STUDY PROTOCOL

Study Title:

A randomized, double-blinded, parallel, placebo-controlled study to investigate the efficacy of *Lb paracasei* [Junlebao Lp. N1115] as a probiotic to enhance gut development in young children

Brief Title:

A Study on Efficacy of Feeding N1115 Probiotic Supplement to Young Children (N1115Baby)

SPONSOR: Shijiazhuang Junlebao Dairy Co.,Ltd.

RESEARCHER: Atlantia Food Clinical Trials Ltd.

Teagasc

Date：01-11-2018

1. STUDY PROTOCOL

Name of Company:	Shijiazhuang Junlebao Dairy Co.,Ltd.
Study Product:	*Lactobacillus paracasei* N1115 versus placebo
Study Centre:	Atlantia Food Clinical Trials Ltd
Study Title:	A randomised, double-blinded, parallel, placebo-controlled study to investigate the efficacy of *Lb paracasei* [Junlebao Lp. N1115] as a probiotic to enhance gut development in young children
Study Design	Randomised, parallel, double-blinded, controlled
Objectives:	**Primary Objectives** - Intestinal health (stool frequency, stool consistency, GI symptoms including flatulence, bloating, cramps and diarrhea) **Secondary Objectives** - Gut microbiota composition - Incidence of UTI - Antibiotic usage
Population:	Children born by Caesarean Section, aged between 6 months and 3-years old
No. of volunteers:	Screen: n = 90 Randomize: n = 60 (30 per group)
Treatment:	*Lactobacillus paracasei* N1115 versus placebo
Duration/No. of visits:	4 Visits (Screening, Weeks 0, 4 &8)
Inclusion Criteria:	To be eligible for inclusion, the subject must fulfill all of the following criteria: 1. Male or female subjects aged ≥ 6 months and ≤ 3 years, 2. Parent/Guardian has given written informed consent, 3. Be in generally good health as determined by the investigator.
Exclusion Criteria:	The presence of any of the following criteria will exclude the subject from participating in the study: 1. Are currently taking probiotics or prebiotics, or have taken them in the past 2 weeks, 2. Unwilling to avoid probiotics / prebiotics for the duration of the study, 3. Have a significant acute or chronic coexisting illness (cardiovascular, gastrointestinal, endocrinological, immunological, metabolic or any condition which contraindicates, in the investigators judgement, entry to the study), 4. Having a condition or taking a medication that the investigator believes would interfere with the objectives of the study, pose a safety risk or confound the interpretation

	of the study results, 5. Subjects have taken antibiotics within the past 3 months (history of antibiotic use in previous 6 months recorded), 6. Subjects may not be receiving treatment involving experimental drugs, 7. If the subject has been in a recent experimental trial, these must have been completed not less than 60 days prior to this study.
Washout:	14 days from probiotics & prebiotics
Optional: **daily eDiary:**	Parent/Guardian would be provided with a tablet PC and be instructed to record daily bowel frequency, stool consistency and GI symptoms (including flatulence, bloating, cramps) of their child. Parent/Guardian would complete the diary daily for the 2 weeks prior to randomization and for the intervention period of 8-weeks.
Analysis	• Fecal microbiota • Fecal water metabolomics • Energy levels of infants-how to monitor it? • Level of antibiotic usage • Salivary cortisol

2. STUDY PROCEDURES

Study preparation:
Design and compile case report form (CRF) and questionnaires. Site initiation visit and training of clinical staff on all aspects of the study will be performed.
Subjects will be randomly assigned to placebo or study product and all study personnel and participants will remain blinded for the duration of the study.
This study will be conducted in accordance with ICH GCP.

The visit windows are set as outlined in the table & text below, but if exceptional circumstances occur then this window can be extended at the discretion of the Principal Investigator. To date there have been no visit window deviations.

STUDY:				
Visit Number	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**
Visit	Screening	Week 0	Week4	Week 8
Window	-28 to -14 days			
Informed Consent	x			
Inc/Excl.	x	x		
Demographic data	x			
Medical History	x			
Anthropometric Measurements (Weight,	x	x	x	x

height, BMI)				
Stool sample				
Saliva sample				
Randomization		x		
Admin Study Product		x	x	
Return Product/Compliance			x	x
Prior/con meds	x	x	x	x
AE/SAE		x	x	x
Optional:				
Daily eDiary (electronic device to capture daily reports/symptoms)	Stool Frequency, Consistency, GI Symptoms (including flatulence, bloating, cramps)			

Sample Collection & Processing:

Stool Samples: Subjects are instructed to collect a stool sample before attending Visit 2, Visit 3 and Visit 4. Each parent/guardian is provided with a stool collection kit with two stool sample containers, and instructed to collect a sample the night before the visit, and again on the morning of the visit. If the child produces a sample on the morning of the visit, the sample collected the previous evening is discarded. Once the sample is delivered to the Atlantia clinic, it is placed in a refrigerator and collected by Teagasc. This collection occurs each day samples are delivered onsite by subjects.

Saliva Samples: A saliva sample is collected from the subject during Visit 2, Visit 3 and Visit 4. The collection is carried out by a research nurse, who is trained according to Teagasc & Atlantia's standard operating procedure for the collection of saliva. The saliva sample is collected by inserting a swab into the child's mouth, letting it rest on the floor of the mouth for 30 seconds, allowing for soakage of any pooled saliva present. The swab is then rubbed on the child's tongue, roof of mouth and left and right buccal mucosa for 10 seconds each. Once collection has finished, the swab is placed into a vial and centrifuged at 1000g for 2 minutes. The separated saliva is aliquoted into a microtube and stored at -80℃. Once all subjects have completed the study and all samples have been collected, the samples will be shipped on dry ice for cortisol analysis.

QC, Data-entry& statistical analysis:

An independent study monitor will randomly review CRFs and source documentation to verify that the study is conducted in accordance with the protocol and GCP. After the last subject's last visit all data from the CRFs will be independently entered, verified and corrected and statistical analysis will be performed. A draft report will be presented to the sponsor and upon required revisions, the final version will be completed.