PREVALENCE OF SHIGA TOXIN-PRODUCING *Escherichia coli* (STEC) IN STOOL SAMPLES FROM STAFF OF MEAT PROCESSING COMPANIES

Rohtraud Pichner, Nadja Brey and Manfred Gareis

Institute for Microbiology and Toxikology, Federal Centre for Meat Research (BAFF) in Alliance Product and Nutrition Research, D-95326 Kulmbach, Germany

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Background:
Shiga toxin-producing *Escherichia coli* (STEC) belong to a group of gut-pathogenic microorganisms, which were made responsible for food-poisoning first in 1982 (CDC, 1982). Ever since there has been a number of outbreaks and sporadic illnesses world wide. First of all, meat-products are of great importance as a zoonotic spread of EHEC. In 1994 raw sausage proved to have redeemed a group disease with EHEC in the United States (CDC, 1994). In 1995 23 cases of HUS (hemolytic uremic syndrome) were reported from Australia after the consumption of raw Bologna sausage (CDC, 1995). Furthermore the significance of the infectious potential via human to human contact, respectively the contamination of food increases.

Objectives:
This work was to investigate the role of staff as a possible source of entry for Shiga toxin-producing *E. coli* in meat processing companies. In this context periodical stool samples of staff members were assayed for STEC with phenotype-oriented (EIA) and genotype-oriented (PCR) methods over a period of 6 (plant II) respectively 21 months (plant I).

Material and Methods:
Sampling: A total of 1041 faecal specimens were obtained monthly from 120 staff members occupied in the production line of two meat processing companies. Plant I employs 22, plant II 100 people in the production line.
Culture methods: The samples were homogenized in buffered peptone solution (PBS) and cultured in modified Tryptic soy broth (mTSB) with Novobiocine for selective enrichment.
ST-screening: The detection of Shiga toxins in sample enrichments was done phenotypically with an enzyme immuno assay for Shiga toxin 1 and 2 (Prospect, Fa. Alexon Trend, USA) and genotypically by PCR with primer pair Mk1/Mk2 according to Karch and Meyer (1989) and Gallien et al. (1996).
Isolation of STEC: EIA-positive and/or PCR-positive enriched stool samples were plated on Sorbitol MacConkey agar (SMAC) and a part of grown colonies tested for Stx genes 1 and 2 by PCR. Then 20 to 30 colonies of positive agars were isolated and examined by PCR.
Characterization of STEC: PCR-positive isolated colonies were examined for their biochemical properties and for their membership to species *E. coli* by api-system (BioMerieux, France). To estimate the potential hazard for human health, the isolated STEC were tested for two further virulence factors: eaeA-gene was detetermined by PCR with the primer pair Sk1/Sk2 according to Schmidt et al. (1993) and EHEC-hämolysin by cultivation on Enterohämolysin agar (Oxoid). The differentiation between Shiga toxin 1 and 2 was done by PCR with primer pair KS7/ KS8 for detection of stx1 according to Schmidt et al. (1994) and primer pair LP43/44 for stx2 (Cebula et al., 1996). The serotyping of STEC was done by Prof. Dr. S. Aleksic (Hamburg).

Results:
1041 faecal samples were examined from 122 employees of two meat processing companies. Results of Shiga toxin (ST)-screening in faecal specimens enrichments are shown in Tab. 1. The prevalence of STEC in stool samples of employees in the production line of meat processing companies is 9% in plant I and 7% in plant II. STEC could be cultivated from both detected shedders in plant I and from 3 of the 7 detected shedders in plant II: Positive screening results were obtained in 51 enriched stool samples from two staff members of plant I and in 7 stool samples from 7 staff members in plant II, with a microbiologically confirmation by isolation of STEC in 26 (5,6%) of the faecal specimens in plant I and 3 (0,5%) in plant II..
The further differentiation of the isolated STEC established following: In plant I Shiga toxin-producing colonies cultivated from one of the two detected shedders possessed only Shiga toxin-gene and belonged to serotype O40:H8. Isolated colonies from the other shedder have the virulence marker Shiga toxin-gene and enterohämolysin and the serotype O91:H-/H14/H21. Furthermore, in this plant a intermittent shedding of STEC over a period of 10 months could be documented. In plant II in one sample Shiga toxin producing isolates (serotype O23:H-) were obtained without eaeA-gene and from two of the Shiga toxin-positive stool-samples isolates with the complete virulence markers (Shiga toxin-gene+, eae-gene+, EHEC-hyl+) and serotypes O103:H2 respectively O26:H- (see Table 2).

Discussion:
The assays of the stools demonstrated clearly that asymptomatic shedders of Shiga toxinogenic *E. coli* occur; this finding supports a role of the staff as an indirect source of STEC. It could not be ruled out in this study, wether the source of infection for staff members was the handling with contaminated meat. Data relating to this problem had not been available until now. Following an enrichment culture, 58 out of 1041 stool samples of 122 employees were Shiga toxin-positive. On the basis of 100 respectively 22 persons the incidence of STEC-positive employees is in plant I 9,0% and in plant II 7.0%. Shiga toxin forming *E. coli* colonies with the complete virulence markers (Shiga toxin-gene+, eae-gene+, EHEC-hly+) were obtained from two of the Shiga toxin-positive stool-samples. Furthermore, an intermittent shedding of STEC over a period of 10 months could be documented. In general, detection and isolation

of STEC is made difficult by the lack of characteristic biochemical markers suitable for selective cultures or for the cultural separation of apathogenic *E. coli*; another difficulty is the loss of extrachromosomal genes during subcultivating (Bockemühl and Karch,1996; Karch et al., 1992). In addition there is a multiplicity of serovars with various combinations of virulence markers (Bockemühl and Karch,1996). The present problem with the definition of EHEC and differentiation between EHEC and STEC, and with the diagnostic methods results in problems with the interpretation of the results and with the safety measures required by law. The Shiga toxine-producing strains which were isolated in the course of this investigation were of a non-clinical origin and yet they either had the complete set of virulence markers (Shiga toxin-gene+, eae-gene+, EHEC-hyl+) or were serovars which are known to have caused human infections.

Conclusions:
These results document the importance of asymptomatic carriers as a possible source of entry for EHEC/STEC in meat producing companies, because three of the isolated serotypes have been connected with human haemorrhagic uremic syndrome or enteritis diseases. Therefore regular examinations of staff are necessary to guarantee product safety. The source of infection for staff members could not be ruled out in this study.

Pertinent literature:
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Tab. 1: Number of employees in the production line of examined faecal specimens, results of STEC-screening by EIA and/or PCR. The percent number belong to the number of employees in production line. Microbiologically confirmation was done by isolation of ST-producing colonies.

	employees			Faecal specimens		
	Examined employees in the production line	ST-screening positive	Microbiologically confirmed *	Examined faecal specimens	ST-screening positive	Microbiologically confirmed
Plant I	22	2 (9,1%)	2 (9,1%)	467	51 (11%)	26 (5,6%)
Plant II	100	7 (7%)	3 (3%)	574	7 (1,2%)	3 (0,5%)
Total	122	9 (7,4%)	5 (4,1)	1041	58 (5,6%)	29 (2,8%)

Tab. 2: Results of further differentiation of isolated STEC

Plant/ Staff member	Shigatoxin			*Eae* (PCR)	EHEC-hly (Blood agar)	Serotype
	Stx1	Stx2	(EIA)			
I/1	-	+	-	-	-	O40:H8
I/2	+	-	+	-	+	O91:H-
	+	-	+	-	+	O91:H14
	+	-	+	-	+	O91:H21
II/1	+	+	+	-	+	O23:H-
II/2	-	+	+	+	+	O26:H-
	+	-	+	+	+	O103:H-
	+	-	+	+	+	O103:H2
II/3	+	-	+	+	+	O103:H-
	+	-	+	+	+	O103:H2