The protective effect of farm milk consumption on childhood asthma and atopy: The GABRIELA study

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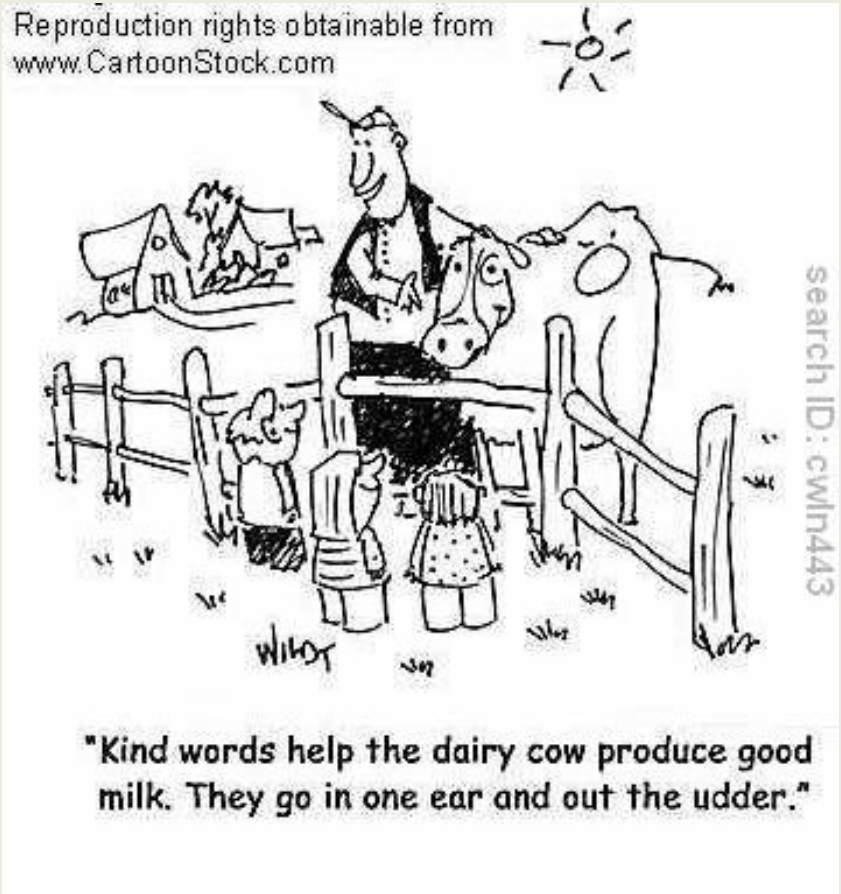
Outline

- Abstract
- Introduction
- Literature Review
- Research Methodology
- Discussions
- Conclusion
- Questions



Abstract

- **Background:** Farm milk consumption has been identified as an exposure that might contribute to the protective effect of farm life on childhood asthma and allergies. The mechanism of action and the role of the particular constituents of farm milk, however, are not yet clear.
- **Objective:** We sought to investigate the farm milk effect and determine responsible milk constituents.
- **Methods:** In rural regions of Germany, Austria, and Switzerland, a comprehensive questionnaire about farm milk consumption and other farm-related exposures was completed by parents of 8,334 school-aged children, and 7,606 of them provided serum samples to assess specific IgE levels. In cow's milk samples collected at the participants' homes, viable bacterial counts, whey protein levels, and total fat content were analyzed. Asthma, atopy, and hay fever were associated to reported milk consumption and for the fist time to objectively measured milk constituents by using multiple regression analyses.

(Loss et al., 2011)

Abstract

- **Results:** Reported raw milk consumption was inversely associated to asthma (aOR, 0.59; 95% CI, 0.46-0.74), atopy (aOR, 0.74; 95% CI, 0.61-0.90), and hay fever (aOR, 0.51; 95% CI, 0.37-0.69) independent of farm exposures. Boiled farm milk did not show a protective effect. Total viable bacterial counts and total fat content of milk were not significantly related to asthma or atopy. Increased levels of the whey proteins BSA (aOR for highest vs lowest levels and asthma, 0.53; 95% CI, 0.30-0.97), alpha-lactalbumin (aOR for interquartile range and asthma, 0.71; 95% CI, 0.52-0.97), and beta-lactoglobulin (aOR for interquartile range and asthma, 0.62; 95% CI, 0.39-0.97), however, were inversely associated with asthma but not atopy.
- **Conclusions:** The findings suggest that the protective effect of raw milk consumption on asthma might be associated with the whey protein fraction of milk.

(Loss et al., 2011)

Introduction

- Childhood asthma and allergies has increased over the years. In the United States 9.4% of children and 8.2% of adults are diagnosed with asthma
- Consistent research has found that children raised on a farm have a reduced risk of asthma, atopy, and hay fever compared to children not raised on a farm.

(CDC, 2010)

Literature Review

- Inverse association of farm milk consumption with asthma and allergy in rural and suburban populations across Europe
  - Farm milk consumption protective of asthma
- Which aspects of the farming lifestyle explain the inverse association with childhood allergy?
  - Reduced risk for asthma with unpasteurized milk consumption

(Waser et al., 2006 & Perkin et al., 2006)

Literature Review

- Many studies have shown a correlation between farm milk, as well as other environmental factors, and lower asthma prevalence
- First to analyze the milk constituents for a possible contributing protective effect
- Grant from European Union Research
  - Conflict of interest
  - Blinding in part of the study

Methodology

- Study design
  - Cross-Sectional Study
- Inclusion Criteria
  - 6-12 year-old school children
  - Living within 5 rural areas of southern Germany, Switzerland, Austria, and Poland
  - Written parent consent for blood sampling, genetic analyses, and dust sampling
  - Questionnaire

(Loss et al., 2011)

Methodology

- Phase I
  - Recruitment questionnaire
    - Farm children
    - Exposed nonfarm children
    - Nonexposed nonfarm children
  - 34,491 eligible participants

(Loss et al., 2011)

Methodology

- Phase II
  - Random sample of 9,668 taken from 34,491
  - Questionnaire about farm-related exposures
    1) Exclusive shop milk exposure
    2) Mixed milk exposure
       - Only boiled farm milk
       - Any unboiled farm milk
         - Daily unboiled farm milk
         - Less than daily unboiled farm milk
         - First unboiled farm milk <1 year of age or during pregnancy
         - First unboiled farm milk >1 year of age
    3) Exclusive farm milk exposure
       - Only boiled farm milk
       - Any unboiled farm milk
         - Daily unboiled farm milk
         - Less than daily unboiled farm milk
         - First unboiled farm milk <1 year of age or during pregnancy
         - First unboiled farm milk >1 year of age

Methodology

- Phase II
  - Additional to the questionnaire, consent from parents for blood sampling, genetic analyses, and dust sampling
    - 7,606 eligible children

(Loss et al., 2011)

Methodology

- Phase III
  - Restricted to Bavaria
  - 1,903 eligible children
    - Asthma
    - Atopy, but no asthma
    - No asthma and no atopy
  - 895 selected for equal sample size

(Loss et al., 2011)

Methodology

- Phase III
  - Milk samples from participants' homes of 800 subjects
    - High heat-treated shop milk (>85 C) (185F)
    - Pasteurized shop milk (not heated to >85 C)
    - Heated farm milk (>72 C) (162F)
    - Raw farm milk (not heated to >72 C)
  - Total fat content, total viable bacterial count, and whey protein levels
    - 222 samples selected for advanced microbiological analyses
  - Laboratory staff blinded to milk type, health and exposure status

(Loss et al., 2011)

Methodology

TABLE I. GABRIEL study population and design

Study module	Study area	Study population	Total no.	Farmer	Exposed nonfarmer	Nonexposed nonfarmer
Phase I	Four centers*	General population	34,491†	n = 4,533	n = 8,666	n = 21,292
↓				↓	↓	↓
Phase II	Four centers*	Subsample stratified by farm exposure	9,668‡	n = 3,477	n = 3,236	n = 2,955
		Parental questionnaires with milk exposure information available	8,334	n = 3,067	n = 2,796	n = 2,471
		IgE measurements and milk exposure information available	7,606§	n = 2,806	n = 2,544	n = 2,256
↓				↓	↓	↓
Phase III	Bavaria	Subsample stratified by exposure and outcome	895‖	n = 298	n = 300	n = 297
		Milk samples available	800¶	n = 274	n = 263	n = 263

*Germany (Bavaria and Baden-Wuerttemberg), Austria (Tyrol), and Switzerland (9 cantons).
†Eligible for phase II: Complete questionnaire plus written informed consent to further analyses were available (Bavaria: n = 11,183: 1,797/2,708/6,678).
‡Selected for phase II: Random selection of stratified (by farm exposure) eligible subjects for phase II (Bavaria: n = 2,573: 1,014/814/745).
§Blood samples with IgE measurements and parental questionnaires with milk exposure information available.
‖Selected for phase III environmental studies: Random selection of stratified (by farm exposure and health outcome) phase III eligible subjects (2,573 Bavarian children).
¶Milk samples and standardized milk documentation sheets available.

(Loss et al., 2011)

Methodology

- Statistical Analysis
  - Associations between milk exposure and health outcomes using weighted multivariate logistic regression models
    - Age, sex, farming status, number of siblings, familial history of asthma or hay fever, study center, breast feeding
  - Associations between heating status of farm milk or measured milk components and asthma and atopy used regression models
  - Results from weighted logistic regression models expressed as adjusted odd ratios with corresponding 95% CI

(Loss et al., 2011)

Phase II Results

- Significantly lower odds ratios for asthma, current asthma, atopy and hay fever for farm milk compared to exclusive shop milk.

- No association with health outcome and only boiled farm milk consumption.

(Loss et al., 2011)

TABLE III. Adjusted association§ of reported milk exposure and asthma, atopy, hay fever, and atopic dermatitis (phase II, n = 8334)

Milk exposure reported in phase II	Asthma, aOR (95% CI)		Current asthma, aOR (95% CI)		Atopy,‖ aOR (95% CI)		Hay fever, aOR (95% CI)		Atopic dermatitis, aOR (95% CI)	
Exclusively shop milk	1.00		1.00		1.00		1.00		1.00	
Mixed milk	0.91	0.78-1.06	0.86	0.71-1.04	0.77	0.67-0.88‡	0.72	0.60-0.87‡	0.97	0.82-1.14
Only boiled farm milk	1.11	0.86-1.44	1.08	0.78-1.50	0.85	0.67-1.08	0.99	0.72-1.36	1.24	0.94-1.64
Any unboiled farm milk	0.84	0.71-1.00*	0.79	0.64-0.97*	0.74	0.64-0.86‡	0.64	0.52-0.78‡	0.88	0.74-1.05
First unboiled farm milk <1 y	0.69	0.57-0.84‡	0.66	0.52-0.84†	0.72	0.61-0.85‡	0.63	0.50-0.79‡	0.71	0.58-0.86†
First unboiled farm milk >1 y	1.08	0.85-1.37	0.98	0.73-1.30	0.78	0.63-0.97*	0.66	0.49-0.88†	1.16	0.90-1.48
Daily unboiled farm milk	0.76	0.61-0.96*	0.69	0.52-0.92*	0.68	0.57-0.82‡	0.60	0.45-0.79‡	0.81	0.65-1.02
Less than daily unboiled farm milk	0.97	0.82-1.15	0.93	0.75-1.14	0.81	0.69-0.94†	0.77	0.63-0.95*	1.03	0.86-1.24
Exclusively farm milk	0.65	0.52-0.81‡	0.64	0.48-0.84†	0.76	0.63-0.92†	0.58	0.44-0.77‡	0.78	0.61-1.00
Only boiled farm milk	1.24	0.82-1.87	1.59	0.98-2.58	0.90	0.60-1.35	1.17	0.68-1.99	1.04	0.54-2.01
Any unboiled farm milk	0.59	0.46-0.74‡	0.55	0.40-0.74‡	0.74	0.61-0.90†	0.51	0.37-0.69‡	0.75	0.59-0.96*
First unboiled farm milk <1 y	0.55	0.43-0.70‡	0.54	0.39-0.73‡	0.74	0.60-0.91†	0.51	0.37-0.71‡	0.72	0.56-0.94*
First unboiled farm milk >1 y	0.61	0.34-1.07	0.42	0.18-0.99*	0.67	0.43-1.07	0.46	0.21-1.01	0.65	0.37-1.12
Daily unboiled farm milk	0.56	0.43-0.73‡	0.51	0.36-0.72‡	0.76	0.61-0.94*	0.53	0.37-0.76‡	0.72	0.55-0.96*
Less than daily unboiled farm milk	0.61	0.43-0.86†	0.59	0.37-0.94*	0.68	0.50-0.92*	0.46	0.29-0.74†	0.77	0.53-1.11

*$P < .05$, †$P < .01$, and ‡$P < .001$.

§aORs with 95% CIs calculated by using weighted logistic regression models adjusted for age, sex, farming status, 2 or more siblings, familial history of asthma or hay fever, breast-feeding, and study center. All models weighted to phase I: n = 34,491.

‖n = 7,606.

(Loss et al., 2011)

Phase III Results

- Highly heated shop milk
  - Lower total fat content, total viable bacterial count, whey protein levels
- Pasteurized shop milk
  - Higher whey protein levels than highly heated shop milk and heated farm milk
- Farm milk
  - Higher whey protein levels and total viable bacterial counts in raw far milk compared to heated farm milk

(Loss et al., 2011)

Phase III Results

- Microbiological analyses
  - Shop milk and heated farm milk
    - Microorganisms detected <15% for all groups except micrococci and staphylococci
  - Raw farm milk
    - 85.2% micrococci and staphylococci
    - 94.1% lactobacilli
    - 63.4% bacilli and bacterial endospores
    - 58.4% psychrotrophic bacteria
    - Pathogenic *Listeria innocua* and *Listeria ivanovii* strains only in 3 unboiled farm milk samples

(Loss et al., 2011)

Phase III Results

- Raw farm milk had a significant inverse association with asthma (p=.04) and current asthma (p=.03), but not with atopy in comparison with high heated shop milk.
- No association with heated farm milk, total fat content, total viable bacterial counts or microbiological counts with asthma or health outcomes.
- Significant inverse association with asthma and current asthma for:
  - alpha-lactalbumin: asthma, P=.03; current asthma, P=.03
  - Beta-lactoglobulin: asthma, P=.03
  - High levels of BSA: asthma, P=.04; current asthma, P=.04

(Loss et al., 2011)

TABLE V. Adjusted association† of asthma or atopy and milk heating status, total fat content, total viable bacterial count, or whey protein levels (phase III)

Milk parameter	No.	Asthma, aOR (95% CI)		Current asthma, aOR (95% CI)		Atopy, aOR (95% CI)	
Milk type and heating status							
Shop milk: high heat–treated	531	1.00		1.00		1.00	
Shop milk: pasteurized	52	0.50	(0.22-1.12)	0.49	(0.19-1.28)	1.28	(0.59-2.75)
Farm milk: heated	60	0.97	(0.49-1.91)	0.90	(0.38-2.16)	0.74	(0.38-1.44)
Farm milk: raw	157	0.58	(0.34-0.99)*	0.45	(0.22-0.93)*	0.90	(0.56-1.45)
Fat content (%)‡							
Lowest tertile	267	1.00		1.00		1.00	
Medium tertile	269	1.13	(0.73-1.75)	1.37	(0.83-2.26)	0.88	(0.57-1.36)
Highest tertile	258	0.98	(0.60-1.59)	0.92	(0.51-1.65)	1.39	(0.88-2.19)
Total viable bacteria (CFU/mL)							
Less than detection limit	326	1.00		1.00		1.00	
Low levels	223	0.94	(0.60-1.48)	0.88	(0.52-1.50)	0.85	(0.55-1.31)
High levels	222	1.02	(0.62-1.69)	0.85	(0.46-1.60)	0.94	(0.58-1.53)
TGF-β2 (ng/mL)‡							
Lowest tertile	247	1.00		1.00		1.00	
Medium tertile	246	1.36	(0.86-2.15)	1.23	(0.72-2.11)	1.07	(0.68-1.67)
Highest tertile	246	0.75	(0.46-1.22)	0.75	(0.42-1.32)	0.98	(0.62-1.55)
Lactoferrin (μg/mL)							
Less than detection limit	497	1.00		1.00		1.00	
Low levels	151	0.83	(0.50-1.37)	0.83	(0.46-1.52)	1.26	(0.78-2.03)
High levels	151	0.72	(0.41-1.26)	0.64	(0.31-1.32)	1.01	(0.62-1.65)
Total IgG (μg/mL)							
Less than detection limit	449	1.00		1.00		1.00	
Low levels	155	0.85	(0.52-1.40)	0.77	(0.42-1.40)	1.08	(0.68-1.73)
High levels	154	0.61	(0.34-1.07)	0.71	(0.35-1.45)	1.32	(0.81-2.17)
BSA (μg/mL)							
Less than detection limit	447	1.00		1.00		1.00	
Low levels	147	0.76	(0.46-1.26)	0.77	(0.42-1.41)	0.95	(0.58-1.55)
High levels	146	0.53	(0.30-0.97)*	0.45	(0.21-0.98)*	0.90	(0.54-1.51)
α-Lactalbumin (μg/mL)§	704	0.71	(0.52-0.97)*	0.67	(0.47-0.97)*	1.07	(0.78-1.48)
β-Lactoglobulin (μg/mL)§	713	0.62	(0.39-0.97)*	0.62	(0.39-1.06)	1.12	(0.74-1.68)

(Loss et al., 2011)

Discussion

- Supportive of previous and current evidence on early farm milk consumption and reduced risk of childhood asthma and allergies
  - Due to *unheated* farm milk
- Whey proteins associated with reduced risk of asthma
  - Sensitive to heat treatment
  - Other milk components not measured must underlie the association of farm milk and atopy
- No association between total viable bacteria or total fat content and health outcomes

(Loss et al., 2011)

Discussion

- Strengths
  - Analyzing several milk compounds and using enzymatic classification for heat treatment of a large number of samples
- Limitations
  - Cross-sectional design, no fatty acid measurements, limited microbial analyses

(Loss et al., 2011)

Conclusion

- Consumption of unboiled farm milk, but not boiled was associated with a reduced risk of asthma, atopy, and hay fever.
- Whey proteins (BSA, alpha-lactalbumin, beta-lactoglobulin) in milk samples were associated with a reduced risk of asthma.
- No association between total viable bacteria count or total fat content of milk and reduced risk of asthma or atopy.
- Since raw milk might contain pathogens, it is not recommended as treatment, yet.

(Loss et al., 2011)

Discussion Questions

- 1) What study design would suggest if another study was done hypothesizing raw farm milk consumption and reduced risk for asthma?
- 2) Would it ever be realistic to recommend the consumption of raw farm milk?
- 3) With farm exposure decreasing in children, is it likely that asthma will increase?

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Questions?