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Original Article

Effects of live versus heat-killed probiotics on acute diarrhea in young children

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Abstract

Background Diarrhea remains one of the major causes of morbidity and mortality in children in developing countries. Probiotics have been shown to be beneficial for decreasing the frequency and duration of diarrhea. However, the reported effects on reducing the duration of diarrhea have been varied.

Objective To compare the effectiveness of live and heat-killed probiotics in 6-60 month-old children with acute diarrhea for decreasing duration and frequency of diarrhea and improving weight gain.

Methods We conducted a randomized, single-blind, controlled trial in children aged 6-60 months with acute diarrhea. Children were randomized into two groups, receiving either live or heat-killed probiotics. All children received standard treatment for diarrhea and probiotics as adjuvant treatment. The primary outcomes were duration and frequency of diarrhea, as well as weight gain. T-test was used for data analysis.

Results There were 165 children with acute diarrhea enrolled in this study. They were divided into 2 groups, with 83 children receiving live probiotics and 82 children receiving heat-killed probiotics. There were no significant differences in diarrheal duration in the two groups. The mean durations of diarrhea in the live and heat-killed probiotic groups were 3.64 (SD 0.85) days and 3.74 (SD 0.73) days (P>0.05), respectively. Mean diarrheal frequencies were also not significantly different, with 3.25 (SD 1.44) times per day in the live probiotic group and 3.26 (SD 1.20) times per day in the heat-killed probiotic group (P>0.05). In addition, mean weight gain was not significantly different, with 241.57 (SD 75.84) g in the live probiotic group and 221.95 (SD 85.38) g in the heat-killed probiotic group (P>0.05).

Conclusion There were no significant differences between live and heat-killed probiotics for reducing duration and frequency of diarrhea, as well as in weight gain in children aged 6-60 months with acute diarrhea. **[Paediatr Indones. 2012;52:249-54]**.

Keywords: *live probiotic, heat-killed probiotic, acute diarrhea*

Acute diarrheal disease remains one of the major causes of morbidity and mortality in children under 5 years of age in Indonesia, with a death rate of 2.3 per 1000 children.[1,2] Treatment of diarrhea consists of replacing lost fluids by means of oral rehydration solutions (ORS). In order to minimize the nutritional impact, treatment aims at shortening the period of fluid losses (diarrhea and vomiting) and duration of diarrhea.[3]

Many studies have been conducted to evaluate adjuvant therapy to fluid and electrolyte management in cases of acute diarrhea. One such possible therapy is probiotics.[4] Probiotics are oral supplements or food products containing a sufficient number of viable microorganisms to alter the microflora of the host. They are now well-known for their beneficial effect in shortening diarrheal duration. The effect of probiotics involves different mechanisms, including direct antagonism to pathogens through competitive inhibition of adhesion to the epithelium and of specific toxins, depletion of nutrients, production of intestinal mucin, bacteriocins or other antimicrobial

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molecules, promoting a "hostile" microenvironment for pathogens, and immune stimulation.[5] Heat-killed (tyndallized) probiotics are sterilized such that they do not produce active metabolites, though they still have effect on human immunity. However, there have been varied results on the effect of probiotics in decreasing duration of diarrhea. A Cochrane review examined 23 random controlled trials and highlighted the beneficial effect of probiotics as an add-on treatment to ORS in shortening the duration of diarrhea (by about 30 hours with RR 0.7).[6] *Lactobacillus acidophillus* (heat-killed lactobacilli) tested in children with acute diarrhea (50% rotavirus-positive), resulted in a similar reduction in duration.[7] These results were consistent with a study by Supriatmo that compared live probiotics (mixed lactic acid bacteria) and heat-killed probiotics (heat-killed lactic acid bacteria) on children with acute diarrhea (mean diarrheal durations of 7.06 days and 5.65 days, respectively). He observed no difference in frequency (3.94 episodes versus 4.08 episodes daily).[8] Different results were reported by Warouw *et al*. in a trial of children with acute diarrhea. Tyndallized probiotics *Lactobacillus sporogenes* and *Lactobacillus acidophilus* showed no differences in reducing the duration of acute diarrhea (3.32 days versus 2.52 days).[9]

The objective of our study was to assess the effectiveness of live compared to heat-killed probiotics for reducing frequency and duration of diarrhea, as well as improving weight gain in children with acute diarrhea.

Methods

We conducted a randomized, single-blind, controlled trial for 10 months (January - October 2010) in the pediatric wards of Soeradji Tirtonegoro Klaten Hospital, Banyumas and Rembang District Hospitals. Subjects were collected by consecutive sampling. The required sample size was calculated to be 165 children, with a power of 80%, $Z\alpha=1.960$ and $Z\beta=0.842$. We included children aged 6-60 months with acute diarrhea, without dehydration or with mild dehydration, who had less than a 7 day-duration of diarrhea, and whose parents consented. We excluded children who received treatment with antibiotics or antidiarrheal medications in the three weeks prior to or during the study, those with bloody diarrhea, malnutrition before entering the hospital, and those who had complications such as severe dehydration, metabolic acidosis, seizures, renal failure, and sepsis. All subjects' parents gave written informed consent.

Medical history was recorded at the time of admission by two pediatric residents and physical examination was performed to assess clinical and nutritional status, as well as the degree of dehydration. Kappa inter-observer disagreement was 0.92 ($P<0.05$). If signs of dehydration were found based on WHO criteria, rehydration was given according to WHO guidelines, with the administration of oral zinc (dose of 1 x 20 mg for 10 days) and continuing feeding. After patients were clinically stabilized, they were randomly allocated into two groups. Group A received live probiotics (*Lacto-B®*: mixed lactic acid bacteria 1 x 10^9) and group B received heat-killed probiotics (*Dialac®*: heat-killed lactic acid bacteria $3x10^{10}$). Subjects were blinded as to which probiotic they received, while researchers and nurses were aware of coding procedures. Codes were opened at the end of the study. All subjects were managed based on standard therapy. Probiotic preparations were prescribed twice per day for five days and administered orally in 20 mL water, according to manufacturers' instructions, so that live and heat-killed probiotics could not be distinguished. Observation was performed every 12 hours, while evaluation and measurement of body weight were done at the end of the study. Frequency of diarrhea (defined as the number of times the patients passed a watery or loose stool per day) and side effects of therapy were recorded by parents or nurses in the study form. All patients were observed for stool passage everyday, including stool consistency, frequency and duration of diarrhea (the time in hours from the first to the last abnormal, loose or liquid stools preceding a normal stool output), as well as weight change between the time the patient consumed probiotic preparations until the stool became solid. Recovery from diarrhea was defined as when the frequency of defecation dropped to less than three times per day. We also investigated safety and tolerability of the probiotics.

The independent variables were live and heat-killed probiotics, while the dependent variable was

the effectiveness of therapy, as measured by the duration and frequency of diarrhea and weight change. Confounding factors were nutritional status, degree of dehydration and duration of diarrhea before time of admission.

The primary outcome measured in this intervention was duration of diarrhea, while the secondary outcome included frequency of diarrhea and body weight change (expressed as a percentage of a child's weight compared to baseline weight).

Data was analyzed with SPSS for Windows 12.0. Continuous variables were compared using the independent t-test and dichotomous variables were compared by the Chi square test. Statistical significance was defined as $P<0.05$ with a 95% confidence interval. This study was approved by the Commission on Medical Research Ethics and Health, Gadjah Mada University Medical School.

Results

We recruited 165 children aged 6-60 months old who were hospitalized with acute diarrhea. They were randomly divided into 2 groups. Live probiotics were given to 83 subjects and heat-killed probiotics were given to 82 subjects. No subjects were lost to follow-up. The study program sequence is shown in **Figure 1**.

The demographic and clinical characteristics of subjects in both groups are shown in **Table 1.**

The decrease in diarrheal frequency to $\leq$ three times per day at days 1 through 5 was analyzed by Pearson's Chi square test, as shown in **Table 2.** There were no statistically significant differences in frequency at any day between the two groups.

The duration and frequency of diarrhea, as well as weight change of the subjects are shown in **Table 3**.

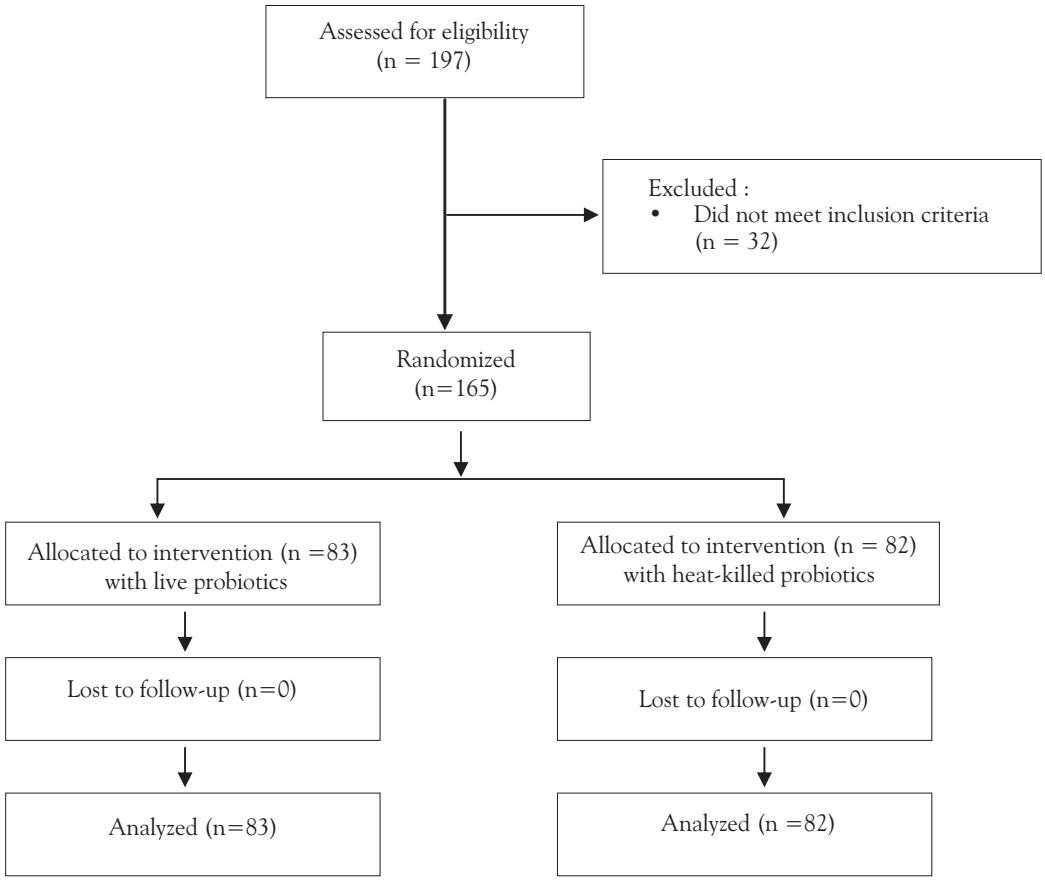

Figure 1. Study flow chart

Table 1. Baseline characteristics of subjects

Characteristics	Live probiotics (n = 83)	Heat-killed probiotics (n=82)
Mean age, months (SD)	17.9 (11.5)	18.3 (13.7)
Sex, n (%)		
Boys	49 (59.0)	57 (69.5)
Girls	34 (41.0)	25 (30.5)
Mean weight at admission, kg (SD)	9.20 (2.2)	8.94 (2.6)
Nutritional status, n (%)		
Well-nourished	63 (75.9)	65 (79.3)
Undernourished	20 (24.1)	17 (20.7)
Breastfed, n (%)		
Exclusively	70 (84.3)	65 (79.3)
Non-exclusively	13 (15.7)	17 (20.7)
Degree of dehydration, n (%)		
Without dehydration	57 (68.7)	55 (67.1)
Mild dehydration	26 (31.3)	27 (32.9)
Mean duration of diarrhea, days (SD)	2.66 (1.4)	2.34 (1.5)

Table 2. Subjects experiencing decreased diarrheal frequency to less than three times per day

	Live probiotics (n=83)	Heat-killed probiotics (n=82)	P
Days, n (%)			
1	2 (2.4)	2 (2.4)	0.06
2	35 (42.1)	31 (37.8)	0.86
3	30 (36.1)	27 (32.9)	0.23
4	12 (14.5)	19 (23.2)	0.84
5	4 (4.9)	3 (3.7)	0.33

Table 3. Duration and frequency of diarrhea and weight gain in both groups

Parameters	Live probiotics (n=83)	Heat-killed probiotics (n=82)	P*
Mean diarrheal duration, days (SD)	3.64 (0.85)	3.74 (0.73)	0.39
Mean episodes of diarrhea, times per day (SD)			
day 1	5.95 (1.92)	6.21 (1.72)	0.37
day 2	4.34 (1.79)	4.3 (1.51)	0.90
day 3	2.5 (1.51)	2.77 (1.37)	0.23
day 4	1.78 (1.09)	1.87 (1.05)	0.68
day 5	1.67 (0.89)	1.14 (0.36)	0.76
Mean weight gain, g (SD)	241.57 (75.84)	221.95 (85.38)	0.12

*t-test

Discussion

Our results show no significant differences between live and heat-killed probiotics in reducing the duration and frequency of diarrhea or in weight gain. An explanation may be that probiotic components produced during fermentation (bacteriocin, peptidoglycan) are included in the tyndallized product, resulting in heat-killed probiotic potency similar to that of live probiotics. Heat-killed probiotics also contain antigens that can stimulate the mucosal immune system when consumed. Heat-killed probiotics are unable to proliferate in the gastrointestinal tract, and they, therefore, do not cause translocation of antibiotic resistance genes, are not affected by stomach acidity, are more stable,

require simpler storage methods and have longer expiration periods.[10]

Our results were consistent with those of Warouw *et al*. They showed in 100 children aged 4-60 months with acute diarrhea, that tyndallized probiotics containing *Lactobacillus sporogenes* and *Lactobacillus acidophilus* were not different in reducing the duration of diarrhea [3.32 (SD 1.5) days vs 2.52 (SD 2.0) days, (P>0.05)].[9] In contrast, Supriatmo had different results when he compared the effects of live probiotics (mixed lactic acid bacteria $1x10^7$) and heat-killed probiotics (heat-killed lactic acid bacteria $3x10^{10}$) in 108 children aged 4-24 months with acute diarrhea. In his study, heat-killed probiotics were better at reducing the duration of diarrhea than live probiotics (7.06 vs 5.65 days, respectively, P=0.042), but he observed no difference in the frequency of diarrhea (3.94 vs 4.08 episodes, respectively, P=0.055). However, these results should be interpreted with caution due to the limitations of the study, as probiotic preparation was not standardized, and it is known that live probiotics will die at high temperatures. Furthermore, the cause of diarrhea was unknown.[8] Simakachom *et al*. examined the provision of heat-killed bacteria with oral rehydration in children with diarrhea. They found that the addition of *L. acidophilus* to the oral rehydration reduced the length of diarrhea. This result may be due to heat-killed bacteria having an inherent ability in the human gut to inhibit pathogenic bacterial processes.[7] Weizman *et al.* reported that baby food supplemented with *L. reuteri* or *B. lactis* had fewer episodes and shorter duration of diarrhea. Lactobacilli may influence the incidence of infections by stimulating nonspecific immunity or enhancing humoral and cellular immune mechanisms. This immunostimulatory effect of bacteria has been shown to prevent recurrent infections in children.[11] Lactic acid bacteria (LAB) have been used as probiotics to manage intestinal disorders such as lactose intolerance, acute gastroenteritis due to rotavirus and other enteric pathogens, adverse effects of pelvic radiotherapy, constipation, inflammatory bowel disease, and food allergies. Increased gut permeability may occur during disease states associated with disturbances of the intestinal microflora and various degrees of inflammation of the intestinal mucosa. To successfully treat these conditions, a probiotic strain should be able to survive gastric acidity, adhere to the intestinal epithelial cells and at least temporarily colonize the intestine.[12]

Although the beneficial effects of probiotic agents for normal hosts, including children with diarrheal illnesses, are well-documented, probiotic therapy may be associated with occasional adverse effects, such as bacteremia, sepsis, or endocarditis, for a select subset of patients, such as immunocompromised or severely debilitated hosts. Rare cases of local or systemic infections including septicemia and endocarditis due to lactobacilli, bifidobacteria or other lactic bacteria have been reported with nearly all patients having had serious underlying fatal diseases which predisposed them to infection. Abnormal heart valves in the case of endocarditis, and the presence of a catheter in cases of septicemia are known risk factors, but other potential weakened conditions such as extremes of age or pregnancy have not been identified as risk factors for LAB or other probiotic infections.[13] In this study, adverse events were not found in either group and the probiotics were well-tolerated. Lands *et al*. reported two cases of sepsis associated with probiotics in adults, but reports of serious infections associated with probiotic lactobacilli are rare. The pathogenesis of infection attributable to *Lactobacillus* species is poorly understood. Adhesion of probiotic strains to the intestinal mucosa and subsequent colonization are considered important prerequisites for probiotic action, because they prolong persistence in the intestine.[14] In a meta-analysis, Cornellius *et al*. concluded that *Lactobacillus* is safe and effective as a therapy for children with acute infectious diarrhea. In general, the use of probiotics is safe, but we should carefully consider the use of probiotics in patients at high risk of systemic infections, such as immunocompromised or critically ill patients.[15]

In conclusion, there were no significant differences between live probiotics and heat-killed probiotics in reducing the duration and frequency of diarrhea, as well as weight gain in children aged 6 – 60 months with acute diarrhea.

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References

1. Kosek M, Bern C, Guerrant RL. The magnitude of the global burden of diarrhoeal disease from studies published 1992-2000. Bulletin of WHO. 2003;81:197-204.
2. Achmadi UF. Harapan pemerintah mengenai peranan kaum professional dalam mendukung program penyakit saluran cerna anak di era otonomi. Proceedings of the 2nd National Congress of Badan Koordinasi Gastroenterologi Anak Indonesia; 2003 July 3-5; Bandung. Jakarta: Badan Penerbit IDAI;2003. p. 17-27.
3. Cucchiara S, Falconieri P, Di Nardo G, Parcelii MA, Dito L, Grandinetti A. New therapeutic approach in the management of intestinal disease: probiotics in intestinal disease in paediatric age. Dig Liver Dis. 2002;34:S44-7.
4. Saavedra JM, Hanna AA, Moore N, Yolken RH. Long term consumption of infant formulas containing live probiotic bacteria: tolerance and safety. AMJ Clin Nutr. 2004;79:261-7.
5. Guandalini S, Pensabene L, Zikri MA, Dias JA, Casali LG, Hoekstra H, et al. Lactobacillus GG administered in oral rehydration solution in children with acute diarrhea: a multicentre European Trial. J Pediatr Gastroenterol Nutr. 2000;30:214-6.
6. Salvatore S, Hauser B, Devreker T, Vieira MC, Luini C, Arrigo S, et al. Probiotics and zinc in acute infectious gastroenteritis in children: are they effective? Nutrition. 2007;23:498-506.
7. Simakachorn N, Pichaipat V, Rithipornpaisarn P, Kongkaew C, Tongpradit P, Varavithya W. Clinical evaluation of the addition of lyophilized, heat-killed Lactobacillus acidophilus LB to oral rehydration therapy in the treatment of acute diarrhea in children. J Pediatr Gastroenterol Nutr. 2000;30:68-72.
8. Supriatmo. Effectivity of live probiotic versus heat-killed probiotic in children with acute diarrhoea. MKN. 2006;39:419-23.
9. Warouw S, Manoppo J, Gunawan S, Kosim S. Comparison between live and tyndallized probiotic effectivities as therapy of acute diarrhea on children aged 4-60 months [dissertation]. [Manado]: Sam Ratulangi University; 2006.
10. Roy D, Boutin Y, Amiot J, Lamoereux M, Martel D, Delcenserie V. Immunomodulatory effect of probiotics in intestinal tract. Curr Issues Mol Biol. 2008;10:37-54.
11. Weizman Z, Ghaleb A, Alsheikh A. Effect of a probiotic infant formula on infections in child care centers: comparison of two probiotic agents. Pediatrics. 2005;115:5-9.
12. Salminen S, von Wright A. Current probiotics-safety assured? Microb Ecol Health Dis.1998;10:68-77.
13. Adams MR, Marteau P. On the safety of lactic acid bacteria from food. Int J Food Microbiol.1995;27:263-4.
14. Lands MH, Shetty AK. Lactobacillus sepsis with probiotic therapy. Pediatrics. 2005;115:181-91.
15. Cornellius W, Feudner C, Michelle MG, Christakis DA. Lactobacillus therapy for acute infectious diarrhea in children: a meta-analysis. Pediatrics. 2002;109:678-84.