Voedsel en Waren Autoriteit
Ministerie van Landbouw, Natuur en Voedselkwaliteit

Detection of STEC in animal feces, on animal hides, and raw meats

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Outline

- Goal
- Materials and methods
- Results
- Points for discussion

Goal

EXPERIMENTAL STUDIES

- Prevalence of STEC O157 and non-O157 (O26, O103, O111, and O145) in/on:
  - » Raw meats
  - » Farms holding veal calves
  - » Cattle 3-24 months of age (if possible), and not being veal calves

- Characterisation of these STEC O157 and non-O157 isolates

PRESENTATION

- Experience with the detection method (ISO/CEN draft TS)

- Experience with the EFSA TS for monitoring STEC on animals and food

Materials and methods

- Raw meats
  - Collected at retail
  - Year-round

- Feces veal calves
  - Collected at the farms
  - Fresh droppings, pooled per 12, max. 5 pooled samples / farm
  - Year-round

- Hides cattle
  - Collected at slaughter (3 slaughterhouses; covering 66% of the national throughput)
  - Sponge samples, 400 cm^2
  - 3-24 months of age (if possible), and not including veal calves
  - April-September

Materials and methods

Raw meats

- *E. coli* O157
  - » Selective enrichment in mTSB+n (20 mg/l), 41,5°C
  - » Immunocapture by VIDAS-ICE
  - » Selective plating onto CT-SMAC & ½CT-CHROM
- STEC
  - » Selective enrichment in mTSB+n (16 mg/l), 37°C
  - » DNA extraction
  - » PCR screening by
    - (1) GeneDisc STEC screening (*stx*, *eae*, O157)
    - (2) GeneDisc EHEC identification (O26, O103, O111, O145)
  - » Isolation onto MacConkey agar, (C)R-MAC (O26), Possé et al.
    - both with and without an IMS step

Materials and methods

Feces veal calves

- *E. coli* O157
  - » Selective enrichment in mTSB+n (20 mg/l), 41,5°C
  - » Immunocapture by VIDAS-ICE
  - » Selective plating onto CT-SMAC & ½CT-CHROM
- STEC
  - » Direct plating onto MacConkey agar, 37°C
  - » DNA extraction
  - » PCR screening by
    - (1) GeneDisc STEC screening (*stx*, *eae*, O157)
    - (2) GeneDisc EHEC identification (O26, O103, O111, O145)
  - » Isolation onto MacConkey agar, (C)R-MAC (O26), Possé et al.
    - both with and without an IMS step

Materials and methods

Hides cattle

- *E. coli* O157
  - Non-selective enrichment in BPW, 41,5°C
  - IMS by Dynabeads anti-*E. coli* O157
  - Selective plating onto CT-SMAC & ½CT-CHROM
- STEC
  - Non-selective enrichment in BPW, 41,5°C
  - DNA extraction
  - PCR screening by
    (1) GeneDisc STEC screening (*stx*, *eae*, O157)
    (2) GeneDisc EHEC identification (O26, O103, O111, O145)
  - Isolation onto MacConkey agar, (C)R-MAC (O26), Possé et al.
    both with and without an IMS step

Results *E. coli* O157 (preliminary)

- Raw meats
  - 0 / 1276

    (pork (576), beef (479), calf (113), lamb (105), sheep (2) horse (1))

- Feces veal calves
  - at farm level: 20 / 130 (15.4%)

- Hides cattle
  - 25 / 341 (7.3%)

Results STEC (preliminary)

Raw meats (n=547)

			No. PCR positive	No. additionally positive for O26 / O103 / O111 / O145 / O157
stx +	eae +	O157 -	1	0 (n=1)
stx +	eae +	O157 +	0	-
stx +	eae -	O157 -	14	
stx -	eae +	O157 -	12	
stx -	eae +	O157 +	0	
stx +	eae -	O157 +	4	
stx -	eae -	O157 +	16	

No. positive for stx and/or eae and/or O157 (%)	47	(8.6)
No. positive for stx & eae (%)	1	(0.2)

No. positive for stx & eae & O26 / O103 / O111 / O145 / O157 (% of those screened for EHEC)	0 / 1 (0)
No. positive for stx & eae & O26 / O103 / O111 / O145 / O157 (% of those screened by PCR)	0 / 547 (0)

Results STEC (preliminary)

Feces veal calves (n=297)

			No. PCR positive	No. additionally positive for O26 / O103 / O111 / O145 / O157
stx +	eae +	O157 -	184	125 (n=177)
stx +	eae +	O157 +	20	20 (n=20)
stx +	eae -	O157 -	36	
stx -	eae +	O157 -	18	
stx -	eae +	O157 +	2	
stx +	eae -	O157 +	5	
stx -	eae -	O157 +	1	

No. positive for stx and/or eae and/or O157 (%) 266 (89.6)
No. positive for stx & eae (%) 204 (68.7)

No. positive for stx & eae & O26 / O103 / O111 / O145 / O157 (% of those screened for EHEC) 145 / 197 (73.6)
No. positive for stx & eae & O26 / O103 / O111 / O145 / O157 (% of those screened by PCR) 145 / 297 (48.8)

Results STEC (preliminary)

Cattle hides (n=224)

			No. PCR positive	No. additionally positive for O26 / O103 / O111 / O145 / O157
stx +	eae +	O157 -	41	39 (n=39)
stx +	eae +	O157 +	55	55 (n=55)
stx +	eae -	O157 -	13	
stx -	eae +	O157 -	43	
stx -	eae +	O157 +	22	
stx +	eae -	O157 +	6	
stx -	eae -	O157 +	10	

No. positive for stx and/or eae and/or O157 (%)	190	(84.8)
No. positive for stx & eae (%)	96	(42.9)

No. positive for stx & eae & O26 / O103 / O111 / O145 / O157 (% of those screened for EHEC)	94 / 94	(100)
No. positive for stx & eae & O26 / O103 / O111 / O145 / O157 (% of those screened by PCR)	94 / 224	(42.0)

Results EHEC (preliminary)

EHEC screening

	raw meats	calf feces	cattle hides
O26	0 / 2 (0)	98 / 198 (49.5)	46 / 107 (43.0)
O103	0 / 2 (0)	19 / 198 (9.6)	94 / 107 (87.9)
O111	0 / 2 (0)	12 / 198 (6.1)	3 / 107 (2.8)
O145	0 / 2 (0)	102 / 198 (51.5)	102 / 107 (95.3)
O157	20 / 547 (3.7)	28 / 297 (9.4)	93 / 224 (41.5)

Results cattle hides:
IMS *E. coli* O157 vs STEC O157 screening

		PCR results of E. coli O157 isolate				PCR results of BPW culture			
IMS O157-CTS	IMS O157-CHA	stx1	stx2	eae	O157	stx1	stx2	eae	O157
10-0142	10-0143	1	1	1	1	1	1	1	1
-	10-0140	1	1	1	1	0	0	1	1
10-0144	10-0145	0	1	1	1	1	1	1	1
-	10-0146	0	1	1	1	1	1	1	1
-	10-0242	0	0	1	1	0	1	0	0
10-0238	10-0241	0	0	1	1	0	0	1	0
10-0239	10-0148	0	0/1	1	1	1	1	1	1
10-0147	10-0150	0	1	1	1	0	0	1	1
-	10-0149	0	1	1	1	1	1	1	1
-	10-0151	0	1	1	1	0	1	1	1
-	10-0166	0	1	1	1	0	0	1	0
10-0169	10-0297	0	1	1	1	0	1	1	0
10-0168	10-0167	0	1	1	1	0	1	1	0
10-0180	-	0	1	1	1	0	1	1	1
10-0248	10-0251	0	1	1	1	0	1	1	0
10-0247	10-0250	0	1	1	1	0	0	1	0
-	10-0255	0	1	1	1	0	0	1	0
-	10-0256	1	1	1	1	0	0	0	0
10-0253	-	0	1	1	1	0	0	1	0
10-0254	10-0257	0	1	1	1	0	1	1	1
n=12	n=18								

Results feces veal calves: VIDAS-ICE vs STEC O157 screening

		PCR results of E. coli O157 isolate				PCR results of MacConkey culture			
VIDAS ICE-CTS	VIDAS ICE-CHA	stx1	stx2	eae	O157	stx1	stx2	eae	O157
10-0011	10-0009	0	1	1	1	0	1	1	1
10-0010	10-0008	0	1	1	1	1	1	1	1
10-0035	10-0036	0	1	1	1	1	1	1	1
10-0037	10-0038	0	1	1	1	0	1	1	1
10-0039	10-0040	0	1	1	1	0	0	1	0
10-0157	10-0165	0	1	1	1	0	1	1	0
10-0156	10-0162	0	1	1	1	1	0	1	0
10-0154	10-0164	0	1	1	1	0	0	1	0
10-0155	10-0161	0	1	1	1	0	1	1	0
10-0153	10-0163	0	1	1	1	0	1	1	0
10-0152	10-0158	0/1	1	1	1	0	1	0	0
10-0240	10-0160	0	0/1	1	1	0	1	1	1
-	10-0159	1	1	1	1	0	1	1	0
10-0177	10-0173	0	1	1	1	0	1	1	1
10-0176	10-0172	0	1	1	1	0	1	1	0
10-0175	10-0171	0	1	1	1	0	1	1	0
10-0174	10-0170	0	1	1	1	0	1	0	0
-	10-0181	0	1	1	1	0	1	1	0
-	10-0182	0	1	1	1	0	1	1	0
10-0179	10-0184	0	1	1	1	0	1	1	0
10-0178	10-0183	0	1	1	1	0	1	1	0
10-0249	10-0252	1	1	1	1	0	0	0	0
n=19	n=22								

Discussion

Calf feces and cattle hides:

- False negative results for *E. coli* O157 with PCR screening?
  - For the detection of *E. coli* O157 there is a need for a concentration of *E. coli*?

- 40-50% of samples of calf feces or cattle hides needs confirmation by isolation, (stx & eae & O26 / O103 / O111 / O145 / O157)

  BUT LOW ISOLATION RATES!
  - More efficient isolation procedure ?

Cattle hides:

- Plates are often overgrown.
  - Why not selective enrichment of cattle hides?

- Less nicely-shaped PCR curves; sometimes difficult to interprete.
  - DNA extraction protocol? BPW enrichment?

Discussion

EHEC SCREENING

- Calf feces: O26 & O145
- Cattle hides: O145 & O103 ….& O26 & O157

- Low detection rate of *E. coli* O111
  - Less present?
  - PCR-related?

  - More selective PCR approach to reduce the number of samples to be screened for the O groups / to be confirmed by isolation of the STEC strains?

Thank you for your attention!