PROBIOTIC SUPPLEMENTATION, KE-99 LACTO CAPSULES IN DENGUE HEMORRHAGIC FEVER I, II, III

A RANDOMIZED PLACEBO CONTROLLED TRIAL

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I BACKGROUND

According to World Health Organization, dengue is the most rapidly spreading mosquito-borne viral disease in the world. In the last 50 years, the incidence has increased 30 fold with increasing geographic expansion to new countries. Between 2001and 2008, more than a million cases were reported in Cambodia, Malaysia, Philippines and Vietnam; the four countries in Western Pacific Region with the highest number of reported cases and deaths.[1]

Dengue fever is a disease caused by a family of viruses that are transmitted by mosquitoes. It is an acute illness of sudden onset that usually follows a benign course with symptoms such as headache, fever, exhaustion, severe muscle and joint pain, swollen glands (lymphadenopathy) and rash. The dengue triad of fever, rash and headache is particularly characteristic. Other symptoms of dengue fever include bleeding gums, severe pain behind the eyes, red palms and soles. Because it is caused by one of four serotypes of virus, it is possible to get dengue fever multiple times. However, an attack of dengue produces immunity for a lifetime to that particular serotype to which the patient was exposed. The cornerstones for the clinical

[1] Republic of the Philippines, Department of Health, Revised Dengue Clinical Case Management Guidelines, 2011, pp1-27

management of dengue hemorrhagic fever rest on fluid therapy, prevention and early recognition of bleeding diatheses and avoidance of complications.

Dengue is an all year round disease in the Philippines. In 2008, Philippines was reported as one of the countries with the highest number of dengue cases and deaths in the Western Pacific Region. In 2010, all regions with cases of dengue and several outbreaks were reported in provinces and municipalities. The cases totaled to 135,355 which is 135% higher compared to 57,636 cases in 2009. The elimination of dengue is the responsibility of everyone, and yet there is no specific treatment for the disease.[2]

Probiotics have been reported to possess clinical properties that affect health and disease. Use of probiotics has been shown to be modestly effective in randomized clinical trials (RCTs) in treating acute viral gastroenteritis; and preventing antibiotic-associated diarrhea in healthy children. [3]

In this present study, a single strain KE 99 Lacto was the probiotic of choice. KE 99 Lacto refers to a strain of *Lactobacillus casei*, one of the good bacteria normal to human intestinal system. It improves intestinal health and strengthens immune system. It restores intestinal balance and repopulates the intestinal wall with beneficial bacteria. It has been shown to be exceptionally effective in killing of pathogenic bacteria. [4]

[2] Republic of the Philippines, Department of Health, Revised Dengue Clinical Case Management Guidelines 2011, Background and Rationale, ADMINISTRATIVE ORDER, No. 2012 - OQOG

[3] Allen SJ,Martinez EG,Gregorio GV,Dans LF. Probiotics for Treating Acute Infectious Diarrhea. Cochrane Database Syst Rev. 2010 Nov 10 ;(11):CD003048. Review

[4] KE-99 Lacto www.probiohealth.com

II REVIEW OF RELATED LITERATURE

A lot of therapeutic options have been studied including the role of multi-strain probiotics as the adjunct treatment in dengue hemorrhagic fever which showed significant results.

A pilot study on Safety and Tolerability of Probiotics in Dengue Hemorrhagic Fever done by Dr. Uy et al, last 2006, showed shorter mean hospital stay, hematocrit normalization after 1.69 day and increase in platelet count at the end of the third day. However, it was an open label case series and included only thirteen children.[5]

Another study published last 2007, The Beneficial Effects of Probiotics Ohhira OMX capsules in Dengue Hemorrhagic Fever Stage II by Dr. Uy et al, showed earlier improvement in temperature, hematocrit and platelet by 2 days, 2.16 days and 5.5 days respectively. [6]

The Efficacy of Multi-Strain Probiotics as Adjunct Treatment in Dengue Hemorrhagic Fever Grade II, a study published last 2012 , 40 children were included ages 5 to 17 years old, showed improvement of platelet count at 4th hospital stay with earlier improvement of abdominal pain and vomiting compared to control group.[7]

This current study focuses on the potential clinical benefits of a single strain probiotic KE 99 Lacto in stages I, II, III.

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[5] Uy, G., Gatcheco, F., Gotos, L., Ruiz., L., Asian Conference on Diarrhoeal Diseases and Nutrition (11th ASCODD) Safety and Tolerability of Probiotics in Dengue Hemorrhagic Fever: A Pilot Study, 2006

[6] Uy,G., Gatcheco, F., Gotos, L., Ruiz., L., The Beneficial Effects of Probiotics, Ohhira Omx Capsules in Grade II Dengue Hemorrhagic Fever, 2nd Congress of Asian Pediatric Research Yokohama Japan, 2007

[7] Authors Unknown, The Efficacy of Multi-Strain Probiotics as Adjunct Therapy for Dengue Hemorrhagic Fever Grade II , Prebiotech Healthcare Philippines, 2012, June 27, Accessed at http://prebiotech.wordpress.com/2012/06/27/the-efficacy-of-multi-strain-probiotics-as-adjunct-therapy-for-dengue-hemorrhagic-fever-grade-ii-2/

OPERATIONAL DEFINITION OF TERMS

- Dengue Grade I – in this study defined as any serologically and clinically confirmed dengue without spontaneous bleeding.
- Dengue Grade II – in this study is defined as any serologically and clinically confirmed dengue with spontaneous bleeding, in addition to Grade I usually in the form of skin hemorrhages.
- Dengue Grade III - in this study is defined as circulatory failure manifested as rapid, weak pulse, narrowing of pulse pressure, or hypotension, with the presence of cold, clammy skin and restlessness.
- Probiotic- in this study refers to single strain KE-99 Lacto documented to have immune boosting effects in three (3) dengue fever clinical trials

III RATIONALE

Dengue fever is an immune-mediated infectious disease. The alleged mechanism by which probiotics induce clinical improvement in dengue patients is still unclear, although local studies have shown a significant improvement in dengue stage II using a multi- strain probiotics. By this, we can use a single strain probiotic (KE-99 Lacto) as an adjunct treatment in dengue stage I, II, III. Secondly, a shortened hospital stay can reduce financial burden of the exorbitant cost of hospital expenses.

V GENERAL OBJECTIVE

To determine the effectiveness of a single strain probiotics (KE-99 Lacto) on the clinical outcomes of children ages 2 to 18 years old admitted for dengue hemorrhagic fever Stages I, II and III .

SPECIFIC OBJECTIVES

1. To compare the baseline clinical profile of subjects in terms of:
    A. Age, sex
    B. Anthropometrics (height, weight, BMI)
    C. Illness duration (days) prior to admission
    D. Signs and symptoms on admission
    E. Co-morbid illness
    F. Laboratory markers (initial white blood cell count, hemoglobin, hematocrit and platelet count)

2. To compare the proportion of children with resolution of clinical signs and Symptoms across disease severity at 24^{th}, 48^{th}, 72^{nd}, and 96^{th} hours in terms of:
    A. Resolution of fever
    B. Bleeding episodes (specific and all sites)
    C. Vital signs (blood pressure, heart rate and respiratory rate)

3. To compare the proportion of children with dengue fever across severity in terms of improved platelet count, hematocrit, hemoglobin, white blood cell count between the two groups at 24^{th}, 48^{th}, 72^{nd} and 96^{th} hours.

4. To compare the length of hospital stay (mean days) and the presence of adverse events between the two groups

VI PATIENTS AND METHODS

6.1 Study Design

This is a double blind, placebo controlled clinical trial involving a single strain probiotic (KE-99 Lacto) as adjunct treatment for dengue I, II and III patients. The coauthor was the randomizer. Principal investigator and patients were unaware of the drugs administered and taken respectively.

6.2 Setting and Duration

The study took place at the Rizal Medical Center, Service Ward, Department of Pediatrics with the duration of three months from August to November 2011.

6.3 Sample Size Estimate

This present study estimated a 20% difference in the proportion of children with improved clinical signs and symptoms and laboratory markers, in favor of the experimental arm (one-tailed test). The minimum number of subjects required to reject the null hypothesis of equality at.05 level of significance, generating a study power of at least 80% and a type II error of only 20%, was 68 per arm. An allowance of 20% was added for attrition. The sample was computed using the Modified Formula of Pocock:

$$n = \frac{P1\,(100-P1) + P2\,(100-P2)}{(P1-P2)^2} \times f\,(\alpha,\beta)$$

Where n is the working sample size

P1- proportion of children with successful outcomes in the experimental arm (95%)

P2- proportion of children with successful outcomes in the placebo (75%)

f (α,β) – is when α=.05 and β=20%, at 95% confidence interval estimate

6.4 Eligibility Criteria

We included children ages 2 to 18 years old with a clinical and laboratory diagnosis of dengue hemorrhagic fever stage I, II, III admitted to this institution, with informed consent and with the capability to take oral medications.

We excluded those with other complicating unstable systemic diseases (cardio-pulmonary, hematologic, autoimmune-mediated, gastrointestinal and neurologic diseases), and children diagnosed with Dengue stage IV

6.5 Sampling and Recruitment of Subjects

Included were children admitted to this institution with a diagnosis of dengue hemorrhagic fever stage I, II, III starting August 2011 until the termination of this study last November 2011.

6.6 Permuted Block Randomization

All subjects who passed the inclusion criteria were randomly assigned to either treatment arm as code A and control arm as code B using permuted blocked randomization. Six blocks were utilized to create allocation sequence with a block size of 4 each. For example, for the block 1 the random sequence for assigning subjects was AABB, block 2 was ABAB, block 3 was BBAA, block 4 was BABA, block 5 was BAAB, block 6 was ABBA, and yields 41 blocks.

6.7 Investigator and Patient Blinding

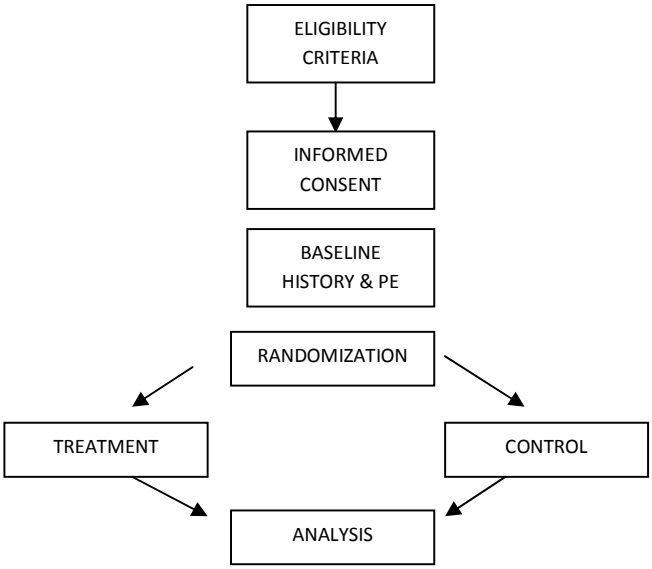
To ensure the avoidance of potential confounders, the primary investigator was blinded of the random sequence of each block. Similarly, patients were unaware of the capsules taken if it contains the probiotic or the placebo.

6.8 Description of Interventions

Probiotics and Placebo Administration

The randomizer prepared the probiotics and placebo, placed in a sachet labeled with the name of the subjects containing 14 capsules each. The primary investigator carried out the probiotic and placebo administration to the patients. Capsules were taken twice a day for 7 days. On the day of discharge, parents were advised to give the capsules to complete the seven-day protocol. Similar instructions were given to the parents of children whose platelet counts normalized before the anticipated seven-day period.

6.9 Flow of the Trial (Figure-1)



Clinical Diagnosis of DHF

The WHO clinical criteria for Dengue I, II and III was utilized. (See table-1). Patients with profound shock were excluded from this study.

Table-1 WHO Clinical Criteria for Assessing Dengue Severity

Stage	Basic Clinical Features	Hemorrhagic Manifestations	Increased Vascular Permeability	Thrombocytopenia
Dengue Fever (Grade I)	Fever with 2 of the following: headache, vomiting, abdominal pain, myalgia, arthralgia, rash	May have positive tourniquet test result and/or spontaneous bleeding*	Not apparent Maximum hematocrit <44%†	May be present
Dengue Hemorrhagic Fever (Stage II,)	Above features with some evidence of bleeding.	Must have positive tourniquet test result and/or spontaneous bleeding*	Present Maximum hematocrit 44%†	Minimum platelet count 100,000/mm3
Dengue Hemorrhagic Fever (Stage III,)	As above	As above	Evidence of hypovolemia	Coagulopathy
Dengue Shock Syndrome (Grade IV)	Rapid weak pulse, narrow pulse pressure (< 20 mmHg)	Hypotension for age,cold clammy, restless	Evidence of profund shock	Disseminated intravascular coagulation

* e.g., skin petechiae, bruising, mucosal/gastrointestinal bleeding.

† The mean haematocrit for children 5–10 years old hospitalized with any medical diagnosis in this institution. We have chosen a cut-off value of 44% as evidence of increased vascular permeability, this value being approximately 20% above the mean for the population.

7.0 Monitoring Endpoints of the Study

The primary endpoints in this study

1.) Clinical improvement of vital signs.

2.) Improvement of blood indices.

3.) Length of overall hospital stay.

Standard nursing care and physician monitoring of patients as indicated in the admission notes was strictly followed. Laboratory parameters hematocrit, platelet and white blood cell count was monitored at 24, 48, 72 and 96th hour from admission. The average values within the particular observation day were recorded as the data for analysis.

7.1 Provisions for Drop-Outs and Protocol Violators

A PROTOCOL VIOLATOR was defined as any dengue patient who was currently randomized to a particular arm of the study but failed to comply with the protocol instructions for patients; those who refuse to take the probiotic and those who inadvertently used another probiotic apart from the trial's treatment arm.

In this case, the patient was terminated from the trial, but still included in the intention-to-treat analysis of the study.

A DROP OUT was any patient currently randomized to any of the trial but showed evidence of allergy to the test drug and showed evidence of non-tolerability to the test drugs.

In this case, the patient was terminated from the trial, but still included in the intention-to-treat analysis of the study.

8.0 Unbinding of Treatment Arms and Termination of the Study

All data were encoded in MICROSOFT EXCEL by a blinded biostatistician. Unmasking of the group assignment occurred only after the analysis was completed.

The enrolment of the last subject in the required sample size and the observation of his or her was the basis for terminating the study protocol.

VII STATISTICAL METHODOLOGY

All analyses were carried out using the intention-to-treat principle using the MEDCALC and STATA version 10 as bio statistical software. Baseline homogeneity of sample was assessed using independent T-test and Chi-Square test for categories. Continuous data was summarized using mean and standard deviation while categories shall be summarized as percentages. Comparison of linear and continuous outcomes will be performed using unpaired T-test while, Chi-Square test or Fisher exact test was used for dichotomous outcomes. Precision was carried out at 95% confidence estimates and all tests of significance were pegged at .05 level.

VIII RESULTS

TABLE-1 Baseline Profile of Dengue Patients, Rizal Medical Center, 2011

Characteristic	Probiotics	Placebo	p-value*
Total Patients	n=81	n=83	
Mean Age (SD)	11.1 (4.6)	11.3 (4.1)	.74
Range	2 yrs-18	2yrs-18	
Age Class			
2 years	1 (1.2)	1 (1.2)	
3-5	10 (12.3)	8 (9.6)	.52
6-8	14 (17.3)	10 (12)	
9-11	17 (21)	21 (25.3)	
12-14	16 (19.8)	20 (24.1)	
15-17	16 (19.8)	21 (25.3)	
18	7 (8.6)	2 (2.4)	
Sex (%)			
Male	37 (44)	48 (58)	.06
Female	44 (56)	35 (42)	
Mean Height in cm (SD)	137.8 (40.4)	139.1 (24)	.81
Range	77 – 414	93-168	
Mean Weight in kg (SD)	35.2 (20)	34.5 (11)	.72
Range	10.4 – 93	12.8 -54	
Body Mass Index (SD)	17.1 (3.9)	17 (2.0)	.87
BMI Classification			
Underweight	15 (18)	3 (4)	.77
Normal	63 (78)	79 (95)	
Overweight	3 (4)	1 (1)	
Mean Duration of Illness (SD)	4.5 (0.87)	4.6 (0.92)	.49
Range	3-6	3-7	
Day of Illness on Admission (SD)	4.5 (0.87)	4.6 (0.91)	.54
Range	2-5	2-6	
Mean Days of Fever (SD)	3.8 (0.97)	4.0 (0.88)	.35
Range	4-7	3-6	

*NS- no significant difference

A total of 164 children qualified the inclusion criteria and were randomized to receive probiotic supplementation (KE-99 Lacto) at one capsule twice a day for seven days (n=81) or placebo (n=83). A summary of the baseline profile is shown (Table-1).

At baseline, there was no statistically significant difference in terms of the mean age (p=.74); sex distribution (p=.06); anthropometric measurements: mean height (p=.81), mean weight (p=.72); mean body mass index (p=.87) and the children's BMI classification (p=.77); the mean duration of illness (p=.49). Both groups has comparable day of illness on admission (p=.54) and mean duration of fever (in days) (p=.35).

TABLE-2 Baseline Laboratory Parameters and Clinical Profile of Dengue Patients, Rizal Medical Center, 2011

Characteristic	Probiotics	Placebo	p-value*
Total Patients	n=81	n=83	
Narrow Pulse Pressure	18 (22)	21 (26)	.77
Febrile Children	23 (20)	19 (23)	.65
Bleeding**			
Nose	3 (4)	6 (7)	.10
Gums	0	2 (3)	
None	78 (96)	75 (90)	
Other Signs and Symptoms			
Headache	13 (16)	17 (20)	.43
Retro-orbital pain	21 (26)	18 (22)	.22
Joint and muscle pains	32 (39)	29 (35)	.19
Abdominal pain	13 (48)	10 (12)	.23
Vomiting	10 (12)	12 (14)	.21
Rashes	11 (13)	9 (11)	.08
Co morbid illness			
URTI, viral	36 (44)	34 (41)	.75
URTI, bacterial	3 (3)	3 (4)	.54
Laboratory Findings			
Mean Hematocrit (SD)	0.41 (.05)	0.41 (.044)	.77
Range	0.31 – 0.50	0.38 – 0.44	
Mean Hemoglobin (SD)	143.6 (18.1)	143.4 (15.8)	.95
Range	138 - 151	128 - 147	
Mean WBC count (SD)	4.7 (1.1)	6.1 (1.4)	.38
Range	3.8 – 10.6	5.6 – 11.7	
Mean Platelet count (SD)	59.6 (22.7)	61.1 (22.4)	.66
Range	1-100	3-119	

*No significant difference, all p-values are > .05, URTI- upper respiratory tract infection, WBC-white blood cell, **May exist in combination

A summary of the baseline laboratory and clinical profile was shown (Table-2). The proportion of children with narrow pulse pressure and fever on admission were not significantly different between the two groups (22% versus 26%, p=.77and 20% versus 23%, p=.65 respectively). There was no statistically significant difference in other signs and symptoms; headache (p=.43), retro-orbital pain (p=.22), joint and muscle pain (p=.19), abdominal pain (p=.23), vomiting (p= .21), rashes (p=.08); co morbid illness: upper respiratory tract infection viral (p=.75), upper respiratory tract infection bacterial (p=.54).

The proportion of children across bleeding sites were not significant (p=.10) Baseline laboratory tests such as the mean hematocrit, hemoglobin, white cell count and platelet count were not significantly different between the two groups (p=.77, p=.95, p=.38, p=.66 respectively).

No drop outs, protocol violator, patient mortality nor adverse events were reported during the course of the study.

TABLE-3 Proportion of Children with Resolution of Signs and Symptoms, Improvement of Blood Indices in Stage I Dengue Hemorrhagic Fever, Rizal Medical Center, 2011

OUTCOME	GRADE I		
	Probiotics n=58 (%)	**Placebo** n=63 (%)	***p*-value**
Resolution of Fever [a]	45 (77.5)	36 (57.1)	**.022***
Improvement in heart rate	56 (96.5)	60 (95.2)	.98
Improvement in systolic/diastolic BP	56 (96.5)	61 (96.8)	.99
Improvement in respiratory rate	56 (96.5)	61 (96.8)	.99
Improvement in platelet count [b]	54 (96.4)	48 (76.2)	**.017***
Improvement in hematocrit [c]	54 (96.4)	48 (76.2)	**.017***
Improvement in hemoglobin [d]	54 (96.4)	48 (76.2)	**.017***
Improvement in WBC count	56 (96.5)	60 (95.2)	.98
Mean hospital stay (± Days)	3.2 ± 2	6.4 ± 1	**.024****

*Significant difference by Z-test of two proportions, **by Independent T-test .
[a]-onset of resolution of fever on the 24th hour of treatment, [b]-Onset of improvement of platelet count on the 72nd hour of treatment, [c]-Onset of improvement of hematocrit on the 72nd hour of treatment-Onset of improvement in hemoglobin noted on the 48th hour of treatment.

Probiotic supplementation was associated with a higher proportion of children with resolution of fever (77.5% versus 57.1%, p=.022) as early as 24th hour of supplementation. Improvement in platelet count was also higher in the probiotic group (96.4% versus 76.2%, p=.017) noted on the 72nd hour of treatment. There was also improvement in hematocrit and hemoglobin (96.4% versus 76.27%, p=.017) noted on the 72nd hour and 48th hour, respectively. The probiotic group was associated with reduced average hospital stay with mean of 3.2 days and 6.4 days, (p=.024). There was no significant difference in terms of white blood cell count and other outcomes mentioned (Table-3).

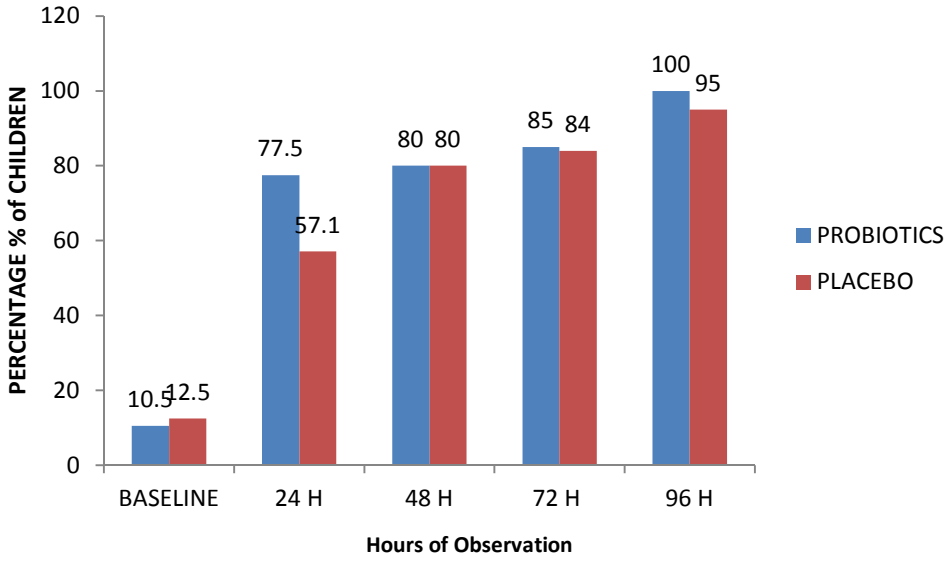
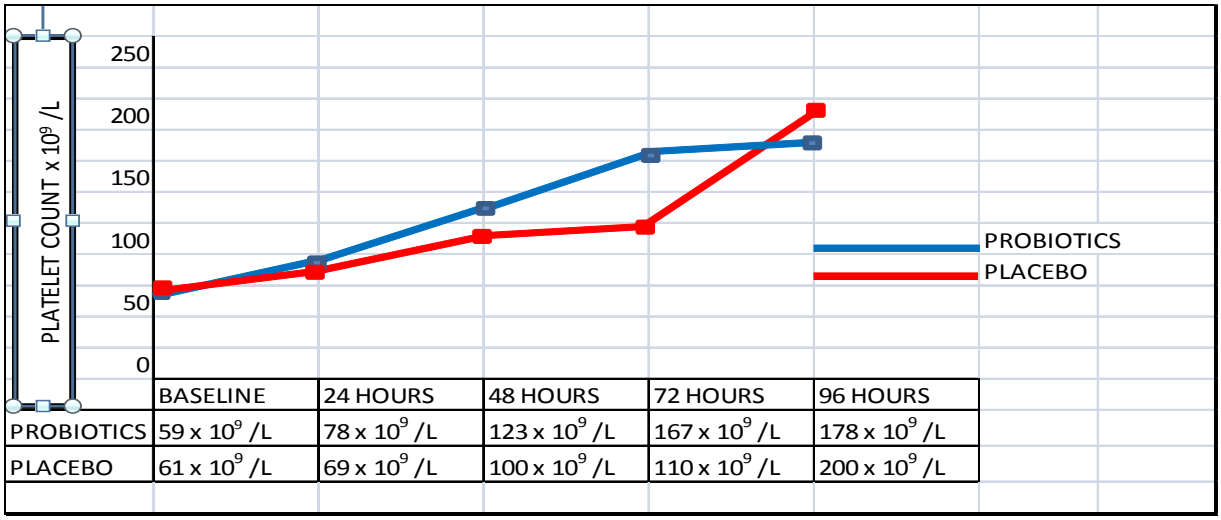


Figure-1 Proportion of Children with Resolution of Fever in DHF Stage I given Probiotics and Placebo

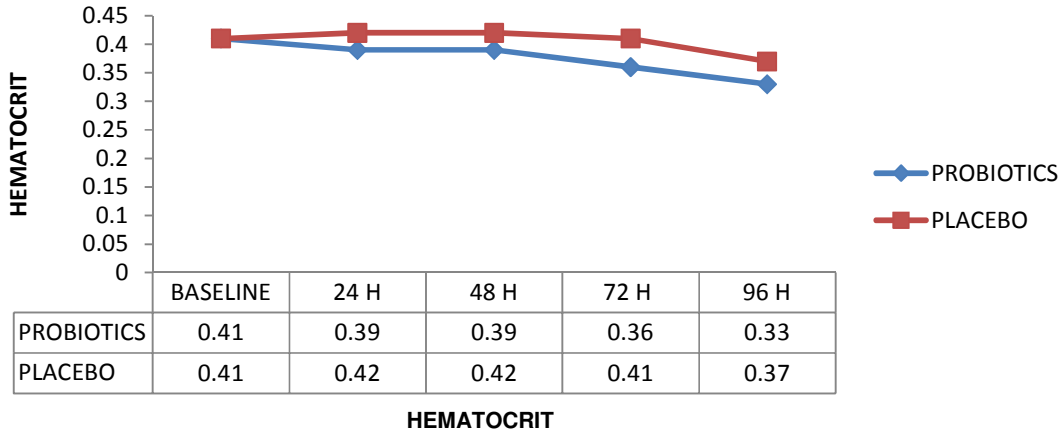
A significantly higher proportion of children with resolution of fever were noted with the probiotic group at 24th hour of observation (77.5% versus 57.1%, p=.022) (Figure-1).



	BASELINE	24 HOURS	48 HOURS	72 HOURS	96 HOURS
PROBIOTICS	59 x 10^9 /L	78 x 10^9 /L	123 x 10^9 /L	167 x 10^9 /L	178 x 10^9 /L
PLACEBO	61 x 10^9 /L	69 x 10^9 /L	100 x 10^9 /L	110 x 10^9 /L	200 x 10^9 /L

Figure-2 Comparison of Actual Platelet Counts in DHF Stage I between Probiotics and Placebo

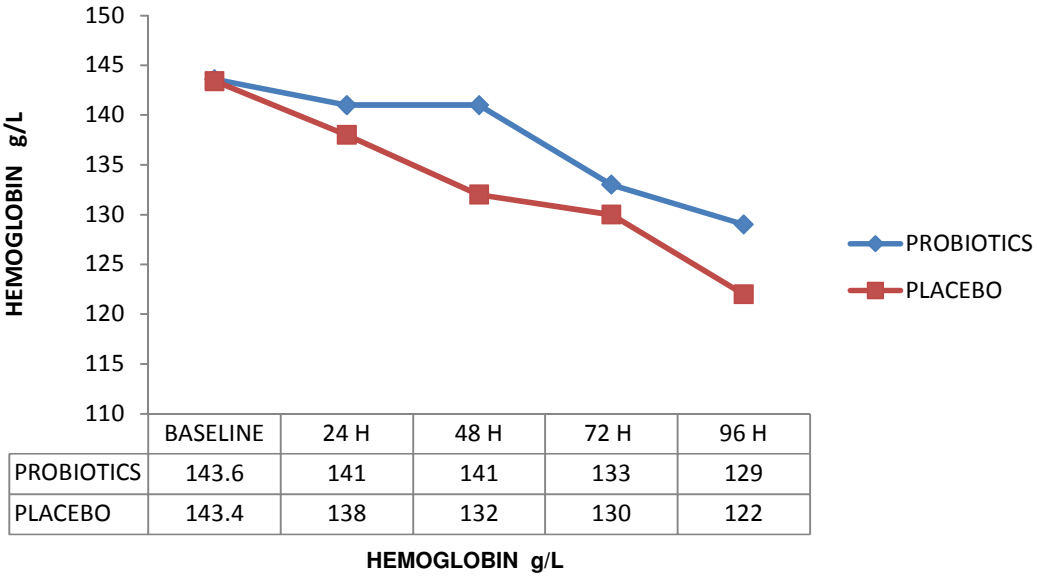
Results showed a significantly higher platelet count among children given probiotics on the 72nd hour when compared to placebo with a mean of 167 and 110, (p=.029) (Figure-2).



	BASELINE	24 H	48 H	72 H	96 H
PROBIOTICS	0.41	0.39	0.39	0.36	0.33
PLACEBO	0.41	0.42	0.42	0.41	0.37

Figure-3 Comparison of Actual Hematocrit in DHF Stage I between Probiotics and Placebo

Hematocrit was significantly lower in the probiotic group starting at 72nd hour of treatment with a mean of 0.36 and 0.41,(p=.013) (Figure-3).



	BASELINE	24 H	48 H	72 H	96 H
PROBIOTICS	143.6	141	141	133	129
PLACEBO	143.4	138	132	130	122

Figure-4 Comparison of Actual Hemoglobin in DHF Stage I between Probiotics and Placebo

Hemoglobin levels were likewise significantly higher among those with probiotics on the 48th hour with mean of 141 and 132, (p=.017) (Figure-4).

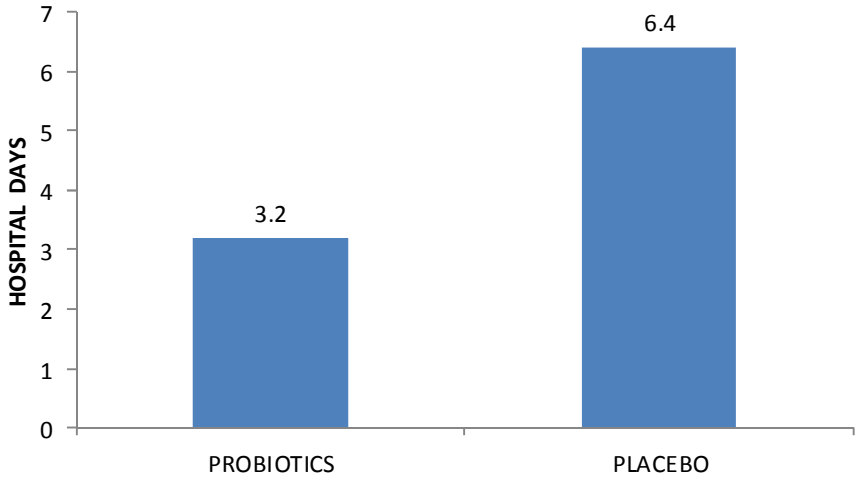


Figure-5 Comparison of Hospital Stay in DHF Stage I given with Probiotics and Placebo

Mean hospital stay was significantly lower among those with probiotics when compared to placebo with a mean of 3.2days and 6.4 days, (p=.024) (Figure-5).

The differences in the proportion of children with improved heart rates, systolic and diastolic blood pressure and respiratory rates were not significantly different. (See Appendix)

TABLE-4 Proportion of Children with Resolution of Signs and Symptoms, Improvement of Blood Indices in Grade II Dengue Hemorrhagic Fever, Rizal Medical Center, 2011

OUTCOME	GRADE II		
	Probiotics n=3 (%)	**Placebo** n=8 (%)	***p*-value**
Resolution of Fever	3 (100)	8 (100)	.99
Improvement in heart rate	3 (100)	8 (100)	.99
Improvement in systolic/diastolic BP	3 (100)	8 (100)	.99
Improvement in respiratory rate	3 (100)	8 (100)	.99
Resolution in Nose Bleeding [a]	3 (100)	1 (12.5)	**.039***
Resolution in Gum bleeding	--	2 (100)	.066
Improvement in platelet count	3 (100)	7 (87.5)	.65
Improvement in hematocrit	2 (66.7)	5 (62.5)	.58
Improvement in hemoglobin	2 (66.7)	5 (62.5)	.58
Improvement in WBC count	2 (66.7)	5 (62.5)	.44
Mean hospital stay (± Days)	6 ± 3.2	7.3 ± 2.1	.76

*Significant difference by Z-test of two proportions, **by Independent T-test
[a]-onset of improvement noted on the 48th hour of treatment

In grade II dengue hemorrhagic fever, probiotic supplementation was associated with a higher proportion of children with resolution of nose bleeding (100% versus 12.5%, p=.039) noted on the 48th hour of treatment (Table-4). There was no significant difference between the two groups in terms of the other outcomes mentioned.

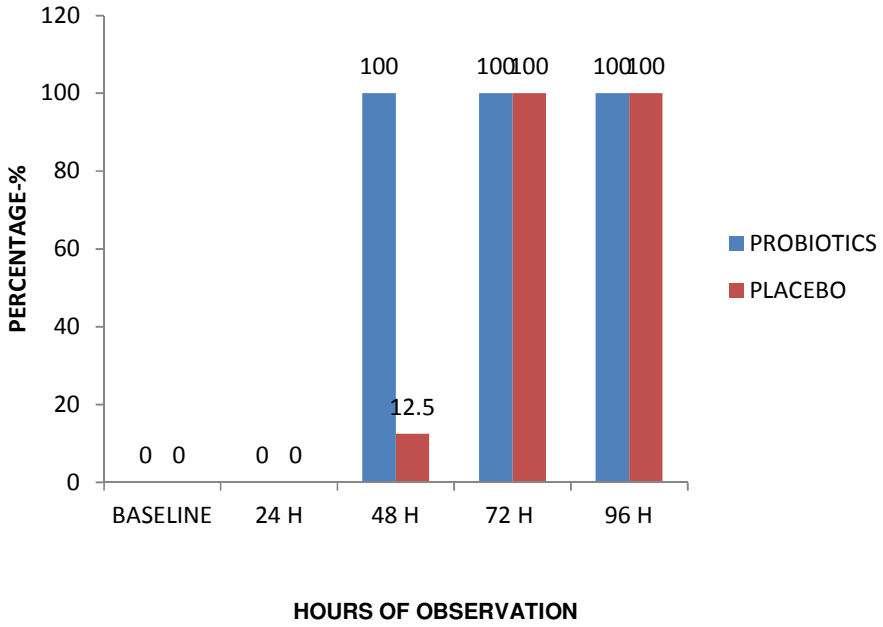


Figure-6 Comparison of Nose-bleeding Resolution in DHF Stage II given with Probiotics and Placebo

Of all the parameters analyzed, only resolution of nose bleeding was significantly higher among those taking probiotics on the 48th hour (100% versus 12.5%, p=.039). The proportion has no difference on the following days (Figure-6).

The differences in the proportion of children with improved heart rates, systolic and diastolic blood pressure and respiratory rates were not significantly different. (See Appendix)

TABLE-5 Proportion of Children with Resolution of Signs and Symptoms, Improvement of Blood Indices in Grade III Dengue Hemorrhagic Fever, Rizal Medical Center, 2011

OUTCOME	GRADE III		
	Probiotics n=20 (%)	**Placebo** n=12 (%)	***p*-value**
Resolution of Fever [a]	20 (100)	9 (75)	**.018***
Improvement in heart rate	20 (100	12 (100)	.99
Improvement in systolic/diastolic BP	20 (100	12 (100)	.99
Improvement in respiratory rate	20 (100	12 (100)	.99
Improvement in platelet count [b]	19 (95)	7 (58.3)	**.001***
Improvement in hematocrit	20 (100)	12 (100)	.13
Improvement in hemoglobin	20 (100)	12 (100)	.13
Improvement in WBC count [c]	17 (85)	8 (66.7)	**.001***
Mean hospital stay (± Days)	5.2 ± 1	8.3 ± 2	**.023****

*Significant difference by Z-test of two proportions, **by Independent T-test
[a]-Onset of resolution of fever noted on the 48th hour of treatment, b-Onset of improvement of platelet count noted on the 72nd hour of treatment, [c]-Onset of improvement, of WBC count noted on the 72nd hour of treatment

In grade III dengue hemorrhagic fever, probiotic supplementation was associated with a higher proportion of children with resolution of fever (100% versus 75%, p=.018) at 48th hour of treatment. There was statistically significant improvement in the percentage of children with improved platelet count (95% versus 58.3%, p=.001) and white blood cell count (85% versus 66.7%, p=.001) noted on the 72nd hour of supplementation. The probiotic group has reduced hospital stay with mean of 5.2 days and 8.3 days, (p=.023). There was no significant difference between the two groups in terms of the other outcomes mentioned (Table-5).

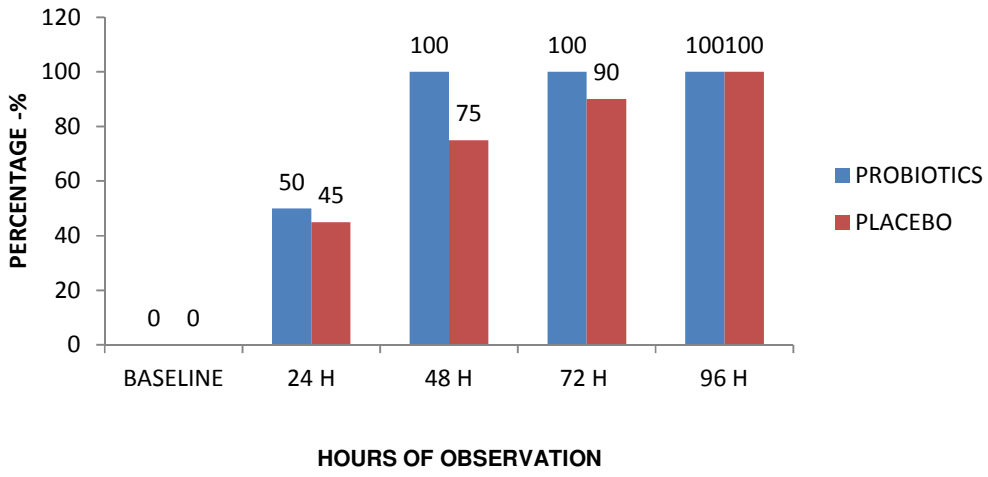
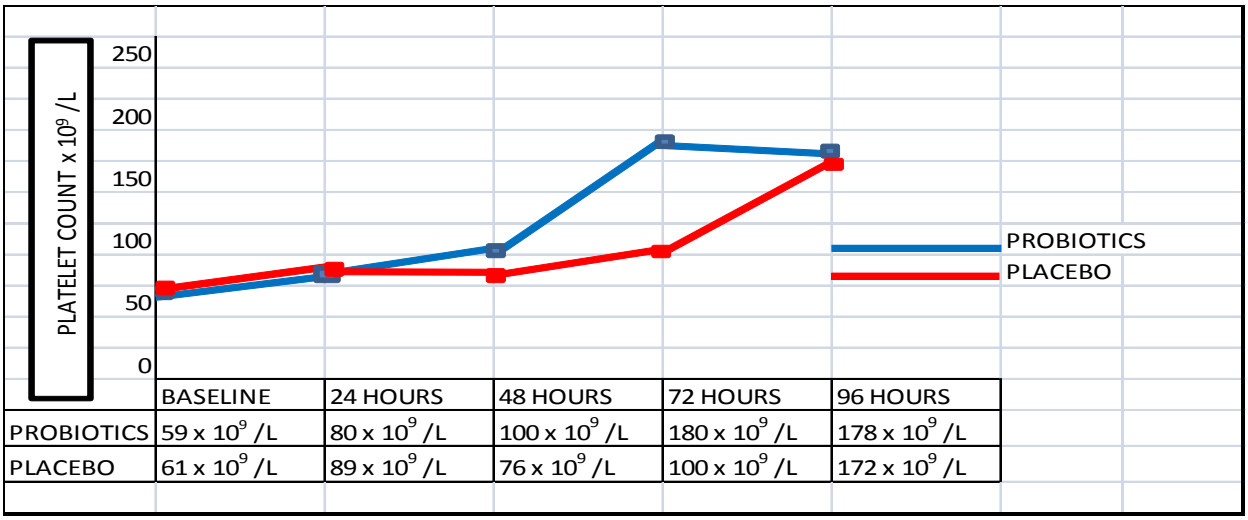


Figure-7 Proportion of Children with Onset of Resolution of Fever in DHF Stage III given with Probiotics and Placebo

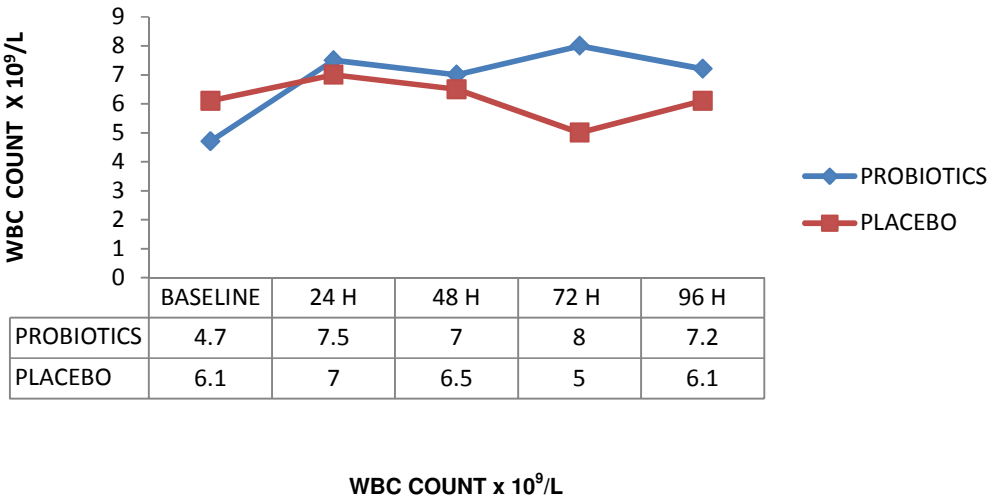
A significantly higher proportion of children with early resolution of fever in the probiotic group on the 48th hour of treatment (100% versus 75%, p=.018) (Figure-7).



	BASELINE	24 HOURS	48 HOURS	72 HOURS	96 HOURS
PROBIOTICS	59 x 10^9 /L	80 x 10^9 /L	100 x 10^9 /L	180 x 10^9 /L	178 x 10^9 /L
PLACEBO	61 x 10^9 /L	89 x 10^9 /L	76 x 10^9 /L	100 x 10^9 /L	172 x 10^9 /L

Figure-8 Comparison of Actual Platelet Counts in DHF Stage III given with Probiotics and Placebo

Children given probiotic started having normalized platelet counts at the 72nd hour of treatment with mean of 180 and 100, (p=.013) (Figure-8).



	BASELINE	24 H	48 H	72 H	96 H
PROBIOTICS	4.7	7.5	7	8	7.2
PLACEBO	6.1	7	6.5	5	6.1

Figure-9 Comparison of Actual White Blood Cell Counts in DHF Stage III given with Probiotics and Placebo

White blood cell counts were significantly higher among those given probiotics during the 72nd hour (Mean 8 versus 5, p=.024) (Figure-9).

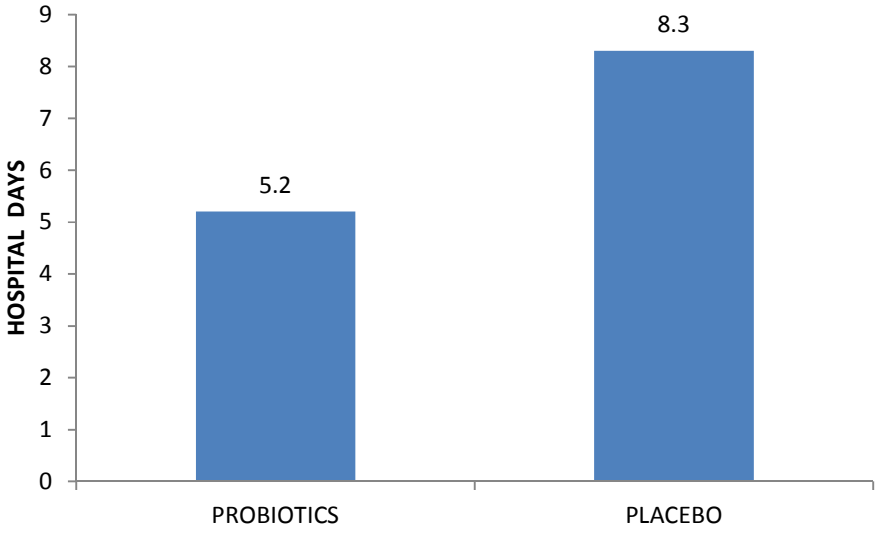


Figure-10 Comparison of Hospital Stay in DHF Stage III given with Probiotics and Placebo

Hospital stay was significantly shorter among those given probiotic when compared to placebo with mean of 5.2 days and 8.3 days, (p=.023) (Figure-10)

The differences in the proportion of children with improved heart rates, systolic and diastolic blood pressure and respiratory rates were not significantly different. (See Appendix)

IX DISCUSSION

This clinical trial utilized KE 99 Lacto *Lactobacillus casei* to regulate immune function in dengue hemorrhagic fever.

The data results showed that single strain probiotic supplementation was associated with higher proportion of children with resolution of fever noted during 24th and 48th hour post treatment at stage I and III respectively. Probiotics in this study possibly reduced the cytopathic effect of viral strain not only destroying the viral entity itself but also blocking the virus infection and inhibit the viral proliferation of the cells. This will result to early resolution of fever leading to slowing down of the inflammatory process which in turn led to the shortened hospital stay as seen in stage I (3.2 versus 6.4, p=.024) and stage III (5.2 versus 8.3, p=.023).

A study of multi-strain probiotics in dengue, showed resolution of fever 2 days earlier than the control group, however, single strain probiotics could not be directly compared. [8] As seen in this data, the proportion of children with fever was significantly low in the treatment group. This supports the findings of other probiotic clinical trials which use fever as an outcome variable.

[8] Uy.G. et.al.2006, Ibid pp. 1

The present study did show a significant difference in the improvement of platelet count in DHF stage I and III both were noted at 72nd hour post treatment. Hematocrit and hemoglobin levels improved in DHF stage I at 72nd and 48th hour post treatment respectively. White blood cell count was improved in grade III at the 72nd hour of supplementation with probiotic.

We could not locate studies that focused on investigating the mechanistic roles of probiotics in augmenting these leukocyte and thrombocyte parameters. One of the major possible mechanisms of probiotic action is through the regulation of host immune response. Studies regarding the biological consequences of probiotics in host immunity suggested that they regulate the functions of systemic and mucosal immune cells and intestinal epithelial cells- thus explaining a wide array of studies on the effect of probiotics in gastrointestinal disease.[9] Dengue fever is an immune-mediated disease and thus may respond to agents which modify the complex interplay of the viral pathogen, its interaction with the hosts thrombocytes and plasma leakage which is responsible for the clinical signs and symptoms of a case

Probiotic supplementation was associated with higher proportion of children with resolution of nose bleeding noted on the 48th hour of treatment. This could be explained by the noticeable rise in platelet counts as the patient improves. Also, probiotics in this study have shown that it could reverse the trend by early normalizing the hematocrit, hemoglobin and increasing platelet counts.

[9] Ng SC, Hart AL, Kamm MA, Stagg AJ, Knight SC. Mechanisms of action of probiotics: recent advances. Inflammatory Bowel Dis. 2009 Feb; 15(2):300-10.

Probiotics probably reduces the likelihood of pathogenic organisms and stabilize the intestinal gut flora by halting the cytokine cascade through down regulation of the inflammatory cells and cytokines. [10] It probably control the overgrowth of potentially pathogenic microorganisms with viral origin by antagonizing noxious or unwanted microorganisms, eliminate toxins and stimulate intestinal immune defense. How these mechanisms are connected to the clinical resolution of signs and symptoms in dengue still warrants close investigation.

[10] Uy.G. et.al. 2006, Ibid pp. 1

X CONCLUSION

The two groups were comparable in terms of baseline demographic and clinical variables.

In DHF I, probiotic supplementation was associated with higher proportion of children with resolution of fever noted as early as 24th hour of treatment. Improvement of platelet count, hemoglobin and hematocrit was also noted at 72nd hour, 72nd hour and 48th hour, respectively. Average hospital stay was shortened to 3.2 days.

In DHF II, probiotic supplementation was associated with higher proportion of children with resolution of nose bleeding noted at 48th hour of treatment.

In DHF III, probiotic supplementation was associated with higher proportion of children with resolution of fever noted at 48th hour of treatment. There was a statistically significant improvement in platelet count and white blood cell count noted at 72nd hour of treatment. Average hospital stay was shortened to 5.2 days.

There was no significant effect on blood pressure, heart rate and respiratory rate.

No adverse event with probiotic supplementation was observed.

XI RECOMMENDATIONS:

It is recommended that the trial be replicated using a higher sample size in all stages of dengue.

XII REFERENCES:

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2 Allen SJ, Martinez EG, Gregorio GV, Dans LF. Probiotics for treating acute infectious diarrhoea. Cochrane Database Syst Rev. 2010 Nov 10;(11):CD003048. Review

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4 KE-99 Lacto www.probiohealth.com

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7 Uy,G., Gatcheco, F., Gotos, L., Ruiz., L., The Beneficial Effects of Probiotics, Ohhira Omx Capsules in Grade II Dengue Hemorrhagic Fever, 2nd Congress of Asian Pediatric Research Yokohama Japan, 2007

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10 Uy.G. et.al.2006, Ibid pp. 1

11 Guillemard E, Tondu F, Lacoin F, Schrezenmeir J. Consumption of a fermented dairy product containing the probiotic Lactobacillus casei DN-114001 reduces the duration of respiratory infections in the elderly in a randomised controlled trial.

12 Giovannini M, Agostoni C, Riva E, Salvini F, Ruscitto A, Zuccotti GV, Radaelli G; Felicita Study Group., A randomized prospective double blind controlled trial on effects of long-term consumption of fermented milk containing Lactobacillus casei in pre-school children with allergic asthma and/or rhinitis., Pediatr Res. 2007 Aug;62(2):215-20.

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14 Ng SC, Hart AL, Kamm MA, Stagg AJ, Knight SC. Mechanisms of action of probiotics: recent advances. Inflamm Bowel Dis. 2009 Feb;15(2):300-10.

15 Uy.G. et.al. 2006, Ibid pp. 1

APPENDIX I

PATIENT'S BASELINE DATA FORM

Code: □ A □ B	ID Number Address:_____ Phone:_____	Name Sex □ Male □ Female	Age ___years ___months
Chief Complaint _____ _____ Baseline History 1. Duration of Illness _____days 2. Day of illness _____	Site of Bleeding □ GIT □ Oral □ Skin □ CNS □ Others □ _____ Tourniquet test □ Positive □ Negative Dengue Blot test □ Positive □ Negative	Baseline vital signs Heart rate=_____ SBP=_____mmHg DBP=_____mmHg RR=_____/min Temp=_____ºC	Baseline Anthropometrics Height _____kg Weight_____cm BMI _____ Nutritional Classification _____
Baseline PE CNS □ Normal □ Abnormal Skin □ Normal □ Abnormal HEENT □ Normal □ Abnormal CVS □ Normal □ Abnormal Pulmo □ Normal □ Abnormal GIT □ Normal □ Abnormal Neuro □ Normal □ Abnormal	Co-Morbid Illness □ AGE □ URTI viral □ URTI bacterial □ CAP Viral □ CAP bacterial □ Diabetes □ Congenital heart disease □ Asthma □ PTB □ Allergy □ Others □ _____ □ _____	Baseline Laboratory Hematocrit_____ Hemoglobin_____ WBC _____/mm^3 Platelet count_____mm^3 Other Labs Serum Na □ Normal □ Abnormal Serum K □ Normal □ Abnormal ABG □ Normal □ Abnormal	Other Labs Chest PA □ Normal □ Abnormal Urinalysis □ Normal □ Abnormal BUN □ Normal □ Abnormal Creatinine □ Normal □ Abnormal Liver Function □ Normal □ Abnormal Others_____

APPENDIX 2

PATIENT OUTCOMES FORM (Day 1-7)

Patient Code □ **Group A** □ **Group B**

Parameter	**Admission**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Fever	□ Febrile □ Afebrile	□ Febrile □ Afebrile	□ Febrile □ Afebrile	□ Febrile □ Afebrile	□ Febrile □ Afebrile	□ Febrile □ Afebrile	□ Febrile □ Afebrile	□ Febrile □ Afebrile
BP	□ Normal □ Abnormal	□ Normal □ Abnormal	□ Normal □ Abnormal	□ Normal □ Abnormal	□ Normal □ Abnormal	□ Normal □ Abnormal	□ Normal □ Abnormal	□ Normal □ Abnormal
Heart rate	□ Normal □ Abnormal	□ Normal □ Abnormal	□ Normal □ Abnormal	□ Normal □ Abnormal	□ Normal □ Abnormal	□ Normal □ Abnormal	□ Normal □ Abnormal	□ Normal □ Abnormal
RR	□ Normal □ Abnormal	□ Normal □ Abnormal	□ Normal □ Abnormal	□ Normal □ Abnormal	□ Normal □ Abnormal	□ Normal □ Abnormal	□ Normal □ Abnormal	□ Normal □ Abnormal
Bleeding	□ Present □ Absent	□ Present □ Absent	□ Present □ Absent	□ Present □ Absent	□ Present □ Absent	□ Present □ Absent	□ Present □ Absent	□ Present □ Absent
Platelet Count*	Exact Value	Exact Value	Exact Value	Exact Value	Exact Value	Exact Value	Exact Value	Exact Value
Hematocrit	Exact Value	Exact Value	Exact Value	Exact value	Exact Value	Exact Value	Exact Value	Exact Value
Hemoglobin	Exact Value	Exact Value	Exact Value	Exact value	Exact Value	Exact Value	Exact Value	Exact Value
White Blood Cell Count	Exact Value	Exact Value	Exact Value	Exact value	Exact Value	Exact Value	Exact Value	Exact Value
Outcomes	□ Alive □ Died □ HAMA	□ Alive □ Died □ HAMA	□ Alive □ Died □ HAMA	□ Alive □ Died □ HAMA	□ Alive □ Died □ HAMA	□ Alive □ Died □ HAMA	□ Alive □ Died □ HAMA	□ Alive □ Died □ HAMA

*Lowest value within a 24 hour period

Total ICU Stay_____(Days) Total Hospital Stay _____(Days)

APPENDIX 3
INFORMED CONSENT

Dear Parent,

Good day !

I am Dr Charon Comia- Capate, a senior pediatric resident of this institution. Dengue fever is serious illness that harms the lives of children. Every effort is taken to reduce the effects of dengue on people's lives.

In search for other alternative and natural options for treating the disease, I come up with an experiment utilizing a natural single strain probiotic named KE-99 Lacto. This natural agent is safe, tolerable and is widely used in, diseases such as diarrhea and pneumonia. I will investigate its therapeutic effect in children with moderate to severe dengue fever.

We would like you to voluntarily participate in this clinical study. It has the following provisions.

1. KE-99 Lacto shall be orally taken twice a day for at least seven (7) days. This is supplied and is free of charge.
2. Laboratory tests such as complete blood count shall be monitored every 24th, 48th, 72nd hour until patient improves.

I am certain that this research will not cause any harm or any untoward effects to your child. Any given information from willing participants will be confidential and each participant's name will not be disclosed to any documents or reports from this study.

Your child's participation in this study is not mandatory. It is the decision of the

parents if they want their children to participate or not. There will be no corresponding special privileges to those who will join and anyone could still avail the usual health services and health benefits we offer regardless they join this study or not.

In cases of an untoward event like an allergy to KE-99 Lacto , we shall take responsible for alleviating its symptoms.

You are free to discontinue from the trial anytime as well as deserving the right not to disclose the reasons from such withdrawal.

For more information regarding this clinical study, please feel free to contact me at telephone number:_____Cell phone:_____

PROOF OF VOLUNTARY PARTICIPATION

I, (state name)_____, the legal parent/guardian of _____, admitted in this institution for dengue fever, having understood well the provisions, objectives, procedures and outcomes of this protocol hereby affix my signature indicating my willing participation to the said clinical trial.

I am fully aware that this is a clinical trial involving a natural probiotic that has been found to be safe and tolerable for kids and that it has been shown to benefit children even in mild dengue fever. I also understand there is no financial incentive for such participation. I am also made aware that Dr. Comia-Capate will take full responsibility of my child's overall welfare during the said course of the clinical trial.

I will not hold Dr. Comia- Capate liable for any untoward incident that may occur during the treatment using the probiotic. Any associated adverse reactions shall be thoroughly investigated.

_____

Name of Parent, Signature Above it

_____

Dr. Charon Comia- Capate

Principal Investigator

MALAYANG PAHINTULOT

Mahal na Magulang,

Magandang araw sa inyo !

Ako si Dr Charon Comia- Capate, isang doktor ng mga bata sa institusyong ito. Ang dengue ay isang seryosong sakit na pumapahamak sa ating kabataan. Ang lahat na pagsisikap ay ginagawa natin upang maibsan ang epekto ng dengue sa buhay ng mga bata.

Upang makahanap ng isang alternatibong solusyon sa paggamot sa dengue, ako ay may pananaliksik sa isang gamot na ang tawag ay KE-99 Lacto Probiotic . Ito ay isang natural na probiotic, na ligtas na gamot na ginagamit para sa pulmonya at pagtatae. Iimbistigahan ko ang epekto ng KE-99 Lacto Probiotic sa dengue na malala.

Ibig ko po kayong imbitahing sumali ng kusang-loob sa pananaliksik na ito. Ang mga sumusunod ay iilang probisyon sa pananaliksik na ito.

1. Ang KE-99 Lacto ay iinumin isang capsula dalawang (2) beses sa isang araw hanggang pitong araw.
2.Eksaminasyon sa dugo tulad ng Complete blood count at platelet count ay kailangan I monitor araw araw hanggang sa pag galling at pag ayos ng kundisyon ng pasyente.

Ang mga pananliksik na ito ay ginawa dito sa atin at ang KE-99 Lacto ay nakita na ligtas sa mga bata. Walang side-effect na nakita rito.

Ako ay nakasisiguro na walang masamang epekto ang KE-99 Lacto sa mga bata. Ang lahat ng impormasyon ay kompidensyal.

Ang paglahok ninyo sa pagsaliksik na ito ay hindi sapilitan. Desisyon ito ng mga magulang. Walang kauukulang espesyal na pribilehiyo ang paglahok rito.

Gagawin ko ang lahat na gamutin ang lahat ng mga pasyenteng may allergy sa KE-99 Lacto.

Kayo din ay may karapatan na umatras sa pananaliksik na ito. Ang mga kadahilanan kung bakit tinanggihan ang pagpapatuloy sa pananaliksik na ito ay iyong karapatan..

Para sa karagdagang ipormasyon, maari kayong sumangguni sa akin sa telepono _____o cellphone_____.

KATUNAYAN NG MALAYANG PAHINTULOT

Ako si _____, ang magulang ni _____na naka-admit sa ospital na ito dahil sa dengue , ay lubos na nauunawaan ang mga layunin, pamamaraan at mga kahihinatnan ng pananaliksik na ito. Ang aking lagda ay patunay na ako ay kusang lalahok sa pananaliksik na ito.

Lubos kong nauunawaan ang probiotic na KE-99 Lacto at ang epekto nito sa aking anak; na ito ay ligtas sa mga bata at may kaukulang benepisyo sa mga batang may dengue.

Nauunawaan ko rin ang si Dr. Comia -Capate ay gagamot sa aking anak kung sakaling may allergy sa KE-99 Lacto. Siya din ang magbabantay sa aking anak kung sakaling may mga side effects ang gamot na ito.

Walang kaugnayan si Dr. Comia- Capate kung sakaling may mga side effects sa KE-99 Lacto habang ito ay binibigay sa aking anak sa loob ng pananaliksik na ito. Ang lahat na kaganapan hinggil sa side effects ng KE-99 Lacto ay iimbistigahan ng mabuti.

_____

Pangalan ng Magulang, Lagda sa ibabaw nito

_____

Dr. Charon Comia- Capate

Punong Tagapanaliksik

APPENDIX-4

EFFECT OF KE-99 LACTO ON VITAL SIGNS

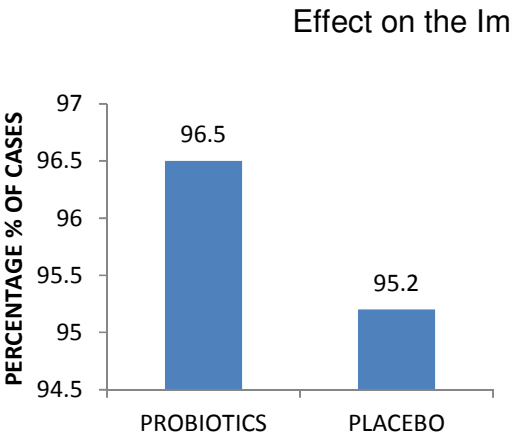

Figure-11 Stage 1 Dengue

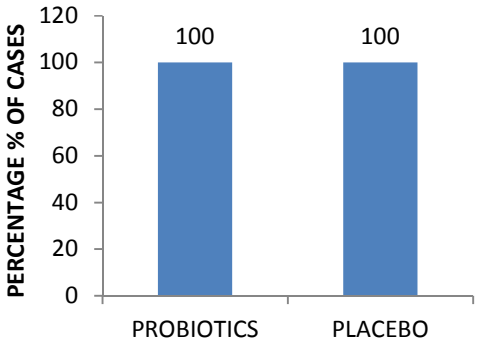

Figure-12 Stage-2 Dengue

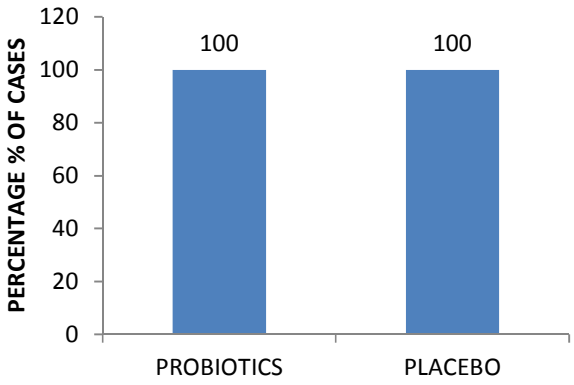

Figure-13 Stage-3 Dengue

APPENDIX-4

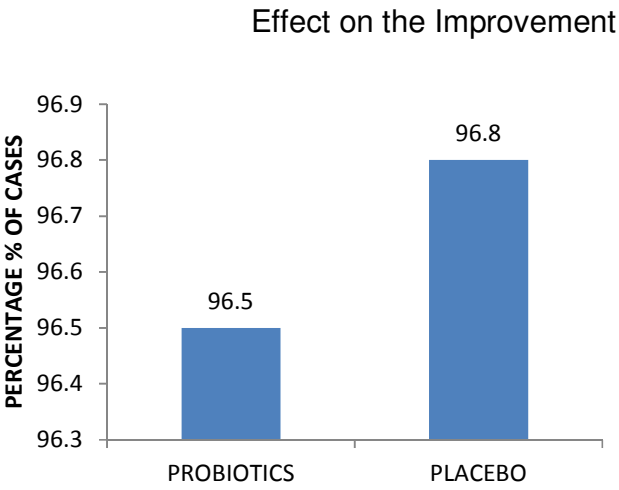

Figure-14 Stage-1 Dengue

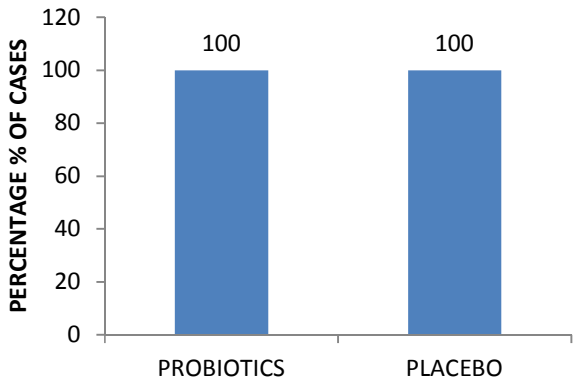

Figure-15 Stage-2 Dengue

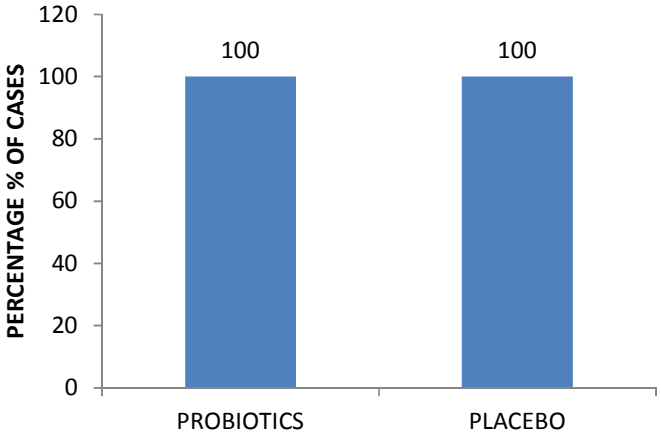

Figure-16 Stage-3 Dengue

APPENDIX-5

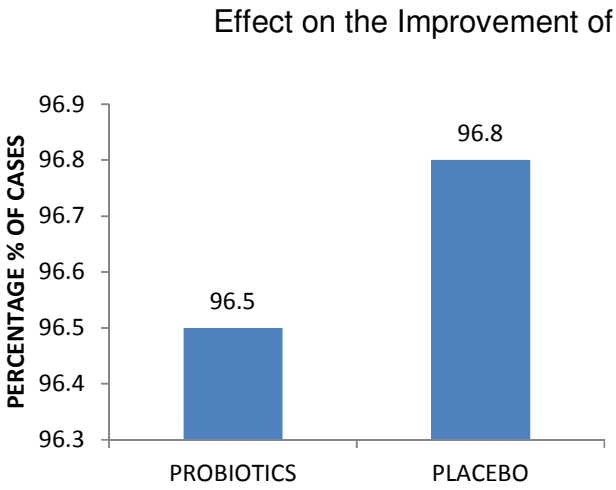

Figure-17 Stage-1 Dengue

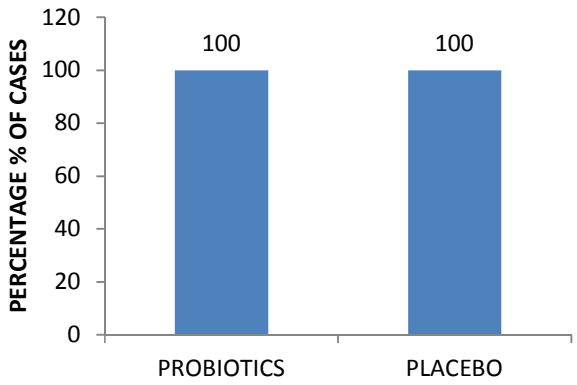

Figure-18 Stage-2 Dengue

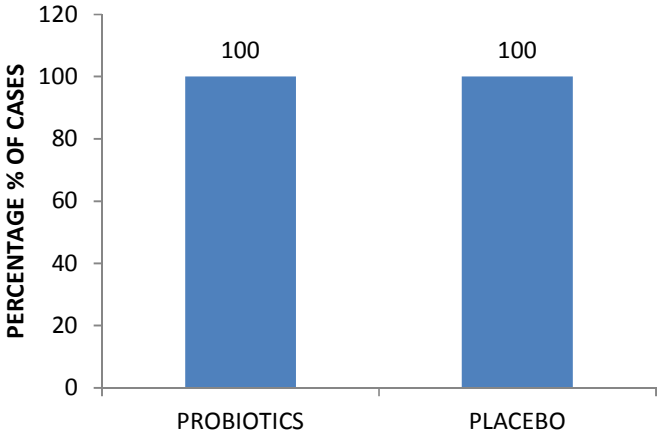

Figure-19 Stage-3 Dengue