Risk versus benefit of raw milk consumption

Dominique Angèle VUITTON

WHO Collaborating Centre; University of Franche-Comté;

25030 Besançon, France

Background: the contradiction between "food safety" and biodiversity, high quality of products, sustainable development, and healthy life style.

Dairy farmers and cheese producers are in the continuing process of answering the somewhat contradictory requirements of consumers for safe, high quality and diversified foods produced in sustainable conditions… at the lowest price. It has turned out that the legitimate requirements of safety from the consumer have eventually evolved in a burden of threats upon producers' activities, once they began being translated in tentative safety regulations. Within the past 10 years, the main concern of numerous producers, with little link with big dairy industry companies, has always been to provide food diversity through a traditional way of producing milk and cheese and to maintain biodiversity while reaching at the same time the very high quality and safety standards requested by the consumers. However, they now have the feeling that their way of doing is not supported in reality by the public policies and that the attitudes of experts and/or authorities may be sometimes quite contradictory.

There is a common misunderstanding on the consumers' claim for safety of foods. Individually of course, the consumer cannot accept any casualty coming from just eating food. However, at the society's level the actual risk could well lay in a continuously increasing prevalence of obesity, diabetes, and immunological/allergic diseases, with a number of cases that currently prevails over food-borne infections of significant severity. Such chronic diseases are less frightening than outbreaks of food-borne diseases; however, they have become a threat to quality of life and life expectancy. The concept of "food safety" has to be re-assessed and products likewise. It has been stressed in various reports by the scientific committees of the European Commission and EFSA that today the objective of food security is almost reached because foods have never been so safe until now. While the zero risk does not exist, it becomes necessary to analyse the ratio between costs and risks versus benefits. In fact, it would be very harmful for the Society to destroy the wealth of safety lying in microbial diversity before having had the opportunity of scientifically demonstrating this potential, because of the urgent need for "zero risk". We all strive to "zero risk" but immediate fight towards "zero risk" may mean immediate threat on sustainable agriculture and the emergence of other types of more insidious risks both for health and for the environment.

Through their activities, the dairy farmers that wish to sell raw milk and the SMEs that are producing raw milk cheese defend a European cultural reality, promote the development of European regions that cannot be involved in other types of industry, contribute to attract tourism and to protect environment and, from land use to production units, are fully involved in the

organisation of the territory in rural areas. Besides the safety of the products, other numerous aspects are worth considering such as quality of landscape, biodiversity, environment and sustainable development, multi-functionality of agriculture, employment and economical activities in difficult regions, other aspects of health (e.g. prevention of chronic diseases) and above all pleasure and happiness of consumers.

HACCP policy and systematic microbiological quality control developed in Europe in the past 15 years have markedly changed the figures of microbial hazards at the farm level. Careful management of milking is currently able to greatly decrease the risk of contamination by pathogens. Raw milk and raw milk cheese producers are aware that the technologies they use are bearing some risks. Having this in mind, they have produced considerable efforts to reduce the hazards all along the production chain, from "farm to fork", from "pasture to product". Intimately, they are convinced "they are working well", for the pleasure and health of the consumer, while producing in harmony with their environment. Conversely, during the same period, outbreaks of food-borne diseases were shown to mostly originate from contamination occurring during food processing, packaging and storage, at processing plant, shop or family level, the percentage of which largely overcame that originating from the initial product, at the producer lever.

It must be said that all reports on food safety have very scientifically and carefully described all potential hazards of raw milk consumption. However, although they mentioned similar potential hazards from pasteurised milk or products, and recognised that raw milk was currently "clean", safe, and actually very rarely involved in food-borne disease outbreaks or sporadic cases, their recommendations were often biased. On one hand, they have reached the conclusion that, despite low contamination by pathogenic agents, the risk of raw milk consumption was high since the "potentially" occurring diseases were "potentially" severe. This did not take into account the production process and the microbiological quality control management currently developed to ensure safety. Following this logic, a lot of other products should be banished from human consumption, and especially long-term preserved ready-to-eat foods. On the other hand, they have simply considered that health benefits from raw milk were not based on scientific assessments and that no "positive" attribute of raw milk was significant; this is not currently accurate.

Pasteurisation: the unique route to milk safety?

It is conventional wisdom that pasteurisation is good for human health as it protects the consumer from infectious agents, especially those transferred from animal faeces or infectious skin lesions, that may contaminate milk at the production level. For many decades, especially when hygienic measures were quite low at the production level, pasteurisation has perfectly achieved its goal of protecting consumers. Pasteurisation came into force to stop milk-borne transmission of TB (*Mycobacterium bovis* and *tuberculosis*) and Brucellosis in the 1920-30ties. Pasteurisation is actually protecting against *Salmonella*, *Campylobacter* and EHEC bacteria that could be transmitted in cow milk, and mainly EHEC and *Campylobacter* for sheep and goat milk. Pasteurisation, however, does not stop some of the potentially hazardous organisms such as *M. paratuberculosis* that some reports have associated with Crohn's disease.

Listeriosis, which may be a severe and even fatal disease in immunosuppressed individuals and in infants born from infected mothers, is a special issue since it is often stressed, including in scientific reports, as "the" main risk when a possible consumption of raw milk or raw milk products is evoked. The general public is well informed of this particular hazard, and in most European countries advertisements to pregnant women regarding listeriosis mostly (and sometimes only) focus onto raw milk and raw milk products. However, *Listeria* is not really relevant when discussing fresh milk unless the producer has clinically sick sheep and goats or feeds the cows low quality silage. Most of the reports regarding the epidemiology of listeriosis in Europe in the past 15 years have shown that listeriosis had the same prevalence in those countries where raw milk and raw milk product consumption was forbidden (such as Norway) and those countries where it was not (such as France) (Antal et al, 2007; Goulet et al, 2006). The recent re-increase of listeriosis prevalence in many European countries (Hof et al, 2007) cannot be explained by an increased consumption of raw milk products, which has concomitantly decreased because of national policies recommending pasteurisation in most of these countries and the fast development of big dairy companies (which sell only pasteurised products) during the same period. Secondary contamination of pasteurised milk products is at the origin of the majority of listeriosis cases when milk or milk products are the suspected food, and pasteurised milk and milk products may contain significant amounts of *Listeria* (Pini and Gilbert, 1988; Maijala et al, 2001; Lewis et a, 2006; Rudol et al, 2001). A higher incidence of *L. monocytogenes* was even observed in cheeses made from pasteurized milk (8.0%) than in cheeses manufactured from raw milk (4.8%) in a German study dealing with red-smeared cheeses of various European origins (Rudol et al, 2001). Highest contamination rates with *Listeria monocytogenes* are currently from fish and sea-food, and especially ready-to-eat smoked salmon (Lattore et al, 2007; Wagner et al, 2007), preserved or hot-treated meat products, and ready-to-eat salads with meat or sea-food ingredients, with extended shelf-lives, produced by authorized companies, and not packaged on the premises, as well as, in the USA, melon or hummus (that are not usually recognized as "at risk" foods) (Nørrung et al, 1999; Little et al, 2007; Varma et al, 2007). Although such foods may account for more than 90% of human listeriosis cases, and raw milk and raw milk products for less than 1%, it is amazing to notice that raw milk and raw milk products are always cited first when public health authorities and/or the Medias communicate on this disease to give advice for prevention... Scientific evidence is sometimes contradictory with risk representation and Media communication!

Bacterial and fungal microflora present in raw milk has become more and more controlled. It is worth mentioning that a recent study showed a lower concentration of Gram negative endotoxin (lipopolysaccharide, LPS) in milk collected in farming families than in milk obtained from rural non-farming families of the same areas (Germany, Switzerland, Austria, France and Finland) (Gehring et al, 2008). There was no difference between farm (raw) milk and shop (pasteurised) milk, regarding endotoxin content. This unexpected result suggest that dairy farmers keeping dairy cows in small production units are more aware of the importance of good hygienic conditions for the quality of the milk.

Are there any substantiated benefits from raw milk consumption?

Advocates for the consumption of non-pasteurised milk and milk products claim that raw milk is preferable to pasteurised milk for several reasons, including: raw milk contains antimicrobial

substances that inhibit the growth of pathogenic bacteria, pasteurisation reduces the vitamin content of milk, pasteurisation reduces the amount of available calcium in milk, raw milk strengthens human health, or pasteurization denatures whey proteins... A recent report on the risks of transmission of microorganisms to humans resulting from the consumption of raw milk and raw cream in Norway, by the Norwegian Scientific Committee for Food Safety, as many other ones, discarded all these assumptions as not scientifically established and thus, non significant. We may totally agree that any claimed effect without appropriate scientific evidence cannot be retained; it is true for drugs (what about traditional medicines or homeopathy?) as well as for nutrients. However, some evidence for a beneficial effect of raw milk and/or raw milk cheese consumption is currently available and should be given.

The microbial intestinal flora has been shown to be an essential component for the development and maintenance of both optimal mucosal immune responses and well balanced systemic immunity. Bacteria can be considered as antigens able to elicit specific systemic and local immune responses. Furthermore, they exert a considerable influence on the number and distribution of the Gut-Associated Lymphoid Tisssue (GALT) cell populations and cytokine balance, and play an important role in the regulation of immune response particularly at the intestinal level. These data have emerged mainly from axenic (germ-free) or gnotobiotic animals, i.e. ex germ-free mice colonized with a known digestive flora. Many circumstances may interfere with the equilibrium of the normal intestinal flora : disease states (infectious agents, intestinal inflammation …), but also other factors (nutrition, stress, antibiotics …) which are also able to unbalance the subtle composition of the microbial flora of the gut in healthy conditions with unknown short-term or even long-term consequences on the host, especially on the immune responses. Some foods are rich in "probiotic" microorganisms which can bring a beneficial effect during their ingestion. Raw milk, fermented milks and cheeses are good representatives of such foods. Biodiversity of raw milk microflora certainly accounts for some of the benefits that were attributed to raw milk cheeses. A marked difference is indeed observed between hard-cooked cheese prepared from raw or from pasteurized milk cheese. Raw milk cheese has higher bacteria counts than pasteurized milk cheese. In pasteurized milk cheeses, mesophilic lactobacilli, propionibacteria and enterococci are lower than the threshold (<100 CFU $g/-^{1}$) while, in raw milk cheeses, they are always present and average 10^{8} , 10^{7} and 10^{4} respectively. Overall, raw milk cheeses have a richer and more diverse microflora than pasteurized milk cheeses (Bertrand et al, 2006). Antibiotics can cause resistant micro-organisms to emerge among intestinal bacteria and can modify the competitive balance between organisms. It has been shown that consumption of hard-cooked cheese was associated with a significant decrease of amoxicillin-resistant enterococci after amoxicillin-clavulanate administration period, with the maximum level of amoxicillin-resistant enterococci falling from 6.2% to 0.03% (Bertrand et al, 2006). Consumption of hard-cooked cheese prepared with raw milk was also shown to significantly decrease *Candida albicans* colonization in volunteers (Millon et al; manuscript in preparation).

Recent studies, using gnotobiotic mice, have brought new insights into the role played by some bacteria which colonize the digestive tract of human newborns just after birth, on the oral tolerance process, stimulation of the protective IgA responses and decrease in IgE production. Oral tolerance plays a major role in the prevention of allergies and its induction depends upon the intestinal microflora (Sudo et al, 1997). The immune-modulating properties of the digestive

microflora have also been shown applicable to humans (Kalliomaki and Isolauri, 2003). Differences in the intestinal flora of children living respectively in Sweden and Estonia, with a marked difference in allergy prevalence have been demonstrated. They have been attributed to their different diet, and especially to the consumption of more "traditional" food (including raw and fermented milk) in Estonia (Björksten, 1997; Björksten et al, 2001; Bottcher et al, 2000). More and more studies have demonstrated the beneficial effects on health of strains of micro-organisms and give hope for other discoveries in this domain, particularly for raw milk and raw milk cheeses, which are rich in non-pathogenic micro-organisms. The preliminary results from a pilot study of nutritional intervention in infants using probiotics are very encouraging since they confirm that this could reverse the increased risk of allergic disease in Scandinavian children (Kalliomaki et al, 2001; 2003; Pessi et al, 2003). Several *Lactobacillus* sp. have been proposed for their ability to modulate immune responses. However some preliminary results suggest that the diversity of the ingested probiotic organisms, more than specific strains, is important to maintain and develop a health-promoting immune balance. It has been suggested that using a combination of multistrain and multispecies probiotics could greatly improve the efficacy of the probiotics (Timmerman et al. 2004). As the main microbiological characteristic of raw milk is the diversity of the microbial species, it thus may be assumed that its consumption could be associated with prevention of allergy.

A positive answer is given by the data obtained from cross-sectional studies developed within the past 15 years which aimed at explaining the impressive increase of a spectrum of diseases associated with the allergic "atopic" condition. This increase has become a Public Health concern in most developed countries, and especially, in Europe, in Scandinavian countries. It has been significantly associated with 1) hygiene improvement in the so-called "western societies", 2) decreased number of siblings in families (that prevents children's immune system to be appropriately stimulated by immunogenic infectious agents), 3) urban environment, and, 4) major changes in the intestinal microflora. Epidemiological studies have especially demonstrated a link between living on a farm for the pregnant mother and the newborn, and/or drinking raw cow milk during pregnancy and for the first year of life, and protection against allergy.

Cross-sectional studies in various European countries, but also in the United States and Canada, have repeatedly shown that children growing up on a farm have a significantly lower prevalence of asthma, hay fever and atopic sensitisation than their peers (Braun-Fahrländer et al, 1999; 2000; von Mutius, 2000; 2004; Ernst and Cormier, 2000; Kaufmann et al, 2002; Kilpelainen et al, 2000; 2002; Riedler et al, 2001; Horak et a;, 2002; Portengen et al, 2002; Rennie et al, 2002; Remes et al, 2003; 2005; Braback et al, 2004; Radon et al, 2004 a and b; Eduard et al, 2004 a and b; Elliott et al, 2004). This protective effect was primarily observed in dairy farms, especially in small and traditional ones where contact of family members to animals is close and frequent (Braun-Fahrlander, 2002). The effect of growing up on a farm as a child was still seen later in adolescence. Likewise, in a Norwegian cross-sectional survey, a lower prevalence of asthma was found among adult farmers (Eduard et al, 2004). Findings from cross-sectional studies in school children in Bavaria, Austria and Finland have suggested that exposure to livestock and unpasteurised milk are relevant exposures, which may induce tolerance towards environmental allergens (Remes et al, 2003); these exposures must, however, occur early in life to confer protection (Riedler et al, 2004). In the ALEX-study, a multi-centre study in rural environments in

Bavaria, Switzerland and Austria, exposure to stables and unpasteurised milk during pregnancy and the first year of life resulted in a significantly lower prevalence of asthma (0.8% versus 11.8%) hay fever (0.8% versus 16.0%) and atopic sensitisation (8.2% versus 32.9%). These effects were independent and synergistic and they remained significant after adjusting for potential confounders: aOR=0.14 [95% CI: 0.04-0.48] for asthma, aOR=0.20 [95% CI 0.08-0.50] for hay fever and aOR=0.32 [95% CI: 0.17-0.62] for atopic sensitisation. Results from a prospective cohort study of 500 children living on a farm compared to 500 rural children non-living on a farm in Austria, Finland, France, Germany and Switzerland, the "Pasture" study, are currently confirming those obtained from cross-sectional studies: an inverse relation of maternal exposure to animal sheds during pregnancy to IgE against seasonal allergens was found in cord blood (Ege et al, 2008); preliminary results indicate that Th1 cytokines are significantly elevated in the cord blood from children living on a farm and this elevation is significantly associated with raw milk consumption by the pregnant mother (The Pasture study group: manuscript submitted).

As of today it remains unclear which exposures within dairy farms are the most beneficial against the development of asthma and allergies; however, consumption of raw milk is the most constant exposure factor, found in nearly all cross sectional studies performed in continental Europe. Similarly its crucial influence was recently found in the United Kingdom: protection against atopy through raw milk consumption was also found in children non-living on a farm and was responsible for a lower frequency of positive allergy skin tests, a reduction of IgE levels by 60% and an increased in interferon-gamma production (Perkin et Strachan, 2006). An inverse relationship between farm raw milk consumption and atopy in urban children was also found in a cross-sectional study in Crete (Barnes et al,) and between raw milk consumption and occurrence of atopic dermatitis in New Zealand (Wickens et al, 2002). Exposure to microbial compounds such as endotoxin and peptidoglycan from bacteria is highly prevalent in stables and other farming environments. Endotoxin is a component of the cell wall of gram-negative bacteria with recognized immunostimulatory capacities. Muramic acid is a component of peptidoglycan in the cell wall of all bacteria. In the ALEX Study, elevated concentrations of both, endotoxin and muramic acid, were found in dust from the child's mattress among farming children as well as non-farming children with regular contact to stables (Braun-Fahrlander et al, 2002; van Strien et al, 2004). Furthermore, endotoxin levels in these indoor dust samples were inversely related to the occurrence of hay fever, atopic asthma and atopic sensitisation, while non-atopic wheeze was significantly negatively associated with muramic acid levels in indoor dusts. However, as mentioned above, farm milk is not different from shop milk regarding endotoxin content (Gehring et al, 2008). A number of other components of raw milk might be of importance for its protective effect against allergy and modulation of the immune response, including the diversity of its bacterial microflora compared to that of pasteurized milk, and aromatic substances, such as terpenes which are highly dependent on the nature of animal feeding (Tornambe et al, 2006) and may be destroyed by pasteurization.

A balanced risk assessment should take into account the beneficial effects of raw milk on the prevention of diseases together with food-borne infections that it may induce. There is now sufficient documented evidence of the link between raw milk consumption by the pregnant mother and her infant and the protection of children against the development of allergic/atopic diseases to consider this beneficial effect seriously. As atopic allergy currently affects 1/4

individuals in the European countries, with an increasing gradient from Mediterranean to Scandinavian countries, the number of allergic people ranges from 60 to 80 million individuals, with several thousand of fatalities, especially due to severe asthma and to anaphylactic shock from food allergy. This has to be compared with the number of fatalities attributable to food-borne infections. The benefit/risk ratio cannot be neglected when recommendations are given by regulatory bodies. Authorisation for market sale of raw milk for human consumption has recently been given in countries where it was previously forbidden (such as Norway) provided that the packaging clearly indicates the "*the product is associated with a marked increased risk of transmission of severe diseases*"... the sentence couldn't be completed by "*but the product is associated with a significant protection against other types of severe diseases*"? That would be "scientifically correct"!

References

Antal EA, Høgåsen HR, Sandvik L, Maehlen J. Listeriosis in Norway 1977-2003. Scand J Infect Dis. 2007;39(5):398-404.

Barnes M, Cullinan P, Athanasaki P, MacNeill S, Hole AM, HarrisJ, et al. Crete: does farming explain urban and rural differences in atopy? Clin Exp Allergy 2001;31:1822–8.

Björksten B. The environment and sensitisation to allergens in early childhood. Pediatr Allergy Immunol 1997;8(supp 10):32–9.

Björksten B, Sepp E, Julge K, Voor T, Mikelsaar M. Allergy development and the intestinal microflora during the first year of life. J Allergy Clin Immunol 2001;108:516–20.

Bottcher MF, Nordin EK, Sandin A, Midtvedt T, Björksten B. Microflora-associated characteristics in faeces from allergic and non-allergic infants. Clin Exp Allergy 2000;30:1590–6.

Braback L, Hjern A, Rasmussen F. Trends in asthma, allergic rhinitis and eczema among Swedish conscripts from farming and non-farming environments. A nationwide study over three decades. Clin Exp Allergy 2004;34:38–43.

Braun-Fahrlander C. Do only European cattle protect from allergies? Allergy 2002;57:1094–6.

Braun-Fahrländer C., Gassner M., Grize L. Neu U., Sennhauser FH, Varonier HS et al.: Prevalence of hay fever and allergic sensitisation in farmer's children and their peers living in the same rural community. Clin.Exp.Allergy 1999; 29:28-34

Braun-Fahrlander C. Allergic diseases in farmers' children. Pediatr Allergy Immunol 2000;11(Suppl 13):19–22.

Braun-Fahrländer C., Riedler J., Herz U., Eder W., Waser M., Grize L. et al.: Environmental exposure to endotoxin and its relation to asthma in school-age children N.Engl.J.Med. 2002;347:869-877

Eduard W., Omenaas E., Bakke PS, Douwes J., Heederik D.: Atopic and non-atopic asthma in farming and a general population Am.J.Int.Med. 2004;46:396-399

Eduard W, Douwes J, Omenaas E, Heederik D. Do farming exposures cause or prevent asthma? Results from a study of adult Norwegian farmers. Thorax 2004;59:381–6.

Ege MJ, Herzum I, Büchele G, Krauss-Etschmann J, Lauener RP, Roponen M, Hyvärinen A, Vuitton DA, Riedler J , Brunekreef B, Dalphin JC, Braun-Fahrländer C, Pekkanen J, Renz H, von Mutius E, and the PASTURE Study group. Prenatal exposure to a farm environment modifies atopic sensitization at birth. J Allergy Clin Immunol 2008 (in press)

Elliott L, Yeatts K, Loomis D. Ecological associations between asthma prevalence and potential exposure to farming. Eur Respir J 2004;24:938–41.

Ernst P, Cormier Y. Relative scarcity of asthma and atopy among rural adolescents raised on a farm. Am J Respir Crit Care Med 2000;161:1563–6.

Goulet V, Jacquet C, Martin P, Vaillant V, Laurent E, de Valk H. Surveillance of human listeriosis in France, 2001-2003. Euro Surveill. 2006;11(6):79-81.

Gehring U, Spithoven J, Schmid S, Bitter S, Braun-Fahrländer C, Dalphin JC, Hyvärinen A, Pekkanen J, Riedler J, Weiland SK, Büchele G, von Mutius E, Vuitton DA, Brunekreef B; on behalf of the PASTURE study group.

Endotoxin levels in cow's milk samples from farming and non-farming families - The PASTURE study. Environ Int. 2008 May 23 (in press)

Hof H, Szabo K, Becker B. [Epidemiology of listeriosis in Germany: a changing but ignored pattern] (in German) Dtsch Med Wochenschr. 2007 Jun 15;132(24):1343-8.

Horak Jr. F, Studnicka M, Gartner C, Veiter A, Tauber E, Urbanek R, et al. Parental farming protects children against atopy: longitudinal evidence involving skin prick tests. Clin Exp Allergy 2002;32:1155–9.

Kalliomaki M, Isolauri E. Role of intestinal flora in the development of allergy. Curr Opin Allergy Clin Immunol 2003;3:15–20.

Kalliomäki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease : a randomised placebo controlled trial. Lancet 2001;357:1076–9.

Kalliomaki M, Salminen S, Poussa T, Arvilommi H, Isolauri E. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. Lancet 2003;361:1869–71.

Kauffmann F, Oryszczyn MP, Maccario J. The protective role of country living on skin prick tests, immunoglobulin E and asthma in adults from the Epidemiological Study on the Genetics and Environment of Asthma, bronchial hyper-responsiveness and atopy. Clin Exp Allergy 2002;32:379–86.

Kilpelainen M., Terho EO, Helenius H., Koskenvuo M.: Farm environment in childhood prevents the development of allergies Clin.Exp.Allergy 2000; 30:201-208

Kilpelainen M, Terho EO, Helenius H, Koskenvuo M. Childhood farm environment and asthma and sensitization in young adulthood. Allergy 2002;57:1130–5.

Latorre L, Parisi A, Fraccalvieri R, Normanno G, La Porta MC, Goffredo E, Palazzo L, Ciccarese G, Addante N, Santagada G. Low prevalence of Listeria monocytogenes in foods from Italy. J Food Prot. 2007 Jun;70(6):1507-12.

Lewis HC, Little CL, Elson R, Greenwood M, Grant KA, McLauchlin J. Prevalence of *Listeria monocytogenes* and other *Listeria* species in butter from United Kingdom production, retail, and catering premises. J Food Prot. 2006 Jul;69(7):1518-26.

Little CL, Taylor FC, Sagoo SK, Gillespie IA, Grant K, McLauchlin J. Prevalence and level of *Listeria monocytogenes* and other *Listeria* species in retail pre-packaged mixed vegetable salads in the UK. Food Microbiol. 2007 Oct-Dec;24(7-8):711-7.

Maijala R, Lyytikäinen O, Autio T, Aalto T, Haavisto L, Honkanen-Buzalski T. Exposure of Listeria monocytogenes within an epidemic caused by butter in Finland. Int J Food Microbiol. 2001 Oct 22;70(1-2):97-109

Nørrung B, Andersen JK, Schlundt J. Incidence and control of *Listeria monocytogenes* in foods in Denmark. Int J Food Microbiol. 1999 Dec 15;53(2-3):195-203.

Perkin MR, Strachan DP. Which aspects of the farming lifestyle explain the inverse association with childhood allergy? J Allergy Clin Immunol 2006;117:1374–81.

Pessi T, Sütas Y, Hurme M, Isolauri E. Interleukin-10 generation in atopic children following oral *Lactobacillus rhamnosus* G.G. Clin Exp Allergy 2000;30:1804–8.

Pini PN, Gilbert RJ. The occurrence in the U.K. of Listeria species in raw chickens and soft cheeses. Int J Food Microbiol. 1988 Jun;6(4):317-26.

Portengen L, Sigsgaard T, Omland O, Hjort C, Heederik D, Doekes G. Low prevalence of atopy in young Danish farmers and farming students born and raised on a farm. Clin Exp Allergy 2002;32:247–53.

Radon K, Ehrenstein V, Praml G, Nowak D. Childhood visits to animal buildings and atopic diseases in adulthood: an agedependent relationship. Am J Ind Med 2004;46:349–56.

Radon K, Windstetter D, Eckart J, Dressel H, Leitritz L, Reichert J, et al. Farming exposure in childhood, exposure to markers of infections and the development of atopy in rural subjects. Clin Exp Allergy 2004;34:1178–83.

Remes ST, Koskela HO, Iivanainen K., Pekkanen J.: Allergen-specific sensitisation in asthma and allergic diseases in children: the study on farmers' and non-farmers' children Clin.Exp.Allergy 2005;35:160-166

Rennie DC, Dosman J, Senthilselvan A. Respiratory symptoms and asthma in two farming populations: a comparison of Hutterite and non-Hutterite children. Can Respir J 2002;9:313–8.

Remes ST, Iivanainen K., Koskela H., Pekkanen J.: Which factors explain the lower prevalence ot atopy amongst farmer's children? Clin.Exp.Allergy 2003;33:427-434

Riedler J, Braun-Fahrlander C, Eder W, Schreuer M, Waser M, Maisch S, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. Lancet 2001;358:1129–33.

Riedler J., Eder W., Schreuer M., Schreuer M.: Riedler J, Eder W, Oberfeld G, Schreuer M. Austrian children living on a farm have less hay fever, asthma and allergic sensitization. Clin Exp Allergy 2000;30:194–200.

Rudol M, Scherer S. High incidence of Listeria monocytogenes in European red smear cheese. Int J Food Microbiol. 2001 Jan 22;63(1-2):91-8

Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. J Immunol 1997;159:1739–45.

Timmerman, H.M., Koning, C.J., Mulder, L., Rombouts, F.M. and Beynen, A.C. (2004) Monostrain, multistrain and multispecies probiotics–A comparison of functionality and efficacy. Int J Food Microbiol 96, 219–233.

Tornambe G, Cornu A, Pradel P, Kondjoyan N, Carnat AP, Petit M, Martin B. Changes in Terpene Content in Milk from Pasture-Fed Cows. J Dairy Sci 2006;89:2309–2319

van Strien RT, Engel R., Holst O., Bufe A., Eder W., Waser M. et al.: Microbial exposure of rural school children, as assessed by level of N-acetyl-muramic acid in mattress dust, and its association with respiratory health. J.AllergyClin.Immunol. 2004;113:860-867

Varma JK, Samuel MC, Marcus R, Hoekstra RM, Medus C, Segler S, Anderson BJ, Jones TF, Shiferaw B, Haubert N, Megginson M, McCarthy PV, Graves L, Gilder TV, Angulo FJ. *Listeria monocytogenes* infection from foods prepared in a commercial establishment: a case-control study of potential sources of sporadic illness in the United States. Clin Infect Dis. 2007 Feb 15;44(4):521-8

von Mutius E.: The environmental predictors of allergic disease J. Allergy Clin. Immunol. 2000;105:9-19

von Mutius E.: Influences in allergy: epidemiology and the environment J.Allergy Clin.Immunol. 2004;113:373-379

Wagner M, Auer B, Trittremmel C, Hein I, Schoder D. Zoonoses Public Health. Survey on the *Listeria* contamination of ready-to-eat food products and household environments in Vienna, Austria. 2007;54(1):16-22.

Wickens K, Lane JM, Fitzharris P, Siebers R, Riley G, Douwes J, et al. Farm residence and exposures and the risk of allergic diseases in New Zealand children. Allergy 2002;57:1171–9.