

Effectivity of Live Versus Heat Killed Probiotic in Children with Acute Diarrhoea

Supriatmo

Departemen Ilmu Kesehatan Anak Fakultas Kedokteran
Universitas Sumatera Utara

Abstract: *Objective: to determine the effectivity of live versus heat killed probiotic in children with acute diarrhea in diarrhea duration and frequency of diarrhoea. Methods: the study was conducted in June 2005-September 2006 in Sari Mutiara Hospital Medan. All charts of children in group age 4-24 months old with diagnosis acute diarrhoea enrolled and divided in to two groups. One group received live probiotic (LP) and other had heat killed probiotic (HKP). Both probiotic consumpt for seven days. The diarrhea duration and frequency diarrhea were noted. Formula milk was discontinued during diarrhea and all children received standard treatment of diarrhea and had probiotic as adjuvant treatment. Results: from the total of 150 children who came to visit pediatric outpatient clinic and pediatric ward, there were 108 children (70.6% boy and 29.4% girl) in group age 4-24 months with acute diarrhoea who meet the inclusion criteria were enrolled in this study. Amount of children who had live probiotic and heat killed probiotic were 54 each. In this study there were 26 (47%) and 25 (44%) in group aged 2-11 months and 28 (53%) and 29 (56%) in group aged 12-24 months in group Live probiotic and Heat killed probiotic, respectively. Boys more than girls. Some dehydration in 48 (82.4%) in group Live probiotic and 27(50%) in group heat killed probiotic. Mother of the almost graduated high school 28 (51.5%), employee 20 (35.3%) and unemployee in 34 (64.7%). All the father were employee, some of them work in informal sectors and 17.6% worked for government. There was significant difference in diarrhoeal duration in group live probiotic compared to heat killed probiotic (7.06 day vs 5.65 day; $p=0.042$), there was no significant differences in frequency diarrhea in both group live probiotic and heat killed probiotic (3.94 episodes vs 4.08 episodes, $p=0.055$). Conclusion: heat killed probiotic looked superior in shorten diarrhea duration compared to live bacteria in children with acute diarrhea, while there was no significant difference in frequency of diarrhea. It is required a longitudinal study with large sample to determine this findings.*

Keywords: acute diarrhea, live probiotic, heat killed probioti, diarrhoeal duration, frequency diarrhea

INTRODUCTION

Diarrhoea remain one of the most causes of morbidity and mortality in children in the developing countries, with one billion episode include 3 up to 5 million death annually.¹⁻³ In Indonesia, reported 60 million episode per year and about 70-80% affected children under five.³⁻⁵ Almost 10% of children with diarrhea under five suffered from bloody diarrhoea, and 15% of them died every year.⁶⁻⁸ The high incidences usually related to low sosio-economic level, crowded area, and poor hygiene and sanitation.⁷⁻⁹

Many research was conducted to find the additional medication beside fluid and electrolyte management in acute diarrhea.^{2,3} All the study goal to make the diarrhea duration shorter but safe without adverse side effect.⁹⁻¹⁴ Recently, one of the most often to study are probiotic using in diarrhea. Probiotic is now well known give beneficial effect to shorten

diarroea duration.^{15,16,17} The term probiotic was derived from the Greek, meaning "for life". The Food and Agriculture Organization of the United Nation (FAO) and the World Health Organization (WHO) have started that there is adequate scientific evidence to indicate that there is potential for probiotic food to provide health benefits and that specific strain are safe for human use.⁹⁻¹⁴ In the past century the beneficial roles of non pathogenic bacteria in the intestinal lumen was described. In the past decade there has been a dramatic increase in scientific work supporting the concept that there are clinical benefits to ingestion specific nonpathogenic organism, that now we called them probiotic. The science develop to find the best probiotic so that the technology emerge the new kind probiotic that tyndalized from live bacteria.^{15,16,17} There is no available data that mention about side effect of probiotic especially as a part of diarrhea management.¹⁸⁻²¹ In

laboratory, the probiotic process with heat killed technology, to collect some parts of probiotic and believe to give similar response as live microbacteria. The question about the live time of packaging probiotic may be answered by this achievement.¹⁹ We run this prospective study to determine the effectivity of live compare to heat killed bacteria in children with acute diarrhea. The all patients received standard treatment of diarrhea include fluid, electrolyte and nutrition.

METHODS

This study was retrospective, we review all the charts of the patients in group age 4-24 months from June 2005 – September 2006 with diagnosis acute diarrhoea who came to visit pediatric outpatient clinic and pediatric ward Sari Mutiara Hospital. Sample size were calculated to ensure that the total sample that enrolled were representative. The sample size with sample power 80%, $Z\alpha=1.960$ and $Z\beta=0.842$ which divided in two group LP and HKP 54 sample each. Inclusion criteria: 1). Children age 4 – 24 months. 2). Children with diagnosis acute diarrhoea (diarrhoea ≤ 14 days) 3) The chart was fulfilled completely by the doctor in charge. 4) Patient was discharged from hospital as doctor permission. Exclusion criteria: 1). Children with persistent diarrhoea and chronic diarrhoea (more than 14 days). 2). Present of parasite in macroscopic or microscopic in stool specimen. 3). Children who already have treatment from other health facilities (in 5 days before came to hospital).

STUDY PROCEDURE

All children with acute diarrhoea (defined as pass watery stool > 3 times per day and occur less than 14 days), age 4 – 24 months from Pediatric outpatient clinic and pediatric ward Sari Mutiara Hospital during June 2005 – September 2006 were recruited to the study. There were 108 cases from 150 cases with diagnosis acute diarrhoea were meet the inclusion criteria and divided into two group.

Group one received Live Probiotic (mixed Lactic Acid Bacteria 1×10^7 cfu/gram) mg/kg/day and group Heat Killed Probiotic (Heat killed Lactic Acid Bacteria 3×10^{10}) once per day for seven days.

In our hospital, probiotic usually prescribed for the patient as a compliment to standard treatment of diarrhea. We give probiotic one sachet per day for seven days or as long as the stay in the hospital. Some time the patients got live probiotic and some of the patients received heat killed bacteria according to supply in pharmacy hospital. The health insurance also covered the probiotic as diarrhea treatment, so that the patients paid nothing for all treatment. There was no antibiotic used in this study and formula milk was discontinued temporary during the diarrhoea..

The patient was defined as recovered from diarrhoea after they passed the formed stool for last 8 hours and the patients were discharged home. The frequency of diarrhea was defined every time the patients passed the watery or loose stool. Data was analysis with SPSS for windows 12.0. Effectivity live probiotic compare to heat killed probiotic were analyzed by t independet test to determine diarrhoea duration in both groups, statically significat if $p < 0.05$.

RESULTS

In this study there were 26 (47%) and 25 (44%) in group aged 2-11 months and 28 (53%) and 29 (56%) in group aged 12-24 months in group Live probiotic and Heat killed probiotic, respectively. Boys more than girls. Some dehydration in 48 (82.4%) in group Live probiotic and 27(50%) in group heat killed probiotic. Mother of the almost graduated high school 28 (51.5%), employee 20 (35.3%) and unemployee in 34 (64.7%). All the father were employee, some of them work in informally and 17.6% worked for government.

Table 1.
Distribution of sample by age, sex, nutritional status

	Live probiotic		Heat killed probiotic	
	n	%	n	%
Age (months)				
2 – 11	26	47.1	25	44.1
12 – 24	28	52.9	29	55.9
Sex				
Boys	34	70.6	34	70.6
Girls	20	29.4	20	29.4
Nutritional status				
Normal	4	11.8	15	44.1
Mild Malnutrition	20	29.4	17	20.6
Moderate Malnutrition	22	35.3	17	20.6
Severe Malnutrition	8	23.5	5	14.7

Table 2.
Distribution of sample by dehydration status, parental education, parental occupation

	Live probiotic		Heat killed probiotic	
	n	%	n	%
Dehydration status				
Without Dehydration	6	17.6	25	44.1
Some Dehydration	48	82.4	27	50
Severe Dehydration	0	0	2	5.9
Parental education				
Father				
Graduate	10	23.5	9	20.5
Under Graduate	1	2.9	2	5.8
Senior High School	30	58.8	28	52.9
Junior High School	2	5.8	9	11.7
Elementary School	6	8.8	6	8.8
Mother				
Graduate	3	8.8	5	14.7
Under Graduate	4	11.7	3	8.8
Senior High School	28	52.9	27	50
Junior High School	6	17.6	4	11.7
Elementary School	3	8.8	5	14.7
Parental Occupation				
Father				
Government Employee	14	26.4	14	26.4
Private Employee	12	20.5	14	26.4
Casual Worker	28	52.9	26	47.1
Mother				
Employee	20	29.4	23	38.2
Unemployee	34	70.5	31	61.7

Table 3.
Diarrhoea duration and frequency diarrhea in group live probiotic and heat killed probiotic

	Live probiotic			Heat killed probiotic			P
	n	Mean	SD	n	Mean	SD	
Diarrhoea duration (day)	54	7.06	2.61	54	5.65	2.38	0.042
Frequency diarrhea (time/day)	54	3.94	1.35	54	4.08	1.24	0.055

Significant if $p < 0.05$

DISCUSSION

The recent study showed that there was significant difference in diarrhea duration between live probiotic and heat killed probiotic. Diarrhoea duration in heat killed probiotic group were shorter than in live probiotic group (5.65 vs 7.06; $p=0.042$). There was no significant difference in frequency diarrhoea in both group LP and HKP (3.94 vs 4.08; $p=0.055$). Simakachorn¹⁸ et al, studied about supplementation of heat killed bacteria with oral rehydration, and found that additional L acidophilus in oral rehydration in children with diarrhea resulted a shorter diarrhoeal duration. This found may be related to the study that revealed the heat killed bacteria has a high adhering ability in human intestinal and inhibits the process of pathogenesis of diarrhoeal bacteria in cultured human intestinal cell. Xiao¹⁹ et al, using heat killed bacteria in chronic diarrhea, and suggested that symptoms of chronic diarrhea may

be relieved with L acidophilus LB by decreasing bowel movement, abdominal pain and distention by improving stool consistency and the feeling of incomplete evacuation.

Weizman²⁰ et al, reported that child care infants fed a formula supplemented with L reuteri or B laci had fewer and shorter episode of diarrhea, with no effect on respiratory illnesses. These effects were more prominent with L reuteri, which has also the only supplement to improve additional morbidity parameters. Montez²¹ et al, described the benefits of probiotic in children with lactose maldigestion, milks inoculate with L acidophilus or with yoghurt culture were associated with decrease symptoms compare with those with uninoculated milk. Lin²² et al, in Taiwan, using inforan as probiotic fed enterally with breast milk reduced the incidence and severity of necrotizing enterocolitis in very low birth weight infants. This study gave more evidence the beneficial of

probiotic to overcome problem beside diarrhea. There was no side effect to provide probiotic in VLBW infants. Vanderhoof,²³ noted that clinical studies have shown that certain probiotics may be useful in treating a variety of diarrhoeal disorder, including rotavirus diarrhea, antibiotic associated diarrhea. Generally the use of probiotic is safe, but patients with high risk of systemic infection such as immuno-compromised or severely ill must be used carefully. Land²⁴ et al, reported two cases of probiotic associated sepsis in adult patients. They mentioned that serious infections attributable to probiotic lactobacilli are extremely rare. Cornelius,²⁵ et al, in their meta-analysis study, concluded that the Lactobacillus is safe and effective as treatment for children with acute infectious diarrhea.

The cause of diarrhoea in this study was unknown. This is one of the limitations of the study beside we cannot control the way of patient to serve the probiotic to their children. May be they can not change the habit that solute the milk or the food with hot boiled water. The live probiotic will die in such high temperature. We realized that there were still many confounding factors that can influence the study out come, and we can not control the parental habit in food serving to their family member including the children. We want to make this study run as naturally in origin. This study cannot explained the role of demographic factors alter diarrhoea duration.

In conclusion, heat killed probiotic looked superior and had a shorter diarrhoea duration compared to live bacteria in children with acute diarrhea, while there was no significant difference in frequency of diarrhoea. May be we have some bias in our outcome as we suggest to do longitudinal study with more large sample to determine our findings. For the next study, it is better if we can isolate the cause of acute diarrhea with stool culture.

REFERENCES

1. Larry K., Pickering, Snyder J.D. Gastroenteritis. In: Behrman RE, Kliegman RM, Jenson HB, penyunting. Nelson Textbook of Pediatric. 16th edition. Philadelphia: WB. Saunders, 2000. p. 765 – 68.
2. Noerasid H., Suraatmadja S., Asnil PO. Gastroenteritis (Diare) Akut. In: Suharyono, Boediarso A, Halimun EM, eds. Gastroenterologi Anak Praktis. Edisi ke 2. Jakarta: Balai Penerbit FK UI, 1994. p. 51 – 76.
3. Ditjen PPM & PPL, Departemen Kesehatan RI. Buku Ajar Diare. Pendidikan Medik Pemberantasan Diare. Jakarta, 1999.
4. Soeparto P., Djupri L.S., Sudarmo S.M., Ranuh RG. Sindroma Diare. 2nd edition. Surabaya: Gramik FK Universitas Airlangga, 1999.
5. Edmundson S.A., Edmundson W.C.. Diarrhoea in India and Indonesia. Available from URL: <http://www.midcoast.com.au/edmundsons/c8> cited in September 2006.
6. Gondwe R., Pruyn N., Torres G., Varma R.. Diarrheal Disease: Prevention and Management. Available from URL: <http://arcz.bumc.bu.edu/IH887/presentation> cited in September 2006.
7. Soenarto Y., Suryono A., Supardi S. Dysentery in children under five year of age: a longitudinal prospective study in primary health care in Indonesia. Paediatr Indones; 41:141 – 8.
8. Soenarto Y., Sebodo T., Suryantoro P., Krisnomurti, Haksokusodo S, Ilyas, et al. Bacteria, parasitic agents and rotaviruses associated with acute diarrhea in hospital inpatient Indonesia children. Transaction Roy Soc Trop Med Hyg 1983; 77: 729 – 30.
9. Isolauri E, Kirjavainen PV, Salminen. Probiotics: a role in the treatment of intestinal infection and inflammation? Gut 2002; 50 (Suppl): 54 – 9.
10. Isolauri E, Juntunen M, Rautanen T, Sillanauke P, Koivula T. A human lactobacillus strain (Lactobacillus casei sp strain GG) promotes recovery from acute diarrhea in children. Pediatrics 1991; 88: 90 – 7.
11. Isolauri E. Probiotic in human disease. Am J Clin Nutr 2001;73(Suppl):1142s-6s.
12. Isibashi N., Yamasaki S. Probiotics and safety. Am J. Clin Nutr 2001; 73 (Suppl):465s-70s.
13. Rolfé RD. The role of probiotic cultures in the control of gastrointestinal health. American Society for Nutritional Science. J Nutr 2000; 130: 396s – 402s.
14. Duc L.H., Hong H.A., Barbosa T.M., Henriques A.O., Cutting S.M.. Characterization of Bacillus probiotic available for human use. Appl Environ Microbiol 2004; 70(4): 2161 – 71.

15. Owehand A.C., Salminen S., Roberts P.J., Ovaska J., Salminen E. Disease dependent adhesion of lactic acid bacteria to the human intestinal mucosa. *Clin Diagn Lab Immunol* 2003; 10(4): 643 – 6.
16. Saavedra J.M., Hanna A.A., Moore N., Yolken R.H. Long term consumption of infant formulas containing live probiotic bacteria: tolerance and safety. *Am J. Clin Nutr* 2004; 79: 261 – 7.
17. Arveola T, Laiho K., Torkelli S., Mykkanen H, Salminen S, Maunula L, Isolauri E. Prophylactic *Lactobacillus GG* reduces antibiotic associated diarrhea in children with respiratory infections: a randomized study. *Pediatrics* 1999; 105(5).
18. Simakachorn N., Pichalpat V., Rithipornpalsarn. 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(1):68 – 72.
19. Xiao S.D., Zhang D.Z., Jiang S.H. Multicenter, randomized controlled trial heat killed *Lactobacillus acidophilus LB* in patients with chronic diarrhea. *Advance in Therapy* 2003;20(5):253-60.
20. Weizman Z, Ghaleb A, Alsheikh A. Effect probiotic infants formula on infections in child care centres: comparison of two probiotic agents. *Pediatrics* 2005;115(1):5-9.
21. Montes RG, Bayles TM, Perman JA, Saavedra JM. Effect of milks inoculated with *Lactobacillus acidophilus* on a yoghurt culture in lactose maldigesting children. *J Dairy Sci* 1995;78:1657-64.
22. Lin HC, Su B.H., Chen A.C., Lin T.W. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. *Pediatrics* 2005;111(1):1 – 4.
23. Vanderhoof J.A. Probiotics: future directions. *Am J. Clin Nutr*; 73 (suppl):1152s-5s.
24. Lands MH, Shetty AK. *Lactobacillus* sepsis with probiotic therapy. *Pediatrics* 2005; 115(1):181 – 81.
25. Cornelli W, Feudtner C, Michelle MG, Christakis DA. *Lactobacillus* therapy for acute infectious diarrhea in children: A meta-analysis. *Pediatrics* 2002; 109: 678 – 84.