ENTRY SOURCES AND ROUTES OF CONTAMINATION OF SHIGATOXIN PRODUCING *ESCHERICHIA COLI* (STEC) IN MEAT PROCESSING COMPANIES

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Background:
Shiga toxin-producing *Escherichia coli* (STEC, synonymous with VTEC) 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 (Enterohemorrhagic Escherichia coli). 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. For meat manufacturing industries, especially for those, who produce short ripened raw sausages, the presence of STEC in companies, in products or in employees can mean a tremendous economical problem. Therefore it has to be searched for solutions which raise the food safety on the one hand, but which give on the other hand help to the industry especially for the safely manufacture of risk-products.

Objectives:
This work was to increase knowledge about entry sources and contamination routes for STEC on the food chain in meat manufacturing industries and to develop strategies in order to avoid contamination and distribution of pathogenic and facultative pathogenic *E. coli* in hygienic critical products (spreadable raw sausages such as "Teewurst").

Methods:
Sampling: Three plants producing raw sausages were inquired. A regularly microbiological analysis for the presence of STEC was done of products, raw material, which was taken for sausage production and of faeces from the staff of processing areas. Furthermore a hygiene monitoring was performed of different regions by taking swab samples from sanitary and processing areas and hand-swab samples from staff of producing and packing areas. Culture methods: All samples were cultured in modified Tryptic soy broth (mTSB) in two steps. Enriched stool samples were plated on Sorbitol MacConkey agar (SMAC). STEC-screening: The detection of shigatoxin in sample enrichments (except stool samples) was done genotypically by PCR with primer pair Mk1/Mk2 according to Karch and Meyer (1989) and Gallien et al. (1996a). For detection of shigatoxin in stool samples a part of grown colonies were tested for Stx genes 1 and 2 by PCR with primer pair Mk1/Mk2. Isolation of STEC: PCR-positive enriched samples were subcultured on SMAC and analyzed again by PCR. Then 20 to 30 colonies of positive agars were isolated and examined by PCR. When isolation of STEC of positive samples was not possible, a colony hybridization was performed with digoxigenine marked probes according to Gallien et al. (1996b).

Results and discussion
From January to December 2001 a total of 6498 samples was analyzed for STEC. In 620 samples (9,5%) positive screening results were found by PCR. 95 of them (1,5%) could confirmed microbiologically by isolation of STEC (Tab. 1). Asymptomatic healthy shedders of STEC were detected in each of the three plants (see Tab. 2a-c). These results document the importance of asymptomatic carriers as a possible source of entry. Therefore regular examinations of staff are necessary to guarantee product safety. Individual results of the plants show 7,1% (plant III) to 12,8% (plant II) screening positive samples. Most of them was found in raw material (38 – 40%), followed by products (13,5 bis 18,4%). 32,8% of meat fluid samples was positive in plant II (Tab. 2b, others). According to these findings raw material is a possible entry source for STEC in the production line. The positive results in the swab samples of processing areas may be a consequence of cross contamination. For final conclusions about entry sources and contamintaion routes for STEC on the food chain in meat manufacturing companies the results of further characterization of isolated STEC are needed (virulence factors, serotypes). These analyses are in progress.

Pertinent literature: CDC (1982): Isolation of *E. coli* O157:H7 from sporadic cases of hemorrhagic colitis – United States. MMWR 31, 580-585. - CDC (1994): *Escherichia coli* O157:H7 outbreak linked to commercially distributed dry-cured salami.-Washington and California 1994. MMWR 43, 213-217. - CDC (1995): Community outbreak of hemolytic uremic syndrome attributable to *Escherichia coli* O111:NM-South Australia. MMWR 44, 550-558. – GALLIEN, P., H. KLIE, K.-W. PERLBERG und D. PROTZ (1996a): Genotypischer Nachweis von Verotoxin-bildenden Krankheitserregern in Kot- und Milchproben von Rindern mittels PCR. Boehringer Mannheim PCR-Bibliographie, 71-73. - GALLIEN, P., H. KLIE, K.-W. PERLBERG und D. PROTZ (1996b): Einsatz von Nylonmembranen zur gezielten Isolierung und Charakterisierung verotoxinbildender Escherichia coli mittels DNA-Sonden. Berl. Münch. Tierärztl. W.schr. 109, 431-433. - KARCH, H., MEYER T. (1989): Single primer pair for amplifying segments of distinct Shiga-like-toxin genes by polymerase chain reaction. J. Clin. Microbiol. 27: 2751-2757.

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Tab. 1: Results of STEC-screening by PCR. Microbiologically confirmation was done by isolation of STEC.

Material	n	Screening positive	Microbiologically confirmation
Hand-swab samples	2078	49 (2,4%)	10 (0,5%)
Faecal specimens	455	34 (7,5%)	17 (3,7%)
Swab samples from sanitary regions	133	3 (2,3%)	0
Swab samples from processing areas	1336	31 (2,3%)	9 (0,7%)
Raw material	607	230 (38%)	10 (1,7%)
Products (short ripened fermented sausages)	1680	213 (13%)	44 (2,6%)
Others (Examination of meat fluids only in plant II, Examination of fumigation spits only in plant I)	209	60 (29%)	5 (2,4%)
Total	**6498**	**620 (9,5%)**	**95 (1,5%)**

Tab. 2a: Kind and number of sample material, results of STEC-screening by PCR in plant I

Material	n	Screening positive	Microbiologically confirmation
Hand-swab samples	499	2 (0,4%)	1 (0,2%)
Faecal specimens	162	16 (9,9%)	9 (5,6%)
Swab samples from sanitary regions	45	0	0
Swab samples from processing areas	401	4 (1%)	2 (0,5%)
Raw material	189	75 (40%)	2 (1,1%)
Products (short ripened fermented sausages)	680	77 (11,3%)	19 (2,8%)
*Others**	26	0	0
Total	**1976**	**174 (8,8%)**	**33 (1,7%)**

* Others: Examination of fumigation spits only in plant I

Tab. 2b: Kind and number of sample material, results of STEC-screening by PCR in plant II

Material	n	Screening positive	Microbiologically confirmation
Hand-swab samples	680	27 (4%)	5 (0,7%)
Faecal specimens	138	13 (9,4%)	8 (5,8%)
Swab samples from sanitary regions	45	3 (6,7%)	0
Swab samples from processing areas	540	24 (4,4%)	4 (0,7%)
Raw material	199	78 (39%)	2 (1%)
Products (short ripened fermented sausages)	429	79 (18,4%)	16 (3,7%)
*Others**	183	60 (32,8%)	5 (2,7%)
Total	**2214**	**284 (12,8%)**	**40 (1,8%)**

* Others: Examination of meat fluids only in plant II

Tab. 2c: Kind and number of sample material, results of STEC-screening by PCR in plant III

Material	n	Screening positive	Microbiologically confirmation
Hand-swab samples	899	20 (2,2%)	4 (0,4%)
Faecal specimens	155	5 (3,2%)	0
Swab samples from sanitary regions	43	0	0
Swab samples from processing areas	395	3 (0,8%)	3 (0,8%)
Raw material	219	77 (38%)	6 (1,7%)
Products (short ripened fermented sausages)	571	57 (13,5%)	9 (1,6%)
Total	**2282**	**162 (7,1%)**	**22 (1%)**