**Healthy Immunity in Children at Childcare Using Probiotics (HICCUP) Study**

**Background**

Respiratory tract infections in children under the age of 5 years are common and are a major cause of hospitalisation and even death. More minor respiratory tract infections often result in children being unable to attend childcare due to the illness, and their parent therefore being unable to attend work in order to care for them.

**The microbiota gut brain axis, inflammation and health**

The human gastrointestinal tract contains a colony of microbes that outnumber the number of cells in the human body. These microbes that include bacteria, fungi and viruses have an important role in physiology and biochemistry in the body and their function has been linked to health and behaviour. Dysbiosis is the term used to broadly refer to negative changes in the microbial balance in the gut. Dysbiosis influences brain chemistry via the microbiota gut brain axis. The gut brain axis refers to multiple bi-directional pathways that link the microbial balance in the gut with brain chemistry These pathways are also interact with the immune system, the neuroendocrine system (particularly the hypothalamic pituitary adrenal axis), the parasympathetic and sympathetic arms of the autonomic nervous system and the vagus nerve [1,2].

**Probiotics, mood and chronic fatigue syndrome**

Probiotic supplementation is one way in which the composition of the gut microbiota can be altered and the effects of dysbiosis reversed hence it has been suggested as a potential treatment for conditions in which dysbiosis and inflammation have been observed [3]. Probiotics are defined as live microorganisms that, when consumed in adequate quantities, confer a health benefit to the host. Previous studies have suggested that supplementation with probiotics can reduce the incidence, duration or severity of viral and cold and flu like illnesses in children, in turn reducing absences from child care [4-7]. However not all studies have reported a significant benefit of probiotics over placebo [6,8-10]. Some studies and recent reviews of the literature suggest that probiotic strains are not equal and that beneficial effects for immunity could vary by strain.

**AIM:**

This study aims to test whether supplementation of children with probiotic HN019 reduces the number of days that the children present with symptoms of a viral illness (cold or flu like symptoms).

**METHODS:**

**Participants:** The study will recruit 144 children aged between 0-5 years who attend a childcare centre for a minimum of two sessions a week.

**Eligibility:** Children will be eligible to participate in the study if they

* Are aged between 12 months and less than 5 years at the time of enrolment
* Attend childcare regularly2 sessions/week or more
* Have not had antibiotics in the last two weeks
* Do not have a sibling enrolled in the study
* Are not on immune suppressant therapy

**Recruitment:** Recruitment will be via online parenting groups and childcare centres. The primary investigator will contact the administrator of online groups to discuss the study and ask permission to post information about the study in their online forum. If approved by the administrator, the advertisement will be posted on the blog/group. Participants will be able to opt-in to the study. The advertisement will also be used for interested childcare centres to place either in electronic form on their website or in print form at the centre.

**Consent:** Participation will be dependent on consent from the parent/caregiver of the preschool aged child. Consent will be recorded electronically via a linked consent form. Consent verification will be automatically sent to the participant via automatic email reply.

**Data collection:** An online database platform will be used to collect initial information about the number of days the child attends childcare, presence of siblings and demographic information. Each week parents will be sent a text or email message (according to their preference) and asked to reply with the number of days their child has had cold and flu like symptoms in the last week.

**Intervention:** Children will be randomly assigned to receive capsules containing either the probiotic Bifidobacterium animalis HN019 (9×109 cfu) or placebo (corn derived maltodextrin). Parents will be asked to give their child one capsule per day for 10 weeks. Capsules can be broken and the powder mixed with liquids or food.

**Safety:** The probiotic strain *Bifidobacterium animalis* HN019 has been safely used in both pregnant women and infants in New Zealand studies [11,12]. Similar to other probiotic supplements, they can be purchased over the counter at health food stores and pharmacies.

Data collection from parents of pre-school aged children will be managed through a secure online database. At the time of registering for the study, parents will complete demographic information about themselves and their child. Parents will also complete a questionnaire collecting information about breastfeeding and allergic history.

Once registered for the study, capsules will be sent to the participants including instructions for how to administer the probiotic/placebo powder.

Each week for the 10 weeks of the study, we will send parents a text or email asking how many days in the previous week their child has had symptoms of a cold or flu such as a runny nose. Parents will be asked to send back a reply with the number of days (between 0 and 7).

**Statistical analysis:** The number of days with cold and flu like symptoms is the primary outcome measure for this study.  A sample size of 144 children attending for 2 sessions/week for the 10-week study period allows for a 90% chance of detecting a reduction of days with symptoms from 4% in the control group to 2% in the treatment group at the 2.5% level of significance.  Allowance for 20% attrition has been made.  An intention-to-treat analysis will be conducted in SAS 9.4 using generalised linear regression to analyse the average number of symptom days per week over the study period between the probiotic and placebo groups.

**Data management Plan:** During this study the researchers will record information about participants including name, address (to send the capsules to), demographic information and information from the study questionnaires. Participants cannot take part in the study if they do not consent to this information being collected.

Identifiable Information

Identifiable information is any data that could identify the participants (e.g. name, date of birth, or address). Only the researchers will have access to this identifiable information. Fonterra who are providing the capsules for the research, but are not funders or sponsors, will NOT have access to this data at any stage.

De-identified (Coded) Information

To make sure the participants’ personal information is kept confidential, information that identifies the participants will not be included in any report generated by the researchers. Instead, the participants will be identified by a study code. Only the primary researcher will keep a list linking these non-identifiable codes with the participants name, so that they can be re-identified by their coded data ONLY if needed, for example if a participant requests removal of their data or access to the data they provided to the researchers.

The results of the study may be published or presented, but not in a form that would reasonably be expected to identify any individual participants.

Future Research Using Information Collected in this Study.

Coded information may be used to inform future research related to interventions for preschool immunity in New Zealand.

Participants will not get reports or other information about any additional research that is done using their coded information.

Security and Storage of Information.

Identifiable information is held on the secure study database during the study data collection, this is password protected and only accessible to the researchers. After the data collection and analysis of study data is complete, data is split into two files, the first containing names, addresses and study code and the second file containing de-identified study information. The rationale for storing a linking file with names and study codes is so that the principal investigator can provide information to individual participants who may request their information in the future. After the study the linkage file and separate de-identified data are electronically stored in password protected form for 10 years in compliance with ethical and data management guidelines before the data is destroyed. All storage will comply with local and/or international data security guidelines.

Risks.

Although efforts will be made to protect the privacy of participants, absolute confidentiality of information cannot be guaranteed. Even with coded and anonymised information, there is no guarantee that participants cannot be identified. The risk of people accessing and misusing participants’ information is currently very small, but may increase in the future as people find new ways of tracing information.

Rights to Access Personal Information.

Participants have the right to request access to their information held by the research team. Participants also have the right to request that any information they disagree with be corrected.

Participants may access other study-specific information before the study is over, but this could result in the participant/s being withdrawn from the study to protect the study’s scientific integrity.

Rights to Withdraw Personal Information.

Participants may withdraw their consent for the collection and use of their information at any time, by informing the primary study researcher.

If participants choose to withdraw their consent, their participation in the study will end.

If the participant consents, the information collected up until their withdrawal from the study will continue to be used and included in the study. Participants may ask for it to be deleted when they withdraw, unless the participant withdraws after the study analyses have been undertaken.

**Non-university collaborators**

**Fonterra:** This is a researcher initiated project, Fonterra Cooperative Limited who manufacture the probiotic *B. animalis* HN019 will supply the probiotics and placebo capsules for this trial.  Both the probiotics and placebo are manufactured to pharmaceutical grade and undergo testing to ensure they comply with safety requirements. As part of the supply agreement, Fonterra will also undertake the randomisation of participants

**Cultural Considerations**

As researchers, we take our responsibilities under Te Tiriti o Waitangi seriously. This study has been designed according to the guidelines set out in Te Ara Tika: Guidelines for Māori Research Ethics. More specifically the following principles have been addressed

Whakapapa- relationships

Ideally the relationship between researchers and participants would be established using some kanohi-ki-te-kanohi (face to face) contact. The online consent and data collection methodology in this study means that face to face contact between the researchers and research participants is not possible at the present time. As researchers we acknowledge that this is not an ideal methodology for building a collaborative relationship between the researchers and participants. In our previous studies that have utilised a similar method of online consent and data collection we have had contact with participants in other ways to try and foster a relationship. In this study we aim to consider the importance of relationship by:

Ensuring all participants receive an email when they register for the study with information encouraging participants to make contact with the researchers if they have questions at any stage and ensuring that the contact details of the researchers are presented in multiple places (confirmation email, participant information sheet, capsule instructions insert). All emails or text messages from participants are responded to personally by the Principal Investigator.

The potential advantage of online consent and data collection is that it reduces the burden of participation by allowing participants to register and take part from any location at a time convenient for them. The study does not require attendance at any clinic visits and can be safely run under any COVID19 alert level.

Tika

Considering the importance of Tika this study will collect ethnicity data according to the recommended prioritisation ethnicity framework. While we do not anticipate having a sample size big enough to analyse results stratified by ethnicity we recognise the importance of collecting this information so that we can 1) describe our sample and evaluate its relevance to the wider group of children in Aotearoa New Zealand and 2) help to inform future studies collecting ethnicity data.

We hope that the online consent and data collection methodology will reduce barriers to participation in our study.

Mana

The right of participants to be fully informed and to give consent. The Participant Information Sheet fully lays out what participation in the study involves. Potential participants are encouraged to discuss participation with whānau. As researchers we also acknowledge our responsibility to treat all participants with respect and aroha and to respect privacy and confidentiality. All data is kept securely and no identifying data is shared with others. Participants have the right to withdraw their data if they wish to.

**Ethical approvals and trial registration**

An application for ethical approval for the study will be made to the Health and Disability Ethics Committee. The trial will be prospectively registered with the Australia and New Zealand Clinical Trials Registry.

**Significance of the Research:** Although there has been a great deal of research interest in the gut microbiota and health, to date, there have been very few studies examining the potential benefit of probiotic supplementation for the reduction of cold and flu like symptoms in pre-school aged children. Those that have been done have yielded conflicting results. If probiotics are found to reduce the incidence of cold and flu like symptoms in pre-school aged children, this may inform future trials of probiotic supplementation, and potentially inform public health policies.

**References**

[1] Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nature reviews. Neuroscience*. 2012;13:701-712.

[2] Mayer EA, Knight R, Mazmanian SK, et al. Gut Microbes and the Brain: Paradigm Shift in Neuroscience. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2014;34:15490-15496.

[3] Hemarajata P, Versalovic J. Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. *Therapeutic Advances in Gastroenterology*. 2013;6:39-51.

[4] King S, Glanville J, Sanders ME, et al. Effectiveness of probiotics on the duration of illness in healthy children and adults who develop common acute respiratory infectious conditions: a systematic review and meta-analysis. *The British journal of nutrition*. 2014;112:41-54.

[5] Leyer GJ, Li S, Mubasher ME, et al. Probiotic Effects on Cold and Influenza-Like Symptom Incidence and Duration in Children. *Pediatrics*. 2009;124:e172-e179.

[6] Laursen R, Hojsak I. Probiotics for respiratory tract infections in children attending day care centers—a systematic review. *Eur J Pediatr*. 2018;177:979-994.

[7] Dekker J, Xu L, , Hong Q , Xiaoyang S. Bifidobacterium animalis subsp. lactis HN019 protects against respiratory tract infections in Chinese infants. *JPGN*. 2017:760.

[8] Cáceres P, Montes S, Vega N, et al. Effects of Lactobacillus rhamnosus HN001 on acute respiratory infections and intestinal secretory IgA in children. *Journal of Pediatric Infectious Diseases*. 2010;5:353-362.

[9] John D Cowden. Probiotics Did Not Prevent Child Care Absences Due to Infection in Danish Study. *NEJM Journal Watch. Pediatrics & Adolescent Medicine*. 2017.

[10] Wang Y, Li X, Ge T, et al. Probiotics for prevention and treatment of respiratory tract infections in children: A systematic review and meta-analysis of randomized controlled trials. *Medicine*. 2016;95:e4509.

[11] Slykerman RF, Hood F, Wickens K, et al. Effect of Lactobacillus rhamnosus HN001 in Pregnancy on Postpartum Symptoms of Depression and Anxiety: A Randomised Double-blind Placebo-controlled Trial. *EBioMedicine*. 2017;24:159-165.

[12] Wickens K, PhD, Black PN, FRACP, Stanley TV, FRCP, et al. A differential effect of 2 probiotics in the prevention of eczema and atopy: A double-blind, randomized, placebo-controlled trial. *Journal of Allergy and Clinical Immunology, The*. 2008;122:788-794.