ENTEROHAEMORRHAGIC E. COLI (EHEC) - RECENT INFORMATION ON A NEW MEDICALLY IMPORTANT ZOONIC AGENT

Prof. Dr. G. Baljer and Dr. L. H. Wieler (Giessen, Germany)

Preface

EHEC are new zoonotic agents. They were first described in 1982. Since then EHEC-epidemics have occured rather frequently worldwide (e.g. USA 1991, Japan 1996, Scotland 1996). In Germany during the last few years EHEC-infections were increasingly noticed in humans. EHEC have become a burning issue of public interest because of exaggerated reporting in the daily press and on television. Instead of giving detailed information the contradictory reporting has, however, mainly caused a feeling of uncertainty. The following article surveys the recent scientific state of knowledge about EHEC.

Classification

EHEC belong to a specific category of E. coli-bacteria displaying specific pathogenic characteristics. In contrast to the majority of E. coli living as commensals in the intestinal tract of humans and animals, the gut also contains E. coli-bacteria with distinctive pathogenic traits. These pathogenic E. coli are classified into 6 categories by means of their virulence features, namely

1) Enterotoxigenic E. coli (ETEC),
2) Enteropathogenic E. coli (EPEC),
3) Enterohaemorrhagic E. coli (EHEC),
4) Enteroinvasive E. coli (EIEC),
5) Enteroaggregative E. coli (EAggEC),
6) Diffusively adhesive E. coli (DAEC).

Among these pathogenic E. coli-strains, EHEC, as the only zoonotic agent, are of tremendous importance. In contrast to the other categories of pathogenic E. coli and the commensalic E. coli they can cause a watery and haemorraghic colitis (HC) followed by haemolytic-uraemic-syndrome (HUS) (tab. 1). From a pathogenic point of view, it is very important that EHEC are able to transduce the genetic information for the production of shigatoxins (syn. verotoxin) by means of phages. Currently two different shigatoxin-types are known in EHEC. Shigatoxin 1 (Stx1) is identical with the toxin produced by dysentery bacteria (Shigella-group) (figure 1). Stx2 is very similar to this toxin with regard to its mode of action and structure, but antigenically it is different. Shigatoxins destroy cells by inhibition of the protein biosynthesis (figure 2). They belong to the most virile biological toxins known today.

Table 1: Definition: Enterohaemorrhagic E. coli (EHEC)

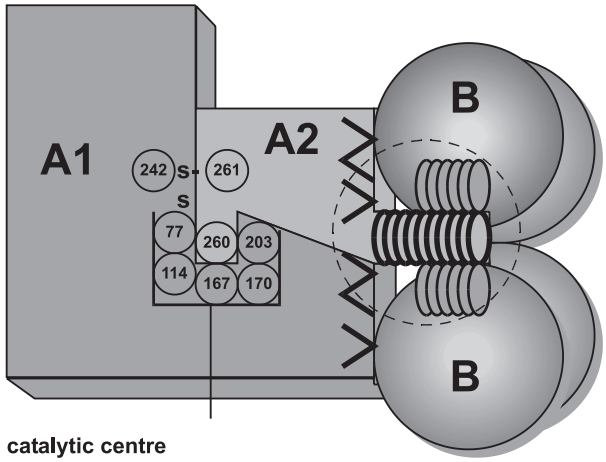
E. coli bakteria causing watery and/or bloody diarrhea in humans as well as haemolytic-ureamic-syndrome (HUS) have the following important virulence-associated factors:

	typical EHEC	atypical EHEC
virulence-associated factors	Stx 1* u./o. Stx 2 LEE** 90 kbp-plasmid	Stx 1 u./o. Stx 2 **?**
serogroup	= e.g. O157, O111, O26, O91, O103, O145 and others	

* shigatoxin (codes for phages)

** Locus of Enterocyte Effacement (chromosomal)

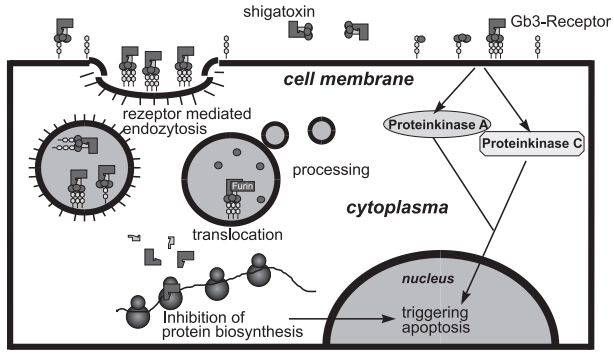
Figure 1: Structure of shigatoxins



A = toxic subunit; B = binding unit

(according to Menge, 1996)

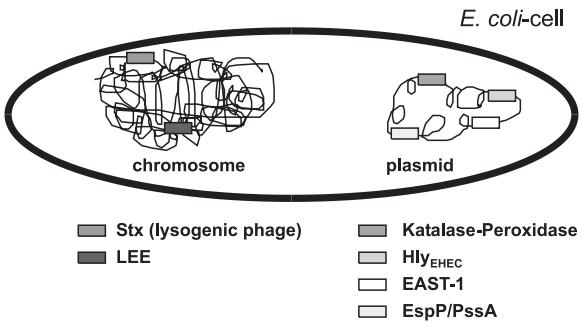
Figure 2: Model of the cellular mode of action of shigatoxin (according to Menge, 1996)



By means of further virulence factors one can distinguish between “typical” and “atypical” EHEC-strains. Typical EHEC harbor the LEE (Locus of Enterocyte Effacement) comprising a group of genes, which give EHEC their so called “attaching and effacing” characteristic, i.e. a) their ability to attach closely to the intestinal epithelial cells and b) to destroy the microvilli. Moreover a 90kbp-plasmid (so called virulence-associated plasmid) codes for a haemolysin (so called EHEC-haemolysin, HlyEHEC), a catalase-peroxidase (KatP), an enterotoxin (EAST-1) as well as a serinprotease (EspP resp. PssA; figure 3).

Typical and atypical EHEC-strains belong to a large number of serogroups. The most important of these are O157, O26, O111, O103, O145, O91 and O22. According to present knowledge typical EHEC of serogroups O157, O111, O26 and O103 are the most virulent ones. In total more than 200 different serovars have been described for EHEC.

Figure 3: Localisation of genes of EHEC-typical virulence-associated factors



E. coli-strains isolated from animals or foodstuffs, which proved to be Stx-positive and whose pathogenicity in humans has not been proven, are often described as shigatoxin-producing E. coli (STEC), and not as EHEC. As any STEC-strain in principle may hide itself behind an EHEC-strain, every STEC-strain is a potential EHEC. Thus in the following only the term "EHEC" is used.

Epidemiology

Together with Salmonella sp., Yersinia sp. and Campylobacter sp., EHEC worldwide rank among the four most important pathogens of foodborne bacterial pathogens. The incidence rates in different countries are summarized in table 2. It should be noted, that several countries have an obligatory registration and in other countries only EHEC O157 is required to be registered.

Table 2: Incidence of EHEC-caused diseases in the year 1996

Country	registered cases total	incidence per 100,000 inhabitants
Germany	314	0.39
UK[1)]	1180	2.03
Spain[1)]	4	0.01
Italy	9	0.02
Netherlands[1)]	10	0.06
* Finland	5	0.01
Denmark	6	0.12
* Austria[1)]	11	0.14
Belgium	52	0.52
* Sweden	118	1.36
U.S.A.[2)]	k.A.	0.03

[1)] figures only refer to EHEC O157
[2)] estimated total figure: appr. 20,000 cases with 250 deaths
* countries in which the disease is notifiable
(according to Eurosurveillance, 1998)

Table 3 illustrates the general increase in the number of registered EHEC-infections. The large outbreaks of recent years in the USA, Japan and Scotland focus people's interest on this topic, but the sporadic EHEC-infections are of even greater medical importance. The number of healthy people shedding EHEC depends on origin and eating habits. People who drink raw milk or who have regular contact with cattle shed EHEC more frequently than other people. In general the number is believed to be smaller than 5 %. The shedding caused by EHEC-diarrhea lasts from 1 week to 5 months (average appr. 17 days).

Table 3: Number of EHEC-caused diseases in the years 1992 to 1996

Country	Number of registered cases					
	1992	1993	1994	1995	1996	1997
Germany	36	32	*	195*	314	573
UK	627	540	685	1138	1180	n.d.a.
Belgium	n.d.a	n.d.a.	29	38	52	n.d.a.
Sweden	0	2	3	114	118	n.d.a.

* figures of 1994 and 1995 were summarized
n.d.a. = no data available (according to Eurosurveillance, 1998)

Since EHEC occurs in humans various studies worldwide have been carried out regarding the prevalence in animals. From the outset cattle have been considered as a possible reservoir. In fact EHEC can be isolated from the faeces of cattle worldwide. A similar distribution is also valid for other ruminants, e.g. sheep and goats and sometimes EHEC were also isolated from the faeces of pigs, horses, hens, pigeons, rabbits, dogs and cats. In countries with EHEC-infections of humans the same strains presumably can also be isolated to a varying extent from animals.

The number of findings of EHEC mainly depends on the detection methods and the number of spot checks. It is impossible to list all findings in animals, already published, in one table. However, to give a survey on the epidemiological situation, table 4 shows several important published data. The isolation rate in cattle varies from 5 to 20 % depending on the use of conventional techniques or molecular biological methods (DNA-hybridization, PCR). By using an immuno-magnetic separation approach to find EHEC O157:H7, the isolation rate increased to more than 50 % in cattle. Similar prevalence rates were also found in faecal samples of sheep and goats. The prevalence in samples of other species is relatively low and varies from 4 to 15 %.

Table 4: Occurrance of EHEC in the faeces of animals after a single examination

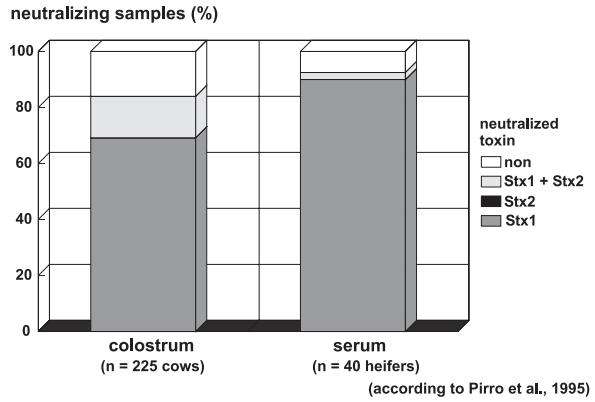
Species	Occurance of	
	EHEC O157	other EHEC
Cattle	11 %[1)]	60 %
Pig	2 %	4 %
Poultry	2 %	12 %
Sheep	4 %	60 %
Turkey	n.d.a.[2)]	7 %
Fish	n.d.a.	10 %
Mussel	n.d.a.	5 %
Dog	n.d.a.	5 %
Cat	n.d.a.	5 %
Pigeon	n.d.a.	15 %

[1)] in single flocks up to 63 %
[2)] n.d.a. = no data available in the literature

The wide-spread occurrence of EHEC in cattle can be confirmed by studies detecting antitoxins in colostrum and serum samples. Antitoxins against shigatoxins were found in 84 % of the colostrum samples of cattle and in 90 % of the serum samples of heifers (figure 4). Due to these data every ruminant is probably infected with EHEC-pathogens during the course of its life and is shedding

them for a short time via the faeces. EHEC in ruminants possibly belong to the resident intestinal flora and in other species to the transient flora.

Figure 4: Frequency of Stx-neutralizing antibodies in cattle (Neutralisation test in the vero-cell-culture; Bavaria, 1989 and 1990)



Because of the high EHEC isolation rates in ruminants EHEC-infections in humans can be explained in part by an EHEC-reservoir in ruminants. The similarity of the described characteristics of EHEC-strains in humans and in ruminants strongly points to this conclusion.

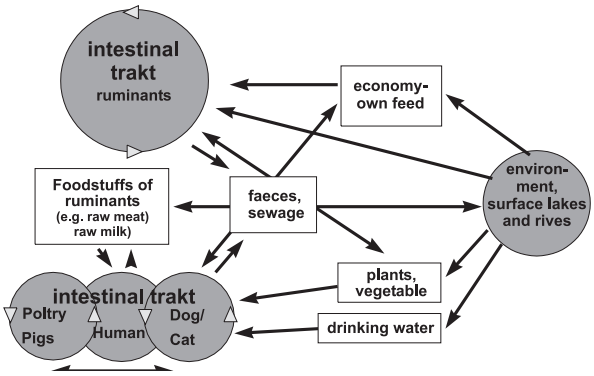
Table 5: Characteristics of EHEC isolated from humans and from livestock animals

Host	Number of serovars	frequent serovars	other frequent characteristics
Human	> 130	O157:H7/H-; O111:H-; O26:H-/H11, O103:H2 O145:H-/H28, O91:H	stx -converted bacteriophages, LEE, Hly_{EHEC}, 90kbp-plasmid,
Cattle	> 150	Ont:H-; O118:H16(H-); O5:H-; O84:H-; O26:H-/H11; O111:H-; O145:H-/H28; O103:H2; O157:H- (H7)	stx converted bacteriophages, LEE, Hly_{EHEC}, 90kbp-plasmid,
Sheep	> 23	O5:H-; O9:H-; O128:H2; OX3:H8, O116:H21; O87:H31	?
Goat	> 5	O5:H-	?

Table 5 shows the close relationship between human and bovine EHEC-strains. Both strains not only correspond regarding to the serovars, but also have numerous other characteristics in common. Examination of more than 200 bovine EHEC-strains showed that appr. 70 % of the strains had the ability of “Attaching and Effacing” (LEE), and the production of EHEC-haemolysin and harbor the virulence-associated plasmid. In contrast EHEC-strains of sheep and goats do not really correspond with human strains regarding the serovars. However, there is a lack of further data of additional EHEC-characteristics for such isolates.

Figure 5 describes the epidemiological situation by taking all available data about EHEC-strains into consideration. Cattle and probably also other ruminants can be considered as the most important reservoir for EHEC-strains. It seems that ruminants accommodate EHEC-strains in the gastrointestinal tract for their lifetime, but they rarely suffer from EHEC-infections. According to recent studies, the rumen plays a significant role as the primary site for EHEC. The rumen is probably the site where phages with stx-genes find an ideal environment for their mulitplication and for the transduction of E. coli-strains of totally different serovars. The EHEC-strains are excreted with the faeces and are directly transmitted to animals and humans via the faecal-oral route or spread by faecal contaminated sewage, foodstuffs etc. It is important to know that EHEC are normally only found in the gastrointestinal tract, i.e. they are not excreted via milk and are not primarily found in organs or meat. Also other species or humans can act as carrier or excretor for a short time. These species, however, do not represent a “real” reservoir.

Figure 5: Main distribution pathways of EHEC



(Baljer and Bauerfeind, 1997)

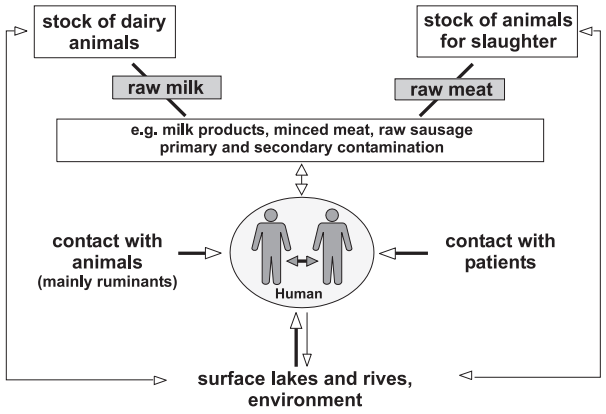
According to the epidemiological situation described above figure 6 shows the ways of transmission of EHEC-pathogens to humans. The infectious sources for humans are:

a) faecal-contaminated foodstuffs (mainly raw milk and raw meat) orginating from ruminants,
b) direct contact with ruminants,
c) faecal-contaminated surface water, vegetables, fruits etc., and
d) direct contact with patients via the faecal-oral route.

Infections in humans can be prevented by sufficient heating and pasteurizing of contaminated foodstuffs and with general hygiene in dealing with infected animals or persons.

The epidemiology of EHEC-pathogens at first sight may be compared with the epidemiology of salmonella, however, there are fundamental differences. Thus the minimum infectious dose for humans for many EHEC-strains is less than 100 bacteria (salmonella: $\geq 10^6$ bacteria). That means that a cooling chain in order to avoid the spreading in foodstuffs does not protect the consumer. In contrast to salmonella, EHEC only get into the foodstuff via faecal contamination, i.e. it is very important to take hygienic steps when producing risk foodstuffs (e.g. raw or full-cream milk, minced meat).

Figure 6: Main transmission pathways of EHEC to humans



(Baljer and Bauerfeind, 1997)

Clinical outcomes

In humans, EHEC mainly cause diarrhea, which may take a slightly watery up to a haemorrhagic course. After an incubation period of appr. 3 to 4 days the disease starts with stomach-convulsions similar to colic with watery diarrhea lasting 1 to 2 days. In half the number of patients vomiting is also observed. Afterwards painful evacuations of bloody stools or only blood occurr. During this time the patients rarely have fever and normally are not dehydrated. The disease generally disappears within 6 to 10 days. Appr. 5 to 10 % of the infected children (< 10 years) and of older people develop haemolytic-uraemic-syndrome (HUS), which occurs appr. 3 to 12 days after the diarrhea started, frequently when the diarrhea stops or even later. HUS is characterised by a quick intravasal haemolysis with a typical fragmentation of the erythrocytes, thrombocytopenia and nephropathy including haematuria and phorphyrinuria. In approximately half of the cases oliguria and sometimes anuria spreads, which requires dialysis. Approximately 10 to 30 % of patients with HUS finally suffer from kidney failure. EHEC-infections in adults in particular cases may lead to the symptom complex of thrombotic-thrombocytopenic-purpura (TTP) mainly involving neurological disturbances.

Diseases in connection with EHEC-infections are not often found in animals. Most infections do not take a clinically apparent course although in calves and piglets, bloody diarrhea caused by EHEC is sometimes described. But the diarrhea first of all is explained by the destruction of the epithelial cells in connection with the EHEC-caused “attaching-and-effacing-lesion”. It seems that shigatoxins, in comparison, only play a minor role in the pathogenesis of EHEC-caused diarrhea in animals. The shigatoxin-producing E. coli-strains, which cause oedema disease in piglets, are an independent group of pathogens which do not belong to EHEC. Oedema disease-causing pathogens produce a distinct variant of shigatoxin (Stx2e) and are no zoonotic agents.

Diagnosis

Diagnostic procedures must relate to specific characteristics of the EHEC-bacteria. The small number of EHEC in the physiological E. coli-flora in the intestine and the large number of possible EHEC-serovars complicate the detection of EHEC-strains in stool samples. It is not longer recommendable to restrict diagnostic procedures to sorbitol-negative strains of the serogroup O157, because in Germany sorbitol-fermenting, and non-O157 strains are involved in half of the EHEC-diseases in humans. A reliable verification of EHEC-infections is finally only possible by combination of cultural, molecular (PCR, DNA-DNA-hybridization) as well as additional serological (antibody determination, toxin detection by means of ELISA) or toxicological detection methods (cell culture). When isolating EHEC, the primary culture should be enriched (4 hours) and then an immuno-magnetic separation carried out. This method providing increased sensitivity is only available up to now for the detection of EHEC O157-strains. The detection of EHEC is very complicated and currently can only be carried out in specialized laboratories. A commercially available ELISA for the detection of the toxins simplifies the diagnosis, but with regard to a direct identification of pathogens it is less suitable.

The postexpositional detection of antibodies is limited to the EHEC-O-antibody determination of important serovars. In diagnosis, the detection of antibodies against shigatoxins is of minor importance, because patients rarely develop detectable antitoxin titers.

Table 6 and 7 give an overview of the indications for an EHEC-diagnosis as well as of present diagnostic procedures.

Table 6: Indications for a microbiological examination of EHEC (DGHM-guidelines; 1997)

1. Haemolytic-uraemic-syndrome (HUS) or thrombotic-thrombocytopenic-purpura
2. Diarrhea and one of the following clinical features:
   - hospitalized children with diarrhea up to the age of 6 years
   - bloody-watery stools
   - haemorrhagic colitis detected by endoscopy
   - nekrotizing enterocolitis
3. By anamnesis: diarrhea (< 1 week) and one of the following clinical features:
   - haemolytic anaemia
   - acute kidney failure
4. Outbreak (2 people and more) in:
   - communal institutions
   - flat-sharing communities
5. Contact of patients with EHEC-infections, resp. with HUS or TTP-patients

Control

Human

The symptomatic treatment of EHEC-diseases predominates. Despite the fact that EHEC in principle are sensitive to antibiotics, the use of antibiotics does not improve the general condition at all. In contrast, it is reported that antibiotics increase the probability of developing HUS. EHEC-caused diarrhea first of all requires the additional supply of liquid and electrolytes and in case of HUS, dialysis, haemofiltration and transfusion are important. In serious cases of HUS the only aid is a lifelong dialysis or a kidney transplant.

At the moment there are no registered vaccines available for prevention and none are expected in the near future. In order to prevent HUS there are first therapeutic steps with a shigatoxin-receptor-analogon (Gb3). This preparation, which is not yet registered, must be taken orally during the diarrhea phase and should prevent the shigatoxin from attaching to the toxin receptor Gb3 in the

Table 7: Method of the microbiological examination of EHEC (DGHM-guidelines; 1997)

A: Identification of the pathogen

1. Bouillon (pre-enrichment)
   - Trypticase Soya Broth + 10-20 mg/l Novobiocin
   - GN-Broth for immunomagnetic separation (up to only O157:H7)
2. Agar culture medium
   Sorbitol-MacConkey-Agar (SMAC) (only non-sorbit-fermenting O157:H7)
   Cefixim-Tellurit-MacConkey-Agar (CT-SMAC) (O157:H7 does not grow) Enterohaemolysin-agar

B: Toxin detection

- Stx-PCR (wash away of colonies or enrichment)
- Stx-ELISA (enrichment)
- Vero-cell-zytoxicity-test (stool)
- Colony-immunoblot

C: Isolation of the pathogen

- Enterohaemolysin agar
- Sorbitol-MacConkey-Agar (SMAC) (only non-sorbitol-fermenting O157:H7)
- Colony-blot-hybridization
- Immunomagnetic separation (up to only O157:H7)

tissue. The prophylactic oral application of antibodies, which are obtained from the colostrum of hyperimmunized cows with high neutralizing antitoxin titers against the shigatoxin is very promising.

As EHEC are only shed via the faeces, it is very important to limit faecal contamination by means of corresponding good hygiene. A sufficient heating of all critical foodstuffs (e.g. raw milk, minced meat) as well as special hygiene regarding the production of these foodstuffs play a significant role. As far as hands are concerned, a strict hygiene must be observed when having contact with EHEC-infected patients or with ruminants in order to prevent smear-infections (human-human, human-animal). As patients shed EHEC for a long period via the faeces (over a period of several weeks), infected people and convalescents should not visit kindergartens, schools, old people's homes, hospitals and food-processing companies.

Animal

In case of diarrhea caused by EHEC the symptoms are treated symptomatically (supply with liquid and electrolytes, diet). Regarding problems with zoonosis it would be desirable to establish ruminant populations being free of EHEC, but this is not realistic because of the wide-spread nature of EHEC. The prevention of faecal contamination as well as decontamination by heating of risk foodstuffs is very important, as EHEC are only shed via the faeces.

Summary

Enterohaemorrhagic E. coli (EHEC)-bacteria are new zoonotic agents known since 1982. EHEC emerged from apathogenic E. coli through a horizontal transfer of genes coding for specific virulence traits. All EHEC-bacteria have the production of one or more types of shigatoxin (syn. verotoxin) in common. Moreover a high percentage of EHEC harbor further virulence characteristics, e.g. the ability to produce a haemolysin or to attach to intestinal epithelial cells and to destroy enterocytes. A large part of EHEC-strains belong to serogroup O157, but other serogroups (e.g. O111, O26, O55, O103) more and more are gaining in importance worldwide.

The gastrointestinal-tract of ruminants is considered as the most important reservoir for EHEC. The number of EHEC isolated from faeces samples of other animals respectively of humans is considerable smaller. Humans are infected orally mainly indirectly by consuming faecal contaminated foodstuffs (e.g. raw milk, minced meat, salad, drinking water) or via animals / humans shedding these pathogens (dirt and smear infection). EHEC-strains are able to survive the stomach-passage or the maturation process of fermented foodstuffs because of their increased acid tolerance.

Typical clinical signs in humans are a watery and sometimes bloody diarrhea (haemorrhagic colitis) with abdominal convulsions. After the onset of diarrhea, haemolytic-uraemic-syndrome (HUS) occurs in 5 to 10 % of cases in infants and elder people as a life-threatening complication with acute kidney failure, microangiopathic haemolytic anaemia and thrombocytopenia as well as, in some cases, neurological symptoms. According to studies in Germany, 4 % of these patients die and approximately 15 % suffer from permanent kidney damage. In animals, EHEC can cause watery, sometimes bloody, diarrhea in the first weeks of life.

The diagnosis of EHEC-infections is difficult because, apart from the production of shigatoxins, no other reliable phenotypic marker, which enables a safe and quick identification of EHEC-strains in a sample, is currently known. The sufficient heating of all critical foodstuffs as well as general hygiene plays a significant role in prevention. Vaccines are not available in the short term. However, immunoglobulin-preparations for the neutralization of shigatoxins are being clinically tested. The antibiotic treatment of EHEC-infections must be approached cautiously. On the one hand it is possible to inactivate the bacteria with antibiotics, but on the other, extraintestinal complications which have occured in the course of antibotic therapy, have been attributed to an increased release of toxins from the bacteria.

Literature

Baljer, G. and Bauerfeind, R. (1997) Neue Erkenntnisse zum Vorkommen und zur Übertragung von enterohämorrhagischen Escherichia coli-Keimen. Vortragszusammenfaßung, S. 102-118, Kongreß des Bundesverbandes der beamteten Tierärzte (Dresden, 24.-25.04.1997)

Baljer, G. and Wieler, L.H. (1995) Infektionen mit enterohämorrhagischen E. coli (EHEC). Hess.Ärztbl. 56, 142-144.

Baljer, G., Wieler, L.H., Bauerfeind, R., Ludwig, S.-B. and Mayr, A. (1990) Nachweis von Vero- (Shiga-like-) toxinbildenden E. coli-Keimen (VTEC) mittels Zellkulturtest und DNA-Hybridisierung bei durchfallkranken Kälbern. Tierärztl.Umschau 45, 71-78.

Beutin, L., Geier, D., Zimmermann, S. and Karch, H. (1995) Virulence markers of Shiga-like toxin-producing Escherichia coli strains originating from healthy domestic animals of different species. J.Clin.Microbiol. 33, 631-635.

Beutin, L., Montenegro, M.A., Orskov, I., Orskov, F., Prada, J., Zimmermann, S. and Stephan, R. (1989) Close association of verotoxin (Shiga-like toxin) production with enterohemolysin production in strains of Escherichia coli. J.Clin.Microbiol. 27, 2559-2564.

Bockemühl, J. and Karch, H. (1996) Zur aktuellen Bedeutung der enterohämorrhagischen Escherichia coli (EHEC) in Deutschland (1994-1995). Bundesgesundheitsblatt 39(8), 290-296.

Karch, H. and Bockemühl, J. (1989) Infektionen durch enterohämorrhagische Escherichia coli (EHEC): ein klinisches und mikrobiologisches Problem und eine Herausforderung für den öffentlichen Gesundheitsdienst. Infekt.Immun. 17, 206-211.

Karmali, M.A. (1989) Infection by verocytotoxin-producing Escherichia coli. Clin. Microbiol. Rev. 2, 15-38.

Levine, M.M. (1987) Escherichia coli that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. J.Infect. Dis. 155, 377-389.

McDaniel, T.K. and Kaper, J.B. (1997) A cloned pathogenicity island from enteropathogenic Escherichia coli confers the attaching and effacing phenotype on E. coli K-12. Mol.Microbiol. 23(2), 399-407.

Menge, Ch. (1996) Untersuchungen zur Wirkung des Shiga-like-Toxins-I (SLT-I) von Escherichia coli auf Immunzellen des Rindes. Vet.Med.Diss., Justus-Liebig-Universität Giessen

Nataro, J.P. and Kaper, J.B. (1998) Diarrheagenic Escherichia coli. Clin.Microbiol. Rev. 11, 142-201.

Pirro, F., Wieler, L.H., Failing, K., Bauerfeind, R. and Baljer, G. (1995) Neutralizing antibodies against Shiga-like toxins from Escherichia coli in colostra and sera of cattle. Vet. Microbiol. 43, 131-141.

Pospischil, A., Mainl, J.G., Baljer, G. and Moon, H.W. (1987) Attaching and effacing bacteria in the intestines of calves and cats with diarrhea. Vet.Pathol. 23, 330-334.

Wieler, L.H. (1997) Bestimmung von Virulenzfaktoren boviner Shiga-Toxin-bildender Escherichia coli-(STEC-) Stämme als Bewertungsgrundlage ihrer klinischen Bedeutung für Rind und Mensch. Vet. Med. Habil. Schrift, Justus-Liebig-Universität Giessen.

Wieler, L.H., Bauerfeind, R. and Baljer, G. (1992) Zur Bedeutung und Diagnostik von Infektionen landwirtschaftlicher Nutztiere mit Shiga-like-Toxin-produzierenden E. coli (SLTEC). Tierärztl.Umschau 47, 524-533.

Wieler, L.H., Vieler, E., Erpenstein, C., Schlapp, T., Steinrück, H., Bauerfeind, H., Byomi, A. and Baljer, G. (1996) Shiga toxin-producing Escherichia coli (STEC) from bovines: association of adhesion with the carriage of eae and other genes. J.Clin.Microbiol. 34, 2980-2984.