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Original paper

Bovine Colostrum in the Treatment of Acute Diarrhea in Children: A Double-Blinded Randomized Controlled Trial

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ABSTRACT

Objectives: To evaluate the effect of bovine colostrum (BC) on the treatment of children with acute diarrhea attending the outpatient clinic.
Methods: This double-blind randomized controlled trial was conducted on 160 children with diarrhea; 80 cases were randomly treated with BC group and 80 cases randomly received placebo (placebo group). All cases were investigated for bacterial causes of diarrhea (*Salmonella spp, Shigella spp, diarrheagenic E. coli (DEC), Campylobacter spp., and Vibrio cholerae*) as well as for *Rotavirus* antigen in stool.
Results: After 48 h, the BC group had a significantly lower frequency of vomiting, diarrhea and Vesikari scoring compared with the placebo group ($p = 0.000$, $p = 0.000$, $p = 0.000$, respectively), whether it was due to *Rotavirus* or *E. coli* infection.
Conclusions: BC is effective in the treatment of acute diarrhea and can be considered as adjuvant therapy in both viral and bacterial diarrhea to prevent diarrhea-related complications

KEYWORDS: bovine, colostrum, diarrhea

INTRODUCTION

Acute diarrhea is a major health problem leading to morbidity and mortality in developing countries. In 2011, it was estimated that 700 000 child deaths were due to diarrhea, with 72% of those deaths taking place in children less than 2 years [1]. Diarrhea is caused by viral, bacterial, parasitic and fungal infections. Viruses are the most common etiology. In 2015, rotaviral enteritis was the most common cause of deaths due to diarrhea under 5 years [2].

Although several rotavirus vaccines have been developed and approved in certain countries [3], it has not yet been encompassed in the national immunization program in Egypt. Thus the development of therapeutic approaches is warranted, because of the severe sequelae associated with rotavirus infection [4].

Current treatments for acute diarrhea in children focus principally on the fluid to prevent dehydration and maintaining feeding [5]. However, this does not ameliorate healing of intestinal mucosa nor shorten diarrheal episodes duration. Zinc supplementation also plays a significant role in mucosal integrity and has been shown to decrease the duration of diarrhea [6]. Many other interventions have been studied for reducing the duration of diarrhea, such as probiotics, prebiotics and racecadotril [7]. Antibiotics may be used in limited types of acute bacterial diarrhea but have no effect on viral causes [8].

Nutritional-based interventions offer a novel therapeutic alternative for the management of acute diarrhea. Breast milk contains pathogen-directed antibodies and other components that help intestinal healing. That is why breastfeeding during diarrheal illness is strongly encouraged by the WHO. Bovine colostrum (BC) provides a similar beneficial role of breast milk [9].

BC is rich in lactoferrin, immunoglobulins, growth and antimicrobial factors that stimulate digestive tract and immune function growth and maturation which are useful for the recovery of diarrhea. The antimicrobial activity of immunoglobulins is by chelation with bacterial and viral antigens [10]. Lactoferrin is an antioxidant, anti-inflammatory, with antimicrobial activities and is involved in the regulation of gastrointestinal tract iron absorption, limiting the bacterial utilization of iron and inhibiting the microbial growth [11, 12].

Since BC has several naturally occurring important nutritional components, and few studies have tried the commercially available BC in the treatment of acute gastroenteritis [13, 14], necrotizing enterocolitis [15, 16] and in gastrointestinal complications in ICU-hospitalized patient [17], the aim of the present study was to investigate the role of BC on the treatment of acute diarrhea in children under the age of 2 years in the attempt to prevent diarrhea-related complications like dehydration.

MATERIALS AND METHODS

This randomized controlled trial registered at Pan African Clinical Trials Registry (Cochrane South Africa) (PACTR201708002507912) and was conducted at University Children's Hospitals; which is a tertiary teaching and referral hospital, on 160 children, aged 6 months to 2 years, diagnosed with acute diarrhea. Informed consent was obtained from the patients' parents or legal guardians. Ethical approval was obtained from the Ethics Committee of The Faculty of Medicine.

Sample size

The sample size of 74 patients per group (148 total) is large enough to detect a difference in the reduction in stool frequency per day between children with acute diarrhea who received BC (70%) and those with acute diarrhea who didn't receive BC (50%) [13], as statistically significant with 80% power and at a significance level of 0.05 [18]. Sample size per group does not need to be increased to control for withdrawal bias [19]. The sample size was calculated using GPower version 3.1.9.2 [20].

Study design

This double-blind randomized controlled trial included 160 children, aged 6 months to 2 years, diagnosed with acute diarrhea, 80 cases were randomly treated with standard therapy of acute diarrhea plus BC (BC group) and 80 cases randomly received standard therapy of acute diarrhea plus placebo (placebo group).

Recruitment

All children were identified and recruited between November 2016 and May 2017 from the emergency room, after taking the informed consent until the desired sample size was achieved. Consecutive children meeting the following inclusion criteria were enrolled.

Inclusion criteria

Included children between 6 months to 2 years old, with acute diarrhea defined as three or more loose, or watery stool for less than 48 h and did not contain blood or mucus.

Exclusion criteria

Children with diarrhea more than 48 h, other infections, malnutrition or who received a prior antibiotic or antidiarrheal treatment were excluded from the study.

Randomization technique

Randomization was made using computer-based permuted block randomization technique and the block size was variable. Allocation sequence/code was concealed from the person allocating the participants to the intervention arms using sealed opaque envelopes.

Blinding

Double-blinded approach was adopted. Masking/blinding was employed to caregivers (providers) and outcome assessors who were blinded to group allocation of patients.

Clinical examination and laboratory investigations

All studied children with acute diarrhea were subjected to:

1. Detailed history taking about: duration and frequency of diarrhea per day, duration and frequency of vomiting per day, presence and grade of fever measured axillary by Celsius, the presence of any associated symptoms of abdominal pain, anorexia, irritability, drowsiness and convulsions. All children were fully examined with special stress on assessment of the degree of dehydration using WHO classification [21]. The severity of diarrhea was recorded according to modified Vesikari Scoring [22].
2. Laboratory investigations including complete blood picture, C-reactive protein (CRP), Serum sodium, serum potassium, renal and liver function tests were done on admission.
3. Microbiological investigations:

a. Stool samples collection and transport: Stool samples were collected in sterile containers and transferred to Alexandria University Hospital Microbiology Laboratory, where they were processed immediately.

b. Bacterial Culture: Stool samples were cultured on MacConkey's agar (Oxoid, UK) for isolation of *Salmonella spp*, *Shigella spp*. and *E. coli*, and on Thiosulfate citrate bile salt sucrose agar (Oxoid UK) for isolation of *Vibrio cholerae*, and on campylobacter selective agar (Oxoid, UK) for the selective isolation of *Campylobacter spp*. Bacterial isolates were identified according to the standard microbiological procedures [23]. The identified *E. coli* bacterial isolates were stored in 15% glycerol-broth for further DNA extraction.

c. Detection of *Rotavirus* antigen (Ag): It was performed using RIDASCREEN *rotavirus* (r-biopharm) qualitative enzyme-linked immunosorbent assay according to the manufacturer's instructions. Samples were considered positive if their extinction was more than 10% above the calculated cutoff.

d. Detection of *diarrheagenic E. coli (DEC)* using two sequential multiplex polymerase chain reaction for detection of eight genes (eae, elt, CVD 432, estA1, estA2-4, ial, bfp, VTcom) present in *typical enteropathogenic E. coli (tEPEC), atypical EPEC, enterotoxigenic E. coli (ETEC), enteroaggregative E. coli (EAEC), enterohemorrhagic E. coli (EHEC)* and *enteroinvasive E. coli (EIEC)*, as previously described [24]. DNA extraction was done from the bacterial sweep of confirmed *E. coli* colonies isolated on the MacConkey's agar plates [25]

Management protocol

The WHO protocol for management of dehydration was applied using oral rehydration solution (ORS), continue feeding and zinc supplementation. Children in BC group received BC sachets: (ImmuGuard®, sachets manufactured by NMI, London, England) for 1 week. It contains a powdered form of the first 6 h BC (3 g/sachet). Each sachet was added to 50 ml of neutral (previously boiled) water with continuous mixing until being dissolved. BC was instructed to be taken on an empty stomach at least 30 min before meals. The sachet contains 65 mg lactoferrin, lactoperoxidase: 2.8 unit and immunoglobulins in the form of 350 mg IgG, 35.3 mg of Ig A and 25.3 mg Ig M. As recommended by the manufacturer, the dose of BC was one sachet per day for children less than 2 years. Children in the control group received an equal dose of placebo, which was identical in physical appearance to BC. All patients were instructed to come for follow up 48 h and after 1 week and were advised to return immediately if the child became sicker, had blood in stool or was unable to have a drink or breastfeed.

The primary outcome was the reduction of frequency and duration of diarrhea (<3 times diarrhea

per day and normal stool consistency were used to determine that diarrhea had stopped) and the stoppage of vomiting and frequency of vomiting per day. The secondary outcomes included the disappearance of fever, assessment of the degree of dehydration with the calculation of modified Vesikari scoring [22]. Data were recorded on admission and after 48 h and after 1 week on follow up visits.

Also, the Vesikari scoring and the time of disappearance diarrhea were assessed in children with *Rota* Ag positive and *E. coli* positive receiving either BC or placebo.

Statistical methodology

Data were collected and entered into the computer using Statistical Package for Social Science program for statistical analysis (version 21) [26]. Data were entered as numerical or categorical, as appropriate. Kolmogorov–Smirnov test of normality revealed significance in the distribution of the variables, so the non-parametric statistics were adopted [27]. Data were described using minimum, maximum, median and inter-quartile range. Categorical variables were described using frequency and the percentage of the total. Comparisons were carried out between two studied independent not-normally distributed subgroups using Mann–Whitney U-test [28]. Chi-square test was used to test the association between qualitative variables. Monte Carlo and Yate's (continuity) correction were carried out when indicated (expected cells less than 5) [29, 30]. An alpha level was set to 5% with a significance level of 95%, and a beta error accepted up to 20% with a power of study of 80%.

RESULTS

The CONSORT diagram for the study is demonstrated in Fig. 1. From a total number of 160 children, 78 (48.8%) were males and 82 (51.25%) were females. Their age ranged from 6 months to 2 years with a mean of 12.3 ± 4.149 months. All studied cases had vomiting and diarrhea. Fever was present among 110 cases (68.75%). Ninety-four cases (58.75%) had no dehydration, 63 cases (39.37%) had some dehydration and three cases (1.87%) had severe dehydration.

Stool cultures were negative for *Salmonella spp.*, *Shigella spp.* and *Vibrio cholerae* in all studied cases. A causative organism was identified in 73 cases (45.6%). Out of 160 diarrhea cases, 40 (25%) were positive for *Rotavirus*, 16 (10%) were positive for *DEC*, while 15 cases (9.3%) had combined *rotavirus* and *E. coli*, and only two cases (1.25%) were positive for *Campylobacter spp*. Thus a total of 56 cases were *Rotavirus* positive. Out of a total of 31 identified *DEC*, 16 (51.6%) were *EAEC*, six (18.8%) were atypical *EPEC*, four (12.5%) were typical *EPEC*, three (9.4%) were *EIEC* and two (6.2%) were *ETEC*.

The BC and placebo groups were similar in the initial basic and clinical condition including the age, sex, residence, feeding type, weight, the frequency and duration of diarrhea, frequency and duration of vomiting, fever, the degree of dehydration and Vesikari scoring, however, the frequency of vomiting was significantly higher in placebo group compared with BC group (Table 1). They received the same appropriate management except for the use of BC or placebo.

After 48 h of treatment, the presence and frequency of vomiting and diarrhea and Vesikari scoring were significantly lower in the BC group vs. the placebo group. The presence of fever was significantly lower in the BC group vs. the placebo group (Table 2).

After 3 days, 75 (93.75%) infants had diarrhea in the placebo group compared to 28 (35.00%) in BC group ($p = 0.000$). After 1-week treatment, 10 (12.50%) children still had diarrhea in the placebo group compared to none in BC group ($p = 0.001$).

The median (IQR) time of the disappearance of vomiting, diarrhea and fever was significantly earlier in the BC group compared with the placebo group. [1.00 (1.00–1.00 days) vs. 3.00 (1.00–3.00 days), $p = 0.000$], [3.00 (3.00–4.00 days) vs. 6.00 (5.00–6.00 days), $p = 0.000$] and [1.00 (0.00–1.00 days) vs. 1.00 (0.00–3.00 days), $p = 0.002$]; respectively.

The Vesikari scoring after 48 h in *Rota* Ag positive patients and *E. coli* positive who received BC was significantly lower compared with the placebo group. The median (IQR) time of the disappearance of diarrhea in *Rota* Ag positive and *E. coli* positive patients who received BC was statistically significantly earlier when compared with the placebo group. (Table 3)

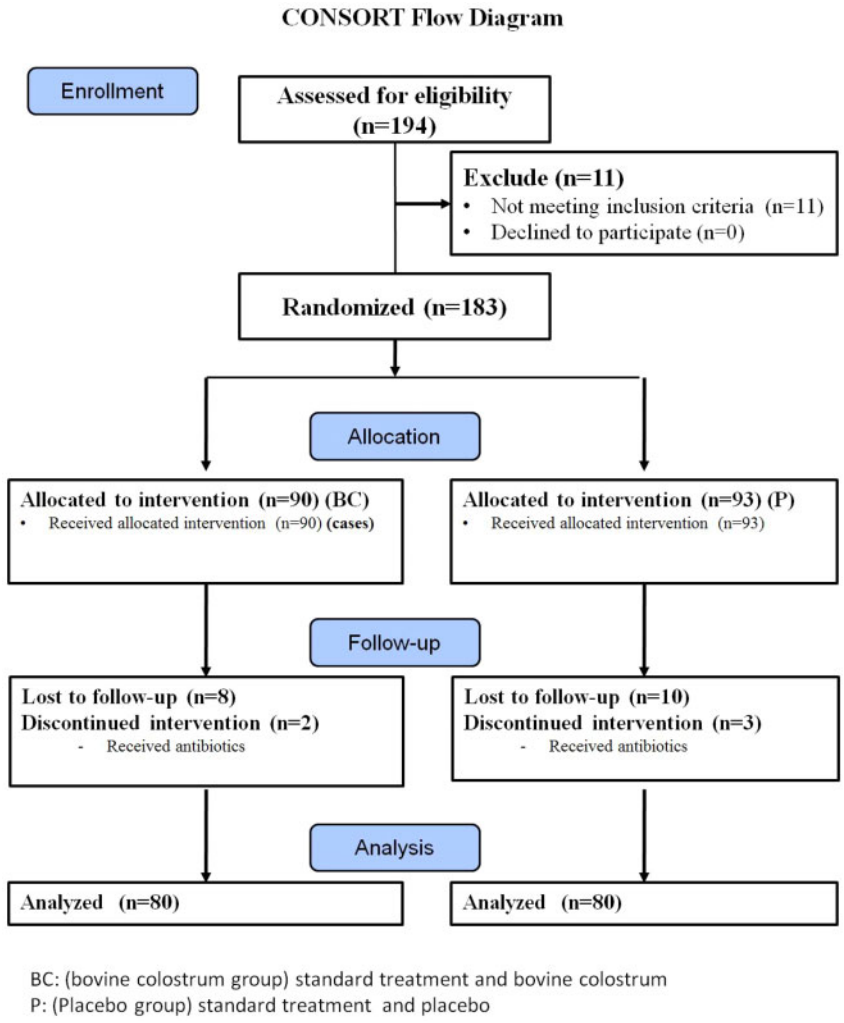

Fig. 1. Consort flow diagram of study design.

DISCUSSION

Over the past several decades, hyperimmune BC (HBC) has been used for treatment as well as for prevention of gastrointestinal infection. It is produced by tedious procedure by cows that have received immunizations against specific disease-causing organisms [31]. Recently, BC is available commercially as a nutraceutical product and its suppliers support its health benefits for treating gastrointestinal disorders, so it is worth to test its effect in one of the developing countries, where diarrhea is still the leading cause of morbidity and mortality.

The present study showed that the presence and frequency of vomiting and diarrhea were significantly lower in the BC group compared with the placebo group. The median frequency of diarrhea and vomiting was also significantly lower after 48 h and stopped after 1 week in the BC group compared with the placebo group. Similarly, El Mashad *et al.* [14] reported that the mean frequency of diarrhea and vomiting after treatment by BC for 5 days was significantly lower compared with the traditional group.

In the current study, the median time of disappearance of diarrhea was significantly earlier in the BC group compared with the placebo group. This result is consistent with that of Suwarba *et al.*, [13] who reported that in the BC group, the time needed

Table 1. Baseline characteristics of both groups before treatment

Baseline characteristics of the patients	BC group ($n = 80$)	Placebo group ($n = 80$)	Significance (p value)
Age (months)			
Min–Max	6.00–24.00	6.00–23.00	Z (MW) = 0.012
Median (IQR)	11.00 (9.00–15.00)	12.00 (9.00–15.00)	$p = 0.990$
Sex			
Male	46 (57.50%)	37 (46.25%)	$X^2 = 2.028$
Female	34 (42.50%)	43 (53.75%)	$p = 0.154$
Type of feeding			
Exclusive Breast feeding	19 (23.75%)	25 (31.25%)	$X^2 = 2.075$
Formula	16 (20.00%)	19 (23.75%)	$p_{(MC)} = 0.371$
Mixed	45 (56.25%)	36 (45.00%)	
Time of initiation of the drug (hours)			
Min–Max	3.00–45.00	3.00–48.00	Z (MW) = 1.327
Median (IQR)	17.00 (9.00–30.00)	21.00 (9.00–36.00)	$p = 0.185$
Duration of vomiting (days)			
1	53 (66.25%)	55 (68.75%)	$X^2 = 0.114$
2	27 (33.75%)	25 (31.25%)	$p = 0.736$
Frequency of vomiting (per day)			
Min–Max	2–7	2–6	Z (MW) = 2.223
Median (IQR)	4 (3–5)	4 (3–4)	$p = 0.026$*
Duration of diarrhea (days)			
1	59 (73.75%)	56 (70.00%)	$X^2 = 0.278$
2	21 (26.25%)	24 (30.00%)	$p = 0.598$
Frequency of diarrhea (per day)			
Min–Max	4–11	4–10	Z (MW) = 1.943
Median (IQR)	7 (6–8)	6 (6–8)	$p = 0.052$ NS
Fever (initial)			
No	29 (36.25%)	21 (26.25%)	$X^2 = 1.862$
Yes	51 (63.75%)	59 (72.75%)	$p = 0.172$
Duration of fever (days)			
1	43 (84.31%)	53 (89.83%)	$X^2 = 0.750$
2	8 (15.69%)	6 (10.17%)	$p = 0.387$
Dehydration			
No	44 (66%)	50 (62.60%)	$X^2 = 1.113$
Some	34 (42.5%)	29 (36.35%)	$p_{(MC)} = 0.561$
Severe	2 (2.5%)	1 (1.25%)	
Vesikari scoring			
Min–Max	8–15	8–14	$Z_{(MW)} = 1.520$
Median (IQR)	11.00 (10.00–13.00)	11.00 (10.00–12.00)	$p = 0.128$

IQR: Inter-quartile range.
MW: Mann–Whitney U-test.
*: Statistically significant ($p < 0.05$).
X^2: Pearson's Chi-Square, MC: Monte Carlo Correction.

Table 2. Comparison between BC group and placebo group regarding clinical presentation at 48 h follow up after treatment

Outcome	BC group ($n = 80$)	Placebo group ($n = 80$)	Significance (p value)
Vomiting			
No	72 (90.00%)	23 (28.75%)	$X^2 = 62.212$
Yes	8 (10.00%)	57 (71.25%)	$p = 0.000$*
Frequency of vomiting (per day)			
Min–Max	0–1	0–3	Z (MW) = 8.061
Median (IQR)	0.00 (0.00–0.00)	0.00 (1.00–2.00)	$p = 0.000$*
Diarrhea			
No	11 (13.75%)	0 (0.00%)	$X^2 = 11.812$
Yes	69 (86.25%)	80 (100%)	$p = 0.001$*
Frequency of diarrhea (per day)			
Min–Max	0–4	2–5	Z (MW) = 7.298
Median (IQR)	2.50 (2.00–3.00)	3.00 (3.00–4.00)	$p = 0.000$*
Fever			
No	74 (92.50%)	52 (65.00%)	$X^2 = 18.077$
Yes	6 (7.50%)	28 (35.00%)	$p = 0.000$*
Vesikari scoring (after 48 h)			
Min–Max	0–7	3–9	Z (MW) = 8.698
Median (IQR)	3.00 (2.00–4.00)	6.00 (4.25–7.00)	$p = 0.000$*
Dehydration			
No	80 (100%)	80 (100%)	NA
Yes	0 (0.00%)	0 (0.00%)	

*: Statistically significant ($p < 0.05$).
X^2: Pearson's Chi-Square.
MW: Mann–Whitney U-test.
NA: Non-applicable statistics (due to exact match).

for defecation frequency changed to less than three times/day achieved significantly earlier than the control group.

The positive effect of BC may be attributable to its high contents of immunoglobulins. IgG neutralizes microbes and their toxins in blood, IgM destroys bacteria and IgE and IgD are potentially antiviral. [32] BC also includes antimicrobial peptides as lactoferrin with antibacterial effects. Lactoferrin is a glycoprotein with antibacterial, antiviral and antifungal effects, lipopolysaccharide binding, and is becoming useful in clinical practice against human diseases [33, 34].

Notably, in the current study, The Vesikari scoring after 48 h in *Rota* Ag positive patients who received BC was significantly lower vs. the placebo group. Moreover, the median time of the disappearance of diarrhea in *Rota* Ag positive patients was significantly earlier in BC group (median 3 vs. 6 days; $p = 0.000$) and on the second day about 18.18% of the children in BC group were no longer suffering from diarrhea. However, 100% of the placebo group still had diarrhea. Similarly, Sarker *et al.* [35] revealed the beneficial nature of HBC by demonstrating a significant decrease in the mean duration of diarrhea in children treated with BC (72.6± 38.9 h) compared with placebo group (96.4± 46.7 h). However, there was a certain important difference in BC composition; in the present study whole BC is used without hyperimmunization with *rotavirus* strains while, Sarker *et al.* [35] used HBC, which contained high titers of 3.6 g of antibodies against four *rotavirus* serotypes; 75% IgG1, 3% IgG2 and 17% Ig A.

TABLE 3. Comparison between Rota Ag positive patients who received BC vs. Rota Ag positive patients who received placebo and between E. coli positive patients who received BC vs. E. coli positive patients who received placebo

Outcome	Rota Ag positive in BC group ($n = 22$)	Rota Ag positive in placebo group ($n = 34$)	*E. coli* positive in BC group ($n = 13$)	*E. coli* positive in placebo group ($n = 18$)
Vesikari scoring (initial)				
Min–Max	8–15	8–13	8–13	8–13
Median	11.00	11.00	11.00	11.00
IQR	10.00–13.00	10.00–12.00	10.50–13.00	10.00–13.00
Significance (p value)	$Z_{(MW)} = 0.555$, $p = 0.579$		$Z_{(MW)} = 0.273$, $p = 0.798$	
Vesikari scoring (after 48 h)				
Min–Max	0–5	3–8	0–7	3–8
Median	3.00	6.00	3.00	7.00
IQR	2.00–3.00	4.00–7.00	2.50–4.00	5.75–8.00
Significance (p value)	$Z_{(MW)} = 5.356$, $p = 0.000^*$		$Z_{(MW)} = 3.910$, $p = 0.000^*$	
Diarrhea (after 48 h)				
No	4 (18.18%)	0 (0.00%)	2 (15.38%)	0 (0.00%)
Yes	18 (81.82%)	34 (100.00%)	11 (84.62%)	18 (100.00%)
Significance (p value)	$X^2_{(Y)} = 4.198$, $p_{(Y)} = 0.040^*$		$X^2_{(Y)} = 0.960$, $p_{(Y)} = 0.327$	
Disappearance of diarrhea after (days)				
Min–Max	2–6	3–9	2–5	4–7
Median	3	6	3	6
IQR	3–3	5–7	3–4	5–7
Significance (p value)	$Z_{(MW)} = 5.345$, $p = 0.000^*$		$Z_{(MW)} = 4.459$, $p = 0.000^*$	

*: Statistically significant ($p < 0.05$).
MW: Mann–Whitney U-test MC: Monte Carlo Correction.
X^2: Pearson's Chi-Square.
Y: Yate's (continuity) correction for Chi-Square test and its p value.

Concerning *E. coli* induced diarrhea, the median day of the disappearance of diarrhea was 3 days in the BC group vs. 6 days in the placebo group. Furthermore, the Vesikari scoring after 48 h in *E. coli* positive patients who received BC was significantly lower compared with the placebo group. The present study results are in accordance with Huppertz *et al.* [36], where the cases were treated with an immunoglobulin preparation, produced from normal BC and having more than 65% immunoglobulin. They reported that the BC treatment significantly reduced stool frequency, in *E. coli* induced diarrhea, when compared with the placebo group ($p = 0.027$). On the other hand Casswall *et al.* [37] found no significant difference among 86 children infected with *E. coli* diarrhea divided into two groups, one group received oral bovine milk concentrate produced from cows hyperimmunized with *ETEC* and *EPEC* strains and the other group received placebo, regarding ORS intake, stool output, frequency of diarrhea, or clearance of *E.coli*, or the duration of diarrhea. The discrepancy between several studies may be due to different sample size, different population and different composition of BC used.

The current study has some limitations: first, not all the etiological agents of diarrhea were investigated. Second, the treatment follows up of *E. coli* or of *rotavirus*-associated diarrhea was assessed only clinically and not microbiologically. Moreover, it was a single-center trial; multicentered randomized clinical trials are warranted to endorse the findings of the present study.

To summarize, our data add additional evidence that BC is effective in the treatment of acute diarrhea and could be used as adjuvant therapy as it reduces both the frequency and the duration of diarrhea. Furthermore, it was effective in the treatment of both viral (*Rotavirus*) and bacterial (*E. coli*) diarrhea. Further multicentered double-blind, placebo-controlled studies with colostrum products are required to focus on its effect in the specific microorganism and extend their therapeutic role in children.

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