

Bovine Colostrum Ameliorates Diarrhea in Infection with Diarrheagenic *Escherichia coli*, Shiga Toxin-Producing *E. coli*, and *E. coli* Expressing Intimin and Hemolysin

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ABSTRACT

Background: Diarrheagenic *Escherichia coli* may cause serious extraintestinal complications, but there is no specific treatment.

Methods: Patients with diarrhea caused by diarrheagenic *E. coli*, specifically Shiga toxin-producing *E. coli* and *E. coli*-expressing intimin and enterohemorrhagic *E. coli*-hemolysin were treated by administration of pooled bovine colostrum, rich in antibodies to Shiga toxin and enterohemorrhagic *E. coli*-hemolysin, in a placebo-controlled, double-blind study. Symptom resolution and fecal excretion of infecting strains were assessed.

Results: No side effects were attributable to colostrum. Stool

frequencies in the group treated with bovine colostrum were significantly reduced compared with those in the placebo group. No effect of therapy on the carriage of the pathogens or on complications of the infection could be demonstrated.

Conclusions: Bovine colostrum is well tolerated and diminishes frequency of loose stools in children with *E. coli*-associated diarrhea. A prospective study should be conducted among a larger number of children with Shiga toxin-producing *E. coli* identified early in illness, to determine the effectiveness of colostrum therapy. *JPGN* 29:452-456, 1999. **Key Words:** Bovine colostrum—Diarrhea—Diarrheagenic *Escherichia coli*—Hemolytic uremic syndrome. © 1999 Lippincott Williams & Wilkins, Inc.

Escherichia coli are important and often overlooked causes of severe diarrhea in children (1-3). Several molecules have been postulated to be critical pathogenicity factors, including Shiga toxins (Stx), produced by *E. coli* O157:H7 and other serotypes of *E. coli* (designated Stx-producing *E. coli* (STEC) (1); intimin, encoded by *eae* (4), which has also been associated with diarrhea, both in epidemiologic (5) and volunteer studies (6) and enterohemorrhagic *E. coli* (EHEC)-hemolysin, which is encoded on the large plasmid of *E. coli* O157:H7 (7,8), which can also be found in *E. coli* that do not produce Stx.

Diarrheagenic *E. coli* can also cause serious extraintestinal complications in children, such as hemolytic uremic syndrome (HUS) which is caused by STEC. Currently available data do not support the use of specific antimicrobial therapy for the treatment of childhood infections caused by STEC or other diarrheagenic *E. coli*

(9). The clinical and economic impact of infection with these bacteria, including hospital admission for dehydration or for HUS, has only recently been elucidated (10).

Colostrum is an important defense against a variety of microbial pathogens. In many mammalian species, these protective factors are transferred from the mother to immunologically naive offspring. In humans, breast feeding during the first months of life decreases infant morbidity and mortality secondary to diarrheal and systemic infections (11). Whole bovine colostrum and immunoglobulin-enriched colostrum fractions have been used in infants and immunocompromised adults to treat or prevent enteric infections (12-14).

We performed an exploratory study to test the hypotheses that bovine colostrum containing antibodies against *E. coli* virulence factors, in particular Stx, intimin, and EHEC-hemolysin, hasten the elimination of a variety of diarrheagenic *E. coli* and reduce the frequency of stools in children infected with these organisms.

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PATIENTS, MATERIALS, AND METHODS

Children with diarrhea whose stool cultures yielded *E. coli* containing *eae* which encodes intimin, in addition to Stx1,

Stx2, or both or EHEC-hemolysin were considered eligible for enrollment. To exclude the presence of typical enteropathogenic *E. coli*, cultured strains had to be negative for the enteropathogenic *E. coli* adherence factor (15).

Screening for the pathogenic *E. coli* strains was performed by polymerase chain reaction (PCR) analysis of overnight stool cultures from sorbitol MacConkey agar to detect the presence of sequences homologous to the genes encoding Stx1, Stx2, intimin, and EHEC-hemolysin (7,16-18). For PCR, colonies grown overnight (approximately 1500 colonies) were harvested in 1 ml of saline solution (0.85% NaCl). The PCR reactions were performed with a commercial system (GeneAmp 9600; Perkin Elmer-Applied Biosystems, Weiterstadt, Germany). Amplifications were carried out in a total volume of 50 μ l containing 15 μ l bacterial suspension (10^6 cells), each deoxynucleoside triphosphate at 200 μ M, 30 pM of each primer, 5 μ l of 10-fold concentrated polymerase synthesis buffer, 1.5 mM MgCl₂, and 2.0 U of DNA polymerase (AmpliTag; Perkin Elmer-Applied Biosystems). The primer sequences and PCR conditions are shown in Table 1. After 30 cycles had been completed, a 5- μ l-aliquot of each PCR sample was analyzed by submarine gel electrophoresis on 1.5% (wt/vol) agarose gel and visualized by staining with ethidium bromide. To distinguish between Stx2 and Stx2c, restriction endonuclease analysis of PCR products obtained with Stx2-specific primers was performed with *Hae*III and *Fok*I, as described (17). To identify colonies of *E. coli* containing these virulence genes in PCR-positive samples, colony-blot hybridization with 100 to 200 individual colonies was performed by using digoxigenin-labeled probes specific for the sequences of Stx1, Stx2, and intimin, respectively, as described (19). Production of Stx by *E. coli* strains was tested by using the Vero cell cytotoxicity assay (20). Enterohemolytic phenotype was verified on enterohemolysin agar (7). All stools were also screened for the presence of other enteropathogenic bacteria by standard culture techniques and for rotavirus antigen by enzyme immunoassay.

Patients (age 1 month to 18 years) admitted to the hospital in either of Würzburg's two children's hospitals because of diar-

rhea caused by *E. coli* were entered into the trial from July 1993 through June 1996. The frequency of isolation of Stx-producing *E. coli* in this population is about 2.8% of all patients with diarrhea (1). Two patients treated for established HUS in the Children's Hospital of the University of Erlangen, Germany, were also entered into the trial. The parents of the children and the adolescent patients were informed about the trial both orally (duration of interview, >60 minutes) and in writing. All parents and adolescents consented in writing to participate.

A complete history, including the time of the onset of diarrhea, a thorough physical examination, and laboratory values including complete blood count, urinalysis, blood gases, serum electrolytes, and other tests were recorded when appropriate. Exclusion criteria were unknown time of onset of diarrhea, a history of bovine milk intolerance, treatment of diarrhea with drugs, and breast-feeding. In addition, patients were excluded from the final evaluation if vomiting interfered with administration of the study medication.

The study medication was either bovine colostrum or placebo. Bovine colostrum concentrate was prepared following the guidelines for the preparation of infant's milk and contained 80% protein with >65% immunoglobulin, mainly IgG (Lactobin, Biotest Pharma, Dreieich, Germany) (21). Bovine colostrum used was from a single batch that originated from more than 100 carefully supervised cows not immunized against *E. coli* strains and contained high titers of neutralizing antibodies against Stx1, Stx2, and EHEC-hemolysin (22). Gelatin (92% protein, Töpfer, Dietmannsried, Germany), an innocuous preparation devoid of antibodies but similar in chemical composition and identical in appearance with bovine colostrum, served as placebo.

Patients meeting the entry criteria and still in the hospital at the time of the bacteriologic diagnosis were randomly allocated to receive either bovine colostrum or placebo administered double-blind as 10 ml daily doses of 7 g before meals for 14 days. Patients were examined every other day during their hospital stays, at least once weekly thereafter for the duration of treatment, and on days 15 (first day after treatment cessation)

TABLE 1. Primers used in polymerase chain reaction assays for the detection of virulence factors in *Escherichia coli* strains grown from stools of children with diarrhea

Virulence factor	Primers	Experimental conditions	References
Shiga toxin 1 (<i>Stx1</i>)	5'-CCC GGA TCC ATG	30 seconds, 94°C	16
	AAA AAA ACA TTA TTA	60 seconds, 52°C	
	ATA GC-3'	40 seconds, 72°C	
	5'-CCC GAA TTC AGC	30 cycles	
	TAT TCT GAG TCA		
Shiga toxin 2 (<i>Stx2</i>)	ACG-3'		
	5'-ATG AAG AAG ATG	30 seconds, 94°C	17
	TTT ATG GCG-3'	60 seconds, 52°C	
	5'-TCA GTC ATT ATT	60 seconds, 72°C	
	AAA CTG CAC-3'	30 cycles	
5'-CCC GAA TTC GGC	30 seconds, 94°C		
Intimin (<i>eae</i>) ^a	ACA AGC ATA AGC-3'	60 seconds, 52°C	18
	5'-CCC GGA TCC GTC	60 seconds, 72°C	
	TCG CCA GTA TTC G-3'	30 cycles	
	5'-GGT GCA GCA GAA	30 seconds, 94°C	
	AAA GTT GTA G-3'	90 seconds, 57°C	
Enterohemorrhagic <i>E. coli</i> -hemolysin (<i>Hly</i>)	5'-TCT CGC CTG ATA	90 seconds, 72°C	7
	GTG TTT GGT-3'	30 cycles	

^a Primers are derived from the conserved region of the gene thus recognizing intimin α , β , and γ .

TABLE 2. Microbiologic characterization of *Escherichia coli* expressing Shiga toxin 1 or 2, Intimin (eae) or EHEC-hemolysin (Hly) isolated from patients with diarrhea and treated with bovine colostrum or placebo

Bovine colostrum		Placebo ^a	
Serovar	Positive PCR indication	Serovar	Positive PCR indication
O8:H-	Hly, eae	O8:H-	Hly, eae
O9:H-	Stx1, Hly, eae	O14:H-	Hly, eae
O25:H14	Stx1, Hly, eae	O26:H-	Hly, eae
O26:H-	Stx1, Hly, eae	O26:H47	Hly, eae
O26:H-	Stx1, Hly, eae	O103:H2	Stx1, Hly, eae
O26:H-	Hly, eae	O156:H25	Hly, eae
O103:H2	Stx1, Hly, eae	O157:H-	Stx2, Hly, eae
O107:H-	Hly, eae	O157:H7	Stx2, Hly, eae
O157:H-	Hly, eae	O157:H7	Stx2, Hly, eae
O157:H-	Stx2, Hly, eae	ONT:H-	Stx1, eae
O157:H7	Stx2, Hly, eae	no isolate	Stx1
Orough:H-	Hly, eae	no isolate	Stx1
		no isolate	Stx2

^a In four patients *E. coli* strains were isolated in which PCR for Shiga toxin sequences 1 or 2 (two patients each) were positive, and stools showed Vero cell cytotoxicity, but the isolate was not tested for the presence of intimin or EHEC-hemolysin and was not serotyped. EHEC, enterohemorrhagic *E. coli*; PCR, polymerase chain reaction; ONT, O antigen not typable.

and 21. While in the hospital, the patients or parents of the patients were instructed in how to follow the study guidelines by specially trained nurses. Stool frequency was noted daily and compared with the stool frequency recorded on diary cards by the parents. Discrepancies between parental report and hospital records were reconciled by interview with the parents. Parents also recorded the consumption of the study medication and other events. Adherence to the study protocol after discharge from hospital was ascertained by telephone calls to the patients' homes.

It was assumed that 15 patients per group in a parallel group setting were sufficient to identify relevant treatment effects and to enable appropriate sample sizes to be determined in subsequent confirmatory studies. Data were analyzed using the Mann-Whitney test or Fisher's exact test. *P* at the 5% level was regarded as significant. The study was approved by the ethics

committee of the Medical Faculty of the University of Würzburg.

RESULTS

Thirty children with diarrhea caused by infection with *E. coli* expressing Stx1, Stx2, or both; intimin; or EHEC-hemolysin were entered into the study. No patient met the exclusion criteria at enrollment. In 1 patient each, *Salmonella enterica* and rotavirus antigen were also found. In three children with Stx-producing *E. coli* infection, study medication was discontinued because of preexisting continuous vomiting: Two of these patients had HUS before entry into the study, whereas the third patient was an infant with severe developmental retardation and wasting secondary to preexisting severe feeding problems. Vomiting in these three children was not considered a side effect of the study medication (two in the bovine colostrum group, one in the placebo group) but to be related to the preexisting illnesses. In the third patient who had HUS before entry into the study, treatment was administered. No patient experienced development of HUS after initiation of study treatment.

Table 2 shows the characterization of the isolated strains of *E. coli* including bacteria of a variety of serotypes. The demographic and clinical data of the patients treated with bovine colostrum (*n* = 13) and those treated with placebo (*n* = 14) showed no obvious or significant differences (Table 3).

The study medication was well tolerated. Six children treated with bovine colostrum and seven administered placebo reported minor symptoms (poor appetite, abdominal colic, and occasional vomiting).

During treatment with bovine colostrum, median stool frequency decreased from three stools per day to one, whereas during treatment with placebo, the median stool frequency did not change during the observation period (*P* < 0.05; Table 4). The treatment period required for a reduction in stool frequency of at least 50% was shorter in patients treated with bovine colostrum (*P* < 0.05).

The excretion of *E. coli* expressing intimin and EHEC-hemolysin by patients treated with bovine colostrum was

TABLE 3. Clinical and demographic data of 27 children with diarrhea caused by infection with *Escherichia coli* expressing Shiga toxin 1 or 2, intimin, or EHEC-hemolysin who were treated with bovine colostrum or placebo

	Bovine colostrum	Placebo
Number of patients	13	14
Male:female	5:8	8:6
Mean age in years (range)	2 (5 months to 18 years)	1 (3 months to 12 years)
Fever measured (<i>n</i> > 38.4°C)	5	9
Vomiting	6	8
Median stool frequency (range) the day before starting study medication	3 (1-20)	2 (1-16)
Median duration in days (range) of diarrhea before study entry ^a	5 (2-17)	5 (2-13)

^a This period includes the time between recognition of diarrhea in the hospital and start of treatment, which was 1 or 2 days. EHEC, enterohemorrhagic *E. coli*.

TABLE 4. Effect of treatment with bovine colostrum or placebo in children with diarrhea caused by infection with *Escherichia coli* expressing Shiga toxin 1 or 2, intimin, or EHEC-hemolysin

	Bovine colostrum	Placebo	P value
Stool frequency at study entry	3 ± 2 (median, 3)	3 ± 4 (median, 2)	
Stool frequency at end of treatment	1 ± 1 (median, 1)	2 ± 3 (median, 2)	
Reduction of stool frequency	2 ± 2 (median, 2)	1 ± 3 (median, 0)	P = 0.027
Elimination of strains expressing virulence factors			
<i>Stx1</i>	4/6 (67) ^a	3/3 (100)	
<i>Stx2</i>	2/2 (100)	3/5 (60)	
<i>eae</i> (intimin)	11/13 (85)	6/10 (60)	
EHEC-hemolysin	11/13 (85)	5/9 (56)	

Data are mean ± SD. EHEC, enterohemorrhagic *E. coli*.

^a Number of patients negative at end of treatment/positive at entry into study (%).

not significantly different from that in patients treated with placebo (Table 4).

DISCUSSION

Bovine colostrum was well tolerated in children infected with diarrheagenic *E. coli*, specifically Shiga toxin-producing *E. coli* and *E. coli* expressing intimin and EHEC-hemolysin. However, two of the three patients in whom vomiting precluded continued administration had HUS. Thus, although it may be reasonable to treat children with HUS with bovine colostrum in an attempt to minimize the absorption of toxins from the bowel, children with HUS sometimes may not tolerate this treatment or other orally administered treatments early in the course because of vomiting. However, therapy with colostrum late in the course of HUS, after the appetite has returned and vomiting has abated, may play a role in reducing the number of bowel movements and thereby reduce potential secondary spread at that point.

Our study was not intended to demonstrate that bovine colostrum prevents HUS in children infected with STEC. However, our demonstration that bovine colostrum reduced the stool frequency in children infected with diarrheagenic *E. coli* suggests that such an intervention may prevent secondary cases of STEC infection. Stx-producing *E. coli* may be excreted for several weeks after acute infection (23). It has been shown that exposure to a family member with diarrhea or other direct contact is a risk factor for infection with STEC, leading to diarrhea and HUS (24,25). Reducing the frequency of diarrhea may curtail transmission of such pathogens in homes and in day care centers if administered earlier in the course of diarrhea than was achieved in this study. However, a larger number of patients and their contacts would be necessary to test these hypotheses.

The interval between hospital admission secondary to diarrhea and initiation of treatment with the study medication was 1 to 2 days. This was because the causative *E. coli* strain had to be grown from stools, identified by PCR, and informed consent obtained for participation in the trial. We do not know whether this delay adversely

affected the efficacy of the bovine colostrum treatment. Therefore, the possibility exists that if colostrum therapy had commenced at the time of presentation, and not 1 or 2 days later, the difference in effects between the two groups may have been even greater.

Antibiotics are not recommended in infections with STEC for several reasons (26): Antibiotic treatment may release Stx, which may be systemically absorbed (27), may increase Stx production (28), may be ineffective in children with STEC-associated enteritis (29), and may increase the risk of development of HUS (30–32). In this regard, it is somewhat reassuring to note that the colostrum did not accelerate clearance of the STEC from the stool of infected patients, as might have been expected had the antibodies elicited an intraintestinal bactericidal effect, with potentially increased toxin release.

In summary, bovine colostrum reduces stool frequency in children infected with diarrheagenic *E. coli* and is well tolerated. These findings warrant extension of this treatment method to larger populations infected with diarrheagenic *E. coli*, ideally, targeting infected children early in the course of their illnesses to determine whether early administration prevents HUS and has an even greater effect on diarrhea than was demonstrated in this study.

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