Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

VKM Report 2016: 02

Health risk assessment of a food supplement containing *Lactobacillus reuteri* Protectis®

Opinion of the Panel on biological hazards of the Norwegian Scientific Committee for Food Safety

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2016: 02
Health risk assessment of a food supplement containing *Lactobacillus reuteri* Protectis®

Opinion of the Panel on biological hazards of the Norwegian Scientific Committee for Food Safety
10.03.2016

ISBN: 978-82-8259-191-1
Norwegian Scientific Committee for Food Safety (VKM)
Po 4404 Nydalen
N – 0403 Oslo
Norway

Phone: +47 21 62 28 00
Email: vkm@vkm.no

www.vkm.no
www.english.vkm.no

Suggested citation: VKM. (2016) Health risk assessment of a food supplement containing *Lactobacillus reuteri* Protectis®. Scientific Opinion of the Panel on biological hazards of the Norwegian Scientific Committee for Food Safety, ISBN: 978-82-8259-191-1, Oslo, Norway.

Health risk assessment of a food supplement containing *Lactobacillus reuteri* Protectis®

Authors preparing the draft opinion

Judith Narvhus (chair), Jørgen Lassen, Danica Grahek-Ogden (VKM staff)

Assessed and approved

The opinion has been assessed and approved by Panel on Biological Hazards. Members of the panel are: Yngvild Wasteson (chair), Karl Eckner, Georg Kapperud, Jørgen Lassen, Judith Narvhus, Truls Nesbakken, Lucy Robertson, Jan Thomas Rosnes, Olaug Taran Skjerdal, Eystein Skjerve, Line Vold, Siamak Yazdankhah

Acknowledgment

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed a working group consisting of VKM members to answer the request from the Norwegian Food Safety Authority/Norwegian Environment Agency. Project leader from the VKM secretariat has been Danica Grahek-Ogden. The members of the working group Jørgen Lassen, Judith Narvhus are acknowledged for their valuable work on this opinion. The Panel on Biological Hazards are acknowledged for comments and views on this opinion.

Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

Table of Contents

Summary

The Norwegian Scientific Commitee for Food Safety (VKM) appointed a working group of experts to answer a request from the Norwegian Food Safety Authority regarding health risk assessment of *Lactobacillus reuteri* Protectis® in a food supplement intended for use by infants and young children. The mandate of this health risk assessment was not to evaluate the health claims related to the products as such health claims are assessed by EFSA.

The specific strain DSM 17938 is a "daughter strain" of the strain ATCC 55730 which was originally isolated from normal human milk. ATCC 55730 harbours two plasmids carrying transferable resistance genes against tetracycline and lincosamides respectively. The "daughter strain" DSM 17938 was established in 2008 by curing the ATCC 55730 for these plasmids, but is in all other respects claimed to be identical to ATCC 55730 and bioequivalence of the two strains has been suggested. The strain DSM 17938 was still resistant to tetracycline (although at a considerably lower level than ATCC 55730) and a number of other antibiotics, but these resistances were all considered being intrinsic by FBO. The absence of possible transferable/mobile genes has, to our knowledge, not been confirmed in later studies.

We are not aware of any data indicating that *L. reuteri* has been the cause of serious human diseases – and none of the studies examined has reported any adverse or undesirable short time effects. It has also been used in preterm infants with dosage corresponding to the actual recommended doses - without reporting any adverse, short term reaction. There is therefore no evidence leading to consider the strain DSM 17938 at the dosage recommended as unsafe.

However, more long-term data are still lacking and the long-term safety for the age groups considered in this assessment cannot be established. As evidence is accruing that the early microbial composition of the infant gut is important for the development of the gut flora and the immune system of the growing child, it is not possible to exclude that a daily supply of a particular bacterial strain over a prolonged period of time to an immature gastro-intestinal tract may have long-term, albeit still unknown, adverse effects on it's development.

As the long-term data are lacking it is not possible to answer whether the amount of the food supplement or the age of the infant or young child is of importance.

However, if later long-term data should reveal any adverse reaction, it is reasonable to assume that the actual age group will be the most vulnerable.

As the safety was not entirely established, the question of whether there are any vulnerable groups (i.e. premature, infants or children with diseases) where there are health risks associated with the intake of *Lactobacillus reuteri* Protectis®, as a food supplement was not considered.

Key words: VKM, risk assessment, Norwegian Scientific Committee for Food Safety, Norwegian Environment Agency, *Lactobacillus reuteri* DSM 17938, probiotic, food supplement, infant, neonate, young children, microbiome

Sammendrag på norsk

Vitenskapskomiteen for mattrygghet (VKM) har på oppdrag fra Mattilsynet etablert en ekspertgruppe for å utarbeide en risikovurdering for bruk av *Lactobacillus reuteri* Protectis® som kosttilskudd beregnet for barn fra «fødselen til 3 år». Det har ikke vært vurderingens mandat å vurdere eventuelle helsepåstander da slike påstander vurderes av EFSA.

Den aktuelle stammen DSM 17938 er en «datterstamme» av stamme ATCC 55730 som opprinnelig ble isolert fra normal human melk. ATCC 55730 inneholder to plasmider med overførbare resistensgener mot henholdsvis tetracyclin og lincosamid. «Datterstammen» DSM 17938 ble etablert i 2008 ved at de to plasmidene ble fjernet, men hevdes i alle andre henseender å være identisk med ATCC 55730. Man har derfor antatt at det foreligger «bioekvivalens» mellom de to stammene.

Stammen DSM 17938 må fortsatt anses å være resistent mot tetracyclin (om enn på et betydelig lavere nivå enn ATCC 55730) og mot en rekke øvrige antibiotika, men disse ble alle antatt å representere iboende, ikke-overførbare resistenser. Fraværet av mulige overførbare/mobile gener har, så langt vi vet, ikke blitt verifisert i senere studier.

Vi er ikke kjent med at *L. reuteri* skal ha vært årsak til alvorlige humane sykdommer – og ingen av studiene som er blitt vurdert her har rapportert om bivirkninger eller uønskete korttidseffekter. Det er derfor ingen grunn til å formode at stammen DSM 17938 i de dosene som anbefales skulle kunne gi opphav til slike effekter.

Det mangler imidlertid fortsatt langtidsdata, og eventuelle langtidseffekter for den aktuelle aldersgruppen («fra fødselen til 3 år») kan derfor ikke vurderes. Det er en klart økende erkjennelse av hvilken avgjørende betydning den tidlige sammensetningen av intestinal mikrobiota hos småbarn kan ha for den senere utviklingen av tarmfloraen og det immunologiske systemet. Man kan derfor ikke utelukke muligheten for at en kontinuerlig tilførsel av en spesifikk stamme over en lengre periode til en fortsatt umoden gastro-intestinal trakt kan ha uønskete, om enn fortsatt ukjente, langtidseffekter på denne utviklingen.

Fordi langtidsdata mangler, er det ikke mulig å besvare spørsmålet om den angitte doseringen av kosttilskuddet er av betydning.

Det er imidlertid rimelig å anta at dersom senere langtidsdata skulle vise uønskede effekter, er det nettopp den aktuelle aldersgruppen som vil vise seg å være mest sårbar.

I og med at muligheten for at en kontinuerlig tilførsel av en spesifikk stamme over en lengre periode til en fortsatt umoden gastro-intestinal trakt kan ha uønskete langtidseffekter ikke var utelukket ble spørsmålet om helserisiko for sårbare grupper ikke vurdert

Abbreviations and/or glossary

Abbreviations

ATCC	American Type Culture Collection
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
EFSA	European Food Safety Authority
FBO	Food business operator
FDA	Food and Drug Administration
GRAS	Generally regarded as safe
ISC	Intestinal stem cells
MIC	Minimal inhibitory concentration
PCR	Polymerase chain reaction
QPS	Qualified presumption of safety
TFEF	Time to full enteral feeds
SOP	Standard operating protocols

Background as provided by the Norwegian Food Safety Authority/ Norwegian Environment Agency

The Norwegian Scientific Committee for Food Safety (VKM) has published several risk- and benefit assessments concerning microorganisms in foods intended for infants and young children.

One of VKMs conclusions from these assessments is that intake of a monoculture of a particular strain, in large quantities over a prolonged period of time, may have unknown adverse effects on infants (0-12 months). At this age, infants are in an immature and vulnerable phase with regard to the establishment of their intestinal flora and the development of the immune system. They therefore also have the highest risk for possible unwanted health effects from a daily intake of microorganisms.

All foods on the Norwegian market shall comply with the requirements of safety in The Food Act paragraph 16. Paragraph 16 is based on article 14 in Regulation (EU) No 178/2002 (General Food Law) which also is incorporated into Norwegian Law. In accordance to article 14 no. 4 c) "the particular health sensitivities of a specific category of consumers where the food is intended for that category of consumers» is of importance when determining whether the product is safe for the group in question, in this case infants and young children.

The objective of the regulation on food supplements is to protect consumers against potential health risks from those products and to ensure that the consumers not are provided with misleading information. The regulation gives provisions on composition, labelling, marketing and distribution of pre-packed food supplements for consumers. The regulation does not deal specifically with microorganisms.

The Norwegian Food Safety Authority (NFSA) has, based on previous assessments by VKM, prohibited the sale of certain foods for infants and young children containing microorganisms.

A food business operator (FBO) distributes "Semper Dråper", a food supplement containing *Lactobacillus reuteri* Protectis®, in Norway. The FBO informs that, by September 2013, 590 million doses of the product have been sold. The importer has provided documentation about the strain in line with "Guidelines for assessment of safety aspects of probiotic (food) products" (VKM Report 2014:5). Further documentation was submitted to support that the strain is safe and suitable for infants and young children, see (1Literature search).

With reference to the criteria for safety, the NFSA requests the VKM for a health risk assessment of the food supplement in question. The supplement is a liquid suspension of *Lactobacillus Reuteri* Protectis®, and marketed for infants and young children.

Terms of reference as provided by the Norwegian Food Safety Authority

The NFSA requests VKM to answer the questions below:

Lactobacillus reuteri Protectis®, provided as a food supplements to infants (0-12 months) and young children (12-36 months):

1. are there any health risks associated to the intake of *Lactobacillus Reuteri* Protectis®, as a food supplement, in healthy infants and young children?
    a. if so, - what kind of risks are associated with the intake of *Lactobacillus reuteri* Protectis®, as a food supplement, in healthy infants and young children?
        i. Is the amount of the food supplement of importance?
        ii. Is the age of the infant or young child of importance?
    b. if not, - are there any vulnerable groups (i.e. premature, infants or children with diseases) where there are health risks associated to the intake of *Lactobacillus reuteri* Protectis®, as a food supplement, in infants and young children?

1 Literature search

Data sources are articles and reports submitted by the FBO.

The following reports and articles have been provided by the FBO:

- Bio Gaia Documentation check list,
- Bio Gaia List of references (Abrahamsson, Jakobsson, Bjorksten, Oldaeus, & Jenmalm, 2013; Ceratto, De Marco, Calabrese, & Savino, 2014; Chau et al., 2015; Di Nardo et al., 2014; Jensen, Grimmer, Naterstad, & Axelsson, 2012; Kleinhans, Jockel-Schneider, Rehn, Schlagenhauf, & Wuerzburg, 2011; Oncel et al., 2015; Rojas et al., 2012; Urbanska & Szajewska, 2014)

Other relevant background papers used in this assessment are previous opinions on probiotics from VKM:

In addition, the following two literature searches were performed in the PUBMED:

Wed Sep 30 06:02:22 2015

Search: (reuteri[Title/Abstract]) AND infants[Title/Abstract] Filters: Review

Returned 9 articles

Wed Sep 30 06:04:55 2015

Search: (reuteri[Title/Abstract]) AND infants[Title/Abstract] Filters: Review

Returned 1 article

1.1 Relevance screening

The titles of all articles were scanned, and for those that were of potential relevance, the abstracts were also inspected. The relevance screening was performed by the members of the ad hoc group, independently. Citations were excluded if they did not relate to the terms of reference. The reference lists in selected citations were scrutinised to identify additional articles or reports, overlooked by the PubMed searches.

2 Introduction

There are no regulatory guidelines for the use of microorganisms as probiotics in Norway. In this assessment, we have defined neonates as children less than one month, infants as children between 1 and 12 months and young children as 12-36 years of age.

This assessment is based on the evaluation of the documentation listed above, under the section Literature. The submitted data provided by the FBO give information regarding the probiotic strain, safety and efficacy of their products.

The provided and obtained information has been used to assess the safety aspects of the microorganism *Lactobacillus reuteri* DSM 17938 in an oil suspension, which may be given in the form of supplementary drops to children from birth to 3 yr.

The mandate of this report is to assess only the safety aspects of the product and not the efficacy of the product or to evaluate health claims.

2.1 The development of intestinal microbiota in the neonates, infants and young children

The gut microbiota of a neonate is initially strongly dependent on the mother's microbiota, the mode of delivery and the birth environment and is subject to ongoing intensive research. The establishment of the microbiota occurs in a stepwise fashion. Studies on mice have shown that the first bacteria to colonise the intestine, even prior to delivery and during the perinatal period ("pioneer bacteria") can modulate gene expression in host intestinal epithelial cells – which may influence the nature of subsequent intestinal colonisation (Endo, Tang, & Salminen, 2015).

In the neonate, initial colonisation with facultative anaerobes such as enterobacteria, lactobacilli and streptococci is rapidly followed by colonisation with anaerobic genera such as *Bifidobacterium*, *Bacteroides*, *Clostridium* in addition to lactic acid bacteria. In breast-fed infants, bifidobacteria has been shown to constitute 60-90% of the total faecal microbiota, while lactobacilli comprise less than 1 % of the total bacterial load (Haarman & Knol, 2006; Koenig et al., 2011). Of the numerous *Lactobacillus* species present, *L. acidophilus, L. paracasei* and *L. casei* dominated in breast-fed infants whereas *L. reuteri* only accounted for approximately 2% (Haarman & Knol, 2006). Some strains of *Bifidobacterium* and *Lactobacillus* may be able to inhibit the growth of pathogens.

In formula-fed infants, the microbiota is more complex with a greater diversity of species and is influenced by the formula composition. Lactic acid bacteria composition in breast-fed and formula-fed infants is similar with *Lactobacillus casei* group microorganisms being common among the lactobacilli (Endo et al., 2015). The healthy intestinal microbiota in

infancy is characterised by a large Gram-positive bacterial population which contains significant numbers of bifidobacteria (Endo et al., 2015).

Evidence is accruing that the early colonisation of the neonatal gut is important for the development of gut flora and the immune system of the growing child (Chassard, de Wouters, & Lacroix, 2014). It has been further suggested that the composition of the gut microbiome is connected to many physiological states, diseases and conditions in both early and later life (Collado, Cernada, Bauerl, Vento, & Perez-Martinez, 2012; Endo et al., 2015). From birth to 24 months, and especially after weaning, 500-1000 bacterial species are normally established in the intestinal tract. This individual intestinal microbiota is continuously influencing the host and the host's immune system, establishing physiological functions and defence mechanisms. The immaturity and vulnerability of the intestinal microbiota and the immune system makes the lowest age groups at the highest risk of unwanted health effects of the daily intake of probiotics (VKM, 2011).

However, knowledge of the true nature and complexity of the gut flora and its diverse roles in health and disease is far from fully understood. Recent research has shown a link between the gut flora and risk of asthma (Arrieta et al., 2015), and the lack, or low numbers, of *Lachnospira, Veillionella, Faecalibacterium and Rothia* in the stools of infants (3 mo) predicted the likelihood of development of signs of asthma by the age of 1 yr. Yu et al. (2015) found, in a mouse study, that the development of the intestinal stem cells (ISC) is associated with methylation of cell DNA and proposed that the gut flora facilitates or guides this process. They suggested that this could have life-long effects on gut health: "Given the profound effects of the gut microbiome in human health and disease, it will be important to determine whether specific bacterial species are involved and whether there is a critical developmental period for the microbiota to influence ISC developmental epigenetics. If so, this may open the possibility for developmentally targeted probiotic therapies to provide lifelong protection against intestinal disease".

Aujoulat et al. (2014) studied the dynamics of the dominant gut flora in 30 very premature infants using PCR – Temporal Temperature Gel Electrophoresis (PCR-TTGE). Early colonization, as indicated from stool specimens, was dominated by *Staphylococcus epidermidis* and *Enterococcus* spp. Later, *Clostridium* spp. gradually became a part of the gut flora. Lactobacilli were not detected.

2.2 Infantile colic and the use of probiotics

Infantile colic is a common condition affecting about 20% of infants under 3 months (Savino et al., 2015). It is characterised by excessive and often inconsolable crying, or fussing, and causes distress to both parents and child. The condition usually resolves itself around 3 months and, despite considerable research for decades, its aetiology has not been unravelled. There are few effective management options (Valerie Sung et al., 2014). It has been suggested that infants with colic are 11-fold more likely to later develop recurring

abdominal pain, as well as an increased risk for allergic diseases and psychological disorders (Savino et al., 2005).

Differences in gut microbiome have been identified in infants with colic compared to healthy controls but it is not certain whether the change in flora is a result of the condition – or whether the condition leads to a change in flora. Probiotics have been used in several trials to ascertain whether their use can lead to less crying. Sung et al. (2014), following a meta-analysis of 12 trials and 1825 infants, concluded that: "Although *L. reuteri* may be effective as treatment for crying in exclusively breast-fed infants with colic, there is still insufficient evidence to support probiotic use to manage colic". Four of the trials assessed in the analysis had studied the effect of *L. reuteri* (either DSM 17938 or ATCC 55730 (the mother strain). No adverse effects were reported. Three of the studies, in which only *L. reuteri* was administered, (Savino et al., 2010; Savino, Pelle, Palumeri, Oggero, & Miniero, 2007; Szajewska, Urbanska, Chmielewska, Weizman, & Shamir, 2014) reported significantly reduced crying time, as subjectively recorded by the parents. The ages of the infants (n=203) in these trials were 3 wk – 3 mo, 2 – 16 wk and under 5 mo, respectively. No adverse effects were reported in these trials.

Sung & al. (2014) carried out a randomised trial, treating 167 infants with colic with the probiotic *L. reuteri* DSM 17938. The infants were either breast-fed or formula fed but contrary to the hypothesis, formula fed infants showed greater crying and fussing in the *L. reuteri* group than the placebo group, whereas the treatment had no effect on crying or fussing time in exclusively breast-fed infants. Shortly after this publication Chau et al. (2015) reported: "Administration of *L. reuteri* DSM 17938 significantly improved colic symptoms by reducing crying and fussing times in breast-fed Canadian infants with colic". This trial was later criticised by Sung (2015) who summed up the present situation by "At this stage it is clear that probiotics cannot be routinely recommended for all infants with colic, and that there is no role for its use in formula-fed infants with colic. However, it is likely that *L. reuteri* DSM 17938 may be effective in certain sub-groups of breast-fed infants with colic. This requires further clarification". Sung also pointed out that "the long term effects of routine probiotic supplementation are unknown".

In a study with a more positive outcome (Mi et al., 2015) *L. reuteri* DSM 17938 was given to 21 breast-fed, or predominantly breast-fed, infants (placebo n=21) who showed a significant reduction in daily crying time from 200.9 ± 6.3 min/day to 32.1 ± 8.3 min/day. No adverse effects were observed during the study period of 4 weeks. A significant decrease in crying time and faecal calprotectin (a marker of inflammation in inflammatory bowel disease) was found following 3 weeks treatment with *L. reuteri* DSM 17938 of breast-fed infants and neonates who were suffering from severe infantile colic.

2.2.1 Prophylactic use of probiotics in preterm infants

Athalye-Jape et al (2015) published a systematic review on the use of *L. reuteri* DSM 17938 as a probiotic for preterm neonates. Six random-controlled trials and 2 observational studies

met the criteria for inclusion. Assessment of these publications indicated that *L. reuteri* DSM 17938 has beneficial effect on feed intolerance in preterm neonates although the authors did note the limitations of their review. These included small number of studies and sample size. In addition, the primary outcomes of the studies did not often include clinically important results such as the occurrence of necrotising enterocolitis, TFEF (time to full enteral feeds) and late onset sepsis.

There are some meta-analyses evaluating the prophylactic use of probiotics for preterm infants, suggesting that different probiotics may have a beneficial effect in the prevention of necrotizing enterocolitis and death (AlFaleh & Anabrees, 2014) The majority of these studies have not included the use of *L. reuteri*. However, Rojas (2012) evaluated the use of *L. reuteri* as a prophylactic probiotic to prevent death and nosocomial infection in preterm infants and concluded that: "although *L. reuteri* did not appear to decrease (significantly) the rate of death or nosocomial infection, the trends suggest a protective role consistent with the literature. Feeding intolerance and duration of hospitalisation were significantly decreased in premature infants <1500 g". In those studies where this was mentioned, no adverse effects of supplementation with *L. reuteri* DSM 17938 were noted.

Chumpitazi and Shulman (2014) however, voiced concern that there are still extremely important areas that require further investigation: "What (if any) are the mechanisms behind the action of probiotics in cases of infantile colic and what is the effect on long-term health? They opined that although there have been no recorded adverse events with this treatment, the long-term effects on health are not clear. Considering the recently-found connection between the gut microbiome and a number of disorders appearing later in life the authors expressed their opinion that the use of probiotics in cases of infantile colic should be subject to long term (years) evaluation to assess for potential long term health consequences."

3 Hazard identification

The hazard identification of this report is implicit in the title of the report and in the terms of reference.

Lactobacillus reuteri is a Gram-positive, heterofermentative rod-shaped lactic acid bacterium.

It belongs to the predominant microflora in cereal fermentation, occurs as a secondary ripening culture in long-ripened cheeses (Ganzle, 2004) and is an inhabitant of the gastrointestinal tract of many mammals, including humans. Strains show evidence of host adaption, but how host-microbe co-evolution influences microbial-derived effects on the host is poorly understood (Spinler et al., 2014). In some mammals, such as pigs, rodents and chicken, *L. reuteri* may be one of the most abundant species present in the gut. In contrast, the prevalence is much lower in humans, where the species is only occasionally found (Walter, Britton, & Roos, 2011). For example Molin & al. (1993) reported that only 4% of human subjects harboured *L. reuteri*. However, as the strains isolated seems to be human-

specific, they are considered as being autochthonous to the human digestive tract (Reuter, 2001)

The ability of strains of *L. reuteri* to produce potent antibacterial compounds, called reuterin, reutericin and reutericyclin, is unique among the lactic acid bacteria. Reuterin is produced during anaerobic metabolism of glycerol and is an antimicrobial substance effective against gram-negative and gram-positive bacteria, yeast, fungi and protozoa, whereas reutericyclin is predominantly active against gram-positive bacteria. These compounds are supposed to contribute to food preservation (Ganzle, 2004).

4 Hazard characterisation

4.1 Identification of the strain

The strain contained in the product under assessment is *Lactobacillus reuteri* DSM 17938, also known as *Lactobacillus reuteri* Protectis ®. According to the website of BioGaia, (biogaia.com) this strain is a "daughter strain" of ATCC 55730, originally isolated from human milk, from which two plasmids carrying resistance genes to tetracycline and lincosamid respectively were removed by non-GMO methods. The strain is deposited in Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH as DSM 17938. The FBO gives references for the origin, PCR characterisation and analysis certificate. Aspects of antibiotic resistance of the "mother strain", and the removal of the relevant genes, have been published (Rosander, Connolly, & Roos, 2008).

According to the FBO, the identity of the strain is confirmed on all culture batches released, using PCR.

The nomenclature used conforms to the current, scientifically recognized names as can be retrieved, for example, from the validation lists published in the International Journal of Systematic and Evolutionary Microbiology.

However, following an application to EFSA in 2009 for the use of a health claim for this organism, the EFSA committee responded: "The Panel considers that, on the basis of the information provided by the applicant, the *L. reuteri* strains DSM 17938 and ATCC PTA 5289 included in the Gum Periobalance™ lozenge and chewing gum as active ingredients are not sufficiently characterised (i.e., the information provided does not allow identification/characterisation of the species and strains used)". However, in 2010 EFSA regarded the "mother strain" ATCC 55730 as sufficiently characterised.

4.2 Characterisation of the strain

4.2.1 Antibiotic resistance

FBO has submitted an internal study: "Antibiotic susceptibility profiles of *Lactobacillus reuteri* strains ATCC 55730 and DSM 17938" - carried out by Swedish National Food Administration in December 2007. The minimum inhibitory concentrations (MICs) were determined by Etest-strips for the following antibiotics: ampicillin, vancomycin, gentamycin, kanamycin, streptomycin, amikacin, netilmicin, tetracycline, erythromycin, clindamycin, chloramphenicol, trimethoprim, linezolid and dalfopristin/quinupristin. As no standard method exists for antibiotic susceptibility testing of lactobacilli, The SOP protocol developed within the ACE-ART project (ACE-ART-ETE¤ST; EU project CT-2004-506214) was applied with the exception that MIC determination was performed after 24 h instead of 48 h incubation.

There are no established sensitivity/resistance MIC levels for *Lactobacillus*, but the resistance against vancomycin and trimethoprim exceeded the antibiotic concentration gradient of the strips of 256 and 32 µg/ml respectively. The MIC for tetracycline was 32 µg/ml, for ampicillin 12 µg/ml, for streptomycin 32 µg/ml and for kanamycin 128 µg/ml. The results indicate that the microbe was resistant to all these antibiotics but the resistance was considered as being intrinsic and not transferable (Rosander et al., 2008).

4.2.2 Intestinal survival

Jensen et al. (2012) investigated a number of potential probiotic lactic acid bacteria, including *L. reuteri* DSM 17938, using common *in vitro* screening assays for transit tolerance in the upper human gastrointestinal tract, adhesion capacity to human intestinal cell lines (using three different cell lines) and effect on epithelial barrier function. All strains tested tolerated the simulated small intestine juice with pancreatin and bile, *L. reuteri* DSM 17938 showed a minimal 0,1 to 0,5-log reduction.

L. reuteri is generally known to have a good adhesion capacity to intestinal cell lines (Wang 2008). The study by Jensen et al. (2012) included four different strains of *L. reuteri*, of which three showed a very high adhesion capacity. However, the actual strain DSM 17938 showed a very poor adhesion capacity to all of the three cell-lines used.

Valeur N. & al. (2004) examined in an *in vivo*-assay the colonization and immunomodulation by *L. reuteri* ATCC 55730 (the "mother strain") in the human gastro-intestinal tract and found that dietary supplementation with this strain induces significant colonisation of the stomach, duodenum and ileum of healthy humans. The colonisation was associated with decreased gastric mucosal histiocyte numbers, increased duodenal B-lymphocyte numbers and a significantly higher amount of CD4-positive T-lymphocytes in the ileal epithelium.

It is difficult to extrapolate in vitro results for bacterial adherence capacity to the situation in the human gastrointestinal tract as the host defence systems, competition with resident

microbiota, mucosal shedding and peristaltic flow are likely to modify the bacterial adhesion (Jensen et al., 2012). It is also not self-evident that any effects connected with the "mother strain" (ATCC 55730) can be extrapolated to the actual used "daughter strain" (DSM 17938).

4.2.3 Haemolysin and toxin production

FBO states that there is no known haemolytic activity or potential for toxin production of species without further documentation. Available literature provided in literature search does not identify such activity or potentials.

4.2.4 *In-vivo* studies

4.2.4.1 Assessment of undesirable short-term side-effects:

L. reuteri has been granted GRAS-status by FDA and QPS-status by EFSA, and, as far as could be establish, has never caused systemic infections in humans. It has been extensively used as a probiotic and several studies have been carried out. No adverse reactions have, to our knowledge, been published.

4.2.4.2 Assessment of undesirable long-term side-effects

Abrahamsen (2013) carried out a seven years follow-up study of his own study from 2007 (Abrahamsen 2007) where probiotic *L. reuteri* ATCC 55730 was given perinatal and as infant supplementation. No severe adverse, short term long-term events were reported. We are not aware of any other long-term studies.

4.3 Product information

4.3.1 Number of viable probiotic bacteria per gram of product / per serving

The FBO state that 5 drops of the product contain 100 million (Log 8) viable cells of *L. reuteri* DSM 17938.

The suggestion for dosage/intake is 5 drops per day for infants from birth to 3 years. On the package, it is stated that the recommended dose should not be exceeded. In addition, it is stated that a doctor should be consulted in the case of infants under one year or pregnant women. The reason(s) for this warning is not stated. No indication is given on the packaging of the rationale for using this product.

4.3.2 Number of recommended daily serving

The recommended dosage/intake is 5 drops per day for infants from birth to 3 years.

4.3.3 Food matrix

The drops may be given directly or mixed with food, without further preparation. The FBO states in the "Documentation check list" that the product is "not to be diluted with hot food". There is, however, no reference on the package concerning the type and temperature of the food or drink.

4.3.4 Storage conditions and shelf life

The product has a shelf life *after opening* of three months at room temperature. The packaged product has a protective atmosphere before opening.

4.4 GRAS and QPS status

L. reuteri has been granted GRAS status by FDA (http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm300525.htm) and QPS-status by EFSA.

4.5 Maintenance of the strain by the FBO

4.5.1 Strain integrity

The FBO has provided internal reports where the analytical procedures for identification and quantification of *L. reuteri* in samples of powder and oil blends containing one strain of *L. reuteri* are given in general terms. The analytical method used is a modified version of ISO 7889.

4.5.2 Viability during storage

The product strain *L. reuteri* DSM 17938 is stored as a freeze-dried preparation and stored at ≤ 18°C. In the unopened, original package, this has a shelf-life of 24 months from the day of manufacture. The number of living *L. reuteri* cells at the end of shelf-life is $\geq 1.5 \cdot 10^{11}$ CFU/g.

4.5.3 Antagonistic or synergistic effects

The FBO states that there are no synergistic or antagonistic effects, but do not refer to any documentation. Available literature provided in the literature search does not identify such effects.

4.6 Consumer group(s)

According to the package label, the drops are intended for use "from birth to 3 years". It is stated that the product should only be used by pregnant women, or children under 1 yr, if

advised by a doctor or a childrens´nurse. The recommended dose should not be exceeded. No further advice or warnings are given.

The FBO has not provided any rationale for the use of this product. Therefor the use for neonates, infants and young children is questionable.

5 Exposure assessment

The recommended daily intake of the product in question is 5 drops per day. According to the FBO, this dose contains at least Log 8 cfu of the strain. In comparison to the amounts of lactic acid bacteria present in mother´s milk, this number of cells is considerably more than would be expected to be naturally ingested. Fernandez et al. (2013) , in a review article, extrapolated results of several authors on the microbiology of breast milk and estimated that an infant would consume between log 5 and log 7 bacteria daily along with the consumption of 800 ml breast milk. On the other hand, the amount of *L. reuteri* contained in a daily dose of the supplement represents less than would be ingested in a 100g serving of probiotic-type yoghurt (Log 9 – 10).

6 Risk characterisation

For characterisation of risk related to the consumption of *L. reuteri* DSM 17938 by neonates, infants and young children VKM has evaluated the following aspects: occurrence of disease, including possible infectivity of the strain in immunocompromised individuals, antibiotic resistance property, toxin production or haemolytic potential of the strain, and assessment of undesirable side effects

6.1 Occurrence of disease:

L. reuteri has been granted GRAS-status by FDA and QPS-status by EFSA.

Infections caused by *Lactobacillus* are considered extremely uncommon among immunocompetent people. The wide distribution of *Lactobacillus* and the few infections they cause, indicates that these bacteria have very low virulence in healthy humans and the lack of pathogenicity seems to extend all age groups. However, such infections do occasionally occur, mainly as bacteraemia, endocarditis and localised infections in patients with severe underlying diseases, mostly the elderly, but children are not excluded. VKM is, however, not aware of any serious human infections reported caused by *L. reuteri* and no adverse, short term reactions have been reported in the "probiotic" studies examined.

6.2 Antibiotic resistance:

The "mother-strain" (ATCC 55730) was found to harbour two plasmids carrying tet(W) tetracycline and Inu(A) lincomycin resistance genes (although clindamycin sensitive) respectively. After removal of the two plasmids, the MIC-value for tetracycline reduced from >256 to 12-16 µg/ml. The gene *tetW* which encodes for resistance against tetracycline in the "mother strain" was not detected in DSM 1938 and whole genome sequencing identified no other variants of *tet* genes. Previously study has also shown the natural intrinsic non-

susceptibility of this species to tetracycline (Egervarn, Danielsen, Roos, Lindmark, & Lindgren, 2007). The MIC for lincomycin was reduced from >16 to 0,25 µg/ml in ATCC 55730 and DSM 17918 respectively (Rosander et al., 2008).

The resistance data for DSM 17938 are only given in an internal report from the FBO (carried out by Swedish National Food Administration in Dec. 2007). No data regarding the sensitivity of lincomycin is given in the study presented by the FBO, although the "mother strain" carried plasmid-associated resistance genes to this antibiotic.

The strain DSM 17938 like the "mother strain" ATCC 55730 is resistant against β-lactam. According to Rosander et al. (2008), the resistance is caused by a number of mutations in the genes encoded PBP1a, and/or PBP2x. The β-lactam resistance gene(s) in *L. reuteri* was considered as non-transferable, by the authors, since they are located chromosomally and no resistance mechanisms to other bacteria was identified.

However, the chromosomally location of resistance gene is not sufficient to consider the gene as non-transferable. In staphylococci, the *mec A* gene that encodes for resistance against methicillin, called Staphylococcal Cassette Chromosome (SCC mec) is chromosomally located but the genetic element is mobile and driven by site specific recombination (Katayama, Ito, & Hiramatsu, 2000). The transfer of β-lactam-resistance gene from *L. reuteri* DSM 17938 to other bacterial species, in particular within the same genera, cannot be discounted.

The safety aspect of *L. reuteri* DSM17938 concerning absence of transferable resistance genes can therefore not be regarded as being sufficiently documented.

Using a number of *in vitro* experiments (*e. g.* fermentation patterns, pathogen inhibition) and data from a clinical trial, to compare strain DSM 17938 and the "mother strain" ATCC 55730, Rosander et al. (2008) concluded that the "daugher" strain retained the properties of "mother" strain. This may indicate that these two strains are "bioequivalent" or "biosimilar". VKM are not aware of guidelines recommending specific experiments required to consider two bacterial species as "bioequivalent" or "biosimilar". The experiments performed by the authors should be considered acceptable.

6.3 Toxin production and haemolytic potential

The producer states that the strain has "No known potential for toxin production" and "No known haemolytic activity", but no references are given. Available literature provided in literature search does not identify such activity or potentials.

6.4 Undesirable side effects

The data provided by the FBO show no adverse or undesirable short term effects. A seven–year follow-up study by Abrahamsson et al (2013) evaluated whether perinatal and infant

supplementation with probiotic *L. reuteri* reduced the prevalence of respiratory allergic disease in school age and to explore whether this supplementation was associated with any long-term side effects. They had in an earlier study (Abrahamsson et al. 2007) found that the prevalence of IgE-associated eczema and the cumulative incidence of IgE-associated allergic diseases were lower in the *L. reuteri* - group than in the placebo-group and significantly so for infants with allergic mothers. However, after 7 years the prevalence of allergic diseases was similar in the probiotic and placebo group, indicating that any possible effect of *L. reuteri* on the immune system had been of transient nature. No severe, adverse, long-term events were reported.

The FBO has not provided any rationale for the use of this product. Therefor the use for neonates, infants and young children is questionable.

A daily dose of a "monoculture" of a particular bacterial strain in large quantities over a prolonged period of time to an age group with an immature intestinal flora may, however, still have unknown long-term adverse effects. The early microbial composition of human gastro-intestinal tract can have long-lasting functional effects. If the supply of a "monoculture" leads to an abnormal colonisation of the infant gut, the result may be that the development of the postnatal immune system is affected, that the postnatal maturation of epithelial cell barrier function is delayed or it can lead to mucosal inflammation that plays a pivotal function in the development of feeding intolerance (Di Mauro et al., 2013)

7 Uncertainties

The degree of confidence in the final estimation of risk depends on the variability, uncertainty, and assumptions identified in all the previous steps. Discrimination between uncertainty and variability is important in subsequent selection of risk management options. Biological variation includes the differences in virulence that exist in microbiological populations and variability in susceptibility within the human population and particular sub-populations. (http://www.fao.org/docrep/005/y1579e/y1579e05.htm). According to EFSA's guidance regarding uncertainties: assessments must state clearly and unambiguously the uncertainties that have been identified and their impact on the overall assessment outcome.

In this assessment, a number of uncertainties have been identified related to:

- the present status of antimicrobial resistance to lincomycin in "daughter strain" is not available
- the role of the food matrix has not been clarified
- it is not demonstrated whether the ATCC 55730 and the DSM17938 strains are "biosimilar"
- the length of administration has not been specified
- lack of data on use in vulnerable groups

8 Conclusions (with answers to the terms of reference)

The Norwegian Food Authority has requested the VKM to answer the following question:

Lactobacillus reuteri Protectis®, provided as a food supplements to infants (0-12 months) and young children (12-36 months):

1. are there any health risks associated to the intake of *Lactobacillus reuteri* Protectis®, as a food supplement, in healthy infants and young children?

The species *L. reuteri* is included in the EFSA-list of bacteria that are presumed to be safe (QPS – qualified presumption of safety) and has been given GRAS-status by FDA. The specific strain DSM 17938 is a "daughter strain" of the strain ATCC 55730 which was originally isolated from normal human milk.

We are not aware of any data indicating that *L. reuteri* has been the cause of serious human diseases – and none of the studies examined has reported any adverse or undesirable short term effects. It has also been used in preterm infants with dosage corresponding to the actual recommended doses - without reporting any adverse, short term reaction. There is therefore no evidence leading to consider the strain DSM 17938 at the dosage recommended as unsafe.

However, more long-term studies are still lacking and the long-term safety following administration to those age groups cannot be established.

   a. if so, - what kind of risks are associated with the intake of *Lactobacillus reuteri* Protectis®, as a food supplement, in healthy infants and young children?

As evidence is accruing that the early microbial composition of the infant gut is important for the development of the gut flora and the immune system of the growing child, it is not possible to exclude that a daily supply of a single particular bacterial strain over a prolonged period of time to an immature gastro-intestinal tract may have long-term, although still unknown, adverse effects on that development.

      i. Is the amount of the food supplement of importance?

This question is not possible to answer because the long-term studies are lacking.

      ii. Is the age of the infant or young child of importance?

This question is not possible to answer because the long-term studies are lacking. However, a daily supply of a single particular bacterial strain over a prolonged period of time to an immature gastro-intestinal tract may have long-term, although still unknown, adverse effects on that development. It is implicit that a greater impact may be expected in a more immature gastro-intestinal tract.

b. if not, - are there any vulnerable groups (i.e. premature, infants or children with diseases) where there are health risks associated with the intake of *Lactobacillus reuteri* Protectis®, as a food supplement, in infants and young children?

This question was not considered because the long-term safety for healthy infants and children was not entirely established.

9 Data gaps

- Sufficient data on factors influencing development of infant gut microbiota and immune system
- Influence of probiotics on infant gut microbiota and immune system
- Long-term data on the effect of the strain in question on the development of the gut microbiota and immune system
- Dose-response study (e.g. animal studies)

10 References

Abrahamsson, T. R., Jakobsson, T., Bjorksten, B., Oldaeus, G., & Jenmalm, M. C. (2013). No effect of probiotics on respiratory allergies: a seven-year follow-up of a randomized controlled trial in infancy. *Pediatr Allergy Immunol, 24*(6), 556-561. doi:10.1111/pai.12104

AlFaleh, K., & Anabrees, J. (2014). Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Evid Based Child Health, 9*(3), 584-671. doi:10.1002/ebch.1976

Arrieta, M.-C., Stiemsma, L. T., Dimitriu, P. A., Thorson, L., Russell, S., Yurist-Doutsch, S., . . . Turvey, S. E. (2015). Early infancy microbial and metabolic alterations affect risk of childhood asthma. *Science translational medicine, 7*(307), 307ra152. Retrieved from <Go to ISI>://MEDLINE:26424567

Athalye-Jape, G., Rao, S., & Patole, S. (2015). Lactobacillus reuteri DSM 17938 as a Probiotic for Preterm Neonates: A Strain-Specific Systematic Review. *JPEN J Parenter Enteral Nutr*. doi:10.1177/0148607115588113

Aujoulat, F., Roudiere, L., Picaud, J.-C., Jacquot, A., Filleron, A., Neveu, D., . . . Jumas-Bilak, E. (2014). Temporal dynamics of the very premature infant gut dominant microbiota. *BMC microbiology, 14*, 325. Retrieved from <Go to ISI>://MEDLINE:25551282

Ceratto, S., De Marco, A., Calabrese, R., & Savino, F. (2014). Atopic disorders, asthma, migraine and BMI Z-score in children treated with *Lactobacillus reuteri* for infantile colic: a post hoc analysis. *JPGN, 58*(Supplement 1).

Chassard, C., de Wouters, T., & Lacroix, C. (2014). Probiotics tailored to the infant: a window of opportunity. *Current opinion in biotechnology, 26*, 141-147. Retrieved from <Go to ISI>://MEDLINE:24469588

Chau, K., Lau, E., Greenberg, S., Jacobson, S., Yazdani-Brojeni, P., Verma, N., & Koren, G. (2015). Probiotics for infantile colic: a randomized, double-blind, placebo-controlled trial investigating *Lactobacillus reuteri* DSM 17938. *The Journal of pediatrics, 166*(1), 74-78. Retrieved from <Go to ISI>://MEDLINE:25444531

Chumpitazi, B. P., & Shulman, R. J. (2014). Prophylactic use of probiotics ameliorates infantile colic. *The Journal of pediatrics, 165*(1), 210. Retrieved from <Go to ISI>://MEDLINE:24973164

Collado, M. C., Cernada, M., Bauerl, C., Vento, M., & Perez-Martinez, G. (2012). Microbial ecology and host-microbiota interactions during early life stages. *Gut Microbes, 3*(4), 352-365. doi:10.4161/gmic.21215

Di Mauro, A., Neu, J., Riezzo, G., Raimondi, F., Martinelli, D., Francavilla, R., & Indrio, F. (2013). Gastrointestinal function development and microbiota. *Ital J Pediatr, 39*, 15. doi:10.1186/1824-7288-39-15

Di Nardo, G., Oliva, S., Menichella, A., Pistelli, R., De Biase, R. V., Patriarchi, F., . . . Stronati, L. (2014). Lactobacillus reuteri ATCC55730 in cystic fibrosis. *Journal of pediatric*

gastroenterology and nutrition, 58(1), 81-86. Retrieved from <Go to ISI>://MEDLINE:24121143

Egervarn, M., Danielsen, M., Roos, S., Lindmark, H., & Lindgren, S. (2007). Antibiotic susceptibility profiles of Lactobacillus reuteri and Lactobacillus fermentum. *Journal of food protection, 70*(2), 412-418. Retrieved from <Go to ISI>://MEDLINE:17340877

Endo, A., Tang, M. L. K., & Salminen, S. (2015). 1.8 Gut microbiota in infants. *World review of nutrition and dietetics, 113*, 87-91. Retrieved from <Go to ISI>://MEDLINE:25906863

Fernandez, L., Langa, S., Martin, V., Maldonado, A., Jimenez, E., Martin, R., & Rodriguez, J. M. (2013). The human milk microbiota: origin and potential roles in health and disease. *Pharmacological research, 69*(1), 1-10. Retrieved from <Go to ISI>://MEDLINE:22974824

Ganzle, M. G. (2004). Reutericyclin: biological activity, mode of action, and potential applications. *Applied microbiology and biotechnology, 64*(3), 326-332. Retrieved from <Go to ISI>://WOS:000220862300004

Haarman, M., & Knol, J. (2006). Quantitative Real-Time PCR Analysis of Fecal Lactobacillus Species in Infants Receiving a Prebiotic Infant Formula. *Applied and environmental microbiology, 72*(4), 2359-2365. doi:10.1128/AEM.72.4.2359-2365.2006

Jensen, H., Grimmer, S., Naterstad, K., & Axelsson, L. (2012). In vitro testing of commercial and potential probiotic lactic acid bacteria. *International journal of food microbiology, 153*(1-2), 216-222. Retrieved from <Go to ISI>://MEDLINE:22177712

Katayama, Y., Ito, T., & Hiramatsu, K. (2000). A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in Staphylococcus aureus. *Antimicrobial agents and chemotherapy, 44*(6), 1549-1555. Retrieved from <Go to ISI>://MEDLINE:10817707

Kleinhans, L., Jockel-Schneider, Y., Rehn, M., Schlagenhauf, U., & Wuerzburg, D. E. (2011). Influence of regular intake of Periobalance® lozenges on plaque control and gingivitis in pregnant women. Abstract 1324.: Perio posters.

Koenig, J. E., Spor, A., Scalfone, N., Fricker, A. D., Stombaugh, J., Knight, R., . . . Ley, R. E. (2011). Succession of microbial consortia in the developing infant gut microbiome. *Proceedings of the National Academy of Sciences of the United States of America, 108 Suppl 1*, 4578-4585. Retrieved from <Go to ISI>://MEDLINE:20668239

Mi, G.-L., Zhao, L., Qiao, D.-D., Kang, W.-Q., Tang, M.-Q., & Xu, J.-K. (2015). Effectiveness of Lactobacillus reuteri in infantile colic and colicky induced maternal depression: a prospective single blind randomized trial. *Antonie van Leeuwenhoek, 107*(6), 1547-1553. Retrieved from <Go to ISI>://MEDLINE:25876529

Oncel, M. Y., Arayici, S., Sari, F. N., Simsek, G. K., Yurttutan, S., Erdeve, O., . . . Dilmen, U. (2015). Comparison of Lactobacillus reuteri and nystatin prophylaxis on Candida colonization and infection in very low birth weight infants. *J Matern Fetal Neonatal Med, 28*(15), 1790-1794. doi:10.3109/14767058.2014.968842

Reuter, G. (2001). The Lactobacillus and Bifidobacterium microflora of the human intestine: composition and succession. *Curr Issues Intest Microbiol, 2*(2), 43-53. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11721280

Rojas, M. A., Lozano, J. M., Rojas, M. X., Rodriguez, V. A., Rondon, M. A., Bastidas, J. A., . . . Arevalo, M. (2012). Prophylactic probiotics to prevent death and nosocomial infection in preterm infants. *Pediatrics, 130*(5), e1113-1120. Retrieved from <Go to ISI>://MEDLINE:23071204

Rosander, A., Connolly, E., & Roos, S. (2008). Removal of antibiotic resistance gene-carrying plasmids from Lactobacillus reuteri ATCC 55730 and characterization of the resulting daughter strain, L. reuteri DSM 17938. *Applied and environmental microbiology, 74*(19), 6032-6040. Retrieved from <Go to ISI>://MEDLINE:18689509

Savino, F., Castagno, E., Bretto, R., Brondello, C., Palumeri, E., & Oggero, R. (2005). A prospective 10-year study on children who had severe infantile colic. *Acta Paediatr Suppl, 94*(449), 129-132. doi:10.1080/08035320510043691

Savino, F., Ceratto, S., Poggi, E., Cartosio, M. E., Cordero di Montezemolo, L., & Giannattasio, A. (2015). Preventive effects of oral probiotic on infantile colic: a prospective, randomised, blinded, controlled trial using Lactobacillus reuteri DSM 17938. *Benef Microbes, 6*(3), 245-251. doi:10.3920/bm2014.0090

Savino, F., Cordisco, L., Tarasco, V., Palumeri, E., Calabrese, R., Oggero, R., . . . Matteuzzi, D. (2010). Lactobacillus reuteri DSM 17938 in infantile colic: a randomized, double-blind, placebo-controlled trial. *Pediatrics, 126*(3), e526-533. doi:10.1542/peds.2010-0433

Savino, F., Pelle, E., Palumeri, E., Oggero, R., & Miniero, R. (2007). Lactobacillus reuteri (American Type Culture Collection Strain 55730) versus simethicone in the treatment of infantile colic: a prospective randomized study. *Pediatrics, 119*(1), e124-130. doi:10.1542/peds.2006-1222

Spinler, J. K., Sontakke, A., Hollister, E. B., Venable, S. F., Oh, P. L., Balderas, M. A., . . . Highlander, S. K. (2014). From Prediction to Function Using Evolutionary Genomics: Human-Specific Ecotypes of Lactobacillus reuteri Have Diverse Probiotic Functions. *Genome Biol Evol, 6*(7), 1772-1789. Retrieved from <Go to ISI>://WOS:000342583200019

Sung, V. (2015). Probiotic interventions in infantile colic. *Current opinion in clinical nutrition and metabolic care, 18*(3), 307-311. Retrieved from <Go to ISI>://MEDLINE:25769063

Sung, V., Cabana, M. D., D'Amico, F., Deshpande, G., Dupont, C., Indrio, F., . . . Tancredi, D. (2014). Lactobacillus reuteri DSM 17938 for managing infant colic: protocol for an individual participant data meta-analysis. *BMJ Open, 4*(12). doi:10.1136/bmjopen-2014-006475

Sung, V., Hiscock, H., Tang, M. L. K., Mensah, F. K., Nation, M. L., Satzke, C., . . . Wake, M. (2014). Treating infant colic with the probiotic Lactobacillus reuteri: double blind,

placebo controlled randomised trial. *BMJ (Clinical research ed), 348*, g2107. Retrieved from <Go to ISI>://MEDLINE:24690625

Szajewska, H., Urbanska, M., Chmielewska, A., Weizman, Z., & Shamir, R. (2014). Meta-analysis: Lactobacillus reuteri strain DSM 17938 (and the original strain ATCC 55730) for treating acute gastroenteritis in children. *Benef Microbes, 5*(3), 285-293. doi:10.3920/bm2013.0056

Urbanska, M., & Szajewska, H. (2014). The efficacy of *Lactobacillus reuteri* DSM 17938 in infants and children: a review of the current evidence. *Eur J Pediatr, 173*(10), 1327-1337. doi:10.1007/s00431-014-2328-0

Valeur, N., Engel, P., Carbajal, N., Connolly, E., & Ladefoged, K. (2004). Colonization and immunomodulation by Lactobacillus reuteri ATCC 55730 in the human gastrointestinal tract. *Applied and environmental microbiology, 70*(2), 1176-1181. Retrieved from <Go to ISI>://MEDLINE:14766603

VKM. (2011). *Assessment of benefits and risks of probiotics in baby foods, F19. Scientific Opinion of the Panel on biological hazards of the Norwegian Scientific Committee for Food Safety. ISBN: 978-82-8259-025-9* Retrieved from

Walter, J., Britton, R. A., & Roos, S. (2011). Host-microbial symbiosis in the vertebrate gastrointestinal tract and the *Lactobacillus reuteri* paradigm. *Proc Natl Acad Sci U S A, 108 Suppl 1*, 4645-4652. doi:10.1073/pnas.1000099107

Yu, X., Huang, Q., & Petersen, F. (2015). History and milestones of mouse models of autoimmune diseases. *Current pharmaceutical design, 21*(18), 2308-2319. Retrieved from <Go to ISI>://MEDLINE:25777764