STUDIES ABOUT THE OCCURRENCE OF SHIGATOXIN-PRODUCING ESCHERICHIA COLI (STEC) IN MEAT PROCESSING COMPANIES

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Introduction

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). Since that time it has been world wide a number of outbreaks and sporadic illnesses. 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 more than 20 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.

Several virulence factors can contribute to the pathogenicity of EHEC/STEC. The major factors, which also define the STEC group, are the Shiga toxins (Stx) comprising Stx 1 and Stx 2 with subtypes (Pierard et al., 1998; Schmidt et al., 2000). Other important virulence factors are the intimin- (encoded by *eae*-gene) and the hemolysin-production (encoded by *hly*-gene) and were closely associated with HUS (Bockemühl and Karch, 1996; Fruth et al., 2002).

Objectives

The aim of the studies was to investigate the contamination of meat products such as short fermented spread able raw sausages with Shiga toxin-producing *Escherichia coli* (STEC) and to determine the entry sources of STEC into meat processing companies.

Methodology

Sampling: From 1997 to 2002 samples were taken from sausage manufacturing plants and local supermarkets during five studies. Origins, kinds and number of the samples are shown in table 1. Stool samples and hand swabs were collected from healthy staff members working in the production line of the plants.

Screening for STEC: The samples were enriched in modified Tryptic soy broth (mTSB) and tested for the encoding gene for shigatoxin production (*stx*) by PCR according to Karch and Meyer (1989). After subcultivation of stx-positive sample

enrichments on Sorbitol MacConkey agar (SMAC) *stx*-positive colonies were isolated and characterised by biochemical methods. STEC confirmed as *E. coli* were further differentiated for other virulence factors by PCR (see table 2) and serotyped.

Results & Discussion

In order to investigate the occurrence of STEC in meat processing companies stool samples (n=1566) and hand swab samples (n=2366) from healthy employees of processing areas, swab samples from sanitary (n=699) and working areas (n=2850), samples of raw material (n=717) and short fermented raw sausages (n=2748) were collected in regular intervals from meat processing companies during five studies (1997 – 2002). STEC were detected in all sample materials except swab samples of sanitary areas.

Most of the STEC were isolated from product samples (1.5%, see figure 1). Some large EHEC-outbreaks were associated with contaminated beef or beef products (Bell et al., 1994). Short fermented raw sausages were involved in one outbreak: In 1995 a total of 23 cases of HUS were reported from Australia after the consumption of raw Bologna sausage (CDC, 1995).

In this work about 7% asymptomatic STEC-shedders (6% to 15%) were detected among the staff (n=233). The serotyping resulted in serotypes, which have been connected with human EHEC-diseases (e.g. O26, O91, and O103). These results are shown in figure 2.

Human shedders are important sources for STEC-infections (Carter et al., 1987; Pavia et al., 1990; Beutin and Niemer, 1995). However, there is a small knowledge about the prevalence of STEC in healthy people. Stephan et al. (2001) studied the occurrence of STEC in employees of food processing companies in Switzerland and found positive results in 3.5 %.

A total of 140 STEC-strains were isolated during the studies. About 58% of them (n=84) possessed the gene for production of shigatoxin 1 (*stx*1), 30% (n=44) of the strains had the *stx* 2-gene and 9% possessed *stx* 1 together with *stx* 2 (n=13). Strains with the *eae*-gene (n=15) were isolated only from stool and product samples (see table 3).

Stx 1 is regarded as less toxic for humans as *stx* 2 and more connected with enteritis than with HUS (Pierard et al., 1998; Fruth et al., 2002). The other important virulence factors closely associated with HUS like the *eae*-gene and the *hly*-gene (Bockemühl and Karch, 1996; Fruth et al., 2002) were found in 10 and 42%, respectively. However, EHEC strains lacking *eae*- and *hly*-gene can also cause diseases in humans (Fruth et al., 2002). Therefore, it is not possible to differentiate high pathogenic EHEC from STEC by analysis of virulence factors. In consequence several authors required to consider food borne STEC as potential EHEC (Azavedo et al., 1994; Bockemühl and Karch 1996).

Conclusions

The results of the studies document the importance of asymptomatic carriers as a possible source of entry for EHEC/STEC into meat producing companies. Therefore regular examinations of staff are necessary to guarantee product safety. The source of infection for staff members could not be ruled out in the studies.

References

Azavedo, De J., Mc Whirter, E., Louie, M., Brunton, J, (1994): Eae-negative verotoxin-producing *Escherichia coli* associated with heamolytic uremic syndrome and hemorragic colitis. In: Karmali, M.A., and Goglio, A.G. (Hrsg.): Recent advances in verocytotoxin producing *Escherichia coli* infections. Amsterdam: Elsevier Science, 265–268.

Bell, B.P., Goldoft, M., Griffin, P.M., Davis, M., Gordon, D.C., Tarr, P.L., Bartleson, C.A., Lewis, J.H., Barett, T.J., Well, J.G. (1994): A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhoea and haemolytic uraemic syndrome from hamburgers. The Washington experience. J. Am. Vet. Med. Assoc. 272, 1349–1353

Beutin, L. And Niemer, U. (1995): Erkennung, Verhütung und Bekämpfung von Infektionen durch enterohaemorrhagische *E. coli* (EHEC). Erfordernisse und Möglichkeiten für das öffentliche Gesundheitswesen. Bundesgesundhbl. 11, 422–427.

Bockemühl, J. And Karch, H. (1996): Zur aktuellen Bedeutung der enterohämorrhagischen *Escherichia coli* (EHEC) in Deutschland (1994–1995). Bundesgesundheitsbl. 8/96, 290-296.

Brunder, W., H. Shcmidt, H. Karch (1996): katP, a novel catalase-peroxidase encoded by the large plasmid of enterohaemorrhagic *Escherichia coli* O157:H7. Microbiol. 142, 3305–3315

Brunder, W., H. Schmidt, H. Karch (1997): *EspP*, a novel extracellular serine protease of enterohaemorrhagic *Escherichia coli* O157:H7 cleaves human coagulation factor V. Molecular Microbiol. 24: 767–778.

Cagney, C., Crowley, H., Duffy, G., Sheridan, J.J., O'brien, S., Carney, E:, Anderson, W., Mcdowell, D.A., Blair, I.S., Bishop, R.H. (2004): Prevalence and numbers of *Escherichia coli* O157:H7 in minced beef and beef burgers from butcher shops and supermarkets in the Republic of Ireland. Food Microbiol. 21, 203–212.

Carter, A.O., Borcyk, A.A., Carlson, J.A.K., Harvey, B., Hockin, J.C., Karmali, M.A., Krishnan, C., Korn, D.A., Lior, H. (1987): A severe outbreak of *Escherichia coli* O157:H7-associated hemorrhagic colitis in a nursing home. N. Engl. J. Med. 12, 1496–1500.

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.

Cebula, T.A., W.I. Payne And P. Feng (1995): Simultaneous identification of strains of *Escherichia coli* serotype O157:H7 and their shiga-like toxin type by mismatch amplification mutation assay-multiplex PCR. J. Clin. Microbiol. 33, 248.

Fruth, A., Prager; R., Friedrich, A., Kuczius, T., Roggentin, P., Karch, H., Ammon, A., Bockemühl, J., Tschäpe. H. (2002): Infektionen des Menschen durch enterohämorrhagische Escherichia coli (EHEC) in der Bundesrepublik Deutschland von 1998 bis 2001. Bundesgesundheitsbl – Gesundheitsforsch – Gesundheitsschutz 45, 715–721.

Hofinger; C., H. Karch, H. Schmidt (1998): Structure and function of plasmid pColD 157 of enterohemorrhagic *Escherichia coli* O157 and its distribution among strains from patients with diarrhea and haemolytic-uremic syndrome. J. Clin. Microbiol. 36: 24–29.

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.

Karch, H., T. Meyer, H. Rüssmann, J. Hessemann (1992): Frequent Loss of Shiga-Like Toxin Genes in Clinical Isolates of *Escherichia coli* upon Subcultivation. Infect. Immun. Vol. 60, No. 8, 3464–3467.

Pavia, A.T., Nichols, C.R., Green, D.P., Tauxe, R.V., Mottice, S., Greene, K.D., Wells, J.G., Siegler, R.L., Brewer, E.D., Hannon, D., Blake, P.A. (1990): Haemolytic uremic syndrome during an outbreak of *Escherichia coli* O157:H7 infections in institutions for mentally retarded persons: clinical and epidemiologic observations. J. Pediatrics 116, 544–551

Pierard, D. Muyldermans, G. Moriau, L. Stevens, D., Lauwers, S. (1998): Identification of new Verocytotoxin type 2 variant B-subunit genes in human and animal *Escherichia coli* isolates. J. Clin. Microbiol. 36, 3317–3322

Rüssmann, H., Kothe, E., Schmidt, H., Franke, S., Harmsen, D., Caprioli, A., Karch, H., 1995. Genotyping of Shiga like toxin genes in non-O157 *Escherichia coli* strains associated with haemolytic uraemic syndrome. J. Med. Microbiol. 42, 404–410

Savarino, S.J., A. Fasano, J. Watson, B.M. Martin, M.M. Levine, S. Guandalini, P. Guerry (1993): Enteroaggregative *Escherichia coli* heat-stable enterotoxin I represents an other subfamily of *E. coli* heat-stable toxin. Proc. Nat. Acad- Soc. USA 90: 3093–3097

Schmidt, H., B. Plaschke, S. Franke, H. Rüssmann, A. Schwarzkopf, J. Hessemann and H. Karch (1993): Differentiation in virulence patterns of *Escherichia coli* possessing eae genes. Med. Microbiol. Immunol. 183, 23–31.

Schmidt, H., H. Rüssmann B., A. Schwarzkopf, A. Aleksic, J. Hessemann And H. Karch (1994): Prevalence of attaching and effacing *Escherichia coli* in stool samples from patients and controls. Zbl. Bact. 281, 201.

Schmidt, H., Beutin, L., Karch, H., 1995. Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157:H7 strain EDL 933. Infect. Immun. 63, 1055–1061

Schmidt, H., H. Henkel, H. Karch (1997a): A gene cluster closely related to type II secretion pathway operons of Gram-negative bacteria is located on the large plasmid of enterohemorrhagic *Escherichia coli* O157 strains. FEMS Microbiol. Lett. 148: 265–272.

Schmidt, H., J. Scheef, C. Janetzki-Mittmann, M. Datz, H. Karch (1997b): An ileX tRNA gen eis located close to the shiga toxin II operon in enterohemorrhagic Escherichia coli O157 and non-O157 strains. FEMS Microbiol. Lett. 149:39–44.

Schmidt, H., Scheff, J., Morabito, S., Caprioli, A., Wieler, L.H., Karch, H. (2000): A new Shiga toxin 2 variant (Stx2f) from *Escherichia coli* isolated from pigeons. *Applied and Environmental Microbiology* **66**, 1205–1208.

Schmitt, C.K., Mckee, M.L., O'brien, A.D., 1991. Two copies of shiga-like-toxin II-related genes common in enterohemorrhagic *Escherichia coli* strains are responsible for the antigenetic heterogenicty of the O157:H- strain E32511. Infect. Immun. 59, 1065–1073

Stephan, R., Ragettli, S., Untermann, F. (2001): Prevalence and characteristics of verotoxin-producing Escherichia coli (VTEC) in stool samples from asymptomatic human carriers working in the meat processing industry in Switzerland. J. Appl. Microbiol. 88, 335–341.

Weinstein, D.L., Jackson, M.P., Samuel, J.E., Holmes, R.K., O'brien, A.D., 1988. Cloning and sequencing of a shiga-like toxin type II variant from an *Escherichia coli* strain responsible for Edema disease of swine. J. Bact. 170, 4223–4230

Tables and Figures

Table 1: Origins, kinds and number of samples

		sample material						
plant	**sampling period**	**stool**	**swabs of sanitary areas**	**hand swabs**	**swabs of working areas**	**raw material**	**meat juice**	**products****
I	4-8/1997	**574** (100)*	**285**	**-**	**522**	**-**	**-**	**-**
II	4/1997-12/1998	**467** (22)*	**256**	**-**	**762**	**-**	**168**	**623**
retail	3/1999-3/2000	**-**	**-**	**-**	**-**	**-**	**-**	**139**
III	10/2001-9-2002	**193** (59)*	**53**	**609**	**473**	**224**	**-**	**813**
IV	10/2001-9-2002	**158** (20)*	**53**	**669**	**624**	**234**	**198**	**501**
V	10/2001-9/2002	**174** (32)*	**52**	**1088**	**469**	**259**	**-**	**672**
Total	1997-2002	**1566** (233)*	**699**	**2366**	**2850**	**717**	**366**	**2748**

* in brackets: analyzed staff members (people, who came in contact with raw material or finished products)
** products: short fermented raw sausages like „Teewurst“

Table 2: Targets, primers, PCR conditions and references

Target	**Primer**	**Reference**
stx	MK1/MK2	Karch and Meyer, 1989
eae	SK1/SK2	Schmidt et al., 1993; Yu and Kaper, 1992
hlyA	hlyA1/hlyA2	Schmidt et al., 1995
stx 1	KS7/KS8	Rüssmann et al., 1995
stx 2	LP43/LP44	Cebula et al., 1995
stx 2, *stx 2c**	GK3/GK4	Schmidt et al., 1994
*after *Hae III*-restriction		Schmitt et al., 1998
stx 2d	VT2-cm/VT2-f	Pierard et al., 1998
stx 2e	slt2v start/slt2v stop	Weinstein et al., 1988
ast A	AstA1/AstA2	Savarino et al., 1993
col D 157	col D1/col D2	Hofinger et al., 1998
esp P	Esp A/Esp B	Brunder et al., 1997
etp D	D1/D13R	Schmidt et al., 1997a
ile X	356/595	Schmidt et al., 1997b
kat P	wkatB/wkatF	Brunder et al., 1996

Table 3: Numbers, origins and virulence factors of the isolated STEC strains

Isolates	Stool	swabs of sanitary areas	THand	swabs of working areas	Raw materials	Meat juices	Products	Total
	sample materials							
n	63	1	11	4	9	3	53	**144**
stx1	53	1	3	2	4	2	19	**84**
stx2	8	-	7	1	6	1	21	**44**
stx1+2	1	-	1	1	-	1	9	**13**
eae	10	-	-	-	-	-	5	**15**
Ehly	39	-	1	1	-	-	19	**60**
astA	15	1	3	1	1	2	12	**35**
colD157	6	-	-	-	-	1	5	**12**
katP	3	-	-	-	-	1	4	**8**
espP	7	-	5	1	3	-	11	**27**
etpD	12	-	-	-	-	-	5	**17**
ileX	1	-	2	-	-	-	4	**7**

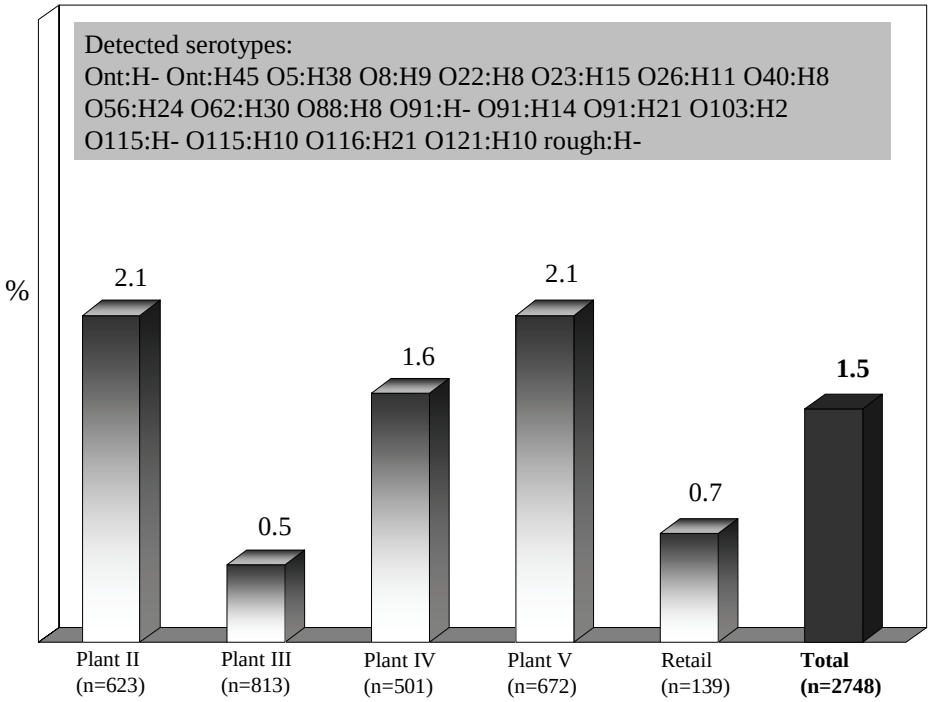

Figure 1: STEC in short fermented raw sausages (1997 – 2002)

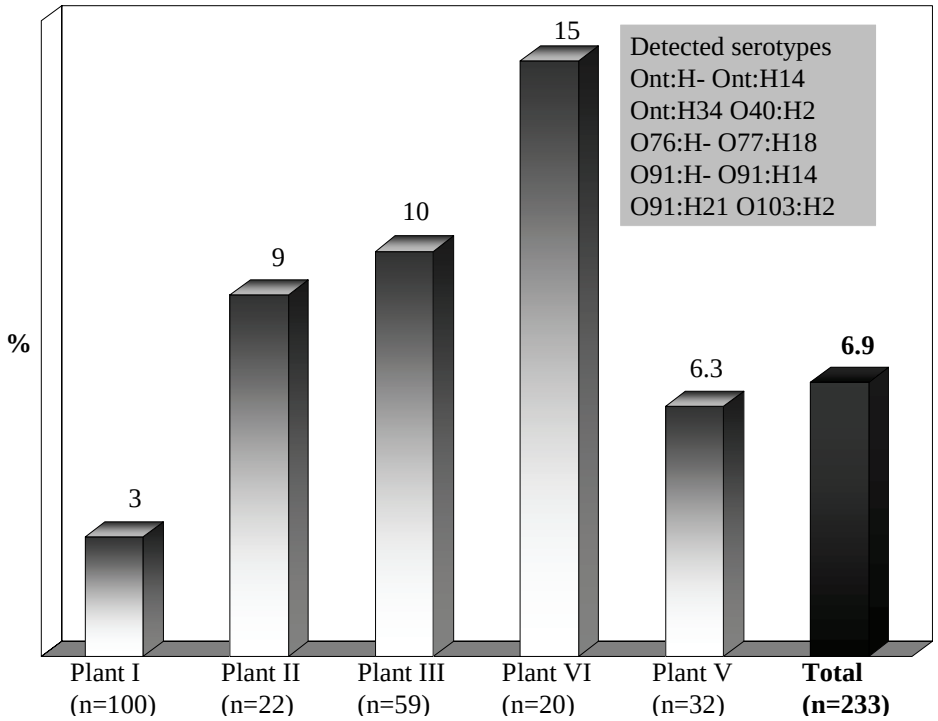

Figure 2: STEC shedders in meat processing plants (1997 – 2002)