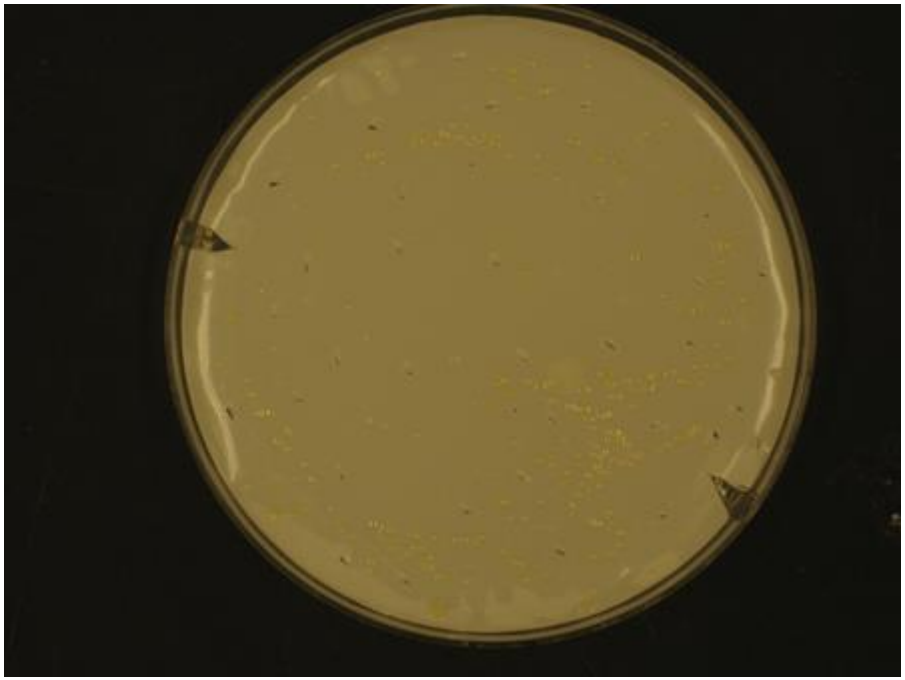
Use of a Shiga Toxin (Stx)-Enzyme-Linked Immunosorbent Assay and Immunoblot for Detection and Isolation of Stx-Producing Escherichia coli from Naturally Contaminated Beef
HEBA NASHED ATALLA, ROGER JOHNSON, SCOTT MCEWEN, R. W. USBORNE, AND C. L. GYLES





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Membrane immunoblot

Capture antibody

- Rabbit polyclonal antibody to verotoxins (VTs) 1 and 2
  - Recognizes A and B subunits of all tested VTs

Detector antibody

- Mouse monoclonal antibody to verotoxin (Shigatoxin) 1,
  - Recognizes the B subunit of all tested VT1s
- Mouse monoclonal antibody to verotoxin (Shigatoxin) 2/2c, Clone F9-7, IgG1
  - Recognizes the B subunits of VT2, VT2c
- Mouse monoclonal antibody to verotoxin (Shigatoxin) 2e,
  - Recognizes the B subunits of VT2e and related VT2 variants from some human isolates
- Mouse monoclonal antibody to verotoxin (Shigatoxin) 2e,
  - Recognizes the A subunit of VT2e, VT2, 2c and most other VT2 variants

Sampling - beef

- Collected between 2010 and 2011
- Most common regions or countries exporting beef to Sweden
- Fresh and frozen meat
- Minced and whole meat

Origin	Number of samples
Irland	39
Other EU countries	96
South America	42
Total	**177**

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Prevalence of STEC on beef

Origin	Number of analyzed samples	Number of (%) samples with confirmed STEC
Irland	**39**	**1 (3)**
The Netherlands	26	6
Germany	35	3
Other[a]	35	7
Total EU countries	**135**	**17 (13)**
Argentina	6	3
Brazil	18	0
Uruguay	18	3
Total South America	**42**	**6 (14)**
Total	**177**	**23 (13)**

[a] Denmark (3), Estonia (1), Italy (3), Lithuania (7), Poland (11), Great Britain (5), Austria (5)

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Results - beef

- Presumptive STEC was found in 46 (26 %) of the 177 beef samples

- STEC was isolated from 23 (13 %) of the177 beef samples
  - 9 (20 %) of 46 samples of minced beef
  - 14 (11 %) of 131 samples of whole beef

- STEC with genes for stx2 and eae was isolated from more than 3 % of the beef samples

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Serotyping

Serotype	Number of isolates
O26:H11	**3**
O22:H8	**2**
O113:H21	**2**
O163:H19	**2**
O178:H19	2
O8:H16	1
O8:H19	**1**
O26:H-	**1**
O88:H8	1
O113:H4	**1**
O148:H8	1
O153:H25	**1**
O157:H-	**1**
O157:H7	**1**
O174:H-	**1**
O179:H8	**1**
OX183:H18[a]	1
OX185:H7[a]	1
ONT:H29[b]	1
ONT:H32[b]	1
ONT:H4[b]	1

- **STEC O26 was the most common serogroup (more than 2 %)**
- **STEC O157 was isolated from two samples (about 1 %)**

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Serotyping was done by Statens Serum Institue (SSI) in Denmark

STATENS SERUM INSTITUT

Serotype	Food category	Origin	stx1	stx2	eae	astA2	toxB	efa1	aaiC	aggR
ONT:H29	Whole beef	Uruguay	-	+	-	-	-	-	-	-
OX183:H18	Minced beef	The Netherlands	+	+	-	-	-	-	-	-
O179:H8	Whole beef	The Netherlands	-	+	-	-	-	-	-	-
OX185:H7	Whole beef	Uruguay	-	+	-	-	-	-	-	-
O26:H11	Whole beef	Germany	+	-	+	+	+	+	-	-
O8:H19	Minced beef	Germany	+	+	-	-	-	+	-	-
O163:H19	Whole beef	Scotland	-	+	-	-	-	-	-	-
O148:H8	Minced beef	Lithuania	-	+	-	-	-	-	-	-
O113:H21	Whole beef	Argentina	-	+	-	-	-	-	-	-
O113:H21	Whole beef	Argentina	+	+	-	-	-	-	-	-
O8:H16	Whole beef	Argentina	+	+	+	-	-	-	-	-
ONT:H32	Whole beef	The Netherlands	-	+	-	-	-	+	-	-
ONT:H4	Minced beef	The Netherlands	-	+	-	-	-	-	-	-
O178:H19	Whole beef	The Netherlands	-	+	-	+	-	-	-	-
O178:H19	Whole beef	Argentina	+	+	-	+	-	+	-	-
O22:H8	Whole beef	Argentina							-	-
O22:H8	Minced beef	Austria							-	-
O113:H4	Minced beef	Poland							-	-
O163:H19	Whole beef	Irland							-	-
O153:H25	Whole beef	Denmark	+	+	-	+	-	+	-	-
O174:H-	Minced beef	Austria	-	+	-	-	-	-	-	-
O88:H8	Whole beef	Germany	-	+	-	+	-	-	-	-
O26:H-	Whole beef	Uruguay	+	-	+	+	+	+	-	-
O26:H11	Minced beef	The Netherlands	+	+	+	+	+	+	-	-
O26:H11	Minced beef	Austria	+	+	+	+	+	+	-	-
O157:H7	Whole beef	Argentina	-	+	+	+	+	+	-	-
O157:H-	Minced beef	Austria	+	+	+	+	+	-	-	-

O157:H7 clade 8

SVA NATIONAL VETERINARY INSTITUTE

Sampling – leafy vegetables

- Collected between 2012 and 2013
- Domestic and imported products
- Available on the Swedish market at the time of sampling

Origin	Number of samples
Sweden	147
EU	365
Third countries	10
Mixed[1]	108
Total	**630**

[1] Mixed contained ingredients from at least two different EU countries, including Sweden

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Results - vegetables

- Presumptive STEC was found in about 11 (2 %) of 630 samples
- No STEC was isolated
- *E. coli* just under 40 %

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Conclusions

- No STEC was isolated in leafy vegetables regardless of origin
- STEC was isolated from more than 10 % of the imported beef samples
- The isolates found belonged to many different combinations of virulence genes (stx1, stx2, eae) and serotypes
- 3 % of the samples contained STEC positive both for stx2 and eae - associated with a higher risk of serious disease

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Next step

- Continue to characterize the isolates by screening for different virulence genes (on going)

- Sampling of Swedish beef during 2015 and the analysis will be done during 2016

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Acknowledgements





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