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A placebo-controlled, randomized, double blind study of a galacto-oligosaccharide in reducing traveller's diarrhoea.

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Background

Traveller's diarrhea (TD) affects nearly one third of all travellers to tropical and subtropical regions. The risk of diarrhea is highest in the beginning of the stay: 62% occurs within the first week. General precautions have no documented effect (Steffen et al., 1983). Prophylactic use of antibacterial agents is cost-effective on a short-term expensive travel, but can give side effects, notably overgrowth syndromes (Ericsson 1998). Furthermore, an extensive use of prophylactic antibiotics in millions of travelers will most likely have a significant impact on emerging antibiotic resistance worldwide. Vaccines against TD would be an environmentally friendly way of prophylaxis. However, because of the great number of different causative agents of TD (Shah 2009), the development of a universal TD vaccine seems unlikely. The most common pathogen causing TD is enterotoxigenic *Escherichia coli* (ETEC), accounting for 30.4% of all TD (Shah 2009). There are two types of enterotoxin produced by *E. coli*, i.e., thermo labile (LT) and thermo stabile (ST) enterotoxin. An oral B-subunit/whole-cell cholera vaccine has been found to reduce the risk of TD caused by LT enterotoxin producing ETEC with 60%, with an overall reduction of TD 31% in controls to 24% in the vaccinated group (Peltola 1991). Overall, 56% of wild-type strains of ETEC are either LT or LT/ST producing. A recent study of a dermal patch vaccine against LT enterotoxin failed to show any protective effect against TD (Steffen et al. 2013). There is a need of an effective, ecologically friendly and affordable way of reducing the risk of TD.

It has been suggested to improve human health by increasing the number of beneficial bacteria in the gut, either by consuming live bacteria, called probiotics, or by prebiotics, which are defined as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon" (Gibson & Roberfroid 1994). Prophylaxis with probiotics (e.g., *Saccharomyces boulardii*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*) seems to have a minor effect on the risk of TD; A metaanalysis of probiotic prophylaxis showed a TD Risk Ratio of 0.85, 95% confidence interval 0.79-0.91, $p < 0.001$ (McFarland 2007). A galacto-oligosaccharide produced by fermenting lactose with a *Bifidobacterium* strain, B-GOS, (Tzortzis et al. 2005) is shown to increase the number of *Bifidobacterium* in the intestinal flora (Depeint et al., 2008). Moreover, there are indications that GOS, apart from its prebiotic properties, may act by reducing the invasion of *Salmonella typhimurium* in host cells (Searle et al., 2009), and it has also been suggested that GOS may reduce the attachment of *Entamoeba histolytica* trophozoites (Jantscher-Krenn 2012). A double-blind study comparing a galacto-oligosaccharide (B-GOS, Bimuno, Clasado Ltd, Milton Keynes, UK) and placebo found that the incidence of traveller's diarrhea in the treatment group was 19/81(23,4%) versus 30/78(38,5%) in the control group, i.e., a 39% reduction in the risk of TD ($P < 0,05\%$) (Drakoularakou et al., 2010). If this is a reproducible finding, GOS could potentially dramatically reduce the risk of TD. This finding need to be confirmed in a larger study, and the purpose of this study is to test if the findings of Drakoularakou et al., 2010 can be confirmed in a larger study. GOS has a mild laxative effect and can

also increase flatulence, but doses less than 12 g per day are usually well tolerated (Niittynen et al. 2007).

Investigational medicinal product (IMP)

Bimuno® GOS is a galactooligosaccharide mixture produced by the transgalactosidic activity of Bifidobacterium bifidum NCIMB 41171 beta-galactosidase . For further details, see the Investigator's brochure (IB). First a syrup is produced, which is then converted to pastilles.

B-GOS is distributed by Bimuno, Clasado Ltd, Milton Keynes, UK, and manufactured by Ernest Jackson & Co. Ltd.

Medicines and Healthcare Regulatory Agency (MHRA)

Certificate No: UK MIA 94 Insp GMP/GDP 94/18415-0023

The manufacturer of the pastilles is:

Ernest Jackson & Company Limited

29 High Street

Credition

Devon

EX17 3AP

United Kingdom

The syrup which then is converted into pastilles is made by:

Groupe LACTALIS

10-20 Rue Adolphe Beck

53089 LAVAL cedex 9

It is considered that it complies with the principles and guidelines of Good Manufacturing Practice laid down in the Directive 2003/94/EC

They are authorized for the production of Human medicinal products,

Hypothesis

We hypothesize that in a placebo-controlled trial, ingestion of GOS will reduce the incidence of traveller's diarrhea.

Study design

Method:

Participants for the study will be recruited among clients at Reisekliniken. All clients will be assessed for eligibility on arrival, and those who are eligible will be given an information sheath (Attachment 1) about the study. During the pre-travel consultation, eligible clients will be asked if they are willing to participate in the GOS study.

Inclusion criteria:

- Healthy male or female

- Age ≥ 16 years
- Travelling to countries with high risk of TD in Asia, Africa and Latin America for 7-15 days
- Willing to participate in the study
- Able to comply with the protocol

Exclusion criteria:

- Any acute or chronic gastrointestinal disease
- Current abdominal discomfort
- Intolerance for lactose or other types of sugar
- Any current medication with antibiotics, antacids or other medication that influences stomach acid production.
- Use of other prebiotics or probiotics (including the sour milk product Biola)

Intervention:

Enrolled subjects will receive Bovine gelatin-based Pastilles containing galacto-oligosaccharide (B-GOS, Bimuno), 0,9 g per pastille, or placebo (maltodextrine), provided by Clasado. The pastilles are declared *halal* and *kosher*. Dosage 3 pastilles per day, from five days before travel until the return day. The pastilles will be packed in blister packages marked with a letter code, of 30 pastilles. The necessary number of additional pastilles of the same letter code will be added. The packages will be labelled with the patients' names (Attachment 2). The participants will be informed that they must only use their own pastilles, and not give them to anyone else.

The study period is from start of ingesting the pastilles five days before departure, until seven days after return.

Three or four of the staff members will be given the responsibility for the information of the participants, registration in the study and dispensing of the study medicine, in the following called the "study staff". A room will be dedicated for the study, in the following called "the study room", which otherwise is only used as a deposit. The study room will be equipped with a computer for the registration of the study. This computer will not be connected to the Internet or Reisklinikken's network. All data in the study will be registered in an Excel-file, in the following called the "GOS-database". After the vaccinators have finished the work the study staff will bring the participants to the study room.

Data on enrollment:

- Name
- Birth date
- Contact information
- Sex
- Itinerary (day out, day home, country/countries)
- Cholera vaccine
- Number of pastilles delivered
- Code and batch number of study drug

The participants will be asked register all episodes of diarrhea in a registration form (Attachment 3). This form must be returned to Reiseklinikken after return, although, if they have had no problems at all, an email or a telephone message to the study staff will be sufficient. They will be informed that they should report any possible side effects of the pastilles to Reiseklinikken. They will be informed that they will be given a free consultation if they are still sick after return. It is easy for everybody to reach Reiseklinikken by telephone during the work hours, or by email. Those who have complaints, i.e, diarrhea, fever or abdominal discomfort within a week after return will be offered a free consultation the first working day after return, ideally, the same day. They will get transport media for stool cultivation of Salmonella, Shigella, Capylobacter and Yersinia, and PCR for Enterotoxigenic E. coli (ETEC-PCR). Supplementary examinations will be done, if clinically indicated, but they will not be a part of the study. Although the participation in the study ends one week after the return, free additional clinical follow-up will, If needed, be provided at Reiseklinikken.

Data that will be registered during the study period:

- Any adverse events/possible side effects of the study drug
- Incidence of diarrhea
- Start and duration of diarrhea
- Maximum bowel movements per day
- Fever
- Stomach pain
- Seeking health care/hospitalisation during travel
- Treatment during travel
- Consultation after return
- Results of microbiological examinations
- Withdrawal from the study, and if available, cause of withdrawal
- Remarks

Consent:

After having received oral and written information, all participants must sign a written consent (Attachment 4).

Withdrawal:

Oral and written information on withdrawal will be provided on enrollment. The participants may at any time withdraw from the study. We will in that case ask them why they want to withdraw

Adverse events:

In the doses used in this study GOS has no known side effects. However, idiosyncratic reactions may occur. Furthermore, there may be previously undiagnosed intolerance of lactose, allergic reactions to the constituents of the pastilles and discomfort of any cause that the participants may attribute to the pastilles. Possible side effects could be diarrhea, nausea, bloating, flatulence and stomach pain, as well as unspecific adverse events, like rash, headache and fever. All adverse events reported by the participants will be registered in the GOS database. If the adverse events start during the five days the participants take the pastilles before travel they should stop taking the pastilles, and be

registered as “discontinued because of adverse events“. They will be followed up by asking when the symptoms stopped after stopping the pastilles. If necessary they will be offered a free consultation at Reiseklinikken. If the adverse events start during travel we must leave to the participants to decide whether they will stop the pastilles or not, but report if they continue the pastilles or not, and when the symptoms disappear. All adverse events will be reported in the year report.

If potentially serious side effects of the GOS-pastilles, e.g. urticaria, is suspected, the pastilles must be stopped immediately, and the incidence will immediately be notified to Relis/Legemiddelverket on the official form. In such cases, the letter code can be sent with the notification to Relis/Legemiddelverket, who can get the information from Clasado if the patient has got GOS or placebo. If preliminary observations indicate unexpected serious adverse effects, we may have to suspend the study, until Legemiddelverket can declare that the study may continue.

The pastilles should be continued if the participants become ill during the travel, also when taking medicines/products that are prohibited before inclusion in the study, but this must be registered in the registration form.

Definition of diarrhea (WHO):

The passage of 3 or more loose or liquid stools per day, or more frequently than is normal for the individual.

End points:

- Incidence of diarrhea
 - fever
 - number of bowel movements
 - duration of traveller’s diarrhea.
- Incidence of pathogens in returned travelers that still have diarrhea

Randomization

The pastilles will be packed with a letter code “A” to “H”, containing either GOS or placebo. The code will be kept by Clasado, and will not be disclosed before the data collection is completed. We will carry out a simple block randomization, with a random list from A and H, where half of the letters represent GOS, the other half placebo. Families or groups travelling together will get individually marked packages of pastilles.

Information

The travelers are offered an information sheet about the study, where they are asked to sign if they are willing to participate in the study. Those who accept to participate will get the appropriate number of pastilles and are told to take the pastilles from five days before departure, until they arrive at home. They will get a form to register any adverse events, diarrhea, number of bowel movements, blood in the stool, and hospital admissions and treatment during or within one week after the travel, when the form will be returned to Reiseklinikken in a closed envelope. If they still have diarrhea when they come home, they will be offered a free consultation at Reiseklinikken, and the stool will be tested for Salmonella, Shigella, Yersinia, Campylobacter and ETEC, in three stool

samples at the department of medical microbiology, Drammen sykehus, Vestre Viken HF. For those who return with diarrhea or get diarrhea within a week after return, we will have to wait for the microbiological results. Therefore, data for each patient will be recorded maximum two weeks after return. If later follow-up is needed it will not be a part of the study, but provided free for the patients.

Compliance issues

Each recruited participant will be instructed by a nurse or medical doctor about the study. They will get an information sheet about the study, telling that they may any time withdraw from the study, but that it is much better that they withdraw before start than during the study. This will also be emphasized by oral information. They will also be instructed in how to complete the "diarrhea log", which will be the most important part of the information, as failure to fill in the log correctly would mean lost results. The participants will have to give a consent that Reiseklinikken may contact them by telephone, mail or email after the travel.

As the pastilles are well tasting, and easy to eat, it should not be a big problem for the participants to take them. However, if two or more are travelling together they may get different preparations, and there would be a risk of taking the wrong pastilles. Therefore, it will be emphasized during the information that each packet of pastilles will be marked with the participants' names and the dosage for each patient, and that it is absolutely necessary for the trial that they take pastilles from their own packages.

Storage of data

All data concerning the study will be registered in the GOS-database on a dedicated, password-protected computer. After publishing the study, the protocol data will be stored anonymized on a CD for fifteen years, according to § 8-2 [Forskrift om klinisk utprøving av legemidler til mennesker FOR-2009-10-30-1321](#). Clinical data from the ones who have diarrhea after return will be stored in Reiseklinikkens database, according to Forskrift om pasientjournal (01.01.2001).

Statistical considerations

To have a 95% chance of getting significant results if the reduction of risk of traveler's diarrhea is from 30% to 22,5%, i.e., 25 % reduction of the risk, we need a Sample size of 800 clients. Postulating that 30% would discontinue the study, we would need to include 1200 clients. Reiseklinikken vaccinates about 12,000 clients per year, and a sufficient number of participants can therefore be recruited from this clinic alone. About one third of our clients would be eligible for the study. Therefore it should be possible to recruit enough clients during a year.

The data will be processed by a Generalized Linear Model, where the incidence, severity and duration of diarrhea, the baseline data, notably the use of cholera vaccine, and the results of microbiological examinations can be treated as separate and interacting effects.

Storing and logistic of the test preparation

The pastilles, containing GOS or placebo, will be stored in a locked room, at room temperature. They will be marked with the code letter A-H. As there will be different amounts of pastilles needed for

each participant, according to age and duration of the travel, i.e., 10-60 pastilles, we cannot know how many pastilles will be needed for the whole project. Considering a delivery time of two weeks we will store pastilles of each letter for at least three weeks use. As there will be only eight different pastilles, which are stored in one room, a visual assessment of the store will be adequate.

Time schedule

- - Start the recruiting of participants medio June 2014
- The recruiting will be ended when 800 completed forms are returned. This should be achieved within December 2014. The forms that are returned after the recruiting is ended will be added to the study.
- Data processing and writing in January 2015.
- Submission during the spring 2015.

Collaborators

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Ethical evaluations

An application to REK sør-øst (2014/149) was sent the 17. February 2014, and the response from REK is dated 7th March 2014. The project is accepted, but some conditions must be met:

1. Minimum age of inclusion is 16 years.
2. A final evaluation and acceptance from Statens Legemiddelverk.
3. The economic appointment between Bimuno and the researchers must be sent REK for orientation.
4. The economic expenses for microbiological examinations must be covered by Reiseklinikken
5. The patients information sheets must be rewritten according to the instructions made by REK, and must be sent to REK

As diarrhea is the most common infectious complaint in travelers, and the only way to find out if GOS is effective in the prevention of diarrhea is a double blind placebo controlled trial, this is a highly relevant study. Furthermore, as 760000 children in poor countries die from diarrhea every year, according to WHO, the results of the study could have an immense effect in international health.

Galactooligosaccharides are chains of three to ten carbohydrate rings. They are naturally occurring substances in human milk, and are added in some milk substitution formulae, and are considered non-toxic and without side effects in the amounts used in this study. They are not routinely used as a prophylactic against travellers' diarrhea in Norway. The probiotics used, which are excluded from the study, are documented to have a poor effect and therefore the study will not significantly exclude any important prophylaxis. Prophylaxis with antibiotics is effective, but not recommended by Norwegian health authorities.

If the participants experience adverse effects they can be registered in a dedicated column for adverse effects in the diarrhea log. Unexpected or serious adverse effects will be reported to RELIS/Legemiddelverket.

All participants will be insured in Legemiddelforikringen.

Economy

The company Clasado will deliver the material free, and will pay for the insurance of 800 participants. The routine examinations of stool samples are tests, and the PCR-test for ETEC will be covered by the researchers. No salary will be paid to the researchers, but Clasado may cover the costs for the microbiological examinations. All the costs with recruiting participants, collecting and treatment of data will be covered by Reiseklinikken.

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produced by the enzymic activity of Bifidobacterium bifidum, reduces Salmonella enterica serovar Typhimurium infection in mice. Journal of Medical Microbiology. 58:37–48.

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WHO health topics: <http://www.who.int/topics/diarrhoea/en/>

Attachments:

1. Informasjonsskriv til mulige deltakere i GOS-studien
2. Etikett
3. Registreringsskjema
4. Samtykkeskjema

We, the investigators, declare that Forskrift om klinisk utprøving av legemidler til mennesker FOR-2009-10-30-1321 and ICH guidelines E6 for GCP are followed.

Oslo, dato

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Lege Gunnar Hasle, prosjektleder

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Lege Ragnhild Raastad

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Lise Heier, statistiker

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Professor Pål A. Jenum

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