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An Introduction to Probiotics

Probiotics are live microorganisms (in most cases, bacteria) that are similar to beneficial microorganisms found in the human gut. They are also called "friendly bacteria" or "good bacteria." Probiotics are available to consumers mainly in the form of dietary supplements and foods. They can be used as complementary and alternative medicine (CAM).[1] To find out more about topics and resources mentioned in this fact sheet, see "For More Information."

Key Points

- People use probiotic products as CAM to prevent and treat certain illnesses and support general wellness.
- There is limited evidence supporting some uses of probiotics. Much more scientific knowledge is needed about probiotics, including about their safety and appropriate use.
- Effects found from one species or strain of probiotics do not necessarily hold true for others, or even for different preparations of the same species or strain.
- Tell your health care providers about any CAM practices you use. Give them a full picture of what you do to manage your health. This will help ensure coordinated and safe care.

What Probiotics Are

Experts have debated how to define probiotics. One widely used definition, developed by the World Health Organization and the Food and Agriculture Organization of the United Nations, is that probiotics are "live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host." (Microorganisms are tiny living organisms—such as bacteria, viruses, and yeasts—that can be seen only under a microscope.)

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[1] CAM is a group of diverse medical and health care systems, practices, and products that are not presently considered to be part of conventional medicine. Complementary medicine is used **together with** conventional medicine, and alternative medicine is used **in place of** conventional medicine. Some health care providers practice both CAM and conventional medicine.

Probiotics are not the same thing as **prebiotics**—nondigestible food ingredients that selectively stimulate the growth and/or activity of beneficial microorganisms already in people's colons. When probiotics and prebiotics are mixed together, they form a **synbiotic**

Probiotics are available in foods and dietary supplements (for example, capsules, tablets, and powders) and in some other forms as well. Examples of foods containing probiotics are yogurt, fermented and unfermented milk, miso, tempeh, and some juices and soy beverages. In probiotic foods and supplements, the bacteria may have been present originally or added during preparation.

Most probiotics are bacteria similar to those naturally found in people's guts, especially in those of breastfed infants (who have natural protection against many diseases). Most often, the bacteria come from two groups, *Lactobacillus* or *Bifidobacterium*. Within each group, there are different species (for example, *Lactobacillus acidophilus* and *Bifidobacterium bifidus*), and within each species, different strains (or varieties). A few common probiotics, such as *Saccharomyces boulardii,* are yeasts, which are different from bacteria.

Some probiotic foods date back to ancient times, such as fermented foods and cultured milk products. Interest in probiotics in general has been growing; Americans' spending on probiotic supplements, for example, nearly tripled from 1994 to 2003.

Uses for Health Purposes

There are several reasons that people are interested in probiotics for health purposes.

First, the world is full of microorganisms (including bacteria), and so are people's bodies—in and on the skin, in the gut, and in other orifices. Friendly bacteria are vital to proper development of the immune system, to protection against microorganisms that could cause disease, and to the digestion and absorption of food and nutrients. Each person's mix of bacteria varies. Interactions between a person and the microorganisms in his body, and among the microorganisms themselves, can be crucial to the person's health and well-being.

This bacterial "balancing act" can be thrown off in two major ways:

1. By antibiotics, when they kill friendly bacteria in the gut along with unfriendly bacteria. Some people use probiotics to try to offset side effects from antibiotics like gas, cramping, or diarrhea. Similarly, some use them to ease symptoms of lactose intolerance—a condition in which the gut lacks the enzyme needed to digest significant amounts of the major sugar in milk, and which also causes gastrointestinal symptoms.

2. "Unfriendly" microorganisms such as disease-causing bacteria, yeasts, fungi, and parasites can also upset the balance. Researchers are exploring whether probiotics could halt these unfriendly agents in the first place and/or suppress their growth and activity in conditions like:

   - Infectious diarrhea
   - Irritable bowel syndrome
   - Inflammatory bowel disease (e.g., ulcerative colitis and Crohn's disease)

- Infection with *Helicobacter pylori (H. pylori)*, a bacterium that causes most ulcers and many types of chronic stomach inflammation
- Tooth decay and periodontal disease
- Vaginal infections
- Stomach and respiratory infections that children acquire in daycare
- Skin infections.

Another part of the interest in probiotics stems from the fact there are cells in the digestive tract connected with the immune system. One theory is that if you alter the microorganisms in a person's intestinal tract (as by introducing probiotic bacteria), you can affect the immune system's defenses.

What the Science Says

Scientific understanding of probiotics and their potential for preventing and treating health conditions is at an early stage, but moving ahead. In November 2005, a conference that was cofunded by the National Center for Complementary and Alternative Medicine (NCCAM) and convened by the American Society for Microbiology explored this topic

According to the conference report, some uses of probiotics for which there is some encouraging evidence from the study of specific probiotic formulations are as follows:

- To treat diarrhea (this is the strongest area of evidence, especially for diarrhea from rotavirus)
- To prevent and treat infections of the urinary tract or female genital tract
- To treat irritable bowel syndrome
- To reduce recurrence of bladder cancer
- To shorten how long an intestinal infection lasts that is caused by a bacterium called *Clostridium difficile*
- To prevent and treat pouchitis (a condition that can follow surgery to remove the colon)
- To prevent and manage atopic dermatitis (eczema) in children.

The conference panel also noted that in studies of probiotics as cures, any beneficial effect was usually low; a strong placebo effect often occurs; and more research (especially in the form of large, carefully designed clinical trials) is needed in order to draw firmer conclusions.

Some other areas of interest to researchers on probiotics are

- What is going on at the molecular level with the bacteria themselves and how they may interact with the body (such as the gut and its bacteria) to prevent and treat diseases. Advances in technology and medicine are making it possible to study these areas much better than in the past.

- Issues of quality. For example, what happens when probiotic bacteria are treated or are added to foods—is their ability to survive, grow, and have a therapeutic effect altered?

- The best ways to administer probiotics for therapeutic purposes, as well as the best doses and schedules.

- Probiotics' potential to help with the problem of antibiotic-resistant bacteria in the gut.
- Whether they can prevent unfriendly bacteria from getting through the skin or mucous membranes and traveling through the body (e.g., which can happen with burns, shock, trauma, or suppressed immunity).

Side Effects and Risks

Some live microorganisms have a long history of use as probiotics without causing illness in people. Probiotics' safety has not been thoroughly studied scientifically, however. More information is especially needed on how safe they are for young children, elderly people, and people with compromised immune systems.

Probiotics' side effects, if they occur, tend to be mild and digestive (such as gas or bloating). More serious effects have been seen in some people. Probiotics might theoretically cause infections that need to be treated with antibiotics, especially in people with underlying health conditions. They could also cause unhealthy metabolic activities, too much stimulation of the immune system, or gene transfer (insertion of genetic material into a cell).

Probiotic products taken by mouth as a dietary supplement are manufactured and regulated as foods, not drugs (for more on this point, see NCCAM's *What's in the Bottle? An Introduction to Dietary Supplements*).

Some Other Points To Consider

- If you are thinking about using a probiotic product as CAM, consult your health care provider first. No CAM therapy should be used in place of conventional medical care or to delay seeking that care.
- Effects from one species or strain of probiotics do not necessarily hold true for others, or even for different preparations of the same species or strain.
- If you use a probiotic product and experience an effect that concerns you, contact your health care provider
- You can locate research reports in peer-reviewed journals on probiotics' effectiveness and safety through the resources PubMed and CAM on PubMed.

NCCAM-Sponsored Research on Probiotics

Among recent NCCAM-sponsored research are the following projects:

- Investigators at Tulane University School of Public Health and Tropical Medicine are developing a clinical trial on the effectiveness of selected probiotic agents to treat diarrhea in undernourished children in a developing country.
- At the Mayo Clinic College of Medicine, researchers have been examining probiotics for possibly decreasing the levels of certain substances in the urine that can cause problems such as kidney stones.

- A team at Tufts-New England Medical Center is studying probiotics for treating an antibiotic-resistant type of bacteria that causes severe infections in people who are hospitalized, live in nursing homes, or have weakened immune systems.

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For More Information

NCCAM Clearinghouse

The NCCAM Clearinghouse provides information on CAM and NCCAM, including publications and searches of Federal databases of scientific and medical literature. Examples of publications include *What's in the Bottle? An Introduction to Dietary Supplements* and *Are You Considering Using CAM?* The Clearinghouse does not provide medical advice, treatment recommendations, or referrals to practitioners.

Toll-free in the U.S.: 1-888-644-6226
TTY (for deaf and hard-of-hearing callers): 1-866-464-3615
Web site: nccam.nih.gov
E-mail: info@nccam.nih.gov

PubMed®

A service of the National Library of Medicine (NLM), PubMed contains publication information and (in most cases) brief summaries of articles from scientific and medical journals. CAM on PubMed, developed jointly by NCCAM and NLM, is a subset of the PubMed system and focuses on the topic of CAM.

Web site: www.ncbi.nlm.nih.gov/entrez
CAM on PubMed: nccam.nih.gov/camonpubmed/

International Bibliographic Information on Dietary Supplements (IBIDS)

A database cosponsored by the NIH Office of Dietary Supplements and a component of the U.S. Department of Agriculture, IBIDS contains citations to articles on dietary supplements from peer-reviewed scientific and biomedical journals and other sources. It draws from several major services, including PubMed.

Web site: www.ods.od.nih.gov/Health_Information/IBIDS_Overview.aspx

Acknowledgments

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Probiotics could improve premature babies' gut health

By Stephen Daniells

14/05/2007- **The risk of necrotising enterocolitis, one of the most common gastrointestinal problems in premature babies, may be cut by 74 per cent by probiotic supplementation, suggests a meta-analysis from Australia.**

The meta-analysis, published in this week's The Lancet, carried out randomised controlled trials including 1393 premature infants and a variety of probiotics. In addition to a reduced risk of necrotising enterocolitis, they also observed a 53 per cent reduction in the risk of mortality.

"The remarkably consistent results, despite the distinct differences in dose, timing, and type of organisms used, suggest that substantial latitude might be available in the choice of an effective probiotic regimen in the design of further trials," wrote the authors.

"If a large well-designed trial confirms our results, it could make a very strong case for the routine use of probiotics in preterm neonates," they added.

The researchers, led by Girish Deshpande from King Edward Memorial Hospital for Women, carried out seven randomised controlled trials on subjects that met their inclusion criteria.

Pooling the data showed that probiotic supplementation reduced the risk of necrotising enterocolitis by 74 per cent, compared to controls. The risk of death was reduced by 53 per cent in the probiotic group, compared to control. The time taken for the premature infants to start full feeding was also reduced by about three days.

"Probiotics might reduce the risk of necrotising enterocolitis in preterm neonates with less than 33 weeks' gestation," concluded the researchers. *"However, the short-term and long-term safety of probiotics needs to be assessed in large trials. Unanswered questions include the dose, duration, and type of probiotic agents (species, strain, single or combined, live or killed) used for supplementation."*

In an accompanying editorial, Parma University's Carlo Caffarelli and Sergio Bernasconi welcomed the research as a positive step.

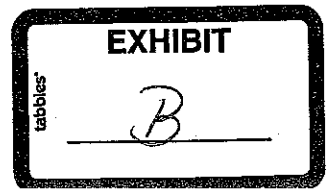
"Over the past few decades, the frequency of the condition has shown no sign of reduction," they wrote. *"Therefore the development of strategies to prevent the disease is a priority."*

However, Caffarelli and Bernasconi cautioned that the results needed to be interpreted with care.

"The meta-analysis by Deshpande and colleagues indicates that a wide range of questions needs to be addressed," they said. *"The analyses were based on clinical trials that tested different probiotics, such as* Lactobacillus acidophilus, L casei GG, L bulgaricus, Bifidobacterium bifidum, B breve, B infants, B lactis, Streptococcus thermophilus, *and* Saccharomyces boulardii. *Each strain is believed to have specific immunomodulatory properties.*

"The limited number of clinical trials does not permit definition of either the optimum strain or dosing regimens. The evidence of the effect of type of feeding and antibiotic therapy on gut colonisation is also unclear. Finally, trials included in the overview show a lack of side-effects," they added.

Source: *The Lancet*



12-18 May 2007, Volume 369, Issue 9573, Pages 1614-1620
"Probiotics for prevention of necrotising enterocolitis in preterm neonates with very low birthweight: a systematic review of randomised controlled trials"
Authors: G. Deshpande, S Rao and S. Patole

Editorial: *The Lancet*
12-18 May 2007, Volume 369, Issue 9573, Pages 1578-1580
"Preventing necrotising enterocolitis with probiotics"
Carlo Caffarelli and Sergio Bernasconi

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Probiotics increase protection against autoimmune disease

By Dominique Patton

12/10/2005- **Probiotic bacteria could not only help fight viruses but they may also protect against autoimmune diseases like diabetes, says Swedish probiotics firm Probi.**

The company has new results from a clinical trial that showed higher numbers of different types of white blood cells after subjects had consumed probiotics.

One of Probi's bacteria, not yet on the market, increased white blood cells that have previously been linked to the protection against type 1 diabetes.

"It's difficult to say how much this would do for a patient at this stage," said Per Bengtsson, the firm's chief executive.

But he added that the results could have an important role in future research into diseases in which the immune system attacks the body, such as diabetes, MS and rheumatism.

Further, the findings show *"for the first time that you can get differential effects on the immune system by eating certain bacteria,"* Bengtsson told NutraIngredients.com.

The findings were reported at the European Conference on Probiotics and their Application in Krakow, Poland last weekend.

In another arm of the trial, carried out on 59 healthy people at the Sahlgrenska Hospital in Göteborg, Sweden, the volunteers consumed a strain of *Lactobacillus plantarum* for five weeks.

The researchers saw a significant increase in white blood cells known to fight disease, including CD4+ and CD8.

"These are markers of how healthy your immune system is," said Bengtsson.

"Since the results are obvious in healthy people, you would imagine that they could be quite significant in people who are ill," he added.

The study is the first clinical trial funded by Probi to investigate effects on the immune system. The firm is planning to present the results to Danone, already working with Probi on a product for gut health.

It will also approach clinical nutrition companies, said Bengtsson.

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Previous page : Probiotics protect against bacterial infection, says study

Probiotics protect against bacterial infection, says study

By Stephen Daniells

20/06/2007- **A newly characterised strain of probiotic bacteria may have potential to kill *Listeria monocytogenes*, an often-lethal pathogen in pregnant women, Irish researchers report.**

The research, published in the *Proceedings of the National Academy of Sciences*, could see pregnant women and a number of other high-risk groups receiving the probiotic to protect them from potential infection.

Lead author Sinead Corr from University College Cork told NutraIngredients.com: *"Our results clearly demonstrate the ability of certain probiotic bacteria to protect against potentially fatal illnesses. More specifically we have shown a role for bacteriocins in protecting against the potentially fatal foodborne pathogen Listeria monocytogenes.*

"As probiotics mechanisms are specific, thorough understanding of their beneficial effects are required. Understanding these mechanisms will enable their use in prevention and treatment of specific illnesses. This study clearly demonstrates how probiotic bacteria may help to improve the health of consumers "

Probiotic products containing 'friendly' bacteria are now well accepted by consumers in many European countries, with putative benefits highlighted for gut and immune health.

Some of the researchers in this new study previously reported that a mixture of five Lactobacillus probiotic strains may reduce food poisoning by salmonella. In that instance, the benefits for gut health were reported to be due to the probiotic bacteria adhering to the walls of the intestine, which inhibits the ability of the pathogenic Salmonella to stick and colonise the gut, thereby reducing the infection

The new study offers an alternative method of protection, with a specific strain of *Lactobacillus salivarius* named UCC118 capable of producing an antibiotic-like compound called a bacteriocin.

When the researchers tested UCC118 in mice infected with two strains of food-borne Listeria monocytogenes, EGDe and LO28, they found that the mice were protected against infection. When the mice were given a mutant form of the probiotic that was unable to produce the Abp118 bacteriocin, no protection against infection was observed

Also, *"Lb. salivarius UCC118 did not offer any protection when mice were infected with a strain of L. monocytogenes expressing the cognate Abp118 immunity protein AbpIM, confirming that the antimicrobial effect is a result of direct antagonism between Lb. salivarius and the pathogen, mediated by the bacteriocin Abp118,"* reported the researchers.

"The results of the UCC work clearly demonstrate a role for bacteriocins in protecting the host against potentially lethal infections. The study is the first to clearly demonstrate a mechanism by which probiotic bacteria may act to help improve the health of consumers," said a release from the university.

Most foods containing probiotic bacteria are found in the refrigerated section of supermarkets as the bacteria is destroyed by heat and other processing conditions.

This has given the dairy sector, already used to handling live bacteria for the manufacture of

yoghurt, a major advantage in probiotic foods - probiotic drinking yoghurts are currently the fastest growing dairy product in Europe

But increasing research has focused on expanding protecting probiotics during processing and expanding the food categories available to prebiotics. Such an avenue of research has led companies like Cell Biotech from Korea using a dual-coating to protect probiotics against oxygen, acid, moisture and high temperatures for use in emerging new product categories such as breakfast cereals and smoothies

Other approaches are also being explored, with scientists looking at improving probiotic viability by using whey protein gel particles, or prebiotic fibres.

Source: *Proceedings of the National Academy of Sciences*
May 1, 2007, Volume 104, Number 18, Pages 7617-7621
"Bacteriocin production as a mechanism for the antiinfective activity of Lactobacillus salivarius UCC118"
Authors: S.C. Corr, Y. Li, C.U. Riedel, P.W. O'Toole, C. Hill, and C.G.M. Gahan

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Probiotics again linked to lower eczema risk

By Stephen Daniells

12/06/2007- **Probiotic supplementation during pregnancy and then for the infants after birth could reduce the incidence of eczema, suggests a new clinical trial from Sweden.**

The study, published in the current issue of the *Journal of Allergy and Clinical Immunology*, reports that, while no significant preventative effect was observed in eczema among all the infants, probiotic-supplemented children of mothers with allergies did experience significant reductions in eczema.

"Although a preventive effect of probiotics on infant eczema was not confirmed, the treated infants had less IgE-associated eczema at 2 years of age and therefore possibly run a reduced risk to develop later respiratory allergic disease," wrote lead author Thomas Abrahamsson from Linkoping University Hospital.

Eczema, also known as atopic dermatitis (AD), is one of the first signs of allergy during the early days of life and is said to be due to delayed development of the immune system. According to the American Academy of Dermatologists it affects between 10 to 20 percent of all infants, but almost half of these kids will 'grow out' of eczema between the ages of five and 15.

The research appears to be in line with a previous study from Finland that reported in 2003 that children who were exposed to the Lactobacillus rhamnosus GG (LGG) bacteria around the time of birth were 40 per cent less likely to develop atopic eczema at four years of age compared with children in a placebo group.

The new double-blind, randomised, placebo-controlled trial included 188 families with allergic disease. Mothers were assigned to receive daily supplements of *Lactobacillus reuteri* (BioGaia, 100 million colony forming units) or placebo from gestational week 36 until the birth of the child. After this point, the infants were then supplemented with the same product for the first 12 months of their lives, and followed until age 24 months.

The incidence of eczema was similar between the two groups, reported Abrahamsson (approximately 35 per cent). The *L. reuteri*-supplemented group however had less IgE-associated eczema during the second year, eight versus 20 per cent, respectively

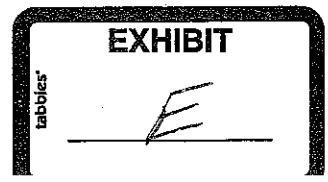
Immunoglobulin E (IgE) is the predominant antibody associated with an allergic response.

Reactivity of the children towards skin prick tests, a common test for allergy, was also less common in the probiotic-supplemented group, and significantly for children of allergy-suffering mothers - 14 versus 31 per cent, respectively.

"Proposed modes of action by probiotics include improved intestinal barrier function, degradation of macromolecules, and influence on the gut immune system," wrote the authors.

"Earlier studies on the effect of lactobacilli on immune cells in animal or in vitro models have shown promotion of Th1-like responses with IFN-gamma, IL-12, and IL-18 activation, which inhibits development of a Th2-like deviation in infants.

"L. reuteri has displayed a slightly different profile than other probiotic bacteria and seems to possess more pronounced anti-inflammatory properties, as demonstrated in animal and human in vitro studies," they said.



"As sensitized infants with eczema have increased risk for later development of allergic asthma and rhinoconjunctivitis, studies on the outcome in older children, as well as possible mechanisms behind this effect, are warranted," they concluded

Source: *Journal of Allergy and Clinical Immunology* (Elsevier)
Volume 119, Issue 5, Pages 1174-1180
"Probiotics in prevention of IgE-associated eczema: A double-blind, randomized, placebo-controlled trial"
Authors: T R. Abrahamsson, T. Jakobsson, M Fageras Bottcher, M Fredrikson, M.C. Jenmalm, B. Bjorksten and G. Oldaeus

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Previous page : Organic baby cereal incorporates probiotics and omega-3

Organic baby cereal incorporates probiotics and omega-3

By Karen Willmer

26/09/2007- **A processor claims to have launched the first organic cereal for babies that is fortified with probiotics and omega-3.**

Nurture claims its Happybellies range of organic oatmeal, rice and multi-grain baby cereals, fortified with probiotics and DHA, will aid the early infant brain and eye development while potentially protecting against the development of future allergies, including those leading to asthma, and eczema.

Products fortified with probiotics are becoming popular due to increasing research indicating the health benefits of "friendly bacteria" that aid in digestion and boost the immune system.

DHA is an omega-3 fatty acid, which has been linked to the healthy development of a baby during pregnancy, and in brain and eye development after birth

Nurture claims that DHA is only available though breast milk or fortified infant formulas, and so the baby will have to get this from other sources once it moves to solid food, making its Happybellies range beneficial.

"The stakes are higher today -- food allergies are increasing in number of affected children and severity, poor eating habits at an early age, deficient immune systems, asthma, attention problems, and early age obesity seems to be more common than ever before," said Nurture's founder Shazi Visram. *"We see the Happybellies line as part of a necessary solution in easing the myriad of health problems children are susceptible to today."*

The company said the cereals are certified as organic in the US and are suitable for vegetarian families because the DHA is obtained from a plant rather than a fish source.

The cereal will be available across the US from October 2008.

contact the editor

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ORIGINAL PAPER

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Inverse association of farm milk consumption with asthma and allergy in rural and suburban populations across Europe

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Clinical and Experimental Allergy

Summary

Background Dietary interventions as a means for atopy prevention attract great interest. Some studies in rural environments claimed an inverse association between consumption of farm-produced dairy products and the prevalence of allergic diseases, but current evidence is controversial.

Objective To investigate whether consumption of farm-produced products is associated with a lower prevalence of asthma and allergy when compared with shop-purchased products.

Methods Cross sectional multi-centre study (PARSIFAL) including 14 893 children aged 5–13 years from five European countries (2823 from farm families and 4606 attending Steiner Schools as well as 5440 farm reference and 2024 Steiner reference children). A detailed questionnaire including a dietary component was completed and allergen-specific IgE was measured in serum.

Results Farm milk consumption ever in life showed a statistically significant inverse association with asthma: covariate adjusted odds ratio (aOR) 0.74 [95% confidence interval (CI) 0.61–0.88], rhinoconjunctivitis: aOR 0.56 (0.43–0.73) and sensitization to pollen and the food mix fx5 (cut-off level of $\geqslant 3.5$ kU/L): aOR 0.67 (0.47–0.96) and aOR 0.42 (0.19–0.92), respectively, and sensitization to horse dander: aOR 0.50 (95% CI 0.28–0.87). The associations were observed in all four subpopulations and independent of farm-related co-exposures. Other farm-produced products were not independently related to any allergy-related health outcome.

Conclusion Our results indicate that consumption of farm milk may offer protection against asthma and allergy. A deepened understanding of the relevant protective components of farm milk and a better insight into the biological mechanisms underlying this association are warranted as a basis for the development of a safe product for prevention.

Keywords allergy, anthroposophy, asthma, children, diet, farming, gastrointestinal microflora, self-production, sensitization

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Introduction

The role of dietary factors in the development of asthma and atopy is still controversial. It has been postulated that the decrease in vegetable consumption and a shift from animal to vegetable fats has contributed to the increase in asthma and allergic diseases over the last decades [1, 2]. Several studies reported positive associations between elevated margarine consumption and childhood atopy risk [3, 4], while studies in rural environments reported an inverse association between consumption of farm-produced dairy products such as yogurt and farm milk and the prevalence of atopy [5–8], allergic rhinitis [5, 8, 9], asthma [5], and atopic dermatitis [8, 9]. However, in a Finnish study among farm and non-farm children, no effect of dairy product consumption and atopy was observed, but regular intake of fresh vegetables, predominantly when grown in the own garden, significantly reduced the risk of atopic sensitization [10]. Thus, current evidence of the relation between farm-produced products and the prevalence of allergic disease is controversial and mechanisms underlying the observed associations are unknown. Experiments demonstrating a beneficial effect of adding apathogenic bacteria (probiotics) to children's diet as a means of preventing atopic dermatitis have [11, 12], however, stimulated the interest in a possible role of microbes associated with the consumption of farm-produced foods.

The large European PARSIFAL study (Prevention of allergy risk factors for sensitization in children related to farming and anthroposophic lifestyle) offered the opportunity to examine specifically the relation between self- or farm-produced products and the prevalence of asthma and allergic diseases in more than 14 000 school-aged children [13]. The study included farm children, children from Rudolf Steiner schools (families with an anthroposophic lifestyle), and reference children from rural and sub-urban areas. The anthroposophic lifestyle includes factors like a restrictive use of antibiotics, antipyretics, and vaccinations, and often a biodynamic diet. An earlier study conducted in an anthroposophic community in Sweden showed a lower prevalence of childhood allergy [14].

The present analyses of the PARSIFAL study specifically addressed the question of whether, first, consumption of self- or farm-produced products, especially farm milk is associated with a lower prevalence of asthma, allergic diseases, and atopic sensitization when compared with shop-purchased products, and second, whether these associations are limited to children from rural environments or explained by other concurrent farm exposures.

Methods

Study population

Within the PARSIFAL study, children aged 5–13 years, from farm families or attending Rudolf Steiner schools, were compared with children from rural non-farming environments (reference for the farm children) and with children from (sub)urban environments attending State schools (reference for the Steiner school children) in Austria, Germany, the Netherlands, Sweden, and Switzerland. Children in Steiner schools often come from families with an anthroposophic lifestyle, which includes a holistic approach to life, education, and medicine. The details of the study design are described elsewhere [13]. In brief, a total of 15 137 (70% participation rate) parents' completed questionnaires were collected. Two hundred and forty-four questionnaires were excluded because children were outside the age limits or main information was missing, leaving 14 893 children for the analyses. In all, 8788 children gave consent for blood sampling and of these 4854 were invited for blood sampling [all children from Austria (806), the Netherlands (691), and Sweden (944) and a random sample of children from Germany (1548) and Switzerland (865) due to the comparatively large number of recruited children]. Four thousand and forty-nine (83.4%) children provided a blood sample and 3979 samples yielded a sufficient volume for allergen-specific IgE measurements. The study was approved by local research ethics committees in each country and informed consent was obtained from the parents of each child.

Parents' questionnaire

The dietary component of the PARSIFAL questionnaire included questions on the frequency of current average consumption of self-produced or directly purchased on a farm- and store-purchased foods. These foods included milk, butter, and other dairy products as well as margarine, eggs, meat, vegetables, and fruits. Response options were restricted to four categories: never, less than once per week, one to six times per week, or once a day or more.

Validity and reproducibility were assessed with 24-h recalls performed as telephone interviews of 493 randomly selected participants from all involved study groups and study areas. The validity for foods consumed with high frequency such as milk, vegetables, and fruits was the highest (positive predictive value above 60%, and negative predictive value above 80%). The reproducibility of reported consumption of milk during the first year of life was concordant among 71% of participants for store-purchased milk and 85% for farm-produced milk. No significant differences were found in the validity of responses between the five countries participating or between the four groups of children.

Besides the dietary component, the parental questionnaire included questions on socio-demographic background, parents' atopic diseases, food avoidance due to allergies in the family, breastfeeding, and the child's height and weight. In addition, information on the child's farm activities as well as the mother's farm exposures during

pregnancy was available. A child who lived on a farm and whose family ran the farm was coded as being a farm child.

The prevalence of diseases and symptoms were assessed by questions of the validated and translated International Study of Asthma and Allergies in Childhood (ISAAC) [15] Children ever diagnosed with asthma, or obstructive bronchitis more than once, were considered to have a doctor's diagnosis of asthma. Current wheezing was defined as having wheezing at least once during the past 12 months. In a subsample of the PARSIFAL study questionnaire, responses on asthma and current wheeze have been validated against a bronchial challenge with hypertonic saline and no significant differences in validity were found between the four study groups [16].

Children diagnosed with hayfever and whoever had the symptoms of hayfever, were considered to have a doctor's diagnosis of rhinoconjunctivitis. Current rhinoconjunctivitis symptoms were defined as sneezing, runny nose, nasal block-up, and itchy eyes during the past 12 months, without having a cold at the same time.

Children with an intermittent itchy rash lasting at least 6 months and who had been diagnosed with atopic/allergic eczema were considered to have a doctor's diagnosis of atopic eczema. Current atopic eczema symptoms was considered present if the child had ever had an itchy rash intermittently for at least 6 months and, in addition, reported an itchy rash in defined locations (bend of the arm/knee, backside of thighs, neck, and around eyes/ears) at any time during the last 12 months.

Allergen-specific immunoglobulin E measurements

All samples were screened with a mix of common inhalant allergens (Phadiatop: birch, timothy, mugwort, *Dermatophagoides pteronyssinus* and *farinae*, cat, dog and hose epithelium and *Cladosporium herbarum*) and a mix of common food allergens (fx5: egg white, milk, fish, wheat, peanut, soya bean) (Pharmacia CAP System; Pharmacia Diagnostics AB, Uppsala, Sweden). Sera that were scored positive in Phadiatop were further analysed separately against *Dermatophagoides pteronyssinus* (house dust mite) and *Lepidoglyphus destructor (storage mite)*, a mix of grass pollen, a mix of tree pollen, cat, and horse epithelium. All analyses were performed centrally at the Department of Clinical Immunology (Karolinska University Hospital, Stockholm, Sweden). Atopic sensitization was defined as allergen-specific IgE $\geq$ 0.35 kU/L. In addition, a cut-off value of 3.5 kU/L was also considered for the analyses. Pollen sensitization was defined as positive grass pollen mix and/or positive tree pollen mix.

Statistics

χ^2 Statistics were used to evaluate differences in dietary habits between farm children and farm reference children as well as between Steiner school children and their reference group. Consumption of products from self-production or directly purchased on a farm was compared with shop-purchased products.

Logistic regression analyses were performed to calculate adjusted odds ratios (aOR) of the association between asthma or allergy outcomes and farm-produced foods. Multivariate models evaluating the effect of each food item on allergy outcomes were adjusted for predefined covariates including study group (farm children, Steiner school children, and the respective reference groups), country, sex, age, mother's and father's reported asthma and/or hayfever, parents' education, maternal smoking during pregnancy, current environmental smoking at home, older siblings, exclusive breastfeeding > 4 months, BMI, and food avoidance due to familial asthma and/or allergy. In a second step, models were mutually adjusted for all food items.

We also calculated stratified estimates for the four study groups. The degree of heterogeneity of the stratum-specific ORs across study groups was evaluated using standard meta-analytic techniques [17]. In models examining the association between farm milk consumption and health outcomes, the timing of consumption (only in the first year of life, only at present, both in the first year of life, and at present), and relevant concomitant farm exposures such as frequency of the child's current visits to animal sheds (less or more than once a week) were additionally tested. In a sensitivity analysis, we also tested whether the effect of farm milk consumption varied across individual countries. Stability of effect estimates was examined by removing each country, one at a time.

To assess the possible influence of allergy-related changes in dietary habits, the respective question was included in all regression models. For the final analyses, we excluded the 469 non-milk-drinking children to avoid potential primary milk avoidance due to allergy-related symptoms at younger ages. Statistical analyses were performed using STATA (version 8.2, Stata Corp LP, College Station, TX, USA).

Results

Table 1 shows the distribution of children's consumption of selected farm-produced or shop-purchased foods across study groups. Although the consumption of farm milk and of self-produced products was most common among farm children, relevant proportions of all other study groups also ate and drank these products.

Table 2 gives the results of the multivariate analyses evaluating the effect of each individual farm-produced food on asthma and allergy adjusting for the predefined covariates (left side). A significant inverse association with a doctor's diagnosed asthma was observed for all

Table 1. Consumption of selected foods according to the study group

	Farm children (%) (n = 2823)	Farm reference (%) (n = 5440)	P-value*	RSS children (%) (n = 4606)	RSS reference (%) (n = 2024)	P-value*
Milk consumption						
Never	5.1	2.8		3.2	1.4	
Shop milk	28.1	77.3		65.7	90.5	
Farm milk (ever)	66.8	19.9	< 0.001	31.1	8.1	< 0.001
Only in the first year of life	6.1	8.3		16.8	3.7	
Only at present	8.9	5.2		6.1	2.8	
Both in the first year and at present	51.9	6.3	< 0.001	8.2	1.7	< 0.001
Butter consumption						
No margarine and no butter	3.0	4.5		3.6	5.4	
Shop-purchased butter only	52.6	75.4		80.4	78.8	
Margarine (exclusively)	5.0	6.2		4.0	7.4	
Butter from farm milk (any)	39.4	14.0	< 0.001	12.0	8.5	< 0.001
Yoghurt consumption						
No	4.6	5.6		4.0	5.5	
Shop purchased only	56.5	77.9		78.2	82.4	
Self-produced or directly purchased on a farm	38.9	16.5	< 0.001	17.8	12.1	< 0.001
Egg consumption						
No	3.7	5.7		4.0	6.1	
Shop purchased only	27.8	55.4		70.3	75.3	
Farm-produced or directly purchased on a farm	68.5	38.9	< 0.001	25.7	18.6	< 0.001
Vegetable or fruit consumption						
No vegetables and no fruits	0.6	1.1		0.7	1.0	
Shop purchased only	15.8	42.8		56.2	67.3	
Self-produced or directly purchased on a farm	83.5	56.1	< 0.001	43.1	31.7	< 0.001
Food avoidance due to allergies in the family	4.9	8.8	< 0.001	17.6	10.3	< 0.001

*P-values are given for the comparison of farm children and Rudolf Steiner School (RSS) children vs. their respective reference groups.

farm-produced products except vegetables and fruits. In addition, farm milk and egg consumption were inversely related to diagnosed rhinoconjunctivitis. When simultaneous adjustment was made for all farm-produced foods (right side), only consumption of farm milk remained significantly and inversely associated with the prevalence of diagnosed asthma, diagnosed rhinoconjunctivitis, and current rhinoconjunctivitis symptoms. None of the food items was significantly associated with atopic eczema and current eczema symptoms.

Table 3 shows the association between farm milk consumption and sensitization to aero and food allergens, adjusted for predefined covariates and all farm-produced foods. Using a cut-off level of 0.35 kU/L, a significant inverse association was found for a sensitization to horse allergen, and associations for sensitization to pollen, cat dander, and to the food mix fx5 tended to be negative, whereas the association with house dust and storage mites tended to be positive. When the more clinically relevant cut-off level of $\geqslant$3.5 kU/L was chosen, the negative association with farm milk consumption became stronger and statistically significant for pollen sensitization and the food mix fx5.

The inverse relation between farm milk consumption and the prevalence of diagnosed asthma, rhinoconjunctivitis, and pollen sensitization was observed in all four study groups without significant heterogeneity (Fig. 1). Similarly, no significant heterogeneity of the effects across study groups was observed for fx5 (p-heterogeneity 0.610) and horse dander (p-heterogeneity 0.465).

When the use of shop-purchased butter and the consumption of butter made out of farm milk was contrasted to margarine consumption, an inverse association was seen between butter consumption and asthma and wheeze even when simultaneously adjusted for farm milk consumption. For asthma: aOR 0.80 (95% CI: 0.65–1.00), 0.62 (0.46–0.84) for shop-purchased and farm-produced butter, respectively. For wheeze aOR 0.84 (0.65–1.06), 0.79 (0.57–1.08) for shop-purchased and farm-produced butter, respectively. No significant associations were observed for other allergy-related health outcomes.

A strong and consistent inverse association was observed for the prevalence of asthma, wheeze, rhinoconjunctivitis (diagnosed and symptoms), pollen sensitization, and fx5 in children who consumed farm milk since their first year of life (Table 4). The inclusion of other relevant concomitant farm activities in the multivariate regression model somewhat attenuated the effects and widened the confidence intervals (CIs) (Table 4), but the estimated protective effect

Table 2. Associations between asthma, rhinoconjunctivitis, and atopic eczema and production type of consumed foods

	Prevalence of health outcomes†, *n* (%) (*N* = 14 424)	Adjusted‡ OR (95% CI) for individual farm-produced foods (consumed ever in life‡) Reference category: shop purchased products					Models simultaneously adjusted for all farm-produced foods				
		Farm milk	Butter from farm milk	Yoghurt from self-production§	Eggs from farm-production§	Vegetables or fruits from self-production§	Farm milk	Butter from farm milk	Yoghurt from self-production§	Eggs from farm-production§	Vegetables or fruits from self-production§
Dr's diagnosis of asthma	1250 (8.9)	0.74*	0.72*	0.81*	0.81*	0.90	0.79*	0.79	1.03	0.90	1.02
		(0.61–0.88)	(0.58–0.90)	(0.67–0.98)	(0.69–0.95)	(0.78–1.04)	(0.65–0.95)	(0.59–1.06)	(0.80–1.33)	(0.74–1.09)	(0.86–1.20)
Current wheezing	1073 (7.6)	0.86	0.89	0.92	0.84*	0.92	0.90	0.97	1.05	0.86	1.00
		(0.72–1.04)	(0.71–1.12)	(0.75–1.12)	(0.71–0.99)	(0.79–1.07)	(0.74–1.10)	(0.71–1.30)	(0.80–1.37)	(0.70–1.06)	(0.84–1.20)
Dr's diagnosis of rhinoconjunctivitis	591 (4.2)	0.56*	0.73	0.87	0.79*	0.87	0.58*	0.81	1.20	0.89	0.98
		(0.43–0.73)	(0.52–1.02)	(0.66–1.15)	(0.63–0.99)	(0.71–1.06)	(0.44–0.76)	(0.53–1.25)	(0.83–1.73)	(0.67–1.19)	(0.78–1.25)
Current rhinoconjunctivitis symptoms	1037 (7.3)	0.70*	0.98	0.95	0.86	0.94	0.70*	1.14	1.04	0.87	1.02
		(0.57–0.85)	(0.77–1.24)	(0.77–1.17)	(0.72–1.03)	(0.80–1.10)	(0.57–0.86)	(0.83–1.56)	(0.78–1.38)	(0.70–1.09)	(0.85–1.22)
Dr's diagnosis of atopic eczema	1436 (10.1)	0.89	0.89	0.98	0.92	0.98	0.91	0.87	1.12	0.92	1.04
		(0.75–1.06)	(0.73–1.09)	(0.82–1.18)	(0.79–1.06)	(0.86–1.13)	(0.76–1.08)	(0.66–1.14)	(0.89–1.43)	(0.77–1.10)	(0.88–1.22)
Current atopic eczema symptoms	1517 (10.7)	0.89	0.94	0.91	0.98	0.91	0.91	1.02	0.92	1.08	0.91
		(0.76–1.05)	(0.77–1.14)	(0.76–1.08)	(0.85–1.13)	(0.80–1.04)	(0.77–1.07)	(0.79–1.32)	(0.73–1.16)	(0.90–1.29)	(0.78–1.06)

* *P*-value < 0.05.

†Four hundred and sixty-nine never-milk-drinking children excluded. Models were limited to 89.2% children without missing values in any of the exposure or outcome variable.

‡Adjusted for study group, country, sex, age, mother's and father's reported asthma and/or hayfever, parent's education, maternal smoking during pregnancy, current environmental smoking at home, older siblings, exclusive breastfeeding > 4 months, BMI, food avoidance due to familial asthma and/or allergy.

§Or directly purchased on a farm.

OR, odds ratio; CI, confidence interval.

Table 3. Adjusted† odds ratios (95% CI) for farm milk consumption ever in life and sensitization against aero and food allergens

	Sensitization cut-off (kU/L)	Prevalence of health outcomes‡, *n* (%) (*N* = 3818)	Farm milk consumption Reference category: shop milk consumption only
Phadiatop	⩾0.35	1 056 (28 1)	1.07 (0.86–1.32)
	⩾3 5	595 (16.0)	0.93 (0.71–1.22)
Food mix fx5	⩾0 35	430 (11 4)	0.80 (0.59–1.07)
	⩾3.5	33 (0.9)	0.42* (0.19–0.92)
Pollen	⩾0 35	702 (18.7)	0 80 (0.62–1.02)
	⩾3.5	348 (9.3)	0 67* (0.47–0.96)
House dust mite	⩾0.35	574 (15.3)	1 24 (0 94–1 62)
	⩾3.5	368 (9.8)	1.35 (0.98–1 87)
Storage mite§	⩾0.35	168 (4 5)	1.22 (0.78–1 92)
Cat dander§	⩾0 35	267 (7.1)	0.86 (0.59–1 25)
Horse dander§	⩾0.35	130 (3.5)	0 50* (0.28–0.87)

*P-value < 0 05

†Adjusted for study group, country, sex, age, mother's and father's reported asthma and/or hayfever, parent's education maternal smoking during pregnancy, current environmental smoking at home, older siblings, exclusive breastfeeding > 4 months, BMI food avoidance due to familial asthma and/or allergy, and all other farm-produced foods in the table.

‡One hundred and sixty-one never milk drinkers were excluded from the analyses

§Due to small numbers of sensitized children, only models with cut-off level 0 35 kU/L were performed.

CI, confidence interval

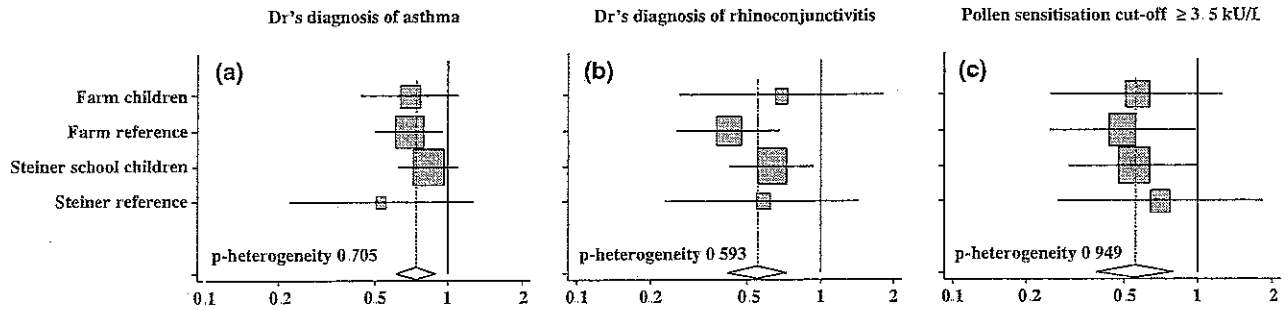

Fig. 1. (a–c) Forest plots of the association between asthma and allergy and consumption of farm milk ever for study groups using meta-analysis. The horizontal lines correspond with the 95% confidence intervals for each study group, with the corresponding box area drawn proportional to the weight for that group in the meta-analysis. The diamond represents the summary estimate. The *P*-value is given for the test of heterogeneity. Adjusted for country, sex, age, mother's and father's reported asthma and/or hayfever, parent's education, maternal smoking during pregnancy, current environmental smoking at home, older siblings, exclusive breastfeeding > 4 months, BMI, food avoidance due to familial asthma and/or allergy.

of farm milk consumption on asthma, rhinoconjunctivitis, and fx5 remained statistically significant.

The effect of being a farm child on asthma [aOR 0.73 (0.59–0.91)], rhinoconjunctivitis [0.42 (0.28–0.62)], and pollen sensitization [0.28 (0.19–0.40)] was only partially explained by farm milk consumption: farm milk adjusted effect of being a farm child on asthma [aOR 0.84 (0 67–1 06)], on rhinoconjunctivitis [aOR 0.55 (0 37–0 83)], and on pollen sensitization [aOR 0.36 (0 24–0 54)].

We also examined whether the observed effect of farm milk consumption was sensitive to the exclusion of specific countries by rerunning the analyses with each country being removed one at a time, but no relevant changes were observed.

Discussion

The analyses of the large cross-sectional study PARSIFAL give evidence of a significant inverse association between farm milk consumption and childhood asthma, rhinoconjunctivitis, sensitization to pollen, a mix of food allergens, and horse dander. Other farm-produced foods were not independently related to asthma and allergy prevalence. Of particular importance is the consistency of the findings across children from farming, rural non-farming, anthroposophic, and (sub)urban environments indicating that farm milk consumption represents a route of exposure that is independent of concomitant exposures to microbial compounds present in animal sheds and farm homes. The

Table 4. Sensitivity analyses for the association between asthma and allergy and farm milk consumption; adjusted OR (95% CI)

	n	Dr's diagnosis of asthma	Current wheezing	Dr's diagnosis of rhinoconjunctivitis	Current rhinoconjunctivitis symptoms	Pollen sensitization cut-off ⩾3.5 kU/L	fx5 cut-off ⩾3.5 kU/L
Timing of farm milk exposure							
Shop milk only (reference category)	9805	1	1	1	1	1	1
Farm milk only in the first year of life	1467	0.79 (0.61–1.01)	0.95 (0.74–1.22)	0.52* (0.35–0.75)	0.69* (0.52–0.90)	0.61* (0.37–1.00)	0.98 (0.69–1.44)
Farm milk only at present	869	0.74 (0.54–1.02)	0.85 (0.61–1.17)	0.58* (0.36–0.94)	0.85 (0.61–1.18)	0.73 (0.42–1.27)	0.86 (0.56–1.33)
Farm milk in the first year and at present	2204	0.67* (0.51–0.88)	0.77 (0.58–1.03)	0.61* (0.40–0.94)	0.60* (0.43–0.83)	0.51* (0.31–0.86)	0.61* (0.42–0.89)
Additional adjustment for the child's current visits to animal sheds							
Shop milk consumption only (Reference category)	9805	1	1	1	1	1	1
Farm milk in the first year and at present	2204	0.74* (0.56–0.98)	0.82 (0.61–1.10)	0.70 (0.45–1.07)	0.68* (0.48–0.94)	0.62 (0.37–1.04)	0.62* (0.42–0.91)

*P-value < 0.05.

†Adjusted for study group, country, sex, age, mother's and father's reported asthma and/or hayfever, parent's education, maternal smoking during pregnancy, current environmental smoking at home, older siblings, exclusive breastfeeding > 4 months, BMI, food avoidance due to familial asthma and/or allergy.

OR, odds ratio; CI, confidence interval.

inverse association was not explained by concurrent farm activities of the child or farm exposures during pregnancy and was most pronounced in children drinking farm milk since their first year of life.

The results of the PARSIFAL study thus confirm an inverse association between farm milk consumption and allergic health outcomes reported previously [5–9]. However, the specific allergic health outcomes associated with farm milk consumption differ between studies. The strong effect on asthma was only reported by the ALEX study, which has been conducted in the same three alpine countries as the PARSIFAL study, but in geographically different and independent study populations [5]. Inverse associations between farm milk consumption and allergic rhinoconjunctivitis have been reported by the ALEX study [5], a study in New Zealand [9], and a recent study in the United Kingdom [8]. The ALEX study and surveys conducted in Crete [7], Northern Germany [6], New Zealand [9], and the United Kingdom [8] reported an inverse association between farm milk consumption and atopy whereas the PARSIFAL study observed an inverse association with most tested allergens, but not mites. No association between farm milk consumption and atopic sensitization was reported by a study conducted among rural children from Finland [10].

Allergen-specific differences in response to environmental exposures have already been reported by a series of farm studies that suggested a stronger association between farm residency and pollen sensitization and no or weak relations with mite sensitization [18–21]. However, other farm studies did not confirm these findings [8, 22, 23]. To evaluate whether the allergen-specific effects associated with farm milk consumption in the PARSIFAL study represent so far unappreciated peculiarities in the biology of allergen/immune system interactions deserves replication of these findings and further investigations of potential mechanisms.

The studies conducted in the United Kingdom and New Zealand reported strong and statistically significant inverse relations between farm or raw milk consumption and atopic eczema that was not observed in the present study. These contrasting findings may in part be explained by the amount of control for food avoidance that differed between the studies. Food avoidance due to a pre-existing allergy may bias the results in cross-sectional analyses. Eleven percent of the PARSIFAL population reported avoiding certain foods due to an existing allergy in one of the family members. Adjusting all regression models for this variable only slightly attenuated the effect estimates of farm milk consumption on asthma or rhinoconjunctivitis. However, the association between farm milk consumption and diagnosed eczema became non-significant when adjustment was made for food avoidance [diagnosed eczema without adjustment for food avoidance (aOR 0.80, 95% CI 0.68–0.95), and current eczema

symptoms (aOR 0.84, 95% CI 0.71–0.98), respectively] When the study population was restricted to those without food avoidance, no significant association between farm milk consumption and diagnosed atopic eczema (aOR 0.90, 95% CI: 0.73–1.10) or current eczema symptoms (aOR: 0.94, 95% CI 0.79–1.13) was observed. We also excluded all non-milk-drinking children because this group may have changed dietary habits due to allergy-related skin or gastrointestinal problems early in life and not report it as deliberate food avoidance. However, this restriction had no strong impact on reported atopic dermatitis or any other allergic health outcome.

At present, we can only speculate about the components of farm milk responsible for the observed protective effect. Farm milk possibly contains different levels or a different composition of pathogenic and nonpathogenic microbes compared with milk purchased in a shop. The health effects of pathogens in raw milk such as salmonella or enterohaemorrhagic *Escherichia coli* (EHEC) are well recognized, and transmission of EHEC through unpasteurized cow's milk continues to cause serious health effects [24]. It is conceivable that the microbial burden of farm milk influences the gut microflora and thus the development of oral tolerance [25, 26]. Recent animal experiments have shown that colonization of germ-free mice with polysaccharide-A-producing *Bacteroides fragilis*, a ubiquitous gut microorganism and an important Gram-negative anaerobe that colonizes the mammalian lower gastrointestinal tract, restored normal cytokine production and established a proper T-helper type 1 (Th1)/Th2 balance for the host [27]. Gut microflora may also regulate immune responses outside the gut as has been evidenced in recent animal experiments. Mice were treated with antibiotics in drinking water, followed by a single oral lavage of yeast (*C. albicans*) [28]. They developed alterations of gastrointestinal bacterial populations and increased yeast numbers in the gastrointestinal microbiota. Subsequent intranasal exposure to mould spores led to an allergic response in the airways that was not observed when exposure occurred without prior alteration of the gut microflora. These results indicate that events in distal mucosal sites may play an important role in regulating immune response in the airways. Commensal microorganisms present in farm milk might therefore be responsible for the decreased risk for respiratory allergies such as asthma and hayfever.

The present study does not allow evaluating the effect of pasteurized vs. raw milk consumption because no objective confirmation of the raw milk status of the farm milk samples was available. Parental answers to a question on consumption of boiled vs. raw farm milk are likely to be biased due to the social desirability of responses because raw milk consumption is not recommended especially for young children. About half of the parents indicated that they usually did not boil the milk before consumption but no differential effects were observed between those boiling and those not boiling the milk. This might be a result of biased parental answers or may indicate that pasteurization is not of key importance because compounds other than microbes may play a role. This interpretation is supported by an analysis of Swiss alpine farm milk from exclusively grass-fed cows showing a higher content of omega-3 fatty acids than milk from cows fed conserved grass such as silage [29]. The relative concentrations of linolenic acid (18 : 3) and eicosapentaenoic acid (20 : 5) and the ratio of eicosapentaenoic acid to arachidonic acid (20 : 4) that is critical for the formation of omega-3-derived eicosanoids were significantly higher in milk from grass-fed cows and in cheese made from this milk [29, 30]. Research into fatty acid effects on allergic diseases has focused on the intake of omega-3 fatty acids that is potentially beneficial, and of omega-6 and trans-fatty acids which might be detrimental to asthma [31]. Elevated margarine consumption, which contributes to the intake of omega-6 fatty acid and of trans-fatty acids has been reported to increase childhood atopy risk in several epidemiological studies [3, 32]. Other studies indicated that full-fat milk and butter was associated with a reduced risk of asthma in young children [33–35]. In the present study, butter compared with margarine consumption was associated with a lower risk for asthma supporting a possible role of fatty acid intake. Future analyses of the farm milk compounds responsible for the beneficial effect therefore have to include fatty acid profiles in addition to microbial compounds.

Several limitations of the study have to be taken into account. First, dietary assessment is based on a limited set of variables that do not provide a complete representation of the child's diet. However, the primary aim of the present analyses was to compare farm-produced vs. shop-purchased products and their effect on allergic diseases and not to evaluate the effect of diet per se. The validity and reproducibility of the present dietary assessment has been shown to be good especially for farm milk consumption. Second, as no measurements of biological compounds of farm milk or other farm-produced products are available, the study reports associations and cannot provide an insight into the mechanism underlying the observed association between farm milk consumption and allergic diseases. Third, as the underlying mechanism of the farm milk effect is not known, the study does not allow to elucidate why consumption of farm milk is associated with different allergic health outcomes in different study populations.

In conclusion, the results of the present study indicate that consumption of farm milk is associated with a lower risk of childhood asthma and rhinoconjunctivitis. These results might be transferred to non-farming populations as they were observed in all subpopulations of the PARSIFAL study. Dietary interventions are an attractive

means for primary prevention. However, raw milk may contain pathogens such as salmonella or EHEC, and its consumption may therefore imply serious health risks [24]. A deepened understanding of the relevant 'protective' components of farm milk and a better insight into the biological mechanisms underlying the reported epidemiological observation are warranted as a basis for the development of a safe product for prevention. At this stage, consumption of raw farm milk cannot be recommended as a preventive measure

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Influence of organic diet on the amount of conjugated linoleic acids in breast milk of lactating women in the Netherlands

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The aim of the present study was to find out whether the incorporation of organic dairy and meat products in the maternal diet affects the contents of the conjugated linoleic acid isomers (CLA) and *trans*-vaccenic acid (TVA) in human breast milk. To this purpose, milk samples from 312 breastfeeding mothers participating in the KOALA Birth Cohort Study have been analysed. The participants had documented varying lifestyles in relation to the use of conventional or organic products. Breast milk samples were collected 1 month postpartum and analysed for fatty acid composition. The content of rumenic acid (the main CLA) increased in a statistically significant way while going from a conventional diet (no organic dairy/meat products, 0·25 weight % (wt%), n 186) to a moderately organic diet (50–90 % organic dairy/meat, 0·29 wt%, n 33, $P=0·02$) and to a strict organic diet (>90 % organic dairy/meat, 0·34 wt%, n 37, $P\leq 0·001$). The levels of TVA were augmented among the participants with a moderately organic diet (0·54 wt%) and those with a strict organic diet (0·59 wt%, $P\leq 0·001$), in comparison with the conventional group (0·48 wt%). After adjusting for covariables (recruitment group, maternal age, maternal education, use of supplements and season) statistical significance was retained in the group of the strict organic dairy users ($P<0·001$ for rumenic acid). Hence, the levels of CLA and TVA in human milk can be modulated if breastfeeding mothers replace conventional dairy and/or meat products by organic ones. A potential contribution of CLA and TVA to health improvement is briefly discussed.

Conjugated linoleic acid: *trans*-Vaccenic acid: Human milk: Organic nutrition

The term conjugated linoleic acid (CLA) describes a mixture of positional and geometric isomers of linoleic acid (C18 : 2*n*-6) which contain a conjugated double-bond system instead of the more common isolated double bonds (for a very recent review see Bhattacharya *et al.* 2006). Rumenic or *cis*-9,*trans*-11-octadecadienoic acid (*cis*9,*trans*11-C18 : 2) is the most common CLA isomer and is often regarded as the biologically most relevant one (Fritsche & Steinhart, 1998). The various CLA are produced in the rumen of ruminant animals mainly by the bacteria *Butyrivibrio fibrisolvens* (Kepler *et al.* 1966; Kim *et al.* 2000) through reactions of isomerization and biohydrogenation. These reactions lead as well to the formation of a wide variety of *trans*- and *cis*-monoenic fatty acids (especially C18 : 1 *trans* isomers). In addition, *trans*-vaccenic acid (*trans*11-C18 : 1, TVA) which originates from linoleic and linolenic acid plays an important role as precursor of rumenic acid. Very recent work has shown that the conversion of TVA in rumenic acid does occur as well in man (Mosley *et al.* 2006). CLA are currently receiving much attention in nutritional research, since there is experimental evidence suggesting that these fatty acids might have anti-carcinogenic, anti-atherosclerotic, anti-diabetic and immune-modulating effects, as well as a favourable influence on body fat composition, i.e. on the proportion of fat tissue to muscle mass (Belury, 2002). Most of this experimental evidence derives from *in vitro* experiments or animal tests (Bhattacharya *et al.* 2006), which justifies the recent interest in clinical trials concerning the relevance of CLA for human health. The newly published reports concerning the effect of CLA supplementation on health-related outcomes have

Abbreviations: CLA, conjugated linoleic acid; FID, free induction decay; TVA, *trans*-vaccenic acid; wt%, weight percentage

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contradicting messages and in some cases an isomer-specific effect on the lipid profile could be shown (for a review see Tricon *et al.* 2005). A double-blind study revealed that the consumption of dairy products naturally enriched in *cis*9,*trans*11-C18 : 2 increases the level of this fatty acid in plasma and cellular lipids (Burdge *et al.* 2005). However, this change did not appear to have a significant effect on the whole blood lipid profile, including several CVD risk parameters (Tricon *et al.* 2006).

It is known that the lipid composition of cow's milk is strongly influenced by the stable conditions and feeding management, with milk from cows held in organic farms (Germany, Italy) containing significantly more CLA than that from their conventionally held counterparts (Jahreis *et al.* 1997; Bergamo *et al.* 2003). Note that farms certified as 'organic' are those in which the use of synthetic inputs, such as synthetic fertilizers and pesticides, preventive veterinary drugs, genetically modified seeds and breeds, most preservatives, additives and irradiation are excluded (htpp://www.ifoam.org/sub/faq.html). Since the major source of CLA in man is the diet, we have hypothesized that the amount of CLA in the milk of breastfeeding women could be augmented by increasing the amount of organic dairy nutrients within their diet. The sources of CLA for man comprise not only dairy products but also ruminant meat (Ritzenthaler *et al.* 2001); therefore, emphasis was put on these two groups of nutrients. In a small pilot study in Switzerland, we have previously found that the milk from breastfeeding women who obtained more than 50 % of the energy content of their diet from organic products had about 30 % higher CLA content at 4 and 40 days post-partum, compared to controls consuming the same mixture (dairy products and meat) of conventional products (Rist *et al.* 2003). The aim of the KOALA Birth Cohort Study, which is being performed in the Netherlands, is to identify factors that influence the clinical expression of atopic disease with a main focus on lifestyle including, among other parameters, dietary habits, breastfeeding and breast milk composition (Kummeling *et al.* 2005). Accordingly, this cohort study comprised persons with alternative lifestyles, including organic food choice, which offered an opportunity to study the effect of organic food intake on the lipid composition of breast milk and, in particular, on the corresponding CLA content.

In summary, we addressed the question whether an organic diet of the mothers can result in increased levels of CLA and TVA in human milk. Accordingly, the relative amounts of these fatty acids were measured in the milk from 312 breastfeeding women following diets with different content of organic dairy and/or meat products. While about 50 % of the study participants consumed conventional food, the remaining women included organic dairy and meat products in their diet. The present results, showing that an organic diet can lead to increased levels of CLA and TVA, are discussed in view of the possible health-favourable properties of these fatty acids.

Subjects and methods

Subjects and collection of breast milk

Breast milk samples were donated by breastfeeding participants to the KOALA study, a prospective birth cohort study described in detail elsewhere (Kummeling *et al.* 2005). Briefly, we recruited participants with varying lifestyles (conventional and alternative). Pregnant women with a conventional lifestyle (n 2343) were recruited from an ongoing prospective cohort study on Pregnancy-related Pelvic Girdle pain in the Netherlands (Bastiaanssen *et al.* 2005). During the same recruitment period (December 2002 to August 2003), pregnant women with an alternative lifestyle, which included the use of organic food (n 491), were recruited through several channels, such as organic food shops, anthroposophic clinicians and midwives, Rudolf Steiner schools and relevant magazines. Finally, 312 (146 from the conventional and 166 from the alternative recruitment group) were enrolled, each donating one sample of breast milk, 1 month post-partum. The study was approved by the Medical Ethical Committee of Maastricht University/Academic Hospital Maastricht, Maastricht, The Netherlands.

Breast milk sampling and extraction of total milk lipids

Mothers received a sterile 50 ml tube (Cellstar PP-test tubes; Greiner Bio-One, Kremsmuenster, Austria) and were instructed to collect the milk sample in the morning, before breastfeeding their child, from the contra-lateral breast (since the last feeding) and to keep the tube in the refrigerator (at approximately 4°C) until it was collected by one of the researchers. If the mother was not able to collect the milk sample by herself (with or without a pumping regimen), an electric breast pump (Medela, Baar, Switzerland) was used with the help of one of the researchers, within the same day. Collection and processing of the breast milk samples occurred on the same day. During transport the milk samples were stored in a cooler (Coleman Company, Inc., Breda, The Netherlands) on packed ice (at approximately 4°C). Fractions for fatty acids analysis were preserved by mixing approximately 2 ml milk with 2 μl butylated hydroxytoluene–methanol (1 : 1, v/v). The samples were stored at −80 °C in plastic storage vials (Sarstedt, Nümbrecht, Germany) at the European Biobank in Maastricht (the Netherlands), until analysis. Lipids were extracted from the 0·2 ml milk samples with 3 ml chloroform–methanol (2 : 1, v/v containing 0·001 % butylated hydroxytoluene) after adding water to improve phase separation and 200 μl of the internal standard (containing approximately 200 μg heptadecenoic acid methyl ester in n-hexane, *cis*10-C17 : 1). The lower organic phase was transferred into a Pyrex glass tube and extraction was repeated twice. The combined organic phases were evaporated to dryness under a nitrogen stream at 40°C.

Fatty acid analysis

The lipid extracts were transmethylated with 5 % potassium methylate solution in methanol for 30 min at 60°C. After cooling to room temperature, 3 ml 0·5 M-methanolic sulphuric acid in methanol were added. Thereafter, the extracts were vortexed and heated at 60°C for 15 min. After cooling, 3 ml saturated sodium chloride solution in water and 2 ml n-hexane phase were added. The newly formed fatty acid methyl esters were then extracted into the n-hexane phase by vortexing. The upper n-hexane phase was transferred after centrifugation into a 4 ml glass vial; the extraction was repeated once. The combined n-hexane phases were

evaporated to dryness under a stream of nitrogen and solved in 500 μl *n*-hexane. Fatty acid methyl esters were analysed by GC–free induction decay (GC–FID) and Ag^+–HPLC essentially as previously described (Müller *et al.* 2005). For the GC–FID analysis, an Agilent 6890 GC (Agilent Technologies, Waldbroon, Germany) equipped with a split/splitless injector at 230°C, a flame ionization detector at 260°C, an autosampler and a CP SIL 88 column (100 m, 0·25 mm, 0·2 μm film thickness; Varian, Darmstadt, Germany) was used. Hydrogen was used as carrier, at a constant flow rate of 1 ml/min. The temperature of the GC oven was set to 70°C for 3 min, increased at 8°C/min up to 180°C, held for 2 min, increased at 4°C/min up to 210°C, held for 4 min, increased at 2°C/min to a final temperature of 240°C and held for 25 min. The data were analysed using the HP Chemstation software (Rev. A08.03); the percentage method which excludes the internal standard was used, to allow a better comparison of the fatty acids among the various samples. Conjugated fatty acid isomers were separated using Ag + –HPLC– diode-array detection. The system consisted of an isocratic Merck-Hitachi L-6000 A HPLC pump equipped with a Waters 717 autosampler (Waters, Eschborn, Germany) and a Waters 996 diode-array detector operated at wavelength between 210·4 and 395·4 nm. Three Chromspher 5 lipid columns (250 mm × 4·6 mm, 5 μm) were used in series with a 50 mm × 4·6 mm pre-column of the same column material (Varian). Propionitrile at 0·02 % in *n*-hexane was used as eluent at a flow rate of 1 ml/min (approximately 80 bar). Millennium^{32} software (Version 3.20; Waters) was used for data analysis. The following CLA isomers were considered in the analysis: *trans*12,*trans*14-, *trans*11,*trans*13-, *trans*10,*trans*12-, *trans*9,*trans*11-, *trans*8,*trans*10-, *trans*7,-*trans*9-, *cis*11,*trans*13-, *trans*10,*cis*12-, *cis*11,*trans*13-, *trans*11,-*cis*13-, *cis*9,*trans*11-, *trans*8,*cis*10-, *cis*11,*cis*13-, *cis*10,*cis*12-, *cis*9,*cis*11- and *cis*8,*cis*10-C18 : 2.

FFQ

The FFQ (Kummeling *et al.* 2005) was included in a self-administered questionnaire in week 34 of the pregnancy. The questionnaire was based on an existing validated one (Grootenhuis *et al.* 1995), which was extended and modified to meet the specific aims of the present study. To make the questionnaire suitable for subjects with a vegetarian, anthroposophic, macrobiotic or other alternative dietary lifestyle, specific foods often used by these groups were included as well. The FFQ consisted of approximately 160 food items, for which the frequency of consumption and portion size were to be estimated. Furthermore, we have asked for information concerning the origin of the various food groups, for each of the three following food categories: dairy products, meat and certain other food items. The study participants had to specify whether the aliments had originated from conventional, organic or biodynamic – a special form of organic agriculture in which emphasis is put on activating the life of the soil by using natural preparations from plant and animal origin – production. The patients who consumed organic (including biodynamic) food were asked whether these constituted <50 %, 50–90 % or >90 % of the food, within the corresponding food group. Since biodynamic foods are expensive, difficult to find and often used as an adjunct to organic foods, we only asked whether subjects used 'any' foods of biodynamic origin, again distinguishing between dairy products, meat and other food groups.

Subjects were classified into four groups distinguished in terms of the origin of the meat and dairy products: (1) conventional (if <50 % of both the meat and dairy they used was of organic origin, or they ate no meat and <50 % of the dairy they used was of organic origin, or they ate no dairy and <50 % of the meat they used was of organic origin); (2) 50–90 % organic (if >50 % of both the meat and dairy they used was of organic origin but <90 % of one of the two was of organic origin, or they ate no meat and 50–90 % of the dairy they used was of organic origin, or they ate no dairy and 50–90 % of the meat they used was of organic origin); (3) >90 % organic (if >90 % of both the meat and dairy they used was of organic origin, or they ate no meat and >90 % of the dairy they used was of organic origin, or they ate no dairy and >90 % of the meat they used was of organic origin); (4) other (including any combination of <50 % meat of organic origin and >50 % dairy of organic origin or vice versa, and missing and inconsistent data). For the purpose of the present study, only those food items which are relevant dietary sources of CLA were documented: milk and milk products, including cheese and butter (nineteen food items), meat and meat products (nineteen food items). Fat intake from these food groups was calculated using the most recent Dutch Food Composition Table (Anonymous, 2001). Calculation of fat intake from meat was limited to that of ruminant cattle such as beef and veal (omitting minced and processed meat because it is often a mixture of beef and pork), lamb and mutton; throughout this paper, we refer to these nutrients as 'meat' only, for simplicity reasons. Since we expected that the fatty acid composition of the breast milk could be influenced by the use of dietary supplements during pregnancy and lactation, the questionnaire administered during the pregnancy and a questionnaire administered at the moment of breast milk sampling included detailed questions on the use of supplements with borage oil or primrose oil (both containing γ-linolenic acid, C18 : 3*n*-6) and fish oil (containing eicosapentaenoic acid, C20 : 5*n*-3; docosapentaenoic acid, C22 : 5*n*-3; docosahexaenoic acid, C22 : 6*n*-3).

Statistical methods

Duplicate values of fatty acids – expressed as weight percentage (wt%) of total fatty acids in breast milk fat – were averaged for each subject, and the resulting mean values were used for further calculations. Mean wt% of rumenic acid and other CLA (total CLA minus rumenic acid) were computed for groups of subjects classified by organic origin of dairy and meat, using Student's *t* test to assess differences between the groups (not assuming equality of variances); a difference between two groups was considered to be statistically significant if $P \leq 0.05$. A linear regression analysis used rumenic acid level and TVA (wt%) as the dependent variables, while the independent variables were the categories of organic or biodynamic origin of dairy and meat and the fat intake from ruminant meat and dairy (g/d). Possible interactions between origin and fat intake were tested by adding interaction terms to the linear regression models. Since we expected CLA levels in fresh dairy products to be higher in summer months,

we also included the season in which the breast milk was sampled in the multivariate analysis, to correct for a possible confounding effect (dichotomized into two periods: December 2002 to May 2003; June 2003 to September 2003). Other covariables in the regression analyses were: recruitment group (conventional/alternative), maternal age, education, and the use of oil supplements during pregnancy or lactation (yes/no). All statistical analyses were done in SPSS version 12.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Of the 312 participants, thirty-three (10·5 %) used 50–90 % meat and dairy of organic origin, while thirty-seven (11·8 %) used more than 90 % of these aliments (Table 1). The subjects differed substantially in terms of recruitment group, and slightly in terms of the month during which breast milk sampling took place, education level and age of the mother, as well as in the use of oil supplements (Table 1). Therefore, we have included these characteristics as covariables in the multivariate linear regression analysis. Only six (3 %) subjects in the conventional group used any meat and dairy of biodynamic origin, whereas, as expected, this percentage was higher in the groups of organic users, increasing up to 30 % use of biodynamic meat and 76 % use of biodynamic dairy in the >90 % organic group (Table 1); the 'other' group had an intermediary position. As shown in Table 1, the total fat intake from the main dietary fat sources included in the FFQ was comparable between the groups, but the percentage contributed by dairy fat was almost twice as high in the groups with 50–90 % (70 %) and >90 % (75 %) meat and dairy of organic origin compared to the conventional group (39 %).

The levels of rumenic acid were higher in the groups of organic meat and dairy users, with an increasing trend going from the 'other' group to the 50–90 % organic meat and dairy group to the >90 % organic meat and dairy group (Table 2). The difference between the levels of rumenic acid in these groups and that in the conventional group was always statistically significant. No such trend was found for other CLA, and the relative amount of all the other CLA (wt%) was slightly lower in the 50–90 % group (Table 2). The mean level of TVA was about twice the level of rumenic acid in breast milk and correlated with rumenic acid (r 0·51, $P<0·001$). Like rumenic acid, TVA content showed an increasing trend over the organic groups relative to the conventional group (Table 2), reaching statistical significance in the >90 % organic meat and dairy group. However, this was not the case for the differences among the other groups, which was probably due to the relatively high standard deviation values. The most abundant fatty acids present in milk are depicted in Table 3, to better understand the context of the mentioned changes in CLA and TVA levels. The increases in the levels of these fatty acids seem to be associated with relative decreases in the levels of *trans*-C18:1 and of C20:4 fatty acids.

Table 1. Relevant characteristics of the study participants†

	Conventional		50–90 % organic meat and dairy		>90 % organic meat and dairy		Other	
Origin of meat and dairy products	*n*	%	*n*	%	*n*	%	*n*	%
Total number	186	100	33	100	37	100	56	100
Recruitment group								
Conventional	126	68	4	12	2	5	14	25
Alternative	60	32	29	88	35	95	42	75
Maternal age (mean and SD)	32·5	3·8	34·2	3·6	35·4	4·0	33·9	3·7
Maternal education								
Low	13	7	2	6	3	8	1	2
Intermediate	70	38	5	15	8	22	12	21
Higher vocational	82	44	12	36	16	43	21	38
University	21	11	14	42	10	27	22	39
Month of sampling								
December to January	40	22	0	0	4	11	12	21
February to March	63	34	11	33	11	30	16	29
April to May	42	23	11	33	7	19	20	36
June to July	33	18	6	18	8	22	7	13
August to September	8	4	5	15	7	19	1	2
Oil supplement use								
No	175	94	31	94	37	100	54	96
Yes	11	6	2	6	0	0	2	4
Use of biodynamic dairy								
None	180	97	28	85	26	70	46	82
Any	6	3	5	15	11	30	10	18
Use of biodynamic meat								
None	180	97	14	42	9	24	32	57
Any	6	3	19	58	28	76	24	43
Fat intake (g/d) from ruminant meat (mean and SD)	3·4	2·6	2·3	2·4	1·5	1·9	3·9	3·2
Fat intake (g/d) from dairy (mean and SD)	17·6	10·4	33·0	14·6	32·8	16·4	23·9	13·2

† The participants (n 312) were distributed by the various groups (conventional, 50–90 % organic, >90 % organic and other) according to the origin of the dairy and meat products included in the corresponding diet. The characteristics of the participants attributed to each of the four groups in terms of number, age, maternal education, month of breast milk sampling, use of oil supplement and use of biodynamic dairy and meat products are depicted.

Table 2. Rumenic acid, other conjugated linoleic acids, *trans*-vaccenic acid and other relevant fatty acid classes in breast milk (as weight percentage (wt%) of total milk fat) by origin of meat and dairy (n 312)†

(Mean values and standard deviations)

	Conventional (n 186)		> 50 % organic meat and dairy (n 33)		> 90 % organic meat and dairy (n 37)		Other (n 57)	
Fatty acids	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Rumenic acid (*cis*9,*trans*11-C18 : 2)	0·25	0·07	0·29*	0·10	0·34***	0·10	0·27	0·11
Other conjugated linoleic acids	0·07	0·03	0·06*	0·03	0·07	0·03	0·07	0·03
trans-Vaccenic acid (*trans*11-C18 : 1)	0·48	0·21	0·54	0·26	0·59***	0·16	0·53*	0·16
LA (C18 : 2*n*-6)	13·73	3·19	13·81	4·28	14·90	4·40	13·06	2·87
Sum of LA derivatives‡	1·33	0·23	1·29	0·19	1·36	0·25	1·30	0·23
LA + LA derivatives	15·06	3·26	15·10	4·28	16·26	4·48	14·37	2·90
α-Linolenic acid (C18 : 3*n*-3)	1·05	0·38	0·89*	0·41	0·82***	0·28	0·93*	0·27
Sum of ALA derivatives§	0·79	0·32	0·77	0·22	0·79	0·43	0·81	0·44
ALA + ALA derivatives	1·84	0·56	1·65*	0·47	1·61*	0·61	1·74	0·48
Total PUFA	18·45	3·53	18·27	4·36	19·51	4·67	17·66	2·95
Total MUFA	40·84	3·08	39·48	4·12	38·57***	3·20	40·28	3·97
Total SFA	40·71	4·33	42·25	5·82	41·91	5·36	42·07	4·75

ALA, α-linolenic acid; LA, linoleic acid

Mean values were significantly different from those of the conventional group (Student's t test): *$P<0·05$; **$P<0·01$; ***$P<0·001$

† For details of procedures, see pp. 736–737.

‡ Sum of LA derivatives includes: C18 : 3*n*-6, C20 : 3*n*-6, C20 : 4*n*-6, C22 : 4*n*-6 and C22 : 5*n*-6

§ Sum of ALA derivatives includes: C18 : 4*n*-3, C20 : 4*n*-3, C22 : 4*n*-3, C22 : 5*n*-3 and C22 : 6*n*-3

After adjusting for covariables (alternative *v.* conventional recruitment group; maternal age; maternal education; use of supplements; winter *v.* summer months), rumenic acid remained statistically significantly higher in the >90 % organic group (*v.* conventional) (Table 4, model A). Fat intake from ruminant meat and from dairy contributed equally to the rumenic acid level in breast milk, but only the result for dairy fat was statistically significant: 0·021 increment of rumenic acid level (as wt% of total milk fat) per 10 g/d increment of daily dairy fat intake, $P<0·001$ (Table 4, model B). When we additionally adjusted for fat intake from dairy and ruminant meat, rumenic acid remained significantly higher in the >90 % organic group compared to the conventional group (Table 4, model C). In addition to the organic origin, dairy fat intake still contributed to the rumenic acid level in breast milk (Table 4, model C). In linear regression analysis of breast milk TVA, results were very similar to those of rumenic acid: TVA (wt% of total milk fat) was statistically significantly higher in the >90 % organic group (*v.* conventional, regression coefficient 0·097, SE 0·040, $P=0·015$). Moreover, TVA was dependent on dairy fat intake in a statistically significant way: 0·023 increment of TVA (as wt% of total milk fat) per 10 g/d increment of daily dairy fat intake (SE 0·009, $P=0·009$), linear regression adjusting for the covariables mentioned earlier. It is worth mentioning that rumenic acid in breast milk peaked in the early summer

Table 3. Most abundant fatty acids in breast milk (as weight percentage (wt%) of total milk fat) by origin of meat and dairy (n 312)†

(Mean values and standard deviations)

	Conventional (n 186)		>50 % organic meat and dairy (n 33)		>90 % organic meat and dairy (n 37)		Other (n 57)	
Fatty acids	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C16 : 0	22·62	2·30	22·79	2·54	22·63	2·86	23·21	2·57
C18 : 2	13·73	3·19	13·81	4·28	14·90	4·40	13·06	2·87
C18 : 0	7·02	1·48	6·60	1·49	6·87	1·15	6·95	1·66
C14 : 0	5·63	1·50	6·65**	1·74	6·42*	1·78	6·20*	1·80
C12 : 0	4·56	1·70	4·95	1·90	4·69	1·52	4·61	1·57
C18 : 3*n*-3	1·05	0·38	0·89*	0·41	0·82***	0·28	0·93*	0·27
*trans*9-C18 : 1	0·61	0·27	0·47***	0·20	0·51*	0·24	0·52**	0·18
C20 : 4	0·53	0·10	0·48***	0·08	0·48**	0·08	0·50*	0·09
*trans*11-C18 : 1	0·48	0·21	0·54	0·26	0·59***	0·16	0·53*	0·16
C18 : 3*n*-6 (di-homo)	0·46	0·11	0·48	0·10	0·53**	0·13	0·47	0·12
C22 : 6	0·42	0·20	0·41	0·16	0·42	0·27	0·44	0·28
C17 : 0	0·31	0·10	0·36***	0·07	0·34	0·12	0·33	0·11
C15 : 0	0·30	0·09	0·38***	0·12	0·40***	0·13	0·37***	0·12
*cis*9,*trans*11-C18 : 2	0·25	0·07	0·29*	0·10	0·34***	0·10	0·27	0·11
C14 : 1	0·23	0·08	0·29**	0·12	0·29*	0·12	0·29***	0·11

Mean values were significantly different from those of the conventional group (Student's t test): *$P<0·05$; **$P<0·01$; ***$P<0·001$

† Only fatty acids present in amounts higher than 0·2 % of total milk fat are shown. For details of procedures, see pp. 736–737

Table 4. Rumenic acid in breast milk as a function of the use of either meat and dairy of organic origin (model A) or dietary fat intake from ruminant meat and dairy products (model B) or both (model C)†

	Model A			Model B			Model C		
Parameter	Coeff	SE	*P*	Coeff	SE	*P*	Coeff	SE	*P*
Origin of meat and dairy‡									
Other	0·005	0·014	0·74				−0·002	0·013	0·88
50–90 % organic	0·019	0·017	0·28				0·000	0·017	0·99
> 90 % organic	0·062	0·017	<0·001				0·045	0·017	0·01
Fat intake from									
Ruminant meat§				0·021	0·017	0·21	0·031	0·017	0·07
Dairy§				0·021	0·004	<0·001	0·019	0·004	<0·001

Coeff linear regression coefficient; SE, standard error of the regression coefficient.

† The difference in rumenic acid (as weight percentage (wt%) of total milk fat) was assumed to vary linearly with one of the two or with both parameters; data from the 312 participants were incorporated in the linear regression. Linear regression models controlled for the following covariables: alternative *v.* conventional group; maternal age; maternal education level; use of supplements; winter *v.* summer months

‡ Coefficients denote the difference in rumenic acid level (wt%) in each group compared to the conventional group.

§ Coefficients denote increase in rumenic acid level (wt%) with 10 g/d increase of fat intake.

months in the conventional and 50–90 % organic meat and dairy users, and somewhat later in the >90 % organic group (data not shown).

Discussion

CLA and TVA are often formed by isomerization and biohydrogenation of dietary linoleic and linolenic acid by microorganisms (mainly *Butyrovibrio fibrisolvens*) living in the rumen of ruminant animals. These reactions lead to the formation of various positional and geometric isomers, which differ substantially in nutritional value (Banni *et al.* 1999). TVA is the major *trans*-fatty acid in ruminant milk fat and an intermediate in the bioconversion of linoleic acid (C18 : 2*n*-6) to rumenic acid (*cis*9,*trans*11-C18 : 2). In man, it can be converted by Δ9 desaturation to rumenic acid (Turpeinen *et al.* 2002), being probably responsible for one-quarter of the human CLA pool (Kuhnt *et al.* 2006). This conversion has been shown to occur in lactating women (Mosley *et al.* 2006); in this precursor study, consumed TVA was converted in rumenic acid which was detectable in the human milk. Other *trans*-fatty acids, such as *trans*-10-octadecenoic acid (*trans*10-C18 : 1) cannot be desaturated. Bertschi *et al.* (2005) have recently described an about 50 % concomitant increase of both TVA and rumenic acid levels in human breast milk after consumption of alpine butter, in comparison with margarine. Since alpine butter had a high content of these fatty acids whereas the tested margarine had hardly any (Bertschi *et al.* 2005), this observation suggests that the CLA and TVA present in human milk have a dietary origin. At least in the Netherlands the main sources of CLA and TVA are of dairy origin (Voorrips *et al.* 2002). In the present work, however, no determination of the content in these fatty acids in the dairy and meat products normally consumed by the mothers of the different groups was performed.

It should be mentioned that industrially produced *trans*-fatty acids, as often incorporated in commercial products, are likely to contribute to pathological situations, such as IHD (Stender & Dyerberg 2004) and type 2 diabetes (Odegaard & Pereira, 2006). This possible contribution is leading several governments to limit the total amount of these fatty acids – mainly elaidic acid (*trans*9-C18 : 1), but to some extent also TVA (*trans*11-C18 : 1) – which can be included in oils and fats. For instance, in the case of the Danish government, this limit has been set to 2 % of the total fat content (Stender *et al.* 2004). The oscillations of the levels of TVA reported in the present study occur therefore within a range which is clearly different from the one of industrially produced *trans*-fatty acids, meaning that no unfavourable effects on human health are to be expected. Furthermore, and as often occurs in natural mixtures, the observed oscillations occur concomitantly to other alterations, namely to an increase in the CLA levels, which are likely to exert beneficial effects on health performance.

We found that the levels of rumenic acid as well as of TVA in breast milk were higher in mothers which included organic dairy and meat products in their diet, in comparison with mothers who had pursued a conventional diet. Furthermore, the extent of the increase in rumenic acid depended on the amount of organic products consumed during the study, with those mothers using almost exclusively (more than 90 %) organic dairy and meat products in their diet having a higher content of this fatty acid in their milk than mothers with a moderately (50–90 %) organic dairy and meat diet. These data corroborate the results of our previous pilot study, which showed that mothers who consumed more than 50 % organic dairy and meat products had higher levels of CLA (Rist *et al.* 2003). Interestingly, it has been shown in a variety of studies (Jahreis *et al.* 1997; Bergamo *et al.* 2003; Gedek, 1980; Dewhurst *et al.* 2003) that the levels of CLA in cow's milk from organic producers in Europe, including the Netherlands (Adriaansen-Tennekes *et al.* 2005), are significantly higher than CLA levels in the milk from conventional producers. Therefore, and since the fat from human breast milk is likely to be of dietary origin (see earlier), we believe that the larger amounts of rumenic acid and TVA in breast milk from the organic groups were due to the corresponding intake of organic dairy and meat products with higher levels of rumenic acid and TVA. This interpretation of the present results is strengthened by the fact that the total fat intake was comparable among the various groups and that the CLA content of the food is very stable and not influenced by storage or processing (Luna *et al.* 2005). The fat intake from meat was five to twenty times lower than that from dairy products; therefore, it is likely that dairy products were more strongly influencing the final fat composition of the milk than the

meat products Much to our surprise, the multivariate analysis found an additional effect of the organic origin on the diet, stronger than that to be expected from the total dairy fat intake.

The CLA levels found in the breast milk from the >90 % organic dairy and meat products consumers were as high as those reached after supplementation with 30 g/d alpine butter for 10 d (Bertschi *et al.* 2005): 0·33 g/100 g milk fat (margarine control: 0·22 g/100 g milk fat). Breast milk levels of rumenic acid in the conventional group (0·25 g/100 g fat) were in the same range as the values previously reported: 0·21 g/100 g fat (Park *et al.* 1999); 0·28 g/100 g total fatty acids (Ritzenthaler *et al.* 2005), and 0·19 and 0·18 g/100 g fat (Jensen *et al.* 1998; Jensen & Lammi-Keefe, 2001). The higher levels, namely 0·4 g/100 g fat, recorded in American (McGuire *et al.* 1997; Innis & King, 1999) and in German mothers (Jahreis *et al.* 1999) can be attributed to a diet which normally includes higher amounts of dairy products and/or meat. The fact that the relative amounts of CLA and TVA correspond to less than 1 % of the total fat should not be taken as indicative of a reduced physiological relevance of these fatty acids. Their mechanism of action is likely to include the production of biologically active compounds and processes of intracellular signalling (Khan & Vanden Heuvel, 2003), and it is typical for molecules participating in such processes that they are present in very small amounts. In this context it is worth mentioning that, although the levels of *n*-3 fatty acids in maternal milk are as well rather low, they have been shown to influence the risk of non-atopic eczema and asthma in the infant (Oddy *et al.* 2006; Wijga *et al.* 2006). Concerning the magnitude of the differences in breast milk CLA levels that we found among the various groups, it might be argued that they are minor. Nevertheless, it should be noted that the level in breast milk reflects CLA intake by maternal diet and could therefore be a marker of placental supply in uterus and possibly of ongoing supply to the child from dietary sources of dairy products and meat shared by the family. Taken together, these factors are likely to represent a lifelong cumulative effect.

The health effects of CLA and TVA on human newborns are still unknown; nevertheless there is promising evidence stemming from animal models and from clinical studies involving human adults. Often, the positive effects of CLA on health parameters revealed themselves stronger in animal models than in clinical studies with man (Bhattacharya *et al.* 2006). One possible explanation for this discrepancy is that, while animal studies have concentrated on very young growing rats or mice clinical studies have exclusively focused on adult man. This strengthens the need for long-term clinical studies starting with very young participants, as is possible within the frame of the KOALA study. An area in which the expectations concerning the CLA effects are relatively high concerns their immunomodulating properties (see review by O'Shea *et al.* 2004). Indeed, in animal models, these fatty acids lead to a reduction of the harmful effects of intranasally administered influenza viruses, and to a reduction of the leukotriene and prostaglandin production which suggests a favourable effect in preventing inflammatory phenomena that are typical of an immediate immune response (O'Shea *et al.* 2004). Moreover, CLA feeding was able to prevent wasting after endotoxin injections (Cook *et al.* 1999). The examination of healthy human volunteers who had been vaccinated against hepatitis B revealed that supplementation with certain CLA isomers resulted in a statistically significant higher level of protective antibodies, indicative of a better immune-responsiveness to the vaccination (Albers *et al.* 2003). CLA supplementation in young healthy men affected the immune function in terms of increased plasma IgA and IgM, and the anti-inflammatory cytokine IL-10, and decreased levels of IgE and the proinflammatory cytokines TNF-α and IL-1γ (Song *et al.* 2005). Dietary studies have indicated a protective effect of butter relative to margarine against allergy and asthma (Bolte *et al.* 2001; Dunder *et al.* 2001; Woods *et al.* 2003). Similarly, a 3-year prospective cohort study found a decreased risk of asthma in children who consumed full cream milk and butter daily, compared to those who did not (Wijga *et al.* 2003). Since butter is normally rich in CLA, this might suggest a positive effect of CLA on the prevention of those diseases. Furthermore, it is known that children who grow up in families with an anthroposophic lifestyle have a reduced risk of atopic diseases compared to those in families with conventional lifestyles (Alm *et al.* 1999; Alfven *et al.* 2006). An anthroposophic lifestyle comprises, besides a restrictive use of antibiotics and few vaccinations, a diet that usually contains raw milk and organic, or more specifically biodynamic, products. Given that an organic diet and organic dairy and/or meat products have a higher CLA content than their conventional counterparts (see earlier), this observation might suggest that CLA consumption could add to a protective effect against atopic diseases.

In conclusion, we show here that the levels of both rumenic acid and TVA in human breast milk were higher in the case of mothers following a diet that contained organic dairy and meat products, in comparison with mothers consuming a conventional diet. In view of the accumulating evidence pointing towards various positive effects of CLA on human health, in particular at a very young age, the present results are highly interesting. Further results of the KOALA Birth Cohort Study, in particular those concerning allergic sensitization and asthma in the children corresponding to the mothers that have participated in the present study, are awaited anxiously.

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USDA ARS United States Department Of Agriculture
Agricultural Research Service

Research Project: MICROBIAL COMPETITIVE EXCLUSION TO REDUCE EPIZOOTIC PATHOGENIC BACTERIA IN SWINE AND CATTLE

Location: **Food and Feed Safety Research**

Title: COLICINS INHIBIT GROWTH OF ESCHERICHIA COLI O157:H7 BUT NOT SALMONELLA IN VITRO

Authors

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Citation: Callaway, T.R., Stahi, C.H., Edrington, T.S., Genovese, K.J., Lincoln, L.M., Anderson, R.C., Lonergan, S.M., Poole, T.L., Harvey, R.B., Nisbet, D.J. 2004. Colicin concentrations inhibit growth of Escherichia coli O157:H7 in vitro. Journal of Food Protection. 67:2603-2607.

Interpretive Summary: Escherichia coli O157:H7 and Salmonella Typhimurium are food-borne pathogens that cause severe human illnesses and inhabit the intestinal tract of food animals. Colicins are antimicrobial proteins produced by certain E. coli strains that inhibit or kill other E. coli. Colicin activity against strains of E. coli O157:H7 and Salmonella were quantified in vitro. Colicin E1 was most effective against E. coli O157:H7 strains, followed by colicin N; colicin A did not affect E. coli O157:H7. Colicin E1 inhibited the growth of E. coli O157:H7 at very low concentrations. In swine, colicin E1 treatment reduced E. coli O157:H7 populations. Because colicins are proteins, in order for them to be an effective strategy to reduce

food borne pathogens in live animals, colicins must be protected from host animal digestion. These potent antimicrobial proteins may potentially provide an effective and environmentally sound pre-harvest strategy to reduce E. coli O157:H7 in food animals.

Technical Abstract: Escherichia coli O157:H7 and Salmonella Typhimurium are food-borne pathogens that cause severe human illnesses and inhabit the intestinal tract of food animals. Colicins are antimicrobial proteins produced by E. coli strains that inhibit or kill other E. coli. In the present study, the efficacy of three pore-forming colicins (E1, N and A) were quantified in vitro against E. coli O157:H7 strains 86-24 and 933 and Salmonella Typhimurium. Colicin E1 and N reduced the growth of E. coli O157:H7 strains, but the efficacy of each colicin varied among strains. Colicin E1 was more effective against both strains of E. coli O157:H7 than colicins A and N, and reduced ($P < 0.05$) populations of E. coli O157:H7 at concentrations less than 0.1 ug/ml. Growth rates, final optical densities, and cell numbers of Salmonella Typhimurium were not affected by colicin treatment. These potent antimicrobial proteins may potentially provide an effective and environmentally sound pre-harvest strategy to reduce E. coli O157:H7 in food animals.

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ORIGINAL ARTICLE

Contamination of milk by enterococci and coliforms from bovine faeces

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Keywords
Aerococcus bovine faeces coliforms, *Enterococcus*, raw milk

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Abstract

Aim: To determine the contribution of enterococci and coliforms from bovine faeces and teats to contamination of raw milk

Methods: Putative enterococci ($n = 301$) and coliforms ($n = 365$) were isolated from bovine faeces ($n = 20$), cows' teats ($n = 20$), the raw milk ($n = 1$) and the milking environment ($n = 4$) on one farm. The clonal relationships of each bacterial group were investigated using Pulsed-Field Gel Electrophoresis of genomic macrorestriction fragments. Representatives of the different clusters of enterococci were identified by molecular techniques including rep-PCR, SDS protein profiling, Fluorescent Amplified Fragment Length Polymorphism (FAFLP), phenylalanyl-tRNA synthase (pheS) sequence analysis and/or 16S rDNA gene sequencing. Coliforms were identified by API 20E strips.

Results: The majority of the bovine faecal enterococcal isolates were identified as a potential new species of *Aerococcus* (100 isolates); *E. faecium* (28 isolates), and *Aerococcus viridans* (28 isolates) were also found. All coliform isolates from the bovine faeces were identified as *Escherichia coli*. The coliforms present in the milk were *Hafnia alvei*, *Serratia liquefaciens*, *Yersinia enterocolitica* and *Enterobacter amnigenus*. No *E. coli*, *Enterococcus* or *Aerococcus* from the bovine faeces were found in the milk. A single clone of *H. alvei* was found in the water, the milking equipment and the milk, suggesting that the water was the source of the organism in the milk. No vancomycin-resistant aerococci or enterococci were found, while most of the isolates tested showed the presence of at least one virulence gene. The milk-sock retained strains that adhered to particulate faecal material. Coliforms were present at approx. 2 orders of magnitude greater than enterococci in the bovine faeces.

Conclusions: The results imply that bovine faeces are not an important source of contamination of raw milk with enterococci or coliforms.

Significance and Impact of the Study: The results confirm those of two previous studies (Gelsomino *et al.* 2001, *Int J Food Microbiol* **71**, 177–188 and Kagkli *et al.* 2007, *Int J Food Microbiol* **114**, 243–251) on two other farms. The three studies show that contamination of milk by enterococci, lactobacilli and coliforms of bovine faecal origin is extremely low. The results also suggest that where raw milk is implicated in food infection, other factors in addition to faecal contamination must be involved

Introduction

Enterococci and coliforms form part of the intestinal microflora of animals and humans (Facklam *et al.* 2002). Because of this, they are generally considered to indicate faecal contamination of animal-derived food, or that the food was produced under poor hygienic conditions. They are also common in environments which can be

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Centers for Disease Control and Prevention

Date: May 8, 2007

From: Enteric Diseases Epidemiology Branch
Division of Foodborne, Bacterial, and Mycotic Diseases, NCZVED/DFBMD

Subject: FOIA 06-0819

To: Pete Kennedy – Sarasota, Florida

Attached is a line list of outbreaks of foodborne illness reported to CDC's National Foodborne Outbreak Surveillance System from 1973 to 2005 associated with milk or milk products. The first line list contains outbreaks indicating milk was the contaminated ingredient or where milk or milk product was indicated as being the implicated food vehicle, excluding unpasteurized milk and cheese often made with unpasteurized milk.

The second line list includes foodborne outbreaks where unpasteurized milk or cheese often made from unpasteurized milk was indicated as being the implicated food.

Food vehicles identified are not necessarily confirmed with statistical or epidemiological evidence. All food vehicles identified by the reporting agency are listed in the line lists.

Division of Foodborne, Bacterial, and Mycotic Diseases
National Center for Zoonotic, Vector-Borne, and Enteric Diseases (NCZVED)

CERTIFIED TO BE A TRUE COPY OF THE ORIGINAL

Ola DiGioia

Certifying Official

Date May 24, 2007

EXHIBIT

tabbies

K

I declare under penalty of perjury that the foregoing is to the best of my knowledge and belief true and correct.

Executed at Atlanta, Georgia

Ola Di Gioia

Ola Di Gioia

State of Georgia)

County of Dekalb)

Subscribed and sworn to before me this 24th day of May, 2007.

Notary Public

Ceci Velasco
My Comm. Expires October 17, 2010
Gwinnett County, Georgia

DEPARTMENT OF HEALTH & HUMAN SERVICES

Office of the Secretary

Office of the General Counsel
Public Health Division
CDC/ATSDR Branch
1600 Clifton Road, N.E., M/S D53
Atlanta, Georgia 30333
(404) 639-7200

AFFIDAVIT

I, OLA DI GIOIA being first duly sworn, deposes and state as follows:

1. I am a Legal Technician in the Office of the General Counsel, Centers for Disease Control and Prevention (CDC), United States Department of Health and Human Services. I make this affidavit upon personal Knowledge, and where indicated, upon the basis of information Communicated to me by employees of the United States because of my Official position.

2. In my position, I am a custodian of and authorized to certify official records of the U.S. Department of Health and Human Services.

3. The attached records, imprinted with the official CDC seal, are true copies of official Records of CDC, an agency of the U. S. Department of Health and Human Services.

4. The document referred to above are part of the official records of the United States Department of Health and Human Services.

Foodborne outbreaks associated with milk, excluding unpasteurized milk and cheese often made with unpasteurized milk, reported to CDC's National Foodborne Outbreak Surveillance System. 1973-2005 (N=47)

Year	Estimated Total	Food	Ingredient	Pathogen
1973	22	Milk	Not indicated	*Salmonella* Dublin
1974	3	Milk	Not indicated	*Salmonella* Dublin
1974	15	Milk	Not indicated	*Staphylococcus aureus*
1975	47	Milk	Not indicated	*Salmonella* Newport
1976	286	Milk	Not indicated	*Yersinia enterocolitica*
1978	66	Milk	Not indicated	*Salmonella* Typhimurium var Cope
1980	8	Milk	Not indicated	*Salmonella* Derby
1980	106	Milk	Not indicated	*Campylobacter* unknown
1981	2	Milk	Not indicated	*Salmonella* Saintpaul
1981	7	Milk	Not indicated	*Salmonella* Group B
1981	17	Milk	Not indicated	*Salmonella* Dublin
1981	14	Milk	Not indicated	*Campylobacter jejuni*
1981	190	Milk	Not indicated	*Campylobacter jejuni*
1981	25	Milk	Not indicated	*Campylobacter jejuni*
1981	103	Milk	Not indicated	*Campylobacter jejuni*
1981	1000	Milk	Not indicated	Suspect *Staphylococcus aureus*
1982	172	Pasteurized milk	Not indicated	*Yersinia enterocolitica*
1982	46	Milk	Not indicated	*Campylobacter jejuni*
1983	61	Milk	Not indicated	*Campylobacter jejuni*
1983	69	Pasteurized milk	Not indicated	*Listeria*
1985	16659	Milk	Not indicated	*Salmonella* Typhimurium
1985	20	Milk	Not indicated	Other chemical
1986	87	Milk	Not indicated	*Campylobacter jejuni*
1989	50	Milk	Not indicated	*Salmonella* Typhimurium
1991	37	Choc. Milk	Not indicated	*Salmonella* Typhimurium
1992	23	Milk	Not indicated	*Campylobacter jejuni*
1993	3	Powdered milk prod	Not indicated	*Salmonella* Tennessee
1994	69	Chocolate milk	Not indicated	*Listeria monocytogenes*

1994	18	Milk	Not indicated	*Escherichia coli* O104:H21
1996	19	Chocolate milk	Not indicated	*Salmonella* unknown
1996	29	Milk	Not indicated	*Campylobacter jejuni*
1998	58	Chile relleno, unspecified	Milk	*Salmonella* Enteritidis
1998	47	Other milk, unspecified	Not indicated	*Salmonella* Typhimurium
1999	2	Milkshake	Not indicated	Suspect *Staphylococcus aureus*
2000	3	Other milk, pasteurized	Not indicated	*Campylobacter jejuni*
2000	2	Blue cheese dressing	Milk	Suspect *Staphylococcus aureus*
2001	8	Milkshake	Not indicated	Hepatitis A
2001	38	Cream, unspecified	Milk	*Salmonella* Newport
2002	1	Cream, pasteurized	Milk	Suspect Other chemical
2002	52	Whole milk, pasteurized	Not indicated	Suspect *Calicivirus norovirus*
2002	7	Chocolate milk, pasteurized	Milk	Other chemical
2002	116	2% milk, pasteurized	Not indicated	*Salmonella* Typhimurium
2003	45	Cake, tres leche	Milk	Suspect *Staphylococcus aureus*
2003	7	Milkshake	Not indicated	Suspect Other chemical
2004	100	Other milk, pasteurized	Not indicated	*Salmonella* Newport
2005	200	1% milk, pasteurized	Milk	*Campylobacter jejuni*
2005	11	Milk/cream	Milk	*Campylobacter jejuni*

Foodborne outbreaks associated with unpasteurized milk and cheese often made with unpasteurized milk reported to CDC's National Foodborne Outbreak Surveillance System, 1973-2005 (N=87)

Year	Estimated Total	Food	Ingredient	Pathogen
1980	105	Raw goat milk	Not indicated	*Salmonella* Typhimurium
1980	22	Raw milk	Not indicated	*Salmonella* Dublin
1981	50	Raw milk	Not indicated	*Campylobacter jejuni*
1982	19	Raw milk	Not indicated	*Salmonella* Typhimurium; *Salmonella* Infantis
1982	16	Raw milk	Not indicated	*Campylobacter fetus; Campylobacter jejuni*
1982	15	Raw milk	Not indicated	*Campylobacter jejuni*
1982	32	Raw milk	Not indicated	*Campylobacter jejuni*
1982	32	Raw milk	Not indicated	*Campylobacter jejuni*
1982	4	Raw milk	Not indicated	*Campylobacter jejuni*
1982	32	Raw milk	Not indicated	*Campylobacter jejuni*
1983	5	Raw milk	Not indicated	*Salmonella* Typhimurium
1983	57	Raw milk	Not indicated	*Campylobacter jejuni*
1983	4	Raw milk	Not indicated	*Campylobacter jejuni*
1983	5	Raw milk	Not indicated	*Campylobacter jejuni*
1983	6	Raw milk	Not indicated	*Campylobacter jejuni*
1983	11	Raw milk	Not indicated	*Campylobacter jejuni*
1983	2	Raw milk	Not indicated	*Campylobacter jejuni*
1983	16	Raw milk, unpasteurized	Not indicated	*Campylobacter jejuni*
1984	16	Raw milk	Not indicated	*Salmonella* Typhimurium
1984	27	Raw milk	Not indicated	*Campylobacter jejuni*
1985	2	Raw milk	Not indicated	*Salmonella* Newport
1985	23	Raw milk	Not indicated	*Campylobacter jejuni*
1985	5	Raw milk	Not indicated	*Campylobacter* unknown
1988	120	Raw milk	Not indicated	*Campylobacter jejuni*
1989	10	Raw goats milk	Not indicated	*Campylobacter jejuni*
1990	42	Raw milk	Not indicated	*Campylobacter jejuni*
1990	13	Raw milk	Not indicated	*Campylobacter* unknown
1990	5	Raw milk	Not indicated	Suspect *Escherichia coli O157:H7*

1990	2	Raw milk	Not indicated	Suspect *Salmonella* Typhimurium
1991	3	Raw goat's milk	Not indicated	*Campylobacter jejuni*
1992	6	Raw milk	Not indicated	*Escherichia coli* O157:H7
1992	50	Raw milk	Not indicated	*Campylobacter jejuni*
1992	11	Raw milk	Not indicated	*Campylobacter* unknown
1993	4	Raw milk	Not indicated	*Escherichia coli* O157:H7
1995	3	Raw, unpasteurized milk	Not indicated	*Salmonella* Typhimurium var Cope
1998	6	Other milk, unpasteurized	Milk	*Campylobacter jejuni*
1998	3	Other milk, unpasteurized	Milk	*Campylobacter* unknown
1998	20	1% milk, unpasteurized	Milk	*Staphylococcus aureus*
1998	2	Other milk, unpasteurized	Milk	*Escherichia coli* O157:H7
1999	2	Other milk, unpasteurized	Not indicated	*Campylobacter* unknown
2000	19	Other milk, unpasteurized	Milk	*Campylobacter jejuni*
2000	2	Other milk, unpasteurized	Milk	*Campylobacter* unknown
2000	11	Other milk, unpasteurized	Milk	*Campylobacter jejuni*
2000	21	Other milk, unpasteurized	Milk	*Campylobacter jejuni*
2000	39	Whole milk, unpasteurized	Milk	*Campylobacter jejuni*
2000	12	Queso fresco, unspecified	Not indicated	*Listeria monocytogenes*
2000	8	Other milk, unpasteurized	Milk	*Campylobacter* other
2000	18	Homemade cheese, unpasteurized	Milk	*Campylobacter jejuni*
2000	42	Other milk, unpasteurized	Milk	*Campylobacter jejuni*
2000	4	Whole milk, unpasteurized	Milk	*Campylobacter jejuni*
2001	202	Other milk, unpasteurized	Not indicated	*Escherichia coli* O157:H7
2001	75	Whole milk, unpasteurized	Milk	*Campylobacter jejuni*
2001	4	Whole milk, unpasteurized	Not indicated	*Campylobacter jejuni*
2001	1	Whole milk, unpasteurized	Not indicated	Suspect *Escherichia coli* O157:H7
2001	4	Homemade cheese, unpasteurized	Milk	*Brucella spp*
2001	38	Queso fresco, unspecified	Milk	*Salmonella* Newport
2001	27	Multiple cheeses, unpasteurized	Milk	*Salmonella* Newport
2002	2	Whole milk, unpasteurized	Not indicated	*Campylobacter jejuni*
2002	46	Other milk, unpasteurized	Not indicated	*Campylobacter jejuni*
2002	13	Whole milk, unpasteurized	Not indicated	*Campylobacter jejuni*

2002	12	Other milk, unpasteurized	Milk	*Campylobacter* unknown
2002	107	Other milk, unpasteurized	Milk	*Salmonella* Typhimurium
2003	2	Other milk, unpasteurized	Not indicated	Suspect *Campylobacter jejuni*
2003	18	Other cheese, unpasteurized	Milk	*Campylobacter jejuni*
2003	11	Queso fresco, unspecified	Not indicated	Suspect *Campylobacter* unknown
2003	6	Other milk, unpasteurized	Not indicated	*Campylobacter jejuni*
2003	3	Other milk, unpasteurized	Not indicated	*Campylobacter jejuni*, *Escherichia coli* O157:H7
2003	12	Queso fresco, unpasteurized	Cheese	*Listeria monocytogenes*
2003	50	Queso fresco, unspecified	Cheese	*Salmonella* Typhimurium
2003	12	Queso fresco, unpasteurized	Milk	*Salmonella* Typhimurium
2004	32	Whole milk, unpasteurized	Not indicated	*Campylobacter jejuni*
2004	3	Queso fresco, unpasteurized	Not indicated	*Escherichia coli* O157:H7
2004	6	Whole milk, unpasteurized	Not indicated	*Campylobacter* unknown
2005	11	Whole milk, unpasteurized; goat milk, unpasteurized	Milk	*Campylobacter jejuni*
2005	3	Other milk, unpasteurized	Not indicated	*Campylobacter jejuni*
2005	4	Whole milk, unpasteurized	Milk	*Campylobacter jejuni*
2005	33	Whole milk, unpasteurized	Milk	*Campylobacter jejuni*
2005	11	Whole milk, unpasteurized	Not indicated	Suspect *Campylobacter jejuni*
2005	13	Whole milk, unpasteurized	Milk	*Campylobacter jejuni*
2005	5	Whole milk, unpasteurized	Milk	*Campylobacter jejuni*
2005	22	Whole milk, unpasteurized	Milk	*Campylobacter jejuni*
2005	12	Queso fresco, unspecified	Milk	Suspect *Salmonella* Group B
2005	3	Queso fresco	Milk	Suspect *Salmonella* Typhimurium
2005	2	Queso fresco	Milk	Suspect *Shigella* unknown
2005	18	Whole milk, unpasteurized	Milk	*Escherichia coli* O157:H7
2005	12	Queso fresco, unpasteurized	Not indicated	*Listeria monocytogenes*
2005	2	Queso fresco, unpasteurized	Not indicated	*Brucella spp*

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Sally Fallon
Weston A Price Foundation,

Friday, 28 December 2007

lt071227 Listeria in past milk doc

RE: Listeriosis

Dear Sally,

This is a sad outcome, but not at all surprising.

1. *Listeria monocytogenes*, the bacterium that causes the disease Listeriosis (foetal deaths and deaths in immuno-compromised humans), **does not survive pasteurisation**

2. However, it is often a passenger on the outside of retail milk containers and the crates and cargons used to transport retail containers; and can be in the milk due to post pasteurisation contamination.

   Outside of retail milk containers. Listeria is a common inhabitant of modern liquid milk plants, particularly the floors and drains of refrigerated storage rooms, where retail containers of pasteurised milk are stored. From the outside of the container to the consumer is not far to jump.

   Studies carried out in Australia and elsewhere have found that most (>60%) of the liquid milk factories have Listeria in the cold room environment. It is part of the microbiota that degrades milk at temperatures below 5°C. At temperatures above 5°C lactic bacteria degrade milk solids and exclude Listeria. The system of using returnable milk crates and palecons (cargons) to hold the retail milk 'bottles' from milk factory to retail supermarket, means that following breakages, leakers, etc, these transport components become contaminated by spoilage microbes that grow at temperatures below 5°C. The cold spoilage microbes are Pseudomonas followed by Listeria. With the push to maintaining colder temperatures for longer shelf life the lactics are loosing the battle in the transport chain and Listeria and its friends are winning.

   Inside the milk container. In Australia and the USA current systems result in every bottle or carton of pasteurised milk being contaminated after pasteurisation. The contaminating spoilage bacteria are water bacteria belonging to the Pseudomonas group, followed by Listeria.

   These bacteria are all killed by pasteurisation but gain access to all pasteurised milks after pasteurisation via filling machines and the associated holding tanks, pumps, valves and pipes that transfer the pasteurised milk to the retail containers. These are cleaned and sanitised to commercial standards but are not sterilised. In

the past this has not been considered a public health problem because:

1. When these spoilage bacteria grow in milk to a level that will cause mild food poisoning the milk has a foul odour, it curdles on heating as added to hot tea or coffee. In this way any consumer can detect that the milk is not suitable to consume.
2. If consumed in large numbers in spoiled milk these bacteria are not pathogens and will only cause vomiting with no lasting infection or toxicity.

3. This story becomes worse when modified milks are studied. Modified milks are products with altered levels of fat, protein, and other components. These products spoil more rapidly than plain whole milk, because they lack the natural antimicrobial systems of whole milk.

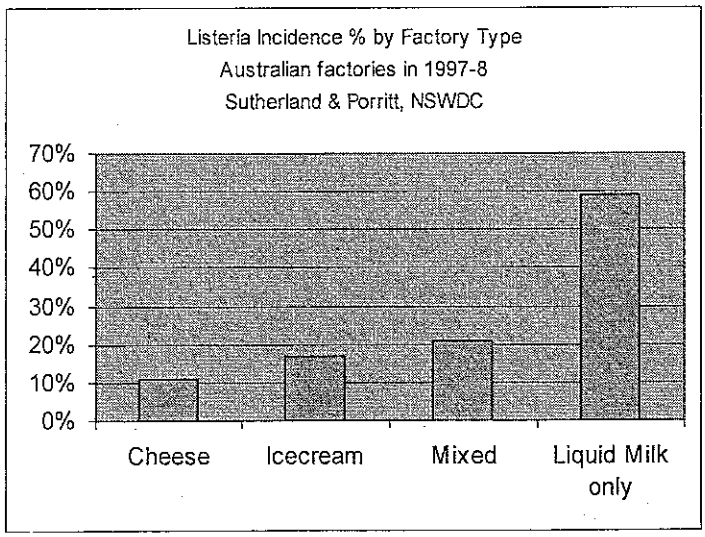
4. Milks with added colours and flavours such as coffee and chocolate often camouflage the spoilage characteristics of milk mentioned above. Consumers then may not realise the milk is spoilt and contains high levels of spoilage bacteria. Consuming a quantity of this spoilt (rotten) milk can make one very ill.

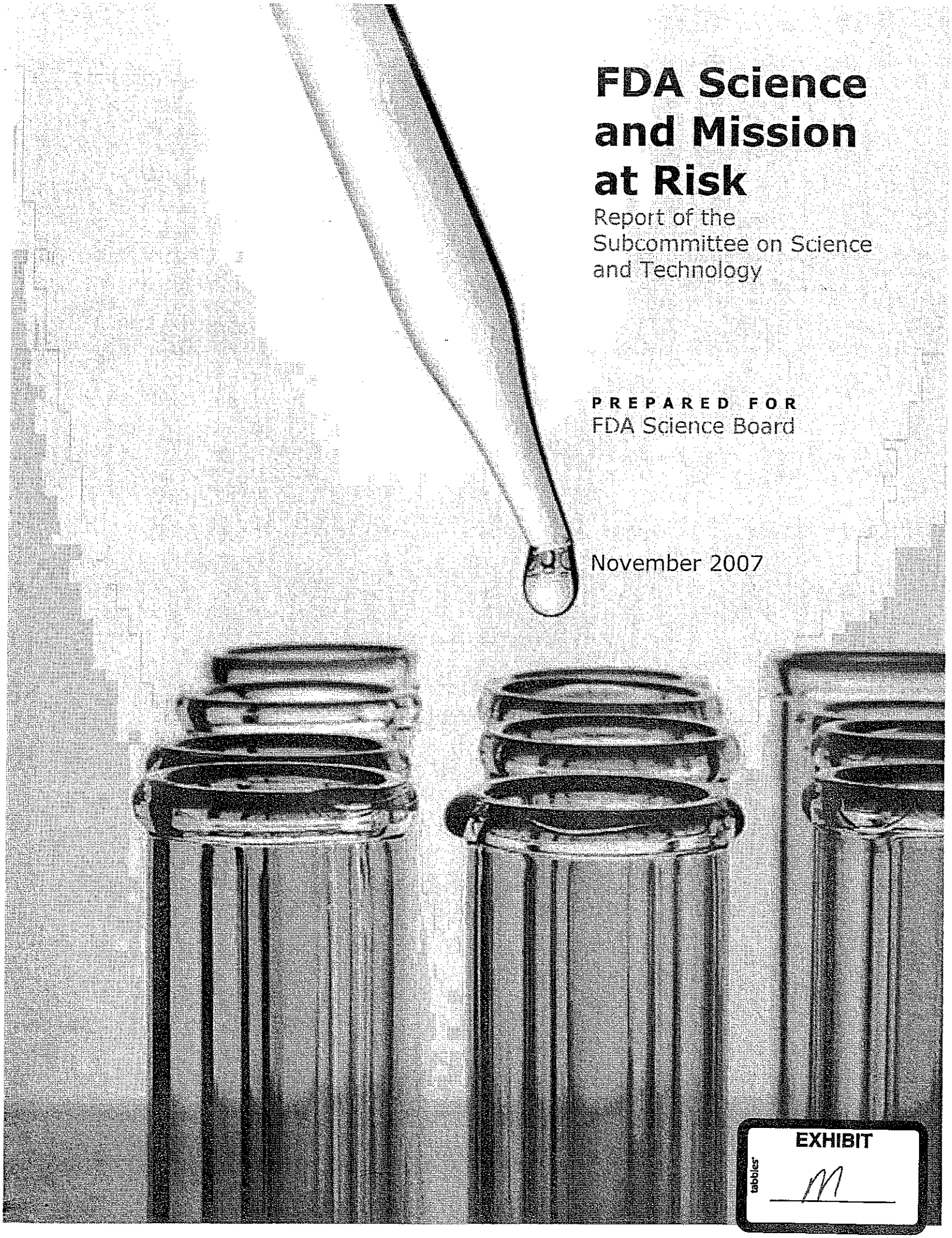
5. The likely sources of Listeria problems at the producing factory are.

   a. Listeria infestation of the chilled water system used to cool down the pasteurised milk. A cross leak in the cooling system to the pasteurised milk results in low level contamination in the pasteurised milk.
   b. Cold room floors/drains and the cold transport systems is contaminated because of milk spillage and the cold temperatures.

Regards

Ron Hull





FDA Science and Mission at Risk

Report of the Subcommittee on Science and Technology

PREPARED FOR
FDA Science Board

November 2007

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Bacteria-Contaminated Milk from Massachusetts Dairy is Blamed for 3 Deaths, Miscarriage

Tuesday , January 08, 2008

Associated Press

BOSTON —

At Whittier Farms dairy, the fifth-generation owners brag of the quality of their Holstein cows and still deliver milk right to your door, in glass bottles. Customers like the products because they are a hormone-free taste of old New England.

But health officials now say three elderly men have died and at least one pregnant woman has miscarried since last June after drinking bacteria-contaminated milk from the dairy's plant in Shrewsbury, about 35 miles west of Boston.

All were infected with listeria, which is extremely rare in pasteurized milk. It is more often found in raw foods, such as uncooked meat and vegetables, and processed foods such as soft cheeses and cold cuts.

The outbreak is believed to be only the third time listeria has ever been linked to pasteurized milk in the United States, said Dr. Alfred DeMaria, state director of communicable disease control.

"We know something is going on; we just don't know what it is," DeMaria said. "We just need to find out how the bacteria is getting into the milk."

Listeria bacteria are often present in manure and are commonly found in soil and water. Pasteurization is supposed to kill listeria.

Tests at the Whittier Farms plant found nothing wrong with its pasteurization process, deepening the mystery.

Health investigators are now looking at the cooling and bottling machinery, including the connecting pipes, for the source of the bacteria, DeMaria said. Investigators have taken about rized cider and coffee-flavored pasteurized milk. When both drinks were tested, only the pasteurized milk — from Whittier Farms — came back positive.

Officials then found listeria in an unopened container at the dairy's store in Shrewsbury.

State health officials would not identify any of the listeria patients because of privacy laws. But health officials in the town of Medway confirmed that the 88-year-old man who died Jan. 3 was John J. Powers, a World War II veteran and retired timekeeper at the Boston Naval Shipyard.

Powers' son bought the milk. The younger Powers had some flu-like symptoms for several days but recovered, while his father — who had other health problems — became ill and died, said Bill Fisher, Medway's health agent.

Two of the victims, a 78-year-old man and a 75-year-old man, died in Worcester County in June and October

A pregnant, 34-year-old woman recovered but suffered a miscarriage. Health officials suspect another pregnant woman, 31, contracted listeriosis from Whittier Farms milk. She recovered, and her baby is doing fine.

Brian Gay, owner of Maple Farm Dairy, has distributed Whittier's milk for about a decade and said many of his customers were shocked by the outbreak. Some canceled their service, Gay said, but most seem willing to return to Whittier when the milk is deemed safe.

"They were bumming out, because they love the product," Gay said.