

The Journal of Allergy and Clinical Immunology: In Practice

Continuous rather than solely early farm exposure protect from hay fever development

--Manuscript Draft--

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Corresponding Author:	Sonali Pechlivanis, PD PhD. Helmholtz Center Munich German Research Center for Environmental Health: Helmholtz Zentrum Munchen Deutsches Forschungszentrum fur Gesundheit und Umwelt Neuherberg , GERMANY
Order of Authors:	Sonali Pechlivanis, PD PhD. Martin Depner, Ph.D. Pirkka V Kirjavainen, Ph.D. Caroline Roduit, M.D. Martin Täubel, Ph.D. Remo Frei, Ph.D. Chrysanthi Skevaki, M.D. Alexander Hose, M.A. M.P.H Cindy Barnig, Ph.D. Elisabeth Schmausser-Hechfellner, B.Sc. Markus J. Ege, M.D. Bianca Schaub, M.D. Amandine Divaret-Chauveau, M.D. Roger Lauener, M.D. Anne M. Karvonen, Ph.D. Juha Pekkanen, M.D. Ph.D. Josef Riedler, M.D. Ph.D. Sabina Illi, Ph.D. Erika von Mutius, M.D. M.Sc.
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Abstract:	<p>Background: An important 'window of opportunity' for early life exposures has been proposed for the development of atopic eczema and asthma.</p> <p>Objective: However it is, unknown whether hay fever with a peak incidence around late school age to adolescence is similarly determined very early in life.</p> <p>Methods: In the PASTURE birth cohort potentially relevant exposures such as farm milk consumption and exposure to animal sheds were assessed at multiple time points from infancy to age 10.5 years and classified by repeated measure latent class analyses (N=769). Fecal samples at age 2 and 12 months were sequenced by 16S rRNA. Hay fever was defined by parental reported symptoms and/or physician's diagnosis of hay fever in the last 12 months using questionnaires at age 10.5 years.</p> <p>Results: Farm children had half the risk of hay fever at age 10.5 years (adjusted odds-ratio (aOR) [95% CI]=0.50 [0.31; 0.79]) compared to non-farm children. While early life events such as gut microbiome richness at age 12 months (aOR=0.66 [0.46; 0.96]) and exposure to animal sheds in the first three years of life (aOR=0.26 [0.06; 1.15]) were determinants of hay fever, the continuous consumption of farm milk from infancy up-to school age was necessary to exert the protective effect (aOR=0.35 [0.17; 0.72]).</p>

	Conclusion: While early life events determine the risk of subsequent hay fever, continuous exposure is necessary to achieve protection. These findings argue against the notion that only early life exposures set long-lasting trajectories.
Additional Information:	
Question	Response
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	<p>authors have no conflict of interest in relation to this work. J.G: reports payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from The journal Pediatric Allergy and Immunology (co-owned by Wiley and EAACI); personal honorarium for serving as Associate Editor. M.K: reports grants or contracts from European Union, German Ministry of Education and Research, German Research Foundation, Infectopharm; consulting fees from Bionorica, Sanofi, Novartis, Bencard; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from ERS, EAACI, ATS, Novartis, Glaxo, Chiesi, Sanofi, Nutricia, Hipp, Allergopharma; patents planned, issued or pending METHOD FOR TESTING A SUBJECT THOUGHT TO HAVE OR TO BE PREDISPOSED TO ASTHMA European patent application 5 EP07301135.5. Other co-authors declares no conflict of interests.</p>
<p>Please enter the text of the Highlights Box, copied from your manuscript document. Each Original Article MUST be accompanied by a highlights box, placed in the text after the Abstract, that provides bulleted answers to the following questions (each answer should be no longer than 35 words):</p> <ol style="list-style-type: none"> 1. What is already known about this topic? 2. What does this article add to our knowledge? 3. How does this study impact current management guidelines? 	<ol style="list-style-type: none"> 1. What is already known about this topic? The protective effects of early life farm exposures and gut microbiome composition on atopic diseases and asthma proposes an important window of opportunity. 2. What does this article add to our knowledge? Early life farm exposures also determine risk of hay fever. However, continuous farm milk consumption is necessary for optimal prevention, thereby arguing against the notion of an early-determined trajectory governing later outcomes. 3. How does this study impact current management guidelines? These results emphasize the preventive potential of continuously drinking unprocessed farm milk for hay fever protection, suggesting carrying out clinical trials to test microbiologically safe cow's milk for protection from hay fever.

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Helmholtz Zentrum München Deutsches Forschungszentrum für
Gesundheit und Umwelt (GmbH), Postfach 11 29, 85758 Neuherberg

Prof. Dr. Michael Schatz
Editor-in-Chief
The Journal of Allergy And Clinical Immunology: In Practice

Dr. Sonali Pechlivanis
PostDoc

Institute of Asthma and Allergy Prevention
Ingolstädter Landsstraße 1
85758 Neuherberg

+49 89/318743783
sonali.pechlivanis@helmholtz-
muenchen.de

11. Oktober 2022

Subject: Submission of the revised manuscript as an "Original article" to The Journal of
Allergy and Clinical Immunology: In Practice

Dear Prof. Dr. Schatz,

Please find enclosed the revised manuscript entitled 'Continuous rather than solely early farm exposure protect from
hay fever development' with manuscript number "INPRACTICE-D-22-00656" along with point-to-point response to the
reviewers and editorial office to be considered for publication as an original article in The Journal of Allergy and Clinical
Immunology: In Practice.

The content of this paper has not been published, nor is it under consideration for publication elsewhere. All the authors
have read the revised manuscript, and approved its submission for publication.

We are of the opinion that the present findings will be of interest to the readers of The Journal of Allergy and Clinical
Immunology: In Practice, and thank you for considering our work for publication. We look forward to your response.

Sincerely yours,



Sonali Pechlivanis

Helmholtz Zentrum München Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH), Ingolstädter Landstr. 1, 85764 Neuherberg,
Telefon +49 89 3187 0, Fax +49 89 3187 3322, info@helmholtz-munich.de | Geschäftsführung: Prof. Dr. med. Dr. h.c. Matthias H. Tschöp, Kerstin Günther |
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Response to the reviewers

Dear Editor,

We thank you, the reviewers and the Editorial Office for the constructive comments that has helped us improve our manuscript. We would like to resubmit the attached revised version of the manuscript to be considered for publication in the JACI: In Practice journal.

Please find our point-to-point response to the questions raised by the reviewers and the editorial office:

COMMENTS FROM REVIEWER #1:

This multi-center birth cohort study examined the association between early farm exposure and hay fever development at the age of 10.5 years. It reported that early life exposure to farm environment could protect against subsequent hay fever, however, the optimal prevention was seen in continuous exposure. This study highlights the significance of continuous exposure to farm environment, rather than only early life exposure in the prevention of hay fever. Following please see some comments:

1. The major concern is the definition of outcome. Because this is a prospective birth cohort, time, or age of hay fever diagnosis and/or symptoms should be available as it stated that some of the children had reported hay fever symptoms or diagnosis at the age 4 or 5 (line 401-403). Incidence of hay fever (onset of the disease) should be analyzed or examined.

We thank the reviewer for pointing this out. Since it is a prospective birth cohort, we have now modified the discussion section lines 446-448 as follows: “In fact, 4.6%, 5.9% and 6.7% of children with data on hay fever at age 10.5 years had already reported symptoms and/or a diagnosis of hay fever at age 4, 5 and 6 years, respectively.”.

At 10.5 years, incidence of hay fever was 7.7% (N=48). In our manuscript, we have already reported the incidence of hay fever at age 10.5 years. The following is written in the results section lines 369-373 “The inverse association of ‘continuous consumption of farm milk’ compared to ‘no consumption of farm milk’ was still observed when using the stringent definition of hay fever (0.41 [0.17; 0.97], 0.04) (Figure E2 Online Repository Text) or incident hay fever at age 10.5 years (0.39 [0.15; 0.99], 0.05, data not shown).”. And in the methods section (Online Repository Text) lines 73-77 “Incident hay fever at 10.5 years (N=48) was defined by parent reported symptoms (itchy, runny, or blocked nose without a cold accompanied by red itchy eyes) and/or a physician’s diagnosis of hay fever in the last 12 months using questionnaires at age 10.5 years and excluding those having hay fever before the age of 10.5 years.”.

2. Among the participants, how many of them have developed other allergic disease, such asthma, eczema, or food allergy? Most importantly, how many children in the no hay fever (controls) have other allergic disease?

The children without hay fever when compared to with hay fever had lower prevalence of asthma (6.2% vs 28.9%), eczema (9.6% vs 36.7%), and food allergy (3.1% vs 21.7%) (Table 1 in the revised manuscript).

We have now added the following regarding other allergic diseases in the Table 1 and in the results section lines 344-346 “Hay fever at the age of 10.5 years was reported in 12.9% children. Of these, 28.9%, 36.7%, and 21.7% had asthma, eczema, and food allergy at age 10.5 years respectively (Table 1).”

3. Because the exposure of interests are repetitive measurements, longitudinal LCA, or repeated measures LCA (RMLCA), an extension of LCA should be applied. Please clarify that RMLCA was used in the analysis.

Thank you for pointing this out. Since the exposures are measured at several time points, we have used repeated measure LCA. We have added the following in the methods section line 293 “We performed repeated measure latent class analyses (RMLCA)” and replaced acronym LCA with RMLCA everywhere in the manuscript.

4. How was the farm and non-farm groups defined? And what was the reason that 193 children did not have hay fever status?

We have now added the following in the methods section lines 253-256 “Children of mothers living on family-run livestock farms at birth of the children were assigned to the farm group. The non-farm group included children of mothers from the same rural areas but not living on a farm (18).”.

Thank you for this hint. Sorry for the confusion regarding 193 children. These are the children who had data on farm exposures i.e. consumption of farm milk and exposure to

animal sheds from pregnancy till 10.5 years (N=962) at least at one time point and hay fever (N=769) at 10.5 years.

At baseline, 1133 children were enrolled into the PASTURE birth cohort. Of them, 778 children participated in the 10.5 years examination and 769 children had data available on hay fever and were included in the present study. Hence, 364 of the original cohort and not 193 children were not included. In the revised version, we have now corrected and modified the results section lines 336-340 and the Table E1 (Online Repository Text) as follows “At 10.5 year follow up 778 children participated in the PASTURE study and 769 have data on hay fever. Comparing the baseline characteristics between included (N=769) and excluded children (N=364) did not show any significant difference except for maternal age at pregnancy, maternal smoking, parental education, and premature birth (Table E1 Online Repository Text).”.

5. It is unclear how mediation analysis of gut microbiome in the association between fam milk consumption and hay fever was conducted. Please specify the analytical method that was used.

We have now added the following in the methods section lines 329-330 “The mediation analysis was conducted through path analysis using maximum likelihood test to estimate the regression parameters in Mplus 8.5 (22).”

6. It was unclear what covariates were included in the multivariate analyses. It is also rather difficult to follow the results (too many figures and tables). It would be helpful to consolidate and reorganize the results to limit the numbers of figures and tables.

The models were adjusted for centers, growing up on a farm and parental asthma and/or atopy. The following is already mentioned in the methods section lines 320-323 “The above models were adjusted for centers and confounders (growing up on a farm and parental asthma and/or atopy) associated with hay fever and exposures in our study. No other confounders i.e. associated with both outcome and exposures were found.”.

As suggested, we have now excluded Figure E5 and Tables (E4, and E5) from the “Online Repository Text” and included the respective OR [95%CI] in the text in the results section lines 377, 382, and 408.

7. In Table E4, the analysis stratified by study centers could not provide valid results due to small numbers in each category. Similarly, the sample size was too small to be interpreted appropriately in the analysis that excluded children having a family history of parental atopy and avoided milk or milk product.

We are aware that the numbers are small. Nevertheless, the effects are comparable in ‘continuous consumption of farm milk’ compared to ‘no consumption of farm milk’ especially in the analysis that excluded the children having a family history of parental

atopy and avoided milk or milk product. As suggested, we have now deleted Table E4 from the "Online Repository Text" and included the text in the results section line 377.

8. The assessment of milk consumption was based on the frequency (daily, 1-6 times a week, less than once a week, or no consumption). Was the volume or size of the milk consumption measured? If not, it was misleading to use "amount" of milk consumption. The "frequency" of milk consumption should be used.

We thank the reviewer for pointing this out. The assessment of milk consumption was based on frequency and not on volume or size of milk consumed. We have now replaced "amount" with "frequency". We do have information on the volume of farm milk consumed. However, this information was not available at three time points (age 12, 18 and 24 months). Hence, the assessment of milk consumption was based on frequency rather than volume of farm milk consumed.

9. Maternal/prenatal exposure to smoking, medication use, maternal age has been associated with allergic disease and hay fever. Was this information available in the PASTURE cohort? It should be considered or addressed.

10. Several other important confounders were not considered, including indicator of socioeconomic status (i.e. household income, parental education, access to health care), second-hand smoking, use of antibiotics, prematurity, birth weight or body mass index of the children.

Thank you for this information. As suggested, we have now addressed these variables (maternal age at pregnancy, maternal smoking, second hand smoking, parental education, use of antibiotic during pregnancy, premature birth, birth weight and use of antibiotics during first year of life; Table 1 and Table E1) in the revised manuscript. However, none of these exposures showed any statistical significant association with hay fever in our study.

COMMENTS FROM REVIEWER #2:

Very nice analysis and manuscript. Only minor comments.

1. Is there a way to calculate a "number needed to treat" re: farm milk's protective effect against hay fever? for example, how many children should drink continuous consumption of farm milk to prevent a diagnosis of hay fever?

As suggested by the reviewer, we have now calculated the Number Needed to Treat using an R-script available at <https://rpubs.com/RatherBit>. However, this was not a randomized placebo-controlled double-blind trial and thus numbers must be taken with some caution. In the revised version, we have added the following in the methods section lines 323-325 "We additionally calculated the Number Needed to Treat (NNT), which is the effectiveness of a treatment on an outcome using an R-script (22).", in the results section lines 394-396 "Additionally, NNT calculated in our study was 7.14, i.e. 7 children would have to drink farm milk continuously from pregnancy by mothers until age 10.5 years in order to prevent hay fever in one child.", and in the discussion section lines 497-499 "Further, the NNT in our study was 7, however, this study is not a randomized

placebo-controlled double-blind trial and thus numbers must be taken with some caution.”.

2. thinking about benefits and risks of farm milk, is there any information to consider any adverse events from drinking farm milk continuously -- for example, were data collected on gastrointestinal illnesses or other possible side effects of farm milk?

In PASTURE study “gastrointestinal illnesses” such as diarrhea was monitored. In the previous study by Loss et al. (1) no clear association of milk consumption with diarrhea in infancy was observed. Similarly, at 10.5 year no elevated risk of diarrhea and farm milk consumption was observed (Table 1 below). We also looked at BMI and did not find any association of farm milk consumption and BMI. Also, the study by Karadag et al. (2) showed no relation of farm milk consumption to eczema or rash.

Table 1.

	Farm milk (Yes) N=290	Farm milk (No) N=474	<i>P-value</i>
Diarrhea			
Yes (N=379)	135 (46.6)	244 (51.5)	
No (N=385)	155 (53.5)	230 (48.5)	0.21
BMI (kg/m ²)*	17.9±2.8	17.6±3.1	0.16

* mean±SD

We have added the following in the revised version of manuscript in the discussion section lines 490-491 “An elevated risk of diarrhea and farm milk consumption at 10.5 years was not observed (data not shown).”, lines 494-497 “Hence, the Milk Against Respiratory Tract Infections and Asthma (MARTHA) an ongoing interventional trial is being carried out to evaluate the preventive effect of minimally treated, i.e. only pasteurized and thus microbiologically safe cow’s milk on upper respiratory tract infections and allergy (34).” and on lines 522-527 “These results emphasize the preventive potential of continuously drinking unprocessed farm milk for hay fever protection. However, the risks associated with raw cow’s milk consumption prohibit its recommendation for daily life. The results of the MARTHA trial however will shed light on potential side effects (34). Further clinical trials based on the present results are warranted.”

3. would you consider adding or highlighting potential next steps or future studies that could build on the current findings?

We have now added the following in the discussion section lines 522-527 “These results emphasize the preventive potential of continuously drinking unprocessed farm milk for hay fever protection. However, the risks associated with raw cow’s milk consumption prohibit its recommendation for daily life. The results of the MARTHA trial however will shed light on potential side effects (34). Further clinical trials based on the present results are warranted.”

4. lines 199-200: current wording implies quality of life (QoL) is a "socio-economic factor", which might be debatable -- could consider deleting "socio-economic factor" to be more clear. Also for this text, to increase generalizability, could consider adding references from outside of Europe, e.g., <https://pubmed.ncbi.nlm.nih.gov/33754932/>

We thank the reviewer for the suggestion. We have now deleted the word "socio-economic factor" and added the suggested literature by Tkacz et al.

5. line 420: consider removing the apostrophe from "it's".

As suggested, we have now removed apostrophe from "it's" (line 465).

COMMENTS FROM REVIEWER #3:

The authors are to be complimented on this very well written and clearly reported study, analyzing the PASTURE cohort. The results are supportive of the study conclusion. I have only two minor suggestions:

1. Study could be strengthened by providing sample size justification,

We thank the reviewer for the suggestion. In our revised version, we have now added the posthoc power calculation in the discussion section lines 508-513, "We performed a posthoc power calculation using SAS and considering $\alpha=0.05$ (two-sided). For our sample size of 650, i.e. in the exposure groups 'continuous consumption of farm milk' and 'no consumption of farm milk' the power of study is over 80% assuming the response

probabilities ranging from 0.02-0.18 for having hay fever in children who consume farm milk and unadjusted OR of 0.24. Thus, our study was well powered to detect a relatively strong effect of farm milk consumption on hay fever.”

2. The study might wish to acknowledge potential caveats in observational study and consider taking more modern causal inference approach in the future.

Yes, the reviewer is right. It is an observational study which has its potential caveats and hence we refer to the MARTHA trial which is an ongoing intervention trial with minimally processed, i.e. only pasteurized cow’s milk. We have added the following in the discussion section lines 491-497 “The results of the present study show protective association of continuous consumption farm milk on hay fever. However, one of the potential caveats of the observation study is finding causality. Hence, the Milk Against Respiratory Tract Infections and Asthma (MARTHA) an ongoing interventional trial is being carried out to evaluate the preventive effect of minimally treated, i.e. only pasteurized and thus microbiologically safe cow’s milk on upper respiratory tract infections and allergy (34).”

COMMENTS FROM THE EDITORIAL OFFICE:

**** Please note that any groups listed as authors must have a conflict of interest statement listed for each group member. Otherwise, please change the author listing to “on behalf of X group.”**

We thank the editorial office for pointing this out. We have now obtained the conflict of interest statement from the members of the PASTURE study group and moved the list of PASTURE study members from the Acknowledgement section to the list of author's lines 39-54. However, I still have a question regarding PASTURE Study group members. Will the PASTURE Study group members be listed in Pubmed? If not, please suggest the possibility of listed them in Pubmed.

**** Revise the abstract to comply with the Journal's structured format, which has five headings: Background, Objective, Methods, Results, Conclusion. When revising your abstract, please keep in mind our length guidelines, which are 250 words or less.**

As suggested, we have now included the five headings in the abstract with the word count of 239.

**** Please provide a Highlight Box that provides bulleted answers (no more than 35 words each) to the following questions:**

We have added the following on lines 207-217

1. What is already known about this topic?

The protective effects of early life farm exposures and gut microbiome composition on atopic diseases and asthma proposes an important window of opportunity.

2. What does this article add to our knowledge?

Early life farm exposures also determine risk of hay fever. However, continuous farm milk consumption is necessary for optimal prevention, thereby arguing against the notion of an early-determined trajectory governing later outcomes.

3. How does this study impact current management guidelines?

These results emphasize the preventive potential of continuously drinking unprocessed farm milk for hay fever protection, suggesting carrying out clinical trials to test microbiologically safe cow's milk for protection from hay fever.

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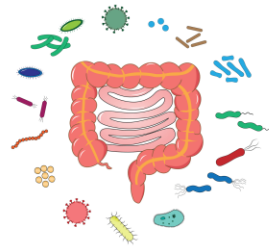
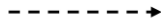
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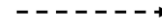
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Continuous consumption of farm milk



Potentially mediated by gut microbiome



Protection of hay fever

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1 **Continuous rather than solely early farm exposure protect from hay fever**
2 **development.**

3

4 Sonali Pechlivanis Ph.D. ¹, Martin Depner Ph.D. ¹, Pirkka V. Kirjavainen Ph.D. ^{2,3},
5 Caroline Roduit M.D. ^{4,5,6}, Martin Täubel Ph.D. ², Remo Frei Ph.D. ^{4,7}, Chrysanthi
6 Skevaki M.D.^{8,9}, Alexander Hose M.A. M.P.H. ¹⁰, Cindy Barnig ^{11,12}, Elisabeth
7 Schmausser-Hechfellner B.Sc. ¹, Markus J. Ege M.D. ^{1,9,10}, Bianca Schaub M.D. ^{9,10},
8 Amandine Divaret-Chauveau M.D. ^{13,14,15}, Roger Lauener M.D. ^{4,6}, Anne M. Karvonen
9 Ph.D. ², Juha Pekkanen M.D. Ph.D. ^{2,16}, Josef Riedler M.D. Ph.D. ¹⁷, Sabina Illi Ph.D. ¹,
10 Erika von Mutius M.D. M.Sc. ^{1,9,10} and the PASTURE Study Group*

11 ¹Institute of Asthma and Allergy Prevention, Helmholtz Zentrum München, German
12 Research Center for Environmental Health, Neuherberg, Germany

13 ²Department of Health Security, Finnish Institute for Health and Welfare, Kuopio,
14 Finland

15 ³Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio,
16 Finland

17 ⁴Christine Kühne Center for Allergy Research and Education (CK-CARE), Davos,
18 Switzerland

19 ⁵Children's Hospital, University of Zürich, Zürich, Switzerland

20 ⁶Childrens Hospital of Eastern Switzerland, St. Gallen, Switzerland

21 ⁷Division of Respiratory Medicine, Department of Paediatrics, Inselspital, University of
22 Bern, Bern, Switzerland

23 ⁸Institute of Laboratory Medicine, Universities of Giessen and Marburg Lung Center
24 (UGMLC), Philipps University Marburg, Marburg, Germany

25 ⁹Member of the German Center for Lung Research, DZL, Germany

26 ¹⁰Dr. von Hauner Childrens Hospital, Ludwig Maximilians University Munich, Munich,
27 Germany

28 ¹¹INSERM, EFS BFC, LabEx LipSTIC, UMR1098, Interactions Hôte-Greffon-
29 Tumeur/Ingénierie Cellulaire et Génique, Univ. Bourgogne Franche-Comté, Besançon,
30 France.

31 ¹²Department of Chest Disease, University Hospital of Besançon, Besançon, France.

32 ¹³Pediatric Allergy Department, Children's Hospital, University Hospital of Nancy,
33 Vandoeuvre les Nancy, France

34 ¹⁴UMR6249 Chrono-environment, University of Bourgogne Franche-Comté, France

35 ¹⁵EA3450 DevAH, Faculty of Medicine, University of Lorraine, Vandoeuvre les Nancy,
36 France

37 ¹⁶Department of Public Health, University of Helsinki, Helsinki, Finland

38 ¹⁷Children's Hospital Schwarzach, Schwarzach, Austria

39 * The members of the PASTURE study group are Johanna Theodorou (Dr. von Hauner
40 Children's Hospital, Ludwig Maximilians University Munich, Munich, Germany; Member
41 of the German Center for Lung Research, DZL, Germany), Andreas Böck (Dr. von
42 Hauner Children's Hospital, Ludwig Maximilians University Munich, Munich, Germany),
43 Harald Renz (Institute of Laboratory Medicine, Philipps University of Marburg, Marburg,
44 Germany; Department of Clinical Immunology and Allergology, Laboratory of
45 Immunopathology, Sechenov University, Moscow, Russia), Petra I. Pfefferle
46 (Comprehensive Biobank Marburg CBBM, Fachbereich Medizin der Philipps Universität
47 Marburg, Marburg, Germany), Jon Genuneit (Pediatric Epidemiology, Medical Faculty,
48 Leipzig University, Germany), Michael Kabesch (Department of Pediatric Pneumology
49 and Allergy, University Children's Hospital Regensburg (KUNO) at the Hospital St.
50 Hedwig of the Order of St. John, University of Regensburg, Regensburg, Germany),
51 Marjut Roponen (Department of Environmental and Biological Sciences, University of
52 Eastern Finland, Kuopio, Finland), and Lucie Laurent (University of Besançon,
53 Department of Respiratory Disease, UMR/CNRS6249 Chrono-environment, University
54 Hospital, Besançon, France).

55 **Corresponding author:**

56 Sonali Pechlivanis, PD Dr.

57 Helmholtz Zentrum München

58 German Research Center for Environmental Health

59 Institute of Asthma and Allergy Prevention

60 Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany

Pechlivanis, 4

61 Telephone: +49 89 3187-43783, Fax: +49 89 4400-54452

62 sonali.pechlivanis@helmholtz-muenchen.de

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185

186 **Abstract**

187 Background: An important 'window of opportunity' for early life exposures has been
 188 proposed for the development of atopic eczema and asthma.

189 Objective: ~~It is not~~ However it is, unknown whether hay fever with a peak incidence around
 190 late school age to adolescence is similarly determined very early in life.

191 Methods: In the PASTURE birth cohort potentially relevant exposures such as farm milk
 192 consumption and exposure to animal sheds were assessed at multiple time points from
 193 infancy to age 10.5 years and classified by repeated measure latent class analyses
 194 (N=769). Fecal samples at age 2 and 12 months were sequenced by 16S rRNA. Hay
 195 fever was defined by parental reported symptoms and/or physician's diagnosis of hay
 196 fever in the last 12 months using questionnaires at age 10.5 years, ~~and for sensitivity~~
 197 ~~analyses (SA) by adding inhalant sensitization to the definition.~~

198 Results: Farm children had half the risk of hay fever at age 10.5 years (adjusted odds-
 199 ratio (aOR) [95% CI]=0.50 [0.31; 0.79]) compared to non-farm children. While early life
 200 events such as gut microbiome richness at age 12 months (aOR=0.66 [0.46; 0.96]) and
 201 exposure to animal sheds in the first three years of life (aOR=0.26 [0.06; 1.15]) were
 202 determinants of hay fever, the continuous consumption of farm milk from infancy up-to
 203 school age was necessary to exert the protective effect (aOR=0.35 [0.17; 0.72]) ~~and SA:~~
 204 ~~aOR=0.41 [0.17; 0.97]).~~

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205 Conclusion: While early life events determine the risk of subsequent hay fever,
206 continuous exposure is necessary to achieve protection. These findings argue against
207 the notion that only early life exposures set long-lasting trajectories.

208 **Highlight box:**

209 **1. What is already known about this topic?**

210 The protective effects of early life farm exposures and gut microbiome composition on
211 atopic diseases and asthma proposes an important window of opportunity.

212 **2. What does this article add to our knowledge?**

213 Early life farm exposures also determine risk of hay fever. However, continuous farm
214 milk consumption is necessary for optimal prevention, thereby arguing against the
215 notion of an early-determined trajectory governing later outcomes.

216 **3. How does this study impact current management guidelines?**

217 These results emphasize the preventive potential of continuously drinking unprocessed
218 farm milk for hay fever protection, suggesting carrying out clinical trials to test
219 microbiologically safe cow's milk for protection from hay fever.

220 ~~Early life farm exposures determine risk of hay fever. However, continuous farm milk~~
221 ~~consumption up to school age is necessary for optimal prevention, thereby arguing~~
222 ~~against the notion of an early-determined trajectory governing later outcomes.~~

223

224 **Keywords:** Childhood, farm milk, farming, gut microbiome, hay fever, animal sheds.

225

226 **Abbreviations:**

227 PASTURE: Protection against Allergy--Study in Rural Environments

228 IgE: immunoglobulin E

229 SPT: skin prick test

230 **RM**LCA: repeated measure latent class analyses

231 q: quintile

232 aOR: adjusted odds ratio

233 95%CI: 95% confidence interval

234 IQR: interquartile range

235 **Introduction**

236 Hay fever is the most common allergic disease worldwide with a prevalence between
237 20-30% (1). The high prevalence has a vast impact on socio-economic-several factors
238 such as quality of life and high healthcare costs_(2, 3). Numerous epidemiological
239 studies have shown the protective effect of early life farm exposures and gut
240 microbiome compositionexposures on asthma, atopy, atopic sensitization, and hay fever
241 (4-11), thus, proposing an important 'window of opportunity' for early life farm exposures
242 and gut microbiome exposures-composition for the protection of atopic diseases and
243 asthma. However, it is unknown whether hay fever with a peak incidence around late
244 school age to adolescence is only determined very early in life or whether later
245 exposure before the onset of disease matters most.

246 The protective "farm-effect" has been attributed to two factors; consumption of
247 unprocessed cow's milk, subsequently termed 'farm milk' and exposure to animal sheds
248 (12-16). Hence, the aim of these analyses is to study the temporal pattern of these
249 protective exposures on hay fever development using the longitudinal data from the
250 PASTURE study. Furthermore, the role of the gut microbiome was investigated.

251 **Methods**252 **Study design and population**

253 PASTURE is a prospective birth cohort study started in 2002 and is conducted in
254 children from rural areas of 5 European countries (Austria, Finland, France, Germany,
255 and Switzerland) (17). The study was designed to evaluate risk and preventive factors
256 for atopic diseases. The study was approved by the local research ethics committees in
257 each country, and written informed consent were obtained from the children's parents.
258 Pregnant women were invited to participate during their third trimester of pregnancy.

259 ~~The women were then classified into farm and non-farm groups.~~The children from the
260 participating women were recruited at birth. Children of mothers living on family-run
261 livestock farms at birth of the children were assigned to the farm group. The non-farm
262 group included children of mothers from the same rural areas but not living on a farm
263 (18). Information were obtained through questionnaires in interviews or self-
264 administered questionnaires from mothers.

265 *Definitions of outcome:*

266 Hay fever was defined by parent reported symptoms (itchy, runny, or blocked nose
267 without a cold accompanied by red itchy eyes) and/or a physician's diagnosis of hay
268 fever in the last 12 months using questionnaires at age 10.5 years. Allergen specific IgE
269 and skin prick test (SPT) were assessed at age 10.5 years (19). Inhalant sensitization
270 was defined as at least one IgE specific to alder, birch, hazel, plantain, mugwort,
271 alternaria, grass, rye, *Dermatophagoides pteronyssinus*, *Dermatophagoides farina*, cat,
272 dog, or horse at levels ≥ 0.7 IU ml⁻¹ or SPT (birch, grass, alternaria, *Dermatophagoides*

273 *pteronyssinus*, *Dermatophagoides farinae*, cat, or dog) ≥ 3 mm. A more stringent
274 definition of hay fever consisting of hay fever plus inhalant sensitization at 10.5 years
275 was used in sensitivity analyses.

276 *Assessment of exposures:*

277 The child's consumption of any farm milk, pasteurized and homogenized milk
278 subsequently termed "processed milk" consumption, and any exposure to animal sheds
279 (cows, pigs, sheep, or horses) at time points 12, 18 months, 2, 3, 4, 5, 6, and 10.5 years
280 were assessed. In addition, maternal any farm milk consumption and animal sheds
281 exposure was assessed during pregnancy and infant's consumption of any farm milk,
282 processed milk and exposure to animal sheds (month 4-12) were obtained on weekly
283 basis by diary. The exposure to animal sheds was further dichotomized based on third
284 quartile (17 weeks) weeks spent on animal sheds as a cut-off.

285 Avoidance of milk or milk products was assessed at the age of 12, 18 months, 2, 3, 4, 5,
286 and 6 years. Additionally, information on ~~the amount~~frequency of farm milk consumption
287 was assessed at the age of 18 months, 2, 3, 4, 5, 6 and 10.5 years of age. ~~Amount~~
288 Frequency of processed milk consumption was assessed at age 10.5 years.

289 *DNA extraction from fecal samples and sequencing analyses:*

290 Fecal samples were collected from children's diapers during the home visit at the age of
291 2 and 12 month. DNA was extracted from homogenized samples and bioinformatics
292 processing were performed as previously described in detail (10). Briefly, α -diversity
293 (i.e. richness and Shannon-index) was calculated as average of multiple times rarefied

294 samples (10). Metabolite levels of short chain fatty acids (SCFA) were measured in
295 fecal samples obtained from 301 children at the age of 12 months (20, 21). Two
296 variables, butyrate and propionate scores were created by modeling SCFA-levels on the
297 relative abundance of all bacterial genera using random forest model in the R-package
298 ranger.

299 **Statistical analyses**

300 We performed repeated measure latent class analyses (RMLCA) using data from
301 pregnancy to age 10.5 years i.e. 9 time points were included separately for exposure to
302 animal sheds, and farm milk consumption (Figure 1(a-b)). The children were allocated
303 to specific exposure classes by their highest posterior probabilities. The analyses were
304 done on children having data at least at 7 of the 9 assessed time points. The optimal
305 number of exposure classes was then determined according to the Bayesian
306 Information Criterion and the labelling of the exposure classes was based on main
307 features of each class.

308 Further as sensitivity analyses, we repeated the farm milk RMLCA, in subgroup of
309 children without a family history of parental asthma and/or atopy and excluding children
310 avoiding milk or milk products at the age 1–6 years as it could introduce confounding by
311 reverse causation, i.e. a positive family history. A farm milk consumption score
312 (Methods section in the Online Repository Text) reflecting the amount-frequency of farm
313 milk consumed was built and divided into quintiles. The quintiles were further
314 categorized as low (q1), intermediate (q2-q4) and high (q5).

315 The associations between hay fever and potential exposures (farm milk exposure
316 classes, animal sheds exposure classes, amount-frequency of farm milk consumption
317 (continuous and quintiles), amount-frequency of processed milk consumption, SCFAs
318 (butyrate score and propionate score) as well as gut microbiome's richness, and
319 Shannon-index) were assessed by logistic regression. We tested the differences in
320 relative abundance of most common single bacterial genera at 2 and 12 months with
321 hay fever by Wilcoxon test (10). The associations between gut microbiome richness and
322 farm milk consumption, processed milk consumption and exposure to animal sheds
323 during infancy was assessed by linear regression. The effect estimates are presented
324 as adjusted odds ratios (aORs) for logistic regression and geometric mean ratios (GMR;
325 calculated by exponentiation of the regression coefficients and their 95% confidence
326 intervals (95%CI)) for linear regression along with their respective 95%CI and a *P value*
327 of 0.05 was considered significant. The above models were adjusted for centers and
328 ~~potential~~-confounders (growing up on a farm and parental asthma and/or atopy)
329 associated with hay fever and exposures in our study. No other ~~potential~~-confounders
330 i.e. associated with both outcome and exposures were found. We additionally calculated
331 the Number Needed to Treat (NNT), which is the effectiveness of a treatment on an
332 outcome using an R-script (22).

333 Furthermore, we conducted mediation analyses to assess whether the associations
334 between farm milk consumption and exposure to animal sheds in infancy (4-12 months)
335 and the risk of hay fever is mediated by gut microbiome features adjusting for centers.
336 The mediation analysis was conducted through path analysis using maximum likelihood

337 [test to estimate the regression parameters in Mplus 8.5](#) (23). The mediating effect is
338 reported as the proportion of the estimated indirect effect to the total effect.

339 The statistical analyses were performed with SAS 9.4 software (SAS Institute, Cary,
340 NC) and Mplus 8.5 software (Muthén & Muthén, Los Angeles, California).

341 **Results**342 **Characteristics of the study population**

343 At 10.5 year follow up 778 children participated in the PASTURE study and 769 have
344 data on hay fever. Comparing the baseline characteristics between included (N=769)
345 and excluded children (N=364) did not show any significant difference except of having
346 contact to dogs at age 2 monthsfor maternal age at pregnancy, maternal smoking,
347 parental education, and premature birth (Table E1 Online Repository Text). In the
348 PASTURE birth cohort, ddata on farm milk consumption and exposure to animal sheds
349 at least at one time point (from pregnancy, age of 12, 18 months, 2, 3, 4, 5, 6, and 10.5
350 years) was available for 962-all these children. Of these, 769 children had information
351 on hay fever at 10.5 years of age. ~~Comparing the baseline characteristics between~~
352 ~~included and excluded children did not show any significant difference except of having~~
353 ~~contact to dogs at age 2 months (Table E1 Online Repository).~~The proportion of
354 children growing up on a farm was 47.7%. Hay fever at the age of 10.5 years was
355 reported in 12.9% children. Of these, 28.9%, 36.7%, and 21.7% had asthma, eczema,
356 and food allergy at age 10.5 years respectively (Table 1). Further, 86.8% were
357 sensitized to inhalant allergens at age 10.5 years (Table 1). Figure E1 (Online
358 Repository Text) shows the proportion of children who were consuming farm milk or
359 were exposed to animal sheds at each time point. The consumption of farm milk by
360 children increased from the age of 1 to 3 years and gradually decreased after age 4
361 years. Similarly, exposure to animal sheds also increased from the age of 1 to 4 years
362 and slightly decreased after age 5 years.

363

364 ***Temporal pattern of the farm-related exposures on hay fever***

365 Children growing up on a farm had half the risk of hay fever as compared to non-farm
366 children (aOR [95%CI], *P value*: 0.50 [0.31; 0.79], 0.003).

367 In a first step, we analyzed the temporal pattern of exposure to animal sheds
368 ('continuous exposure to animal sheds', 'only early exposure to animal sheds', 'only late
369 exposure to animal sheds' and 'no exposure to animal sheds'; Figure 1(a)) on hay fever
370 development. Of these categories, 'only early exposure to animal sheds' showed an
371 inverse association when compared to 'no exposure to animal sheds' which however
372 did not reach statistical significance (0.26 [0.06; 1.15], 0.08) (Table E2 Online
373 Repository [Text](#)). When adjusting this model for consumption of farm milk exposure
374 classes, the results remained unchanged (Table E2 Online Repository [Text](#)).

375 We then analyzed the temporal pattern of consumption of farm milk in similar categories
376 'continuous consumption of farm milk', 'only early consumption of farm milk', 'only late
377 consumption of farm milk' and 'no consumption of farm milk' (Figure 1(b)). The
378 strongest inverse association was found for the 'continuous consumption of farm milk'
379 as compared to 'no consumption of farm milk' (0.35 [0.17; 0.72], 0.004) exposure class
380 (Figure 2 and Table E3 Online Repository [Text](#)). In contrast, 'only early consumption of
381 farm milk' showed no significant effect on hay fever. The inverse association of
382 'continuous consumption of farm milk' compared to 'no consumption of farm milk' was
383 still observed when using the stringent definition of hay fever (0.41 [0.17; 0.97], 0.04)
384 (Figure E2 Online Repository [Text](#)) or incident hay fever at age 10.5 years (0.39 [0.15;

385 0.99], 0.05, data not shown). ~~Furthermore, stratification by center yielded similar effects,~~
 386 ~~thus replicating the findings in the independent PASTURE populations (Table E4 Online~~
 387 ~~Repository).~~ Since confounding by reverse causation might have biased our findings,
 388 we ran a sensitivity analysis in the subgroup of children without a family history of
 389 parental asthma and/or atopy and excluded children avoiding milk or milk products at
 390 the age 1–6 years. This did not change the inverse association with hay fever (Table E4
 391 ~~Online Repository~~ 0.21 [0.06; 0.78], 0.02, data not shown).

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392 We next assessed the association of the ~~amount~~ frequency of farm milk consumption
 393 i.e. whether frequently drinking ~~more~~ farm milk has a dose-response effect on hay fever.
 394 The highest compared to the lowest quintile of farm milk consumption was inversely
 395 associated with hay fever (0.37 [0.16; 0.84], 0.02), whereas the intermediate group (q2-
 396 q4; ~~0.63 [0.37; 1.10], 0.10~~) showed a similarly inverse but non-significant association
 397 (~~Table E5 Online Repository~~). Similar results were obtained when using ~~amount~~
 398 frequency of farm milk consumption score as a continuous variable (data not shown).

399 We further investigated if consumption of processed milk shows similar effects as
 400 consumption of farm milk (Figure E3(a) Online Repository Text). Consumption of 'high
 401 farm and low processed milk' was inversely associated with hay fever (0.24 [0.09; 0.66],
 402 0.006), however, the consumption of processed milk attenuated the farm milk effect
 403 when both farm milk and processed milk were consumed ('mixed consumption of farm
 404 and processed milk' (0.43 [0.19; 0.96], 0.04) (Figure E3(b) and Table E3 Online
 405 Repository Text). Furthermore, daily consumption of shop milk at the age of 10.5 years
 406 showed association in positive direction with hay fever (Figure E4 Online Repository
 407 Text).

408 Additionally, NNT calculated in our study was 7.14, i.e. 7 children would have to drink
 409 farm milk continuously from pregnancy by mothers until age 10.5 years in order to
 410 prevent hay fever in one child.

411 **Early life effect of gut microbiome on hay fever**

412 We investigated the role of the early life gut microbiome by relating bacterial
 413 composition, richness, Shannon-index (at age 2 and 12 months) and SCFA to hay
 414 fever.

415 We did not find any significant differences in relative abundance of most common
 416 bacterial genera at 2 and 12 months with subsequent hay fever at 10.5 year (data not
 417 shown). Also, richness and Shannon-index of bacteria at 2 months were not associated
 418 with hay fever at 10.5 years (Figure 3). However, the bacterial richness of the gut
 419 microbiome at 12 months was inversely associated with hay fever (aOR [95%CI], *P*
 420 *value*: 0.66 [0.46; 0.96], 0.03, Figure 3). Shannon-index at 12 months also showed an
 421 inverse non-significant trend for hay fever (0.71 [0.49; 1.04], 0.08, Figure 3). The SCFAs

422 butyrate (*1.00 [0.92; 1.09], 0.99*) and propionate scores (*0.97 [0.90; 1.05], 0.50*) were in
 423 turn not associated with hay fever (*data not shown*) (Figure E5). We reasoned that
 424 consumption of milk and exposure to animal sheds may shape the gut microbiome, in
 425 particular its richness. Consumption of farm milk (aGMR [95%CI]: 1.20 [1.03; 1.40], *P*
 426 *value*=0.02) and exposure to animal sheds (aGMR [95%CI]: 1.19 [1.01; 1.40], *P*
 427 *value*=0.04) in the first year of life increased gut microbiome richness (Figure 4). In turn,
 428 no association was observed for consumption of processed milk (Figure 4). Since both,
 429 farm milk consumption and exposure to animal sheds during infancy (4-12 months)
 430 showed significant associations with gut microbiome richness at 12 months, we

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431 performed a mediation analysis including unexposed and children exposed to both in
432 infancy. The mediation analysis revealed that part (18.4%) of the total protective effect
433 of farm milk consumption and exposure to animal sheds in the first year of life on hay
434 fever was mediated by gut microbiome richness (P value=0.03, Figure 5). The number
435 of children only being exposed to animal sheds or farm milk, respectively, was too low
436 to allow separate mediation analyses.

437 **Discussion**

438 In the PASTURE birth cohort, the continuous consumption of farm milk throughout age
439 10.5 years, but neither the only early nor the only late exposure alone was significantly
440 associated with reduced risk of hay fever at age 10.5 years. In contrast, exposure to
441 animal sheds only exerted a trend towards protection early in life. Both exposures, farm
442 milk and animal sheds, early in life increased gut microbiome richness at age 12
443 months, which partly explained the protective effect of these exposures on hay fever.

444 The human gut microbiome composition plays an important role in shaping the
445 development of the immune system (24). There is some evidence that the gut
446 microbiome diversity in the first years of life may protect from atopic sensitization. In the
447 population based CHILD cohort, the Shannon-index at age 3 months was associated
448 with protection from atopic sensitization at 1 year (8). However, in a Swedish study the
449 Shannon-index in early infancy was not associated with allergic rhinoconjunctivitis and
450 SPT at age 7 years (25). Our analyses likewise do not confirm this very early 'window of
451 opportunity' since gut microbiome richness and Shannon-index at age 2 month was
452 unrelated to hay fever development.

453 In contrast, gut microbiome richness at the age of 1 year was inversely associated with
454 hay fever at age 10.5 years. We have previously shown in the PASTURE cohort in
455 agreement with others that the compositional structure of the gut microbiome undergoes
456 very significant changes from early age when most infants are breastfed to age 12
457 months ~~and~~ when most foods have been introduced into a child's diet (10, 11).

458 Nevertheless, an inverse association of gut microbiome richness at age 1 year with an

459 outcome much later in life at age 10.5 years may seem surprising. This long-term
460 association may be attributable to an earlier onset of disease. In fact, ~~9.24.6%~~, 5.9%
461 and ~~15.66.7%~~ of children with data on hay fever at age 10.5 years had already reported
462 symptoms and/or a diagnosis of hay fever at age 4, 5 and ~~5-6~~ years, respectively.
463 Furthermore, early alterations of the composition of the gut microbiome may shape its
464 subsequent development towards an adult-like compositional structure in the first 3
465 years of life (26). Unfortunately, no fecal samples have been collected at later time
466 points in the PASTURE cohort.

467 The production of the SCFAs butyrate and propionate measured at 12 months of age
468 has been reported previously as determinants of protection against atopic sensitization
469 at age 6 years (20). In our study, no relation between the SCFAs butyrate and
470 propionate with hay fever was found. Furthermore, no association with single taxa was
471 seen. Thus, different facets of the early development of the gut microbiome composition
472 may matter for different clinical outcomes.

473 Of the environmental exposures investigated in these analyses, the continuous, but
474 neither the early nor the late, consumption of farm milk was seen to protect from hay
475 fever development. Moreover, a dose-response effect was found corroborating the
476 strength of the observation. Interestingly, this protective effect was partly mediated by
477 gut microbiome richness which may suggest that a continued exposure to unprocessed
478 cow's milk may increase gut microbiome richness beyond the age of 12 months and
479 thereby confer ~~it's~~ its protective effect.

480 Continuous exposure also implies repeated exposures. The novel concept of trained
481 immunity may lend itself to mechanistic speculations since phenomena like LPS
482 tolerance are based on the necessity of repeated rather than single exposures (27).
483 A potential explanation for the differential effect of unprocessed versus processed cow's
484 milk is grounded in the observation that most farm children drink their milk unboiled. In
485 fact, too few children received only boiled, i.e. heat treated farm milk over the study
486 period to allow meaningful stratified analyses. A number of population-based and
487 experimental studies have stressed the potential importance of heat-treatment of cow's
488 milk for the loss of protective effects (16, 28-31). Whether alterations of the milk
489 microbiome or denaturation and loss of function of milk (whey) proteins underlie these
490 findings awaits further elucidation.

491 Exposure to animal sheds during early years showed an inverse, albeit non-significant
492 effect on hay fever. This is in contrast to previous farm studies showing stronger effects
493 (12, 32). The discrepancy might be attributable to important differences in the definition
494 of exposure to animal sheds used in the PASTURE study, which only assessed
495 exposure to any animal sheds without differentiating between cows, pigs, sheep and
496 horses. The nature of animal exposure may however matter. While exposure to cow
497 sheds showed a significant protective effect on hay fever and asthma (12), sheep sheds
498 and keeping of hares and rabbits were risk factors for wheezing and asthma
499 respectively in the PARSIFAL farm study (33).

500 The main strength of this study is its longitudinal design, which enabled us to assess the
501 exposures at several time points before the assessment of the outcome. ~~The study~~
502 ~~population comes from five European countries; however, the consumption of farm milk~~

503 ~~effect was replicable in all the PASTURE centers, thus substantiating the observations.~~
504 ~~Likewise, e~~Excluding children with parental asthma and/or atopy and who were avoiding
505 milk or milk products showed similar inverse associations with hay fever consequently
506 arguing against confounding by reverse causation. An elevated risk of diarrhea and
507 farm milk consumption at 10.5 years was not observed (data not shown). The results of
508 the present study show protective association of continuous consumption farm milk on
509 hay fever. However, one of the potential caveats of the observation study is finding
510 causality. Hence, the Milk Against Respiratory Tract Infections and Asthma (MARTHA)
511 an ongoing interventional trial is being carried out to evaluate the preventive effect of
512 minimally treated, i.e. only pasteurized and thus microbiologically safe cow's milk on
513 upper respiratory tract infections and allergy (34). Further, the NNT in our study was 7,
514 however, this study is not a randomized placebo-controlled double-blind trial and thus
515 numbers must be taken with some caution. One of the drawbacks of the study is the
516 missing data on hay fever at 10.5 years. However, comparing the baseline
517 characteristics between included and excluded children did not show any significant
518 difference except ~~of for maternal age at pregnancy, maternal smoking, parental~~
519 ~~education, and premature birth~~having contact to dogs at age 2 months. However,
520 adjusting for these variables ~~analyses for contact to dogs~~ did not change the results
521 (data not shown). Another drawback is the small number in the “only early” and “only
522 late” exposure groups that shows protective non-statistical significant effect on hay
523 fever. However, using the RMLCA approach our study could identify these small groups
524 manifesting that these types of habits i.e. farm milk consumption or exposure to animal
525 sheds do exist. We performed a posthoc power calculation using SAS and considering

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526 $\alpha=0.05$ (two-sided). For our sample size of 650, i.e. in the exposure groups 'continuous
527 consumption of farm milk' and 'no consumption of farm milk' the power of study is over
528 80% assuming the response probabilities ranging from 0.02-0.18 for having hay fever in
529 children who consume farm milk and unadjusted OR of 0.24. Thus, our study was well
530 powered to detect a relatively strong effect of farm milk consumption on hay fever.

531 In summary, the results of the present study demonstrate that continuous exposure of
532 the main determinant, i.e. farm milk consumption but neither only early nor only late
533 exposure alone conferred protection from hay fever development. The early
534 compositional structure of the gut microbiome at age 1 year, but not age 2 month, did
535 however in part mediate this protective effect. One might speculate that continuous
536 consumption of unprocessed cow's milk may also increase gut microbiome richness at
537 later ages, but we do not have data to support this notion. Overall, the findings
538 presented herein do not support the notion of an early-determined trajectory where only
539 early exposures in the first months of life would govern later outcomes. These results
540 emphasize the preventive potential of continuously drinking unprocessed farm milk for
541 hay fever protection. However, the risks associated with raw cow's milk consumption
542 prohibit its recommendation for daily life. The results of the MARTHA trial however will
543 shed light on potential side effects (34). Further clinical trials based on the present
544 results are warranted.

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663 **Figure legends**

664 **Figure 1.** Types of exposure classes.

665 Solution for repeated measure latent classes defined by different exposures, which are a)
666 exposure to animal sheds, and b) farm milk consumption in the PASTURE children. Numbers in
667 parentheses indicate the total number of children in each class.

668 **Figure 2.** Associations of farm milk exposure classes with hay fever at age 10.5 years.

669 Associations of farm milk exposure classes with hay fever at age 10.5 years. Models are adjusted
670 for centers, growing up on a farm, and parental atopy. The forest plot represent the adjusted odds
671 ratios (aOR) with 95% confidence intervals [95% CI].

672 **Figure 3.** Association of gut microbiome richness, and Shannon-index at the age of 2 and 12
673 months with hay fever at 10.5 years.

674 Association of gut microbiome richness, and Shannon-index at months 2 (hay fever/total:
675 59/439) and 12 (hay fever/total: 79/633) with hay fever at 10.5 years. Models are adjusted for

676 centers, growing up on a farm, and parental atopy. The association with hay fever is shown as
677 aOR per-interquartile-range of the probability along with 95% CI.

678 **Figure 4.** Association of consumption of farm milk, consumption of processed milk, and
679 exposure to animal sheds in infancy with gut microbiome richness at month 12.

680 Association of consumption of farm milk (N=624), consumption of processed milk (N=624) and
681 exposure to animal sheds (N=617) with richness at 12 months. Models are adjusted for centers,
682 growing up on a farm, and parental atopy. The forest plot represent the adjusted geometric mean
683 ratios with 95% CI.

684 **Figure 5.** Mediation analysis.

685 Mediation analysis of the protective effect of consumption of farm milk and exposure to animal
686 sheds in infancy on hay fever mediated by gut microbiome richness at 12 months adjusting for
687 centers (N=466). The figure shows the direct (β_1), indirect (β_2) and total (β) effects as well as
688 their respective 95% CI from the path model. The proportion of the mediated (indirect) effect
689 was 18.4%.

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699 **Table 1:** Description of the study population

Characteristic	All (N=769)	Hay fever (N=99 (12.9%))	No hay fever (N=670 (87.1%))	<i>P value</i>
	<u>N (%) / Total</u>	<u>N (%) / Total</u>	<u>N (%) / Total</u>	
Farm child (yes)	367 (47.7) / <u>768</u>	31 (31.3) / <u>99</u>	336 (50.2) / <u>670</u>	0.0005
Exposure to cats at age of 2 months (yes)	199 (26.0) / <u>767</u>	19 (19.2) / <u>99</u>	180 (27.0) / <u>668</u>	0.11
Exposure to dogs at age of 2 months (yes)	147 (19.2) / <u>766</u>	17 (17.2) / <u>99</u>	130 (19.5) / <u>667</u>	0.68
<u>Maternal age at pregnancy (years) †</u>	<u>31.2±4.5 (N=769)</u>	<u>31.4±4.4 (N=99)</u>	<u>31.2±4.5 (N=670)</u>	<u>0.52</u>
<u>Maternal smoking (yes)</u>	<u>96 (12.5) / 766</u>	<u>16 (16.5) / 97</u>	<u>80 (12.0) / 669</u>	<u>0.25</u>
<u>Second hand smoking (yes)</u>	<u>33 (4.3) / 764</u>	<u>3 (3.1) / 98</u>	<u>30 (4.5) / 666</u>	<u>0.79</u>
<u>Parental education (yes)</u>				<u>0.13</u>
<u>Low</u>	<u>62 (8.1) / 764</u>	<u>3 (3.1) / 97</u>	<u>59 (8.9) / 667</u>	
<u>Medium</u>	<u>280 (36.7) / 764</u>	<u>39 (40.2) / 97</u>	<u>241 (36.1) / 667</u>	
<u>High</u>	<u>422 (56.7) / 764</u>	<u>55 (56.7) / 97</u>	<u>367 (55.0) / 667</u>	
<u>Use of antibiotics during pregnancy (yes)</u>	<u>204 (27.0) / 755</u>	<u>26 (26.5) / 98</u>	<u>178 (27.1) / 657</u>	<u>1.00</u>

Parental atopy (yes)	416 (54.4)/ <u>765</u>	72 (73.5)/ <u>98</u>	344 (51.6)/ <u>667</u>	<0.0001
Mode of delivery (normal)	624 (81.9)/ <u>762</u>	82 (83.7)/ <u>98</u>	542 (81.6)/ <u>664</u>	0.68
<u>Premature birth (yes)</u>	<u>11 (1.4)/769</u>	<u>1 (1.0)/99</u>	<u>10 (1.5)/670</u>	<u>1.00</u>
<u>Birth weight (kg) †</u>	<u>3.4±0.44 (N=605)</u>	<u>3.4±0.5 (N=82)</u>	<u>3.4±0.4 (N=523)</u>	<u>0.81</u>
Breast feeding 2 months (yes)	711 (92.7)/ <u>767</u>	90 (90.9)/ <u>99</u>	621 (93.0)/ <u>668</u>	0.41
Gender (female)	366 (47.7)/ <u>768</u>	42 (42.4)/ <u>99</u>	324 (48.4)/ <u>669</u>	0.28
Having siblings (yes)	494 (64.2)/ <u>769</u>	60 (60.6)/ <u>99</u>	434 (64.8)/ <u>670</u>	0.43
<u>Use of antibiotics during first year of life (weeks) †</u>	<u>0.03±0.3 (N=746)</u>	<u>0.01±0.1 (N=97)</u>	<u>0.03±0.4 (N=649)</u>	<u>0.86</u>
Doctor's diagnosis of hay fever (yes)	36 (4.7)/ <u>769</u>	36 (36.4)/ <u>99</u>	NA	NA
Inhalant sensitization (IgE≥0.7 kU/L or SPT≥3mm) at 10.5 years	259 (49.6)/ <u>522</u> /522*	66 (86.8)/-76*	193 (43.3)/446*	<0.0001
<u>Concomitants</u>				
<u>Asthma (yes)</u>	<u>69 (9.0)/764</u>	<u>28 (28.9)/97</u>	<u>41 (6.2)/667</u>	<u><0.0001</u>
<u>Eczema (yes)</u>	<u>100 (13.1)/763</u>	<u>36 (36.7)/98</u>	<u>64 (9.6)/665</u>	<u><0.0001</u>
<u>Food allergy (yes)</u>	<u>41 (5.5)/746</u>	<u>21 (21.7)/97</u>	<u>20 (3.1) /649</u>	<u><0.0001</u>

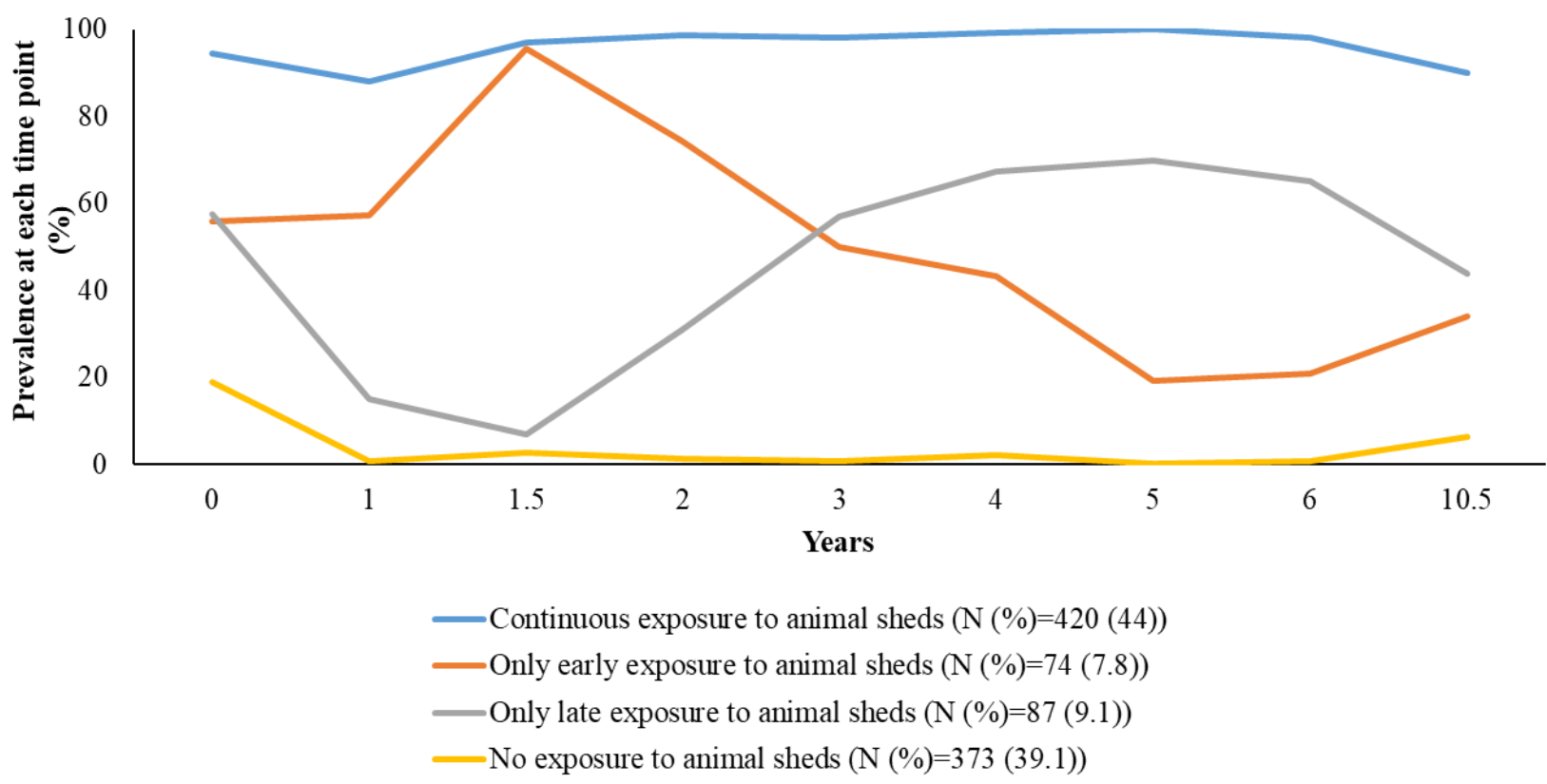
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701 The categorical variables are presented as frequency (percentage) and the continuous variables as †mean †: mean±standard deviation; -median (quartile 1; quartile702 3). The test for differences between the groups are χ^2 or Fischer's Exact test for categorical variables and Mann Whitney U test for continuous variables.

703 Farm child was defined as “Children of mothers living on family-run livestock farms were assigned to the farm group. The non-farm group included children of
 704 mothers from the same rural areas but not living on a farm”. Exposure to pets at the age of 2 months (cats and dogs) was defined by asking “if you have cats?”,
 705 “if you have dogs?” and “if they stay indoors in the house?””. Maternal smoking during pregnancy was defined using the following questions “Have you in
 706 your life smoked more than 5 packs of cigarettes?” Or “Have you quit smoking in the meantime?” and if yes “Was it during this pregnancy?”. Smoking by father,
 707 “Have you in your life smoked more than 5 packs of cigarettes?” Or “Do you still smoke?”. Second hand smoking “How many cigarettes are on average per day
 708 were smoked in your house by other people?” If greater than 1 then second hand smoking was defined as 1 else 0. Parental education was defined as low (less
 709 than 10 years), medium (10 years) and high (greater than 10 years). Parental atopy was defined as doctor’s diagnosis of hay fever, atopic dermatitis, or asthma
 710 ever in mother or father. Use of antibiotics during pregnancy was defined by asking “Have you taken antibiotics since the beginning of pregnancy?” Or “Have
 711 you taken any antibiotics during this pregnancy?”. Child was defined as premature if the child was born before the completion of 37 weeks of pregnancy. Use of
 712 antibiotics by a child during first year of life was defined as “Total No. of weeks with antibiotics ingested”. Breastfeeding at the age of 2 months (yes or no) was
 713 defined by asking “if you have ever breastfed?”. ~~Parental atopy was defined as doctor’s diagnosis of hay fever, atopic dermatitis, or asthma ever in mother or~~
 714 ~~father.~~ SPT: skin prick test. Inhalant sensitization was defined as at least one IgE specific to alder, birch, hazel, plantain, mugwort, alternaria, grass, rye,
 715 *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, dog, or horse at levels $\geq 0.7 \text{ IU ml}^{-1}$ or SPT (birch, grass, alternaria, *Dermatophagoides*
 716 *pteronyssinus*, *Dermatophagoides farinae*, cat, or dog) $\geq 3 \text{ mm}$. Serum specific IgE and SPT was not measured in the Austrian study center, hence only sub-
 717 sample N=522 was included. ~~* sensitized to inhalant allergens/total.~~ Asthma was defined as a physician’s diagnosis of asthma or recurrent obstructive bronchitis
 718 established until 10.5 years. Eczema and food allergy were defined as physician diagnoses at least once until the age of 10.5 years. NA: not applicable.

Figure 1.

a)



b)

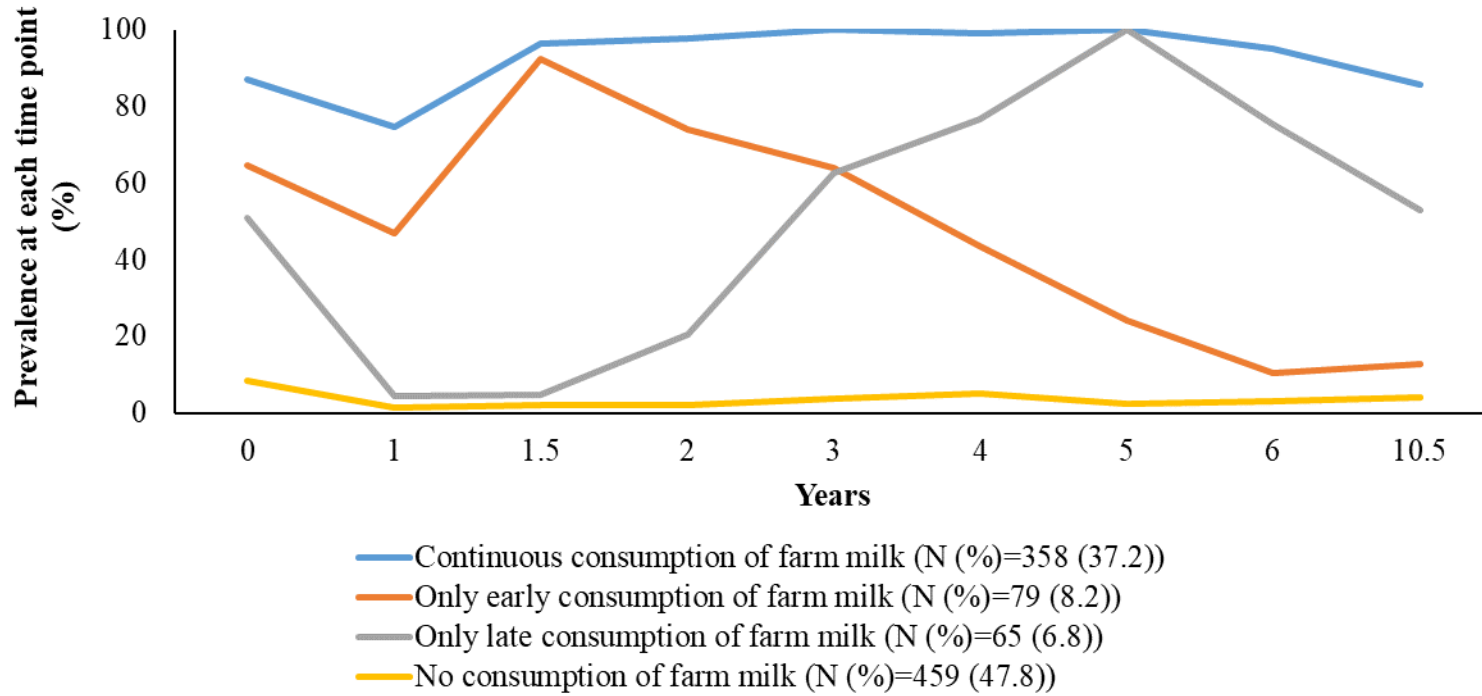


Figure 2

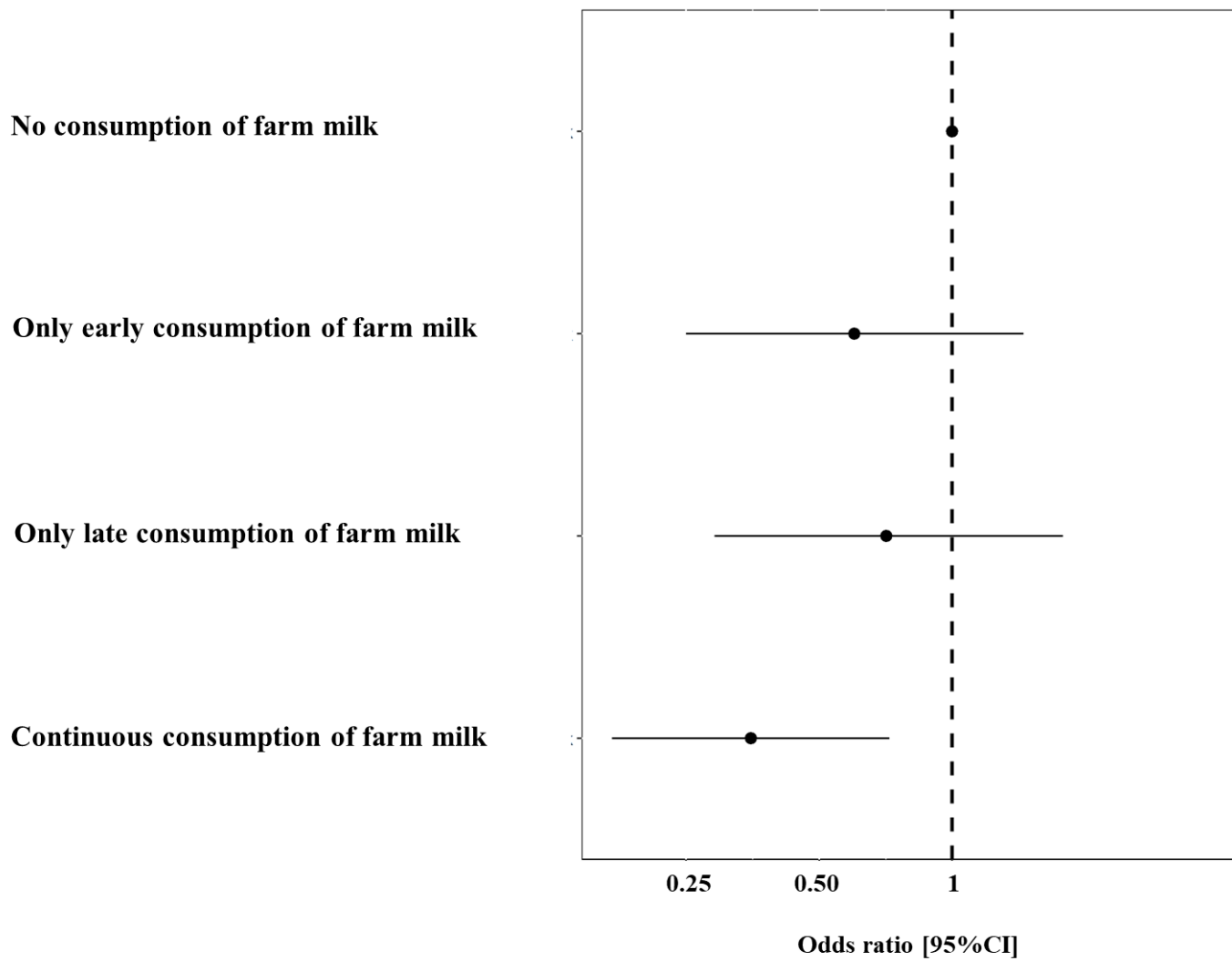


Figure 3

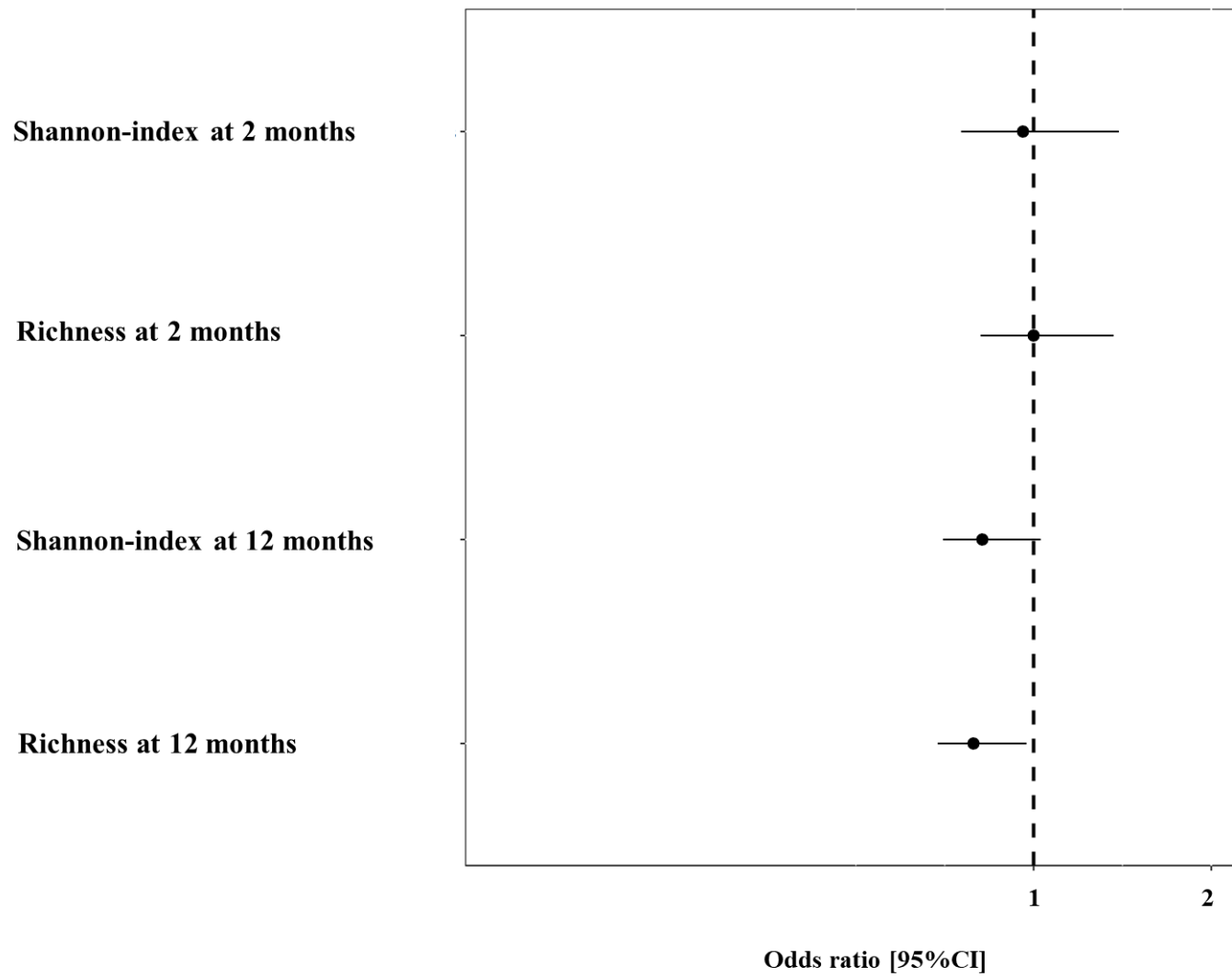


Figure 4.

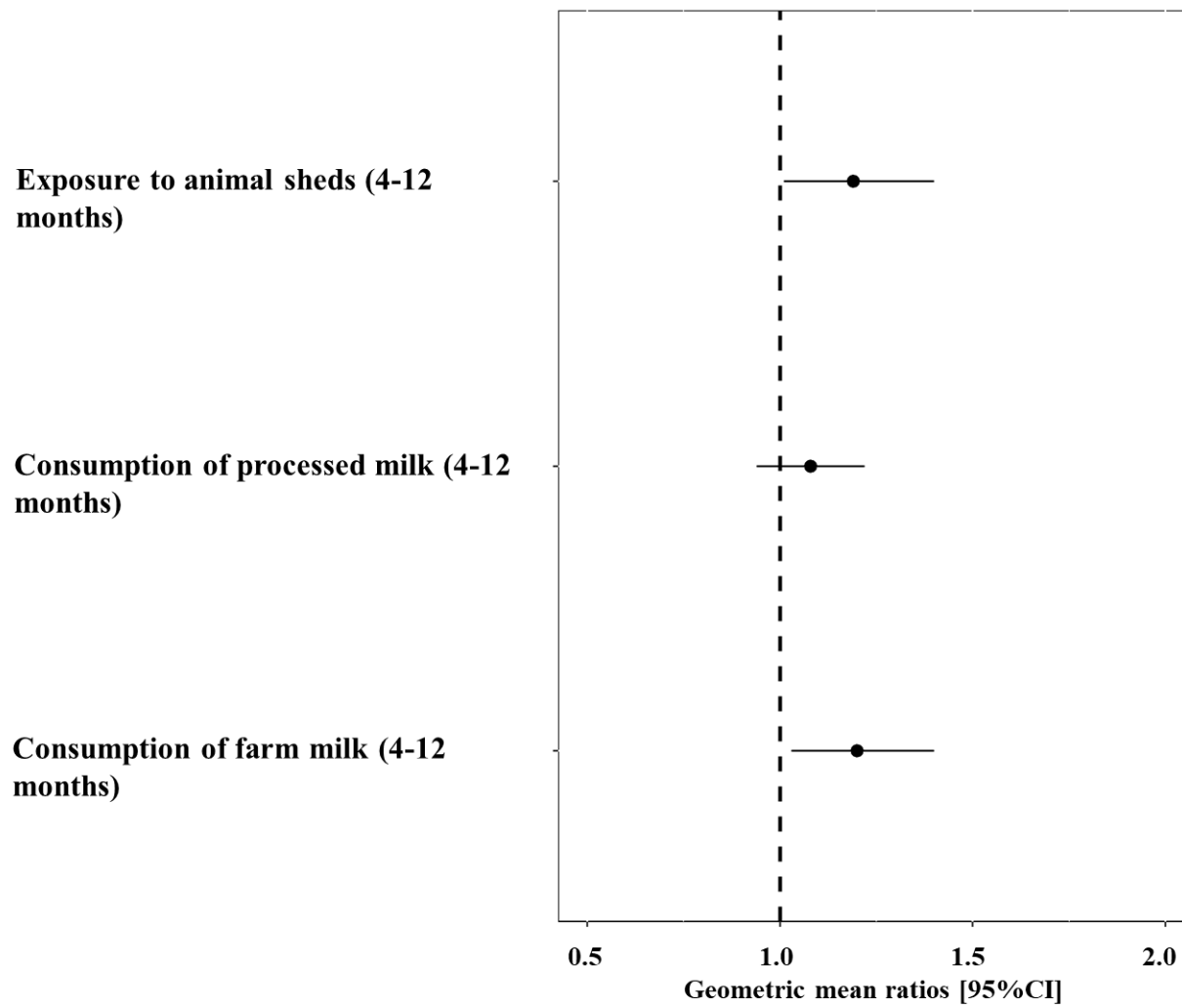
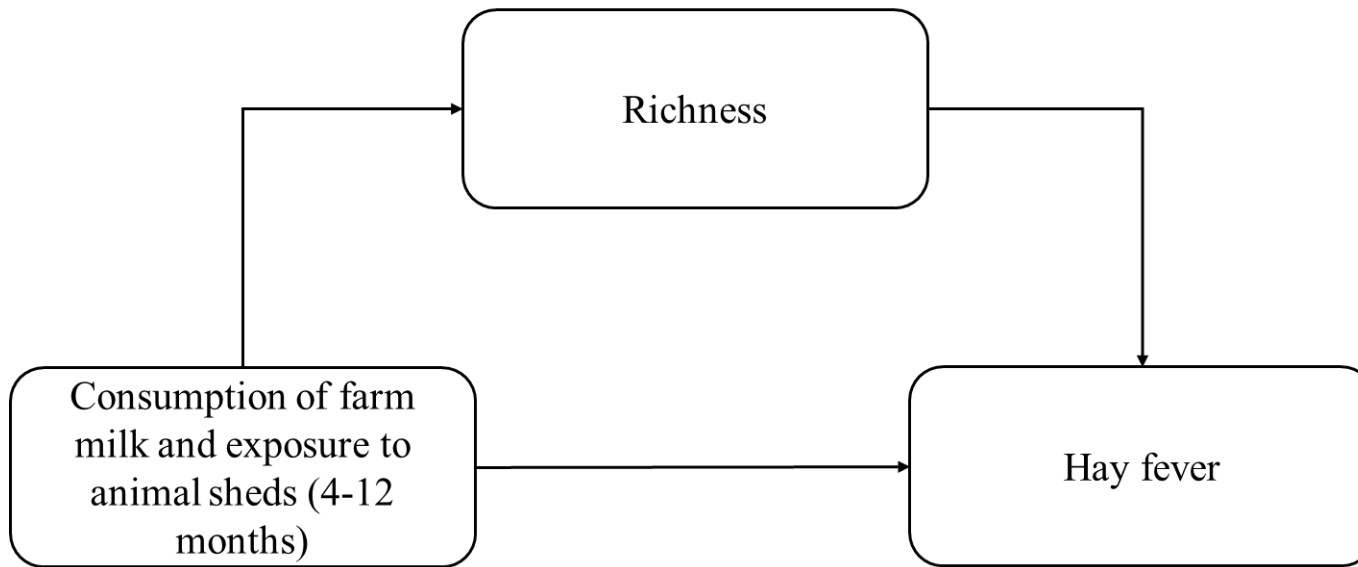


Figure 5.

Total effect, β [95%CI]= -0.98 [-1.88; -0.08]; *P value*=0.03

Indirect effect, β_2 [95%CI]= -0.18 [-0.36; -0.004]; *P value*=0.03



Direct effect, β_1 [95%CI]=-0.80 [-1.70; 0.10]; *P value*=0.08

1 **Online Repository Text**

2 **Continuous rather than solely early farm exposure protect from hay fever development.**

3 Sonali Pechlivanis Ph.D.¹, Martin Depner Ph.D.¹, Pirkka V. Kirjavainen Ph.D.^{2,3}, Caroline
4 Roduit M.D.^{4,5,6}, Martin Täubel Ph.D.², Remo Frei Ph.D.^{4,7}, Chrysanthi Skevaki M.D.^{8,9},
5 Alexander Hose M.A. M.P.H.¹⁰, Cindy Barnig^{11,12}, Elisabeth Schmausser-Hechfellner B.Sc.¹,
6 Markus J. Ege M.D.^{1,9,10}, Bianca Schaub M.D.^{9,10}, Amandine Divaret-Chauveau M.D.^{13,14,15},
7 Roger Lauener M.D.^{4,6}, Anne M. Karvonen Ph.D.², Juha Pekkanen M.D. Ph.D.^{2,16}, Josef
8 Riedler M.D. Ph.D.¹⁷, Sabina Illi Ph.D.¹, Erika von Mutius M.D. M.Sc.^{1,9,10} and the PASTURE
9 Study Group*

10 ¹Institute of Asthma and Allergy Prevention, Helmholtz Zentrum München, German Research
11 Center for Environmental Health, Neuherberg, Germany

12 ²Department of Health Security, Finnish Institute for Health and Welfare, Kuopio, Finland

13 ³Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland

14 ⁴Christine Kühne Center for Allergy Research and Education (CK-CARE), Davos, Switzerland

15 ⁵Children's Hospital, University of Zürich, Zürich, Switzerland

16 ⁶Childrens Hospital of Eastern Switzerland, St. Gallen, Switzerland

17 ⁷Division of Respiratory Medicine, Department of Paediatrics, Inselspital, University of Bern,
18 Bern, Switzerland

19 ⁸Institute of Laboratory Medicine, Universities of Giessen and Marburg Lung Center (UGMLC),
20 Philipps University Marburg, Marburg, Germany

21 ⁹Member of the German Center for Lung Research, DZL, Germany

22 ¹⁰Dr. von Hauner Childrens Hospital, Ludwig Maximilians University Munich, Munich,
23 Germany

24 ¹¹INSERM, EFS BFC, LabEx LipSTIC, UMR1098, Interactions Hôte-Greffon-
25 Tumeur/Ingénierie Cellulaire et Génique, Univ. Bourgogne Franche-Comté, Besançon, France.

26 ¹²Department of Chest Disease, University Hospital of Besançon, Besançon, France.

27 ¹³Pediatric Allergy Department, Children's Hospital, University Hospital of Nancy, Vandoeuvre
28 les Nancy, France

29 ¹⁴UMR6249 Chrono-environment, University of Bourgogne Franche-Comté, France

30 ¹⁵EA3450 DevAH, Faculty of Medicine, University of Lorraine, Vandoeuvre les Nancy, France

31 ¹⁶Department of Public Health, University of Helsinki, Helsinki, Finland

32 ¹⁷Children's Hospital Schwarzach, Schwarzach, Austria

33 * The members of the PASTURE study group are Johanna Theodorou (Dr. von Hauner
34 Children's Hospital, Ludwig Maximilians University Munich, Munich, Germany; Member of the
35 German Center for Lung Research, DZL, Germany), Andreas Böck (Dr. von Hauner Children's
36 Hospital, Ludwig Maximilians University Munich, Munich, Germany), Harald Renz (Institute of
37 Laboratory Medicine, Philipps University of Marburg, Marburg, Germany; Department of

38 [Clinical Immunology and Allergology, Laboratory of Immunopathology, Sechenov University,](#)
39 [Moscow, Russia\), Petra I. Pfefferle \(Comprehensive Biobank Marburg CBBM, Fachbereich](#)
40 [Medizin der Philipps Universität Marburg, Marburg, Germany\), Jon Genuneit \(Pediatric](#)
41 [Epidemiology, Medical Faculty, Leipzig University, Germany\), Michael Kabesch \(Department](#)
42 [of Pediatric Pneumology and Allergy, University Children's Hospital Regensburg \(KUNO\) at the](#)
43 [Hospital St. Hedwig of the Order of St. John, University of Regensburg, Regensburg, Germany\),](#)
44 [Marjut Roponen \(Department of Environmental and Biological Sciences, University of Eastern](#)
45 [Finland, Kuopio, Finland\), and Lucie Laurent \(University of Besançon, Department of](#)
46 [Respiratory Disease, UMR/CNRS6249 Chrono-environment, University Hospital, Besançon,](#)
47 [France\).](#)

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52 Corresponding author:

53 Sonali Pechlivanis, PD Dr.

54 Helmholtz Zentrum München

55 German Research Center for Environmental Health

56 Institute of Asthma and Allergy Prevention

57 Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany

58 Telephone: +49 89 3187-43783, Fax: +49 89 4400-54452

59 sonali.pechlivanis@helmholtz-muenchen.de

60

61 Methods:

62 *Questionnaires:*

63 Information were collected through mothers using questionnaires in interviews or self-
64 administered questionnaires within the third trimester of pregnancy and when the children were
65 2, 12, 18 months of age and then at the age of 2, 3, 4, 5, 6, and 10.5 years. Using weekly and
66 monthly diaries and questionnaires from the 8th to 53rd weeks of age, additional information on
67 child's health, nutrition and farm-related exposures were collected (E1, E2).

68 *Definitions of outcome:*

69 Inhalant sensitization at 10.5 years was defined as at least one IgE specific to alder, birch, hazel,
70 plantain, mugwort, alternaria, grass, rye, *Dermatophagoides pteronyssinus*, *Dermatophagoides*
71 *farina*, cat, dog, or horse at levels $\geq 0.7 \text{ IU ml}^{-1}$ or SPT (birch, grass, alternaria, *Dermatophagoides*
72 *pteronyssinus*, *Dermatophagoides farinae*, cat, or dog) $\geq 3 \text{ mm}$. Serum specific IgE and SPT was
73 not measured in the Austrian study center. Serum specific IgE was assessed using the
74 semiquantitative Allergy Screen test panel for atopy (Mediwiss Analytic, Moers; Germany) (E3).
75 As described before, SPTs were performed on the anterior part of the forearm using a
76 Stallerpoint® (Stallergenes, Antony, France) (E4). Incident hay fever at 10.5 years (N=48) was
77 defined by parent reported symptoms (itchy, runny, or blocked nose without a cold accompanied
78 by red itchy eyes) and/or a physician's diagnosis of hay fever in the last 12 months using

79 questionnaires at age 10.5 years and excluding those having hay fever before the age of 10.5
80 years.

81

82 *Assessment of exposures:*

83 Socioeconomic and lifestyle factors, farm-related exposures, health status of women, their
84 husbands and their children were assessed through questionnaires in interviews or self-
85 administered questionnaires to the mothers within the third trimester of pregnancy and when the
86 children were 2, 12, 18 months of age and then at the age of 2, 3, 4, 5, 6, and 10.5 years.

87 Maternal smoking during pregnancy was defined using the following questions “Have you in
88 your life smoked more than 5 packs of cigarettes?” Or “Have you quit smoking in the
89 meantime?” and if yes “Was it during this pregnancy?”. Smoking by father, “Have you in your
90 life smoked more than 5 packs of cigarettes?” Or “Do you still smoke?”. Second hand smoking
91 was defined by asking “How many cigarettes are on average per day were smoked in your house
92 by other people?” If greater than one then second hand smoking was defined as 1 else 0. Parental
93 education was defined as low (less than 10 years), medium (10 years) and high (greater than 10
94 years). Parental atopy (yes or no) was defined as doctor’s diagnosis of hay fever, atopic
95 dermatitis, or asthma ever in mother or father. Use of antibiotics during pregnancy was defined
96 by asking “Have you taken antibiotics since the beginning of pregnancy?” Or “Have you taken
97 any antibiotics during this pregnancy?”. Child was defined as premature if the child was born
98 before the completion of 37 weeks of pregnancy. Use of antibiotics by a child during first year of
99 life was defined as “Total number of weeks with antibiotics ingested”. Further, breastfeeding at
100 age of 2 months (yes or no) was defined by asking “if you have ever breastfed?”, exposure to
101 pets at age of 2 months (cats and dogs) was defined by asking “if you have cats?”, “if you have

102 dogs?” and “if they stay indoors in the house?”, and data on having siblings (yes or no) were also
103 collected. Further, asthma was defined as a physician’s diagnosis of asthma or recurrent
104 obstructive bronchitis established until 10.5 years. Eczema and food allergy were defined as
105 physician diagnoses at least once until the age of 10.5 years.

106 Additionally, the amount-frequency of farm milk consumption at each time point by a child
107 (daily, 1-6 times a week, less than once a week or no consumption) was further weighted as
108 follows: weight of 3 was assigned for daily consumption, a weight of 2 for 1-6 times a week, a
109 weight of 1 for consumption less than once a week and 0 for no consumption). The weights over
110 the years were then summed up as farm milk consumption score representing the amount
111 frequency of farm milk consumed. Since data on amount-frequency of processed milk
112 consumption was available only at age 10.5 years, instead of constructing a score it was
113 categorized as daily, 1-6 times a week and no (less than once a week or no) consumption of
114 processed milk.

115 *DNA extraction from fecal samples and sequencing analyses:*

116 Briefly, the fecal samples were frozen within 10 minutes of collection, and stored at -20°C until
117 further processing. Targeted DNA amplifications using primers targeting the V4 region of the
118 16S rRNA gene were performed. The amplicon sequencing was done on Illumina MiSeq
119 instrument producing 250-bp paired end sequences as described previously (E5). Sequencing
120 processing was done using QIIME2-2018.6 (Quantitative Insights Into Microbial Ecology) and
121 reads were denoised using DADA2 (E6, E7). Samples were rarefied at the minimum sequence
122 numbers 1,029. Rarefaction and calculation of richness and Shannon-index was iterated 1,000
123 times and the resulting measures of α -diversity were then averaged (E5). As described
124 previously, SCFA levels were modeled by the relative abundance of bacterial genera in children

125 with available SCFA measurements using the “predict” function of R-package ranger (E5).
126 These prediction models were then applied to predict SCFA production scores (butyrate and
127 propionate) in the entire population.

128 Statistical analyses:

129 *Repeated measure ~~Latent~~ latent classes (exposure classes)*

130 Using farm milk consumption and processed milk consumption exposures together, 3 types of
131 farm and processed milk exposure classes were identified: i) ‘high farm and low processed milk’,
132 ii) ‘mixed consumption of farm and processed milk’, and iii) ‘low farm and high processed milk’
133 (Figure E2(a)). The children were allocated to specific exposure classes by their highest posterior
134 probabilities. The optimal number of exposure classes was then determined according to the
135 Bayesian Information Criterion. Further, the labelling of the exposure classes was based on main
136 features of each class. The analyses were done on children having data at least at 7 of the 8
137 assessed time points for the combined farm and processed milk consumption.

138 The associations between hay fever and farm and shop milk consumption exposure classes was
139 assessed by logistic regression. The above model was adjusted for centers and ~~potential~~
140 confounders, (growing up on a farm, and parental asthma and/or atopy). We tested the
141 differences in relative abundance of most common single bacterial genera at 2 and 12 months
142 with hay fever by Wilcoxon test, main associations ($p < 0.05$) were then confirmed in logistic
143 regression models using center-log-ratio-transformed variables. Gut microbiome richness and
144 Shannon-index at 2 and 12 months were transformed by dividing the original variable by their
145 respective interquartile range (IQR: IQRrichness_2m: 8.07, IQRShannon-index_2m: 0.75,
146 IQRrichness_12m: 15.9 and IQRShannon-index_12m: 0.75) and the new variables were then

147 included in the regression models (logistic regression to test the association with outcome hay
148 fever at 10.5 years and linear regression to test the associations between consumption of farm
149 milk, consumption of processed milk and exposure to animal sheds in infancy). The association
150 with hay fever is then represented as adjusted odds ratio per IQR of the probability.

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169 **Figure E1.** Proportion of farm milk consumption and exposure to animal sheds over time in the
170 PASTURE children with data on hay fever at 10.5 years (N=769).

171 **Figure E2.** Association of farm milk consumption exposure classes with the stringent definition
172 of hay fever

173 Models are adjusted for centers, growing up on a farm, and parental atopy. The forest plot
174 represent the aOR with 95%confidence intervals [95%CI].

175 **Figure E3.** Farm and processed milk consumption exposure classes

176 a) Solution for repeated measure latent classes defined by farm and processed milk consumption
177 in the PASTURE children. Numbers in parentheses indicate the total number of children in each
178 class. b) Association of farm and processed milk consumption exposure classes with hay fever.
179 Models are adjusted for centers, growing up on a farm, and parental atopy. The forest plot
180 represent the aOR with 95%CI.

181 **Figure E4.** Association of the amount-frequency of processed milk consumption at the age of
182 10.5 years with hay fever at 10.5 years in the PASTURE children.

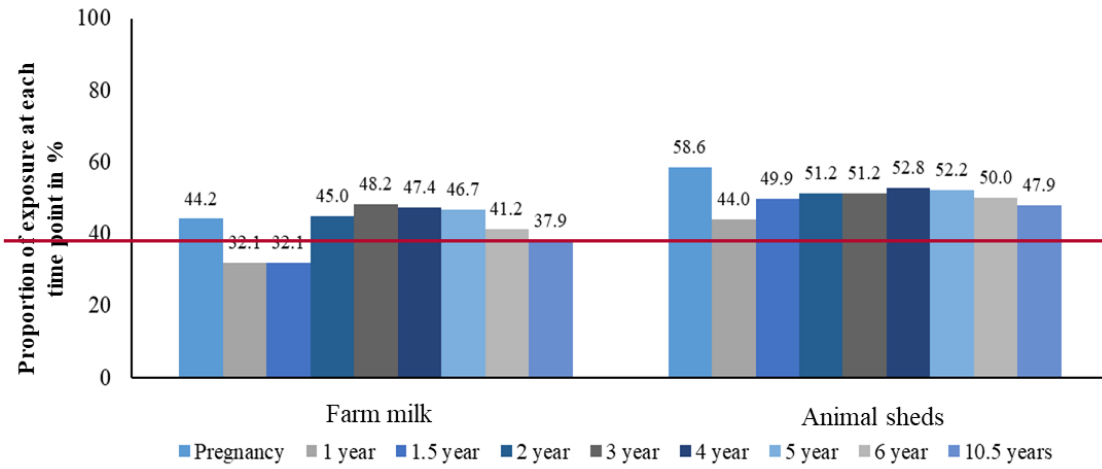
183 Model is adjusted for centers, growing up on a farm, and parental atopy. The forest plot represent
184 the aOR with 95%CI.

185 ~~**Figure E5.** Association of the short chain fatty acid butyrate and propionate scores at 12 months
186 with hay fever at 10.5 years.~~

187 ~~Model is adjusted for centers, growing up on a farm, and parental atopy (hay fever (%)/Total: 79
188 (12.5)/633). The forest plot represent the aOR with 95%CI.~~

189

Figure E1.



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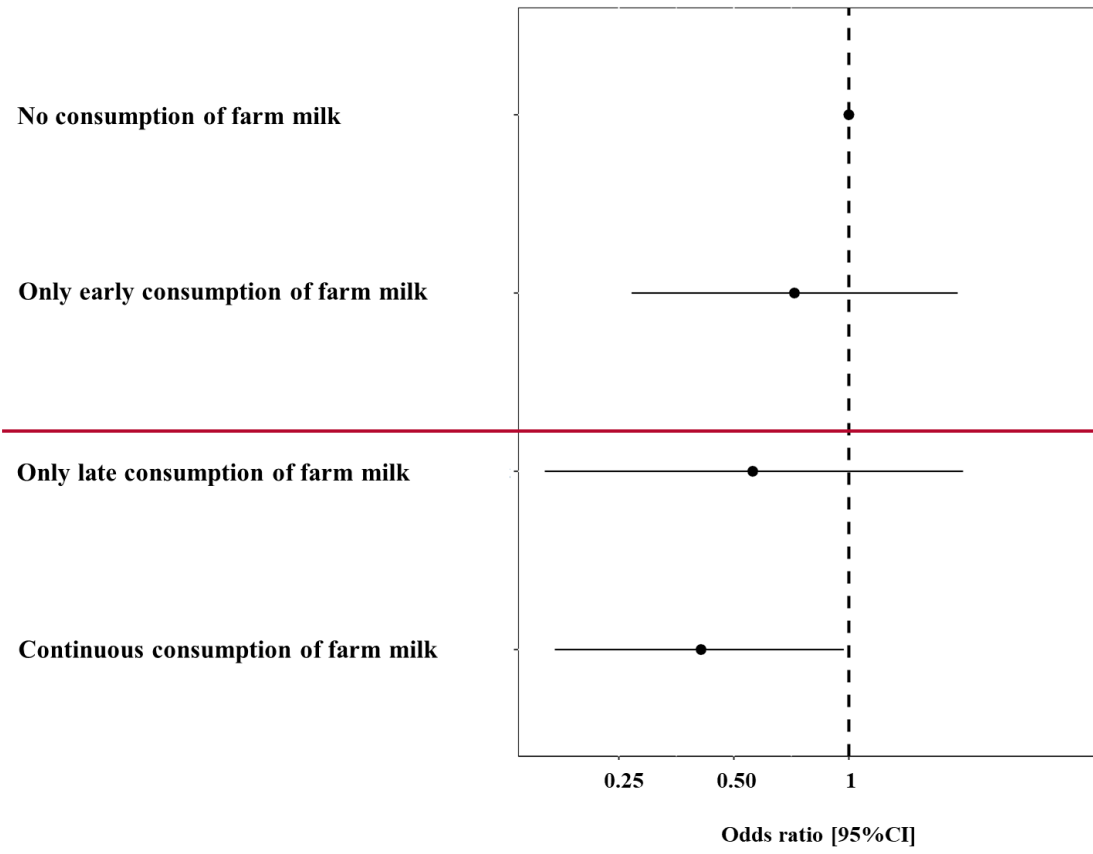
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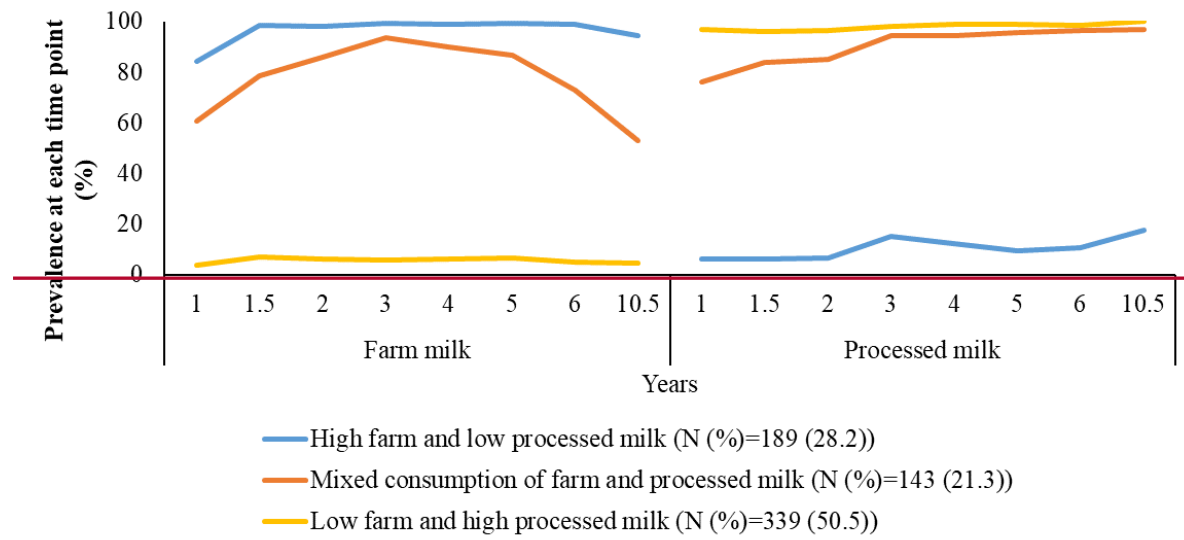
195 **Figure E2**



196

197 **Figure E3.**

198 **(a)**



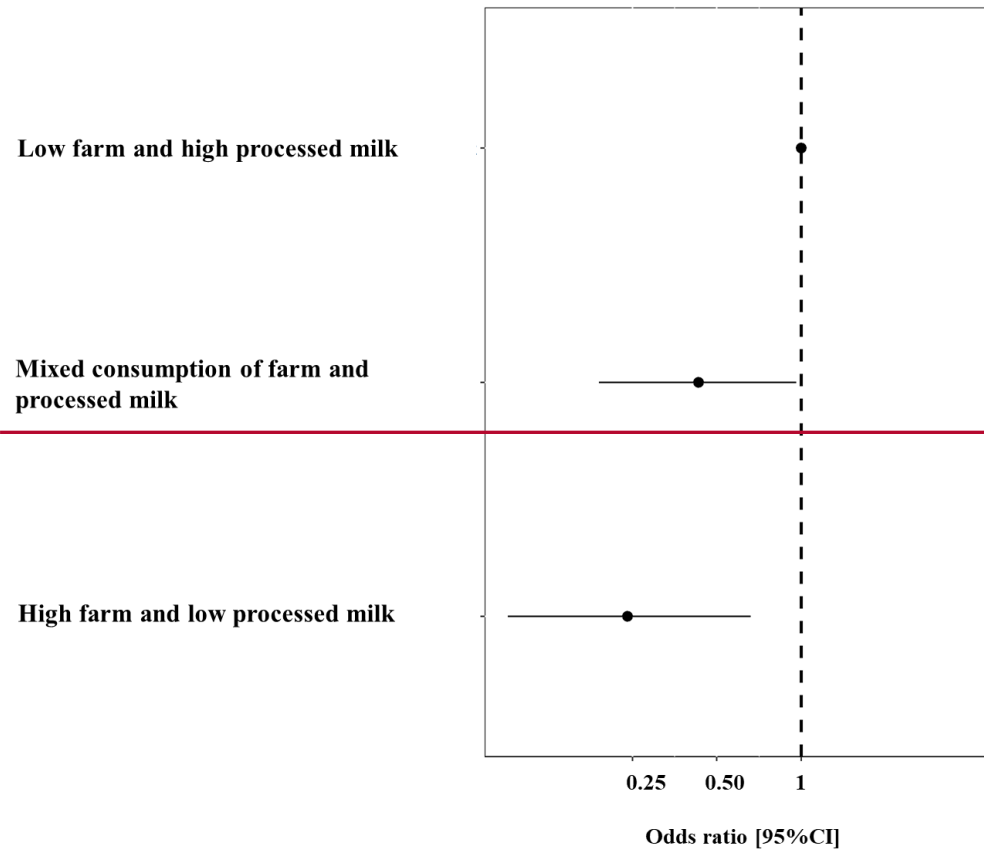
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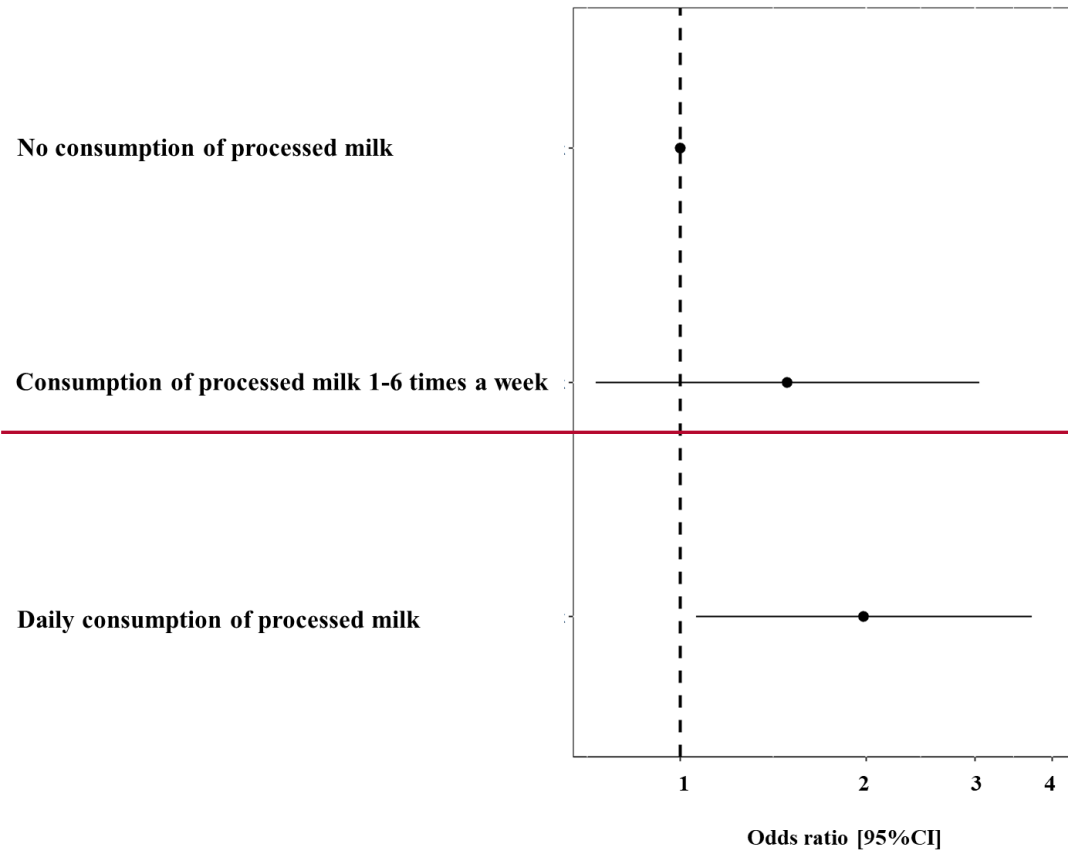
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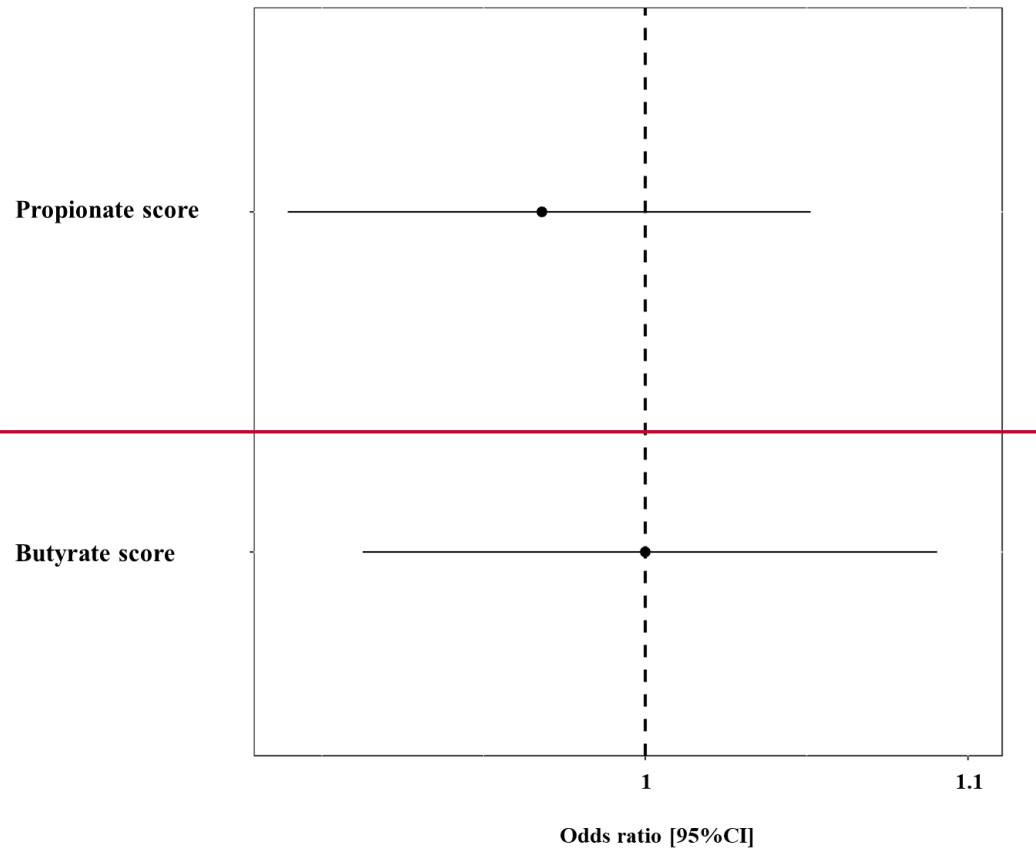
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205 **Figure E4.**



206

207 **Figure E5.**



208

209 **Table E1.** Description of the included and ~~not in~~excluded study population

Characteristic	Included in the study (N=769)	Excluded in the study (N= 193 364)	<i>P value</i>
	N (%) / <u>Total</u>	N (%) / <u>Total</u>	
Farm child (yes)	367 (47.7) / <u>769</u>	98 164 (50.8 45.0) / <u>364</u>	<u>0.417</u>
Exposure to cats at age of 2 months (yes)	199 (26.0) / <u>767</u>	582 (262 5.9) / <u>323</u>	<u>0.7888</u>
Exposure to dogs at age of 2 months (yes)	147 (19.2) / <u>766</u>	244 8 (140 .9) / <u>322</u>	<u>0.1008</u>
<u>Maternal age at pregnancy (years) †</u>	<u>31.3±4.5 (N=769)</u>	<u>30.2±5.0 (N=364)</u>	<u><0.003</u>
<u>Maternal smoking (yes)</u>	<u>96 (12.5) / 766</u>	<u>62 (17.0) / 363</u>	<u>0.04</u>
<u>Second hand smoking (yes)</u>	<u>33 (4.3) / 764</u>	<u>16 (5.0) / 322</u>	<u>0.63</u>
<u>Parental education (yes)</u>			
<u>Low</u>	<u>62 (8.1) / 764</u>	<u>63 (18.1) / 349</u>	
<u>Medium</u>	<u>280 (36.7) / 764</u>	<u>146 (41.8) / 349</u>	
<u>High</u>	<u>422 (55.2) / 764</u>	<u>140 (40.1) / 349</u>	<u><0.001</u>
<u>Use of antibiotics during pregnancy (yes)</u>	<u>204 (27.0) / 755</u>	<u>85 (26.1) / 326</u>	<u>0.77</u>
Parental atopy (yes)	416 (54.4) / <u>765</u>	92 176 (47 52.7) / <u>334</u>	<u>0.1160</u>

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Mode of delivery (normal)	624 (81.9)/ <u>762</u>	155-267 (81.83.24) /320	<u>0.8366</u>
<u>Premature birth (yes)</u>	<u>11 (1.4)/769</u>	<u>50 (13.7)/364</u>	<u><0.0001</u>
<u>Birth weight (kg) †</u>	<u>3.4±0.4 (N=605)</u>	<u>3.4±0.44 (N=239)</u>	<u>0.31</u>
Breast feeding 2 months (yes)	711 (92.7)/ <u>767</u>	175-294 (9091.70) /323	<u>0.3627</u>
Gender (female)	366 (47.7)/ <u>768</u>	101-166 (5250.39) /326	<u>0.2636</u>
Having siblings (yes)	494 (64.2)/ <u>769</u>	119-227 (6162.43) /364	<u>0.510</u>
<u>Use of antibiotics during first year of life (weeks) †</u>	<u>0.03±0.3 (N=746)</u>	<u>0.02±0.1 (N=286)</u>	<u>0.50</u>

210

211 The categorical variables are presented as frequency (percentage) and the continuous variables as †: ~~median±standard deviation (quartile 1; quartile 3)~~. The test
212 for differences between the groups are χ^2 test for categorical variables and Mann Whitney U test for continuous variables. Farm child was defined as “Children of
213 mothers living on family-run livestock farms were assigned to the farm group. The non-farm group included children of mothers from the same rural areas but not
214 living on a farm”. ~~Parental atopy was defined as doctor’s diagnosis of hay fever, atopic dermatitis, or asthma ever in mother or father.~~ Exposure to pets at the age
215 of 2 months (cats and dogs) was defined by asking “if you have cats?”, “if you have dogs?” and “if they stay indoors in the house?”. Maternal smoking during
216 pregnancy was defined using the following questions “Have you in your life smoked more than 5 packs of cigarettes?” Or “Have you quit smoking in the
217 meantime?” and if yes “Was it during this pregnancy?”. Smoking by father, “Have you in your life smoked more than 5 packs of cigarettes?” Or “Do you still
218 smoke?”. Second hand smoking “How many cigarettes are on average per day were smoked in your house by other people?” If greater than 1 then second hand
219 smoking was defined as 1 else 0. Parental education was defined as low (less than 10 years), medium (10 years) and high (greater than 10 years). Parental atopy
220 was defined as doctor’s diagnosis of hay fever, atopic dermatitis, or asthma ever in mother or father. Use of antibiotics during pregnancy was defined by asking
221 “Have you taken antibiotics since the beginning of pregnancy?” Or “Have you taken any antibiotics during this pregnancy?”. Child was defined as premature if the
222 child was born before the completion of 37 weeks of pregnancy. Use of antibiotics by a child during first year of life was defined as “Total No. of weeks with
223 antibiotics ingested”. Breastfeeding at the age of 2 months (yes or no) was defined by asking “if you have ever breastfed?”. ~~Parental atopy was defined as doctor’s~~

224 ~~diagnosis of hay fever, atopic dermatitis, or asthma ever in mother or father.~~ Asthma was defined as a physician's diagnosis of asthma or recurrent obstructive
225 bronchitis established until 10.5 years. Eczema and food allergy were defined as physician diagnoses at least once until the age of 10.5 years. NA: not applicable.

226 **Table E2.** Association between animal sheds exposure classes with hay fever at 10.5 years.

	Hay fever/Total	OR [95% CI], <i>P value</i>
Model 1		
No exposure to animal sheds	56 (18.0)/312	1
Only early exposure to animal sheds	2 (3.9)/52	0.26 [0.06; 1.15], <i>0.08</i>
Only late exposure to animal sheds	9 (12.5)/72	0.88 [0.40; 1.96], <i>0.76</i>
Continuous exposure to animal sheds	31 (9.4)/329	1.14 [0.50; 2.64], <i>0.75</i>
Model 2		
No exposure to animal sheds	56 (18.0)/312	1
Only early exposure to animal sheds	2 (3.9)/52	0.32 [0.07; 1.44], <i>0.14</i>
Only late exposure to animal sheds	9 (12.5)/72	1.04 [0.46; 2.36], <i>0.92</i>
Continuous exposure to animal sheds	31 (9.4)/329	1.94 [0.79; 4.74], <i>0.15</i>

227 Model 1: adjusted for centers, growing up on a farm, and parental atopy. Model 2: adjusted for centers, farm milk consumption exposure classes, and parental
 228 atopy. The number of children included in the analyses are different to that shown in Figure 1(a) due to the missing values of hay fever at year 10.5 years.

229

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232 **Table E3.** Proportion of children in each exposure classes.

Exposure classes	Hay fever (%) / Total
Farm milk	
No consumption of farm milk	70 (18.9) / 371
Only early consumption of farm milk	7 (10.9) / 64
Only late consumption of farm milk	6 (11.8) / 51
Continuous consumption of farm milk	15 (5.4) / 279
Farm milk and processed milk	
Low farm milk and high processed milk	49 (16.2) / 302
Mixed consumption of farm milk and processed milk	9 (7.1) / 127
High farm milk and low processed milk	7 (4.3) / 162

233 Numbers in parentheses indicate percent of children with hay fever in each exposure class. The number of children included in the analyses are different to that
 234 shown in Figure 1(b) and Figure E3(a) due to the missing values of hay fever at year 10.5 years.

235 **Table E4.** Association between the farm milk exposure classes in different strata with hay fever at 10.5 years.

	Hay fever (%) / Total	OR [95% CI], <i>P</i> value
Center Austria [*]		
—No consumption of farm milk	6 (11.3)/53	†
—Only early consumption of farm milk	0 (0)/8	NA
—Only late consumption of farm milk	1 (9.1)/11	0.78 [0.09; 7.24], 0.83
—Continuous consumption of farm milk	2 (3.3)/61	0.27 [0.05; 1.38], 0.11
Center Finland [*]		
—No consumption of farm milk	25 (26.6)/94	†
—Only early consumption of farm milk	3 (13.0)/23	0.41 [0.11; 1.51], 0.18
—Only late consumption of farm milk	3 (37.5)/8	1.66 [0.37; 7.44], 0.51
—Continuous consumption of farm milk	2 (5.4)/37	0.16 [0.04; 0.70], 0.02
Center France [*]		
—No consumption of farm milk	13 (17.3)/75	†
—Early consumption of farm milk	1 (11.1)/9	0.60 [0.07; 5.19], 0.64
—Late consumption of farm milk	0 (0)/6	NA
—Continuous consumption of farm milk	5 (7.9)/63	0.41 [0.14; 1.23], 0.11
Center Germany [*]		
—No consumption of farm milk	15 (18.5)/81	†
—Only early consumption of farm milk	2 (22.2)/9	1.26 [0.24; 6.67], 0.79

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—Only late consumption of farm milk	2 (12.5)/16	0.63 [0.13; 3.07], 0.56
—Continuous consumption of farm milk	3 (4.9)/61	0.23 [0.06; 0.83], 0.02
Center Switzerland*		
—No consumption of farm milk	12 (17.1)/70	†
—Only early consumption of farm milk	1 (6.7)/15	0.35 [0.04; 2.88], 0.33
—Only late consumption of farm milk	0 (0)/10	NA
—Continuous consumption of farm milk	3 (5.1)/59	0.26 [0.07; 0.97], 0.04
Excluding children having a family history of parental atopy and avoided milk or milk products**		
—No consumption of farm milk	17 (13.8)/123	†
—Only early consumption of farm milk	1 (3.7)/27	0.25 [0.03; 2.09], 0.20
—Only late consumption of farm milk	2 (8.3)/24	0.63 [0.13; 3.13], 0.57
—Continuous consumption of farm milk	5 (3.0)/168	0.21 [0.06; 0.78], 0.02

236 *: crude model. Models are adjusted for **: centers, and growing up on a farm.

237

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241 **Table E5.** Association of the amount of the farm milk consumption over time with hay fever at 10.5 years

	OR [95% CI], <i>P</i> value	242
Intermediate (28 (10.3)/272) vs Low (60 (19.5)/308)	0.63 [0.37; 1.10], 0.10	243
High (10 (5.4)/185) vs Low (60 (19.5)/308)	0.37 [0.16; 0.84], 0.02	244

245 Models are adjusted for centers, growing up on a farm, and parent parental atopy.

1 **Continuous rather than solely early farm exposure protect from hay fever**
2 **development.**

3
4 Sonali Pechlivanis Ph.D. ¹, Martin Depner Ph.D. ¹, Pirkka V. Kirjavainen Ph.D. ^{2,3},
5 Caroline Roduit M.D. ^{4,5,6}, Martin Täubel Ph.D. ², Remo Frei Ph.D. ^{4,7}, Chrysanthi
6 Skevaki M.D.^{8,9}, Alexander Hose M.A. M.P.H. ¹⁰, Cindy Barnig ^{11,12}, Elisabeth
7 Schmausser-Hechfellner B.Sc. ¹, Markus J. Ege M.D. ^{1,9,10}, Bianca Schaub M.D. ^{9,10},
8 Amandine Divaret-Chauveau M.D. ^{13,14,15}, Roger Lauener M.D. ^{4,6}, Anne M. Karvonen
9 Ph.D. ², Juha Pekkanen M.D. Ph.D. ^{2,16}, Josef Riedler M.D. Ph.D. ¹⁷, Sabina Illi Ph.D. ¹,
10 Erika von Mutius M.D. M.Sc. ^{1,9,10} and the PASTURE Study Group*

11 ¹Institute of Asthma and Allergy Prevention, Helmholtz Zentrum München, German
12 Research Center for Environmental Health, Neuherberg, Germany

13 ²Department of Health Security, Finnish Institute for Health and Welfare, Kuopio,
14 Finland

15 ³Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio,
16 Finland

17 ⁴Christine Kühne Center for Allergy Research and Education (CK-CARE), Davos,
18 Switzerland

19 ⁵Children's Hospital, University of Zürich, Zürich, Switzerland

20 ⁶Childrens Hospital of Eastern Switzerland, St. Gallen, Switzerland

21 ⁷Division of Respiratory Medicine, Department of Paediatrics, Inselspital, University of
22 Bern, Bern, Switzerland

23 ⁸Institute of Laboratory Medicine, Universities of Giessen and Marburg Lung Center
24 (UGMLC), Philipps University Marburg, Marburg, Germany

25 ⁹Member of the German Center for Lung Research, DZL, Germany

26 ¹⁰Dr. von Hauner Childrens Hospital, Ludwig Maximilians University Munich, Munich,
27 Germany

28 ¹¹INSERM, EFS BFC, LabEx LipSTIC, UMR1098, Interactions Hôte-Greffon-
29 Tumeur/Ingénierie Cellulaire et Génique, Univ. Bourgogne Franche-Comté, Besançon,
30 France.

31 ¹²Department of Chest Disease, University Hospital of Besançon, Besançon, France.

32 ¹³Pediatric Allergy Department, Children's Hospital, University Hospital of Nancy,
33 Vandoeuvre les Nancy, France

34 ¹⁴UMR6249 Chrono-environment, University of Bourgogne Franche-Comté, France

35 ¹⁵EA3450 DevAH, Faculty of Medicine, University of Lorraine, Vandoeuvre les Nancy,
36 France

37 ¹⁶Department of Public Health, University of Helsinki, Helsinki, Finland

38 ¹⁷Children's Hospital Schwarzach, Schwarzach, Austria

39 * The members of the PASTURE study group are Johanna Theodorou (Dr. von Hauner
40 Children's Hospital, Ludwig Maximilians University Munich, Munich, Germany; Member
41 of the German Center for Lung Research, DZL, Germany), Andreas Böck (Dr. von
42 Hauner Children's Hospital, Ludwig Maximilians University Munich, Munich, Germany),
43 Harald Renz (Institute of Laboratory Medicine, Philipps University of Marburg, Marburg,
44 Germany; Department of Clinical Immunology and Allergology, Laboratory of
45 Immunopathology, Sechenov University, Moscow, Russia), Petra I. Pfefferle
46 (Comprehensive Biobank Marburg CBBM, Fachbereich Medizin der Philipps Universität
47 Marburg, Marburg, Germany), Jon Genuneit (Pediatric Epidemiology, Medical Faculty,
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49 and Allergy, University Children's Hospital Regensburg (KUNO) at the Hospital St.
50 Hedwig of the Order of St. John, University of Regensburg, Regensburg, Germany),
51 Marjut Roponen (Department of Environmental and Biological Sciences, University of
52 Eastern Finland, Kuopio, Finland), and Lucie Laurent (University of Besançon,
53 Department of Respiratory Disease, UMR/CNRS6249 Chrono-environment, University
54 Hospital, Besançon, France).

55 **Corresponding author:**

56 Sonali Pechlivanis, PD Dr.

57 Helmholtz Zentrum München

58 German Research Center for Environmental Health

59 Institute of Asthma and Allergy Prevention

60 Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany

61 Telephone: +49 89 3187-43783, Fax: +49 89 4400-54452

62 sonali.pechlivanis@helmholtz-muenchen.de

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185

186 **Abstract**

187 Background: An important 'window of opportunity' for early life exposures has been
188 proposed for the development of atopic eczema and asthma.

189 Objective: However it is, unknown whether hay fever with a peak incidence around late
190 school age to adolescence is similarly determined very early in life.

191 Methods: In the PASTURE birth cohort potentially relevant exposures such as farm milk
192 consumption and exposure to animal sheds were assessed at multiple time points from
193 infancy to age 10.5 years and classified by repeated measure latent class analyses
194 (N=769). Fecal samples at age 2 and 12 months were sequenced by 16S rRNA. Hay
195 fever was defined by parental reported symptoms and/or physician's diagnosis of hay
196 fever in the last 12 months using questionnaires at age 10.5 years.

197 Results: Farm children had half the risk of hay fever at age 10.5 years (adjusted odds-
198 ratio (aOR) [95% CI]=0.50 [0.31; 0.79]) compared to non-farm children. While early life
199 events such as gut microbiome richness at age 12 months (aOR=0.66 [0.46; 0.96]) and
200 exposure to animal sheds in the first three years of life (aOR=0.26 [0.06; 1.15]) were
201 determinants of hay fever, the continuous consumption of farm milk from infancy up-to
202 school age was necessary to exert the protective effect (aOR=0.35 [0.17; 0.72]).

203 Conclusion: While early life events determine the risk of subsequent hay fever,
204 continuous exposure is necessary to achieve protection. These findings argue against
205 the notion that only early life exposures set long-lasting trajectories.

206 **Highlight box:**

207 **1. What is already known about this topic?**

208 The protective effects of early life farm exposures and gut microbiome composition on
209 atopic diseases and asthma proposes an important window of opportunity.

210 **2. What does this article add to our knowledge?**

211 Early life farm exposures also determine risk of hay fever. However, continuous farm
212 milk consumption is necessary for optimal prevention, thereby arguing against the
213 notion of an early-determined trajectory governing later outcomes.

214 **3. How does this study impact current management guidelines?**

215 These results emphasize the preventive potential of continuously drinking unprocessed
216 farm milk for hay fever protection, suggesting carrying out clinical trials to test
217 microbiologically safe cow's milk for protection from hay fever.

218

219 **Keywords:** Childhood, farm milk, farming, gut microbiome, hay fever, animal sheds.

220

221 **Abbreviations:**

222 PASTURE: Protection against Allergy-Study in Rural Environments

223 IgE: immunoglobulin E

224 SPT: skin prick test

- 225 RMLCA: repeated measure latent class analyses
- 226 q: quintile
- 227 aOR: adjusted odds ratio
- 228 95%CI: 95% confidence interval
- 229 IQR: interquartile range

230 **Introduction**

231 Hay fever is the most common allergic disease worldwide with a prevalence between
232 20-30% (1). The high prevalence has a vast impact on several factors such as quality of
233 life and high healthcare costs (2, 3). Numerous epidemiological studies have shown the
234 protective effect of early life farm exposures and gut microbiome composition on
235 asthma, atopy, atopic sensitization, and hay fever (4-11), thus, proposing an important
236 'window of opportunity' for early life farm exposures and gut microbiome composition for
237 the protection of atopic diseases and asthma. However, it is unknown whether hay fever
238 with a peak incidence around late school age to adolescence is only determined very
239 early in life or whether later exposure before the onset of disease matters most.

240 The protective "farm-effect" has been attributed to two factors; consumption of
241 unprocessed cow's milk, subsequently termed 'farm milk' and exposure to animal sheds
242 (12-16). Hence, the aim of these analyses is to study the temporal pattern of these
243 protective exposures on hay fever development using the longitudinal data from the
244 PASTURE study. Furthermore, the role of the gut microbiome was investigated.

245 **Methods**

246 **Study design and population**

247 PASTURE is a prospective birth cohort study started in 2002 and is conducted in
248 children from rural areas of 5 European countries (Austria, Finland, France, Germany,
249 and Switzerland) (17). The study was designed to evaluate risk and preventive factors
250 for atopic diseases. The study was approved by the local research ethics committees in
251 each country, and written informed consent were obtained from the children's parents.
252 Pregnant women were invited to participate during their third trimester of pregnancy.
253 The children from the participating women were recruited at birth. Children of mothers
254 living on family-run livestock farms at birth of the children were assigned to the farm
255 group. The non-farm group included children of mothers from the same rural areas but
256 not living on a farm (18). Information were obtained through questionnaires in interviews
257 or self-administered questionnaires from mothers.

258 *Definitions of outcome:*

259 Hay fever was defined by parent reported symptoms (itchy, runny, or blocked nose
260 without a cold accompanied by red itchy eyes) and/or a physician's diagnosis of hay
261 fever in the last 12 months using questionnaires at age 10.5 years. Allergen specific IgE
262 and skin prick test (SPT) were assessed at age 10.5 years (19). Inhalant sensitization
263 was defined as at least one IgE specific to alder, birch, hazel, plantain, mugwort,
264 alternaria, grass, rye, *Dermatophagoides pteronyssinus*, *Dermatophagoides farina*, cat,
265 dog, or horse at levels $\geq 0.7 \text{ IU ml}^{-1}$ or SPT (birch, grass, alternaria, *Dermatophagoides*
266 *pteronyssinus*, *Dermatophagoides farinae*, cat, or dog) $\geq 3 \text{ mm}$. A more stringent

267 definition of hay fever consisting of hay fever plus inhalant sensitization at 10.5 years
268 was used in sensitivity analyses.

269 *Assessment of exposures:*

270 The child's consumption of any farm milk, pasteurized and homogenized milk
271 subsequently termed "processed milk" consumption, and any exposure to animal sheds
272 (cows, pigs, sheep, or horses) at time points 12, 18 months, 2, 3, 4, 5, 6, and 10.5 years
273 were assessed. In addition, maternal any farm milk consumption and animal sheds
274 exposure was assessed during pregnancy and infant's consumption of any farm milk,
275 processed milk and exposure to animal sheds (month 4-12) were obtained on weekly
276 basis by diary. The exposure to animal sheds was further dichotomized based on third
277 quartile (17 weeks) weeks spent on animal sheds as a cut-off.

278 Avoidance of milk or milk products was assessed at the age of 12, 18 months, 2, 3, 4, 5,
279 and 6 years. Additionally, information on frequency of farm milk consumption was
280 assessed at the age of 18 months, 2, 3, 4, 5, 6 and 10.5 years of age. Frequency of
281 processed milk consumption was assessed at age 10.5 years.

282 *DNA extraction from fecal samples and sequencing analyses:*

283 Fecal samples were collected from children's diapers during the home visit at the age of
284 2 and 12 month. DNA was extracted from homogenized samples and bioinformatics
285 processing were performed as previously described in detail (10). Briefly, α -diversity
286 (i.e. richness and Shannon-index) was calculated as average of multiple times rarefied
287 samples (10). Metabolite levels of short chain fatty acids (SCFA) were measured in

288 fecal samples obtained from 301 children at the age of 12 months (20, 21). Two
289 variables, butyrate and propionate scores were created by modeling SCFA-levels on the
290 relative abundance of all bacterial genera using random forest model in the R-package
291 ranger.

292 **Statistical analyses**

293 We performed repeated measure latent class analyses (RMLCA) using data from
294 pregnancy to age 10.5 years i.e. 9 time points were included separately for exposure to
295 animal sheds, and farm milk consumption (Figure 1(a-b)). The children were allocated
296 to specific exposure classes by their highest posterior probabilities. The analyses were
297 done on children having data at least at 7 of the 9 assessed time points. The optimal
298 number of exposure classes was then determined according to the Bayesian
299 Information Criterion and the labelling of the exposure classes was based on main
300 features of each class.

301 Further as sensitivity analyses, we repeated the farm milk RMLCA, in subgroup of
302 children without a family history of parental asthma and/or atopy and excluding children
303 avoiding milk or milk products at the age 1–6 years as it could introduce confounding by
304 reverse causation, i.e. a positive family history. A farm milk consumption score
305 (Methods section in the Online Repository Text) reflecting the frequency of farm milk
306 consumed was built and divided into quintiles. The quintiles were further categorized as
307 low (q1), intermediate (q2-q4) and high (q5).

308 The associations between hay fever and potential exposures (farm milk exposure
309 classes, animal sheds exposure classes, frequency of farm milk consumption

310 (continuous and quintiles), frequency of processed milk consumption, SCFAs (butyrate
311 score and propionate score) as well as gut microbiome's richness, and Shannon-index)
312 were assessed by logistic regression. We tested the differences in relative abundance
313 of most common single bacterial genera at 2 and 12 months with hay fever by Wilcoxon
314 test (10). The associations between gut microbiome richness and farm milk
315 consumption, processed milk consumption and exposure to animal sheds during infancy
316 was assessed by linear regression. The effect estimates are presented as adjusted
317 odds ratios (aORs) for logistic regression and geometric mean ratios (GMR; calculated
318 by exponentiation of the regression coefficients and their 95% confidence intervals
319 (95%CI)) for linear regression along with their respective 95%CI and a *P value* of 0.05
320 was considered significant. The above models were adjusted for centers and
321 confounders (growing up on a farm and parental asthma and/or atopy) associated with
322 hay fever and exposures in our study. No other confounders i.e. associated with both
323 outcome and exposures were found. We additionally calculated the Number Needed to
324 Treat (NNT), which is the effectiveness of a treatment on an outcome using an R-script
325 (22).

326 Furthermore, we conducted mediation analyses to assess whether the associations
327 between farm milk consumption and exposure to animal sheds in infancy (4-12 months)
328 and the risk of hay fever is mediated by gut microbiome features adjusting for centers.
329 The mediation analysis was conducted through path analysis using maximum likelihood
330 test to estimate the regression parameters in Mplus 8.5 (23). The mediating effect is
331 reported as the proportion of the estimated indirect effect to the total effect.

332 The statistical analyses were performed with SAS 9.4 software (SAS Institute, Cary,
333 NC) and Mplus 8.5 software (Muthén & Muthén, Los Angeles, California).

334 **Results**

335 ***Characteristics of the study population***

336 At 10.5 year follow up 778 children participated in the PASTURE study and 769 have
337 data on hay fever. Comparing the baseline characteristics between included (N=769)
338 and excluded children (N=364) did not show any significant difference except for
339 maternal age at pregnancy, maternal smoking, parental education, and premature birth
340 (Table E1 Online Repository Text). Data on farm milk consumption and exposure to
341 animal sheds at least at one time point (from pregnancy, age of 12, 18 months, 2, 3, 4,
342 5, 6, and 10.5 years) was available for all these children. Of these, 769 children had
343 information on hay fever at 10.5 years of age. The proportion of children growing up on
344 a farm was 47.7%. Hay fever at the age of 10.5 years was reported in 12.9% children.
345 Of these, 28.9%, 36.7%, and 21.7% had asthma, eczema, and food allergy at age 10.5
346 years respectively (Table 1). Further, 86.8% were sensitized to inhalant allergens at age
347 10.5 years (Table 1). Figure E1 (Online Repository Text) shows the proportion of
348 children who were consuming farm milk or were exposed to animal sheds at each time
349 point. The consumption of farm milk by children increased from the age of 1 to 3 years
350 and gradually decreased after age 4 years. Similarly, exposure to animal sheds also
351 increased from the age of 1 to 4 years and slightly decreased after age 5 years.

352 ***Temporal pattern of the farm-related exposures on hay fever***

353 Children growing up on a farm had half the risk of hay fever as compared to non-farm
354 children (aOR [95%CI], *P* value: 0.50 [0.31; 0.79], 0.003).

355 In a first step, we analyzed the temporal pattern of exposure to animal sheds
356 ('continuous exposure to animal sheds', 'only early exposure to animal sheds', 'only late
357 exposure to animal sheds' and 'no exposure to animal sheds'; Figure 1(a)) on hay fever
358 development. Of these categories, 'only early exposure to animal sheds' showed an
359 inverse association when compared to 'no exposure to animal sheds' which however
360 did not reach statistical significance (0.26 [0.06; 1.15], 0.08) (Table E2 Online
361 Repository Text). When adjusting this model for consumption of farm milk exposure
362 classes, the results remained unchanged (Table E2 Online Repository Text).

363 We then analyzed the temporal pattern of consumption of farm milk in similar categories
364 'continuous consumption of farm milk', 'only early consumption of farm milk', 'only late
365 consumption of farm milk' and 'no consumption of farm milk' (Figure 1(b)). The
366 strongest inverse association was found for the 'continuous consumption of farm milk'
367 as compared to 'no consumption of farm milk' (0.35 [0.17; 0.72], 0.004) exposure class
368 (Figure 2 and Table E3 Online Repository Text). In contrast, 'only early consumption of
369 farm milk' showed no significant effect on hay fever. The inverse association of
370 'continuous consumption of farm milk' compared to 'no consumption of farm milk' was
371 still observed when using the stringent definition of hay fever (0.41 [0.17; 0.97], 0.04)
372 (Figure E2 Online Repository Text) or incident hay fever at age 10.5 years (0.39 [0.15;
373 0.99], 0.05, data not shown). Since confounding by reverse causation might have
374 biased our findings, we ran a sensitivity analysis in the subgroup of children without a
375 family history of parental asthma and/or atopy and excluded children avoiding milk or
376 milk products at the age 1–6 years. This did not change the inverse association with hay
377 fever (0.21 [0.06; 0.78], 0.02, data not shown).

378 We next assessed the association of the frequency of farm milk consumption i.e.
379 whether frequently drinking farm milk has a dose-response effect on hay fever. The
380 highest compared to the lowest quintile of farm milk consumption was inversely
381 associated with hay fever (0.37 [0.16; 0.84], 0.02), whereas the intermediate group (q2-
382 q4; 0.63 [0.37; 1.10], 0.10) showed a similarly inverse but non-significant association.
383 Similar results were obtained when using frequency of farm milk consumption score as
384 a continuous variable (data not shown).

385 We further investigated if consumption of processed milk shows similar effects as
386 consumption of farm milk (Figure E3(a) Online Repository Text). Consumption of 'high
387 farm and low processed milk' was inversely associated with hay fever (0.24 [0.09; 0.66],
388 0.006), however, the consumption of processed milk attenuated the farm milk effect
389 when both farm milk and processed milk were consumed ('mixed consumption of farm
390 and processed milk' (0.43 [0.19; 0.96], 0.04) (Figure E3(b) and Table E3 Online
391 Repository Text). Furthermore, daily consumption of shop milk at the age of 10.5 years
392 showed association in positive direction with hay fever (Figure E4 Online Repository
393 Text).

394 Additionally, NNT calculated in our study was 7.14, i.e. 7 children would have to drink
395 farm milk continuously from pregnancy by mothers until age 10.5 years in order to
396 prevent hay fever in one child.

397 ***Early life effect of gut microbiome on hay fever***

398 We investigated the role of the early life gut microbiome by relating bacterial
399 composition, richness, Shannon-index (at age 2 and 12 months) and SCFA to hay
400 fever.

401 We did not find any significant differences in relative abundance of most common
402 bacterial genera at 2 and 12 months with subsequent hay fever at 10.5 year (data not
403 shown). Also, richness and Shannon-index of bacteria at 2 months were not associated
404 with hay fever at 10.5 years (Figure 3). However, the bacterial richness of the gut
405 microbiome at 12 months was inversely associated with hay fever (aOR [95%CI], *P*
406 *value*: 0.66 [0.46; 0.96], 0.03, Figure 3). Shannon-index at 12 months also showed an
407 inverse non-significant trend for hay fever (0.71 [0.49; 1.04], 0.08, Figure 3). The SCFAs
408 butyrate (1.00 [0.92; 1.09], 0.99) and propionate scores (0.97 [0.90; 1.05], 0.50) were in
409 turn not associated with hay fever (data not shown). We reasoned that consumption of
410 milk and exposure to animal sheds may shape the gut microbiome, in particular its
411 richness. Consumption of farm milk (aGMR [95%CI]: 1.20 [1.03; 1.40], *P value*=0.02)
412 and exposure to animal sheds (aGMR [95%CI]: 1.19 [1.01; 1.40], *P value*=0.04) in the
413 first year of life increased gut microbiome richness (Figure 4). In turn, no association
414 was observed for consumption of processed milk (Figure 4). Since both, farm milk
415 consumption and exposure to animal sheds during infancy (4-12 months) showed
416 significant associations with gut microbiome richness at 12 months, we performed a
417 mediation analysis including unexposed and children exposed to both in infancy. The
418 mediation analysis revealed that part (18.4%) of the total protective effect of farm milk
419 consumption and exposure to animal sheds in the first year of life on hay fever was
420 mediated by gut microbiome richness (*P value*=0.03, Figure 5). The number of children

421 only being exposed to animal sheds or farm milk, respectively, was too low to allow
422 separate mediation analyses.

423 **Discussion**

424 In the PASTURE birth cohort, the continuous consumption of farm milk throughout age
425 10.5 years, but neither the only early nor the only late exposure alone was significantly
426 associated with reduced risk of hay fever at age 10.5 years. In contrast, exposure to
427 animal sheds only exerted a trend towards protection early in life. Both exposures, farm
428 milk and animal sheds, early in life increased gut microbiome richness at age 12
429 months, which partly explained the protective effect of these exposures on hay fever.

430 The human gut microbiome composition plays an important role in shaping the
431 development of the immune system (24). There is some evidence that the gut
432 microbiome diversity in the first years of life may protect from atopic sensitization. In the
433 population based CHILD cohort, the Shannon-index at age 3 months was associated
434 with protection from atopic sensitization at 1 year (8). However, in a Swedish study the
435 Shannon-index in early infancy was not associated with allergic rhinoconjunctivitis and
436 SPT at age 7 years (25). Our analyses likewise do not confirm this very early 'window of
437 opportunity' since gut microbiome richness and Shannon-index at age 2 month was
438 unrelated to hay fever development.

439 In contrast, gut microbiome richness at the age of 1 year was inversely associated with
440 hay fever at age 10.5 years. We have previously shown in the PASTURE cohort in
441 agreement with others that the compositional structure of the gut microbiome undergoes
442 very significant changes from early age when most infants are breastfed to age 12
443 months when most foods have been introduced into a child's diet (10, 11).
444 Nevertheless, an inverse association of gut microbiome richness at age 1 year with an

445 outcome much later in life at age 10.5 years may seem surprising. This long-term
446 association may be attributable to an earlier onset of disease. In fact, 4.6%, 5.9% and
447 6.7% of children with data on hay fever at age 10.5 years had already reported
448 symptoms and/or a diagnosis of hay fever at age 4, 5 and 6 years, respectively.

449 Furthermore, early alterations of the composition of the gut microbiome may shape its
450 subsequent development towards an adult-like compositional structure in the first 3
451 years of life (26). Unfortunately, no fecal samples have been collected at later time
452 points in the PASTURE cohort.

453 The production of the SCFAs butyrate and propionate measured at 12 months of age
454 has been reported previously as determinants of protection against atopic sensitization
455 at age 6 years (20). In our study, no relation between the SCFAs butyrate and
456 propionate with hay fever was found. Furthermore, no association with single taxa was
457 seen. Thus, different facets of the early development of the gut microbiome composition
458 may matter for different clinical outcomes.

459 Of the environmental exposures investigated in these analyses, the continuous, but
460 neither the early nor the late, consumption of farm milk was seen to protect from hay
461 fever development. Moreover, a dose-response effect was found corroborating the
462 strength of the observation. Interestingly, this protective effect was partly mediated by
463 gut microbiome richness which may suggest that a continued exposure to unprocessed
464 cow's milk may increase gut microbiome richness beyond the age of 12 months and
465 thereby confer its protective effect.

466 Continuous exposure also implies repeated exposures. The novel concept of trained
467 immunity may lend itself to mechanistic speculations since phenomena like LPS
468 tolerance are based on the necessity of repeated rather than single exposures (27).
469 A potential explanation for the differential effect of unprocessed versus processed cow's
470 milk is grounded in the observation that most farm children drink their milk unboiled. In
471 fact, too few children received only boiled, i.e. heat treated farm milk over the study
472 period to allow meaningful stratified analyses. A number of population-based and
473 experimental studies have stressed the potential importance of heat-treatment of cow's
474 milk for the loss of protective effects (16, 28-31). Whether alterations of the milk
475 microbiome or denaturation and loss of function of milk (whey) proteins underlie these
476 findings awaits further elucidation.

477 Exposure to animal sheds during early years showed an inverse, albeit non-significant
478 effect on hay fever. This is in contrast to previous farm studies showing stronger effects
479 (12, 32). The discrepancy might be attributable to important differences in the definition
480 of exposure to animal sheds used in the PASTURE study, which only assessed
481 exposure to any animal sheds without differentiating between cows, pigs, sheep and
482 horses. The nature of animal exposure may however matter. While exposure to cow
483 sheds showed a significant protective effect on hay fever and asthma (12), sheep sheds
484 and keeping of hares and rabbits were risk factors for wheezing and asthma
485 respectively in the PARSIFAL farm study (33).

486 The main strength of this study is its longitudinal design, which enabled us to assess the
487 exposures at several time points before the assessment of the outcome. Excluding
488 children with parental asthma and/or atopy and who were avoiding milk or milk products

489 showed similar inverse associations with hay fever consequently arguing against
490 confounding by reverse causation. An elevated risk of diarrhea and farm milk
491 consumption at 10.5 years was not observed (data not shown). The results of the
492 present study show protective association of continuous consumption farm milk on hay
493 fever. However, one of the potential caveats of the observation study is finding
494 causality. Hence, the Milk Against Respiratory Tract Infections and Asthma (MARTHA)
495 an ongoing interventional trial is being carried out to evaluate the preventive effect of
496 minimally treated, i.e. only pasteurized and thus microbiologically safe cow's milk on
497 upper respiratory tract infections and allergy (34). Further, the NNT in our study was 7,
498 however, this study is not a randomized placebo-controlled double-blind trial and thus
499 numbers must be taken with some caution. One of the drawbacks of the study is the
500 missing data on hay fever at 10.5 years. However, comparing the baseline
501 characteristics between included and excluded children did not show any significant
502 difference except for maternal age at pregnancy, maternal smoking, parental education,
503 and premature birth. However, adjusting for these variables did not change the results
504 (data not shown). Another drawback is the small number in the "only early" and "only
505 late" exposure groups that shows protective non-statistical significant effect on hay
506 fever. However, using the RMLCA approach our study could identify these small groups
507 manifesting that these types of habits i.e. farm milk consumption or exposure to animal
508 sheds do exist. We performed a posthoc power calculation using SAS and considering
509 $\alpha=0.05$ (two-sided). For our sample size of 650, i.e. in the exposure groups 'continuous
510 consumption of farm milk' and 'no consumption of farm milk' the power of study is over
511 80% assuming the response probabilities ranging from 0.02-0.18 for having hay fever in

512 children who consume farm milk and unadjusted OR of 0.24. Thus, our study was well
513 powered to detect a relatively strong effect of farm milk consumption on hay fever.
514 In summary, the results of the present study demonstrate that continuous exposure of
515 the main determinant, i.e. farm milk consumption but neither only early nor only late
516 exposure alone conferred protection from hay fever development. The early
517 compositional structure of the gut microbiome at age 1 year, but not age 2 month, did
518 however in part mediate this protective effect. One might speculate that continuous
519 consumption of unprocessed cow's milk may also increase gut microbiome richness at
520 later ages, but we do not have data to support this notion. Overall, the findings
521 presented herein do not support the notion of an early-determined trajectory where only
522 early exposures in the first months of life would govern later outcomes. These results
523 emphasize the preventive potential of continuously drinking unprocessed farm milk for
524 hay fever protection. However, the risks associated with raw cow's milk consumption
525 prohibit its recommendation for daily life. The results of the MARTHA trial however will
526 shed light on potential side effects (34). Further clinical trials based on the present
527 results are warranted.

528

529

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625

626 **Figure legends**627 **Figure 1.** Types of exposure classes.

628 Solution for repeated measure latent classes defined by different exposures, which are a)
629 exposure to animal sheds, and b) farm milk consumption in the PASTURE children. Numbers in
630 parentheses indicate the total number of children in each class.

631 **Figure 2.** Associations of farm milk exposure classes with hay fever at age 10.5 years.

632 Associations of farm milk exposure classes with hay fever at age 10.5 years. Models are adjusted
633 for centers, growing up on a farm, and parental atopy. The forest plot represent the adjusted odds
634 ratios (aOR) with 95% confidence intervals [95%CI].

635 **Figure 3.** Association of gut microbiome richness, and Shannon-index at the age of 2 and 12
636 months with hay fever at 10.5 years.

637 Association of gut microbiome richness, and Shannon-index at months 2 (hay fever/total:
638 59/439) and 12 (hay fever/total: 79/633) with hay fever at 10.5 years. Models are adjusted for
639 centers, growing up on a farm, and parental atopy. The association with hay fever is shown as
640 aOR per-interquartile-range of the probability along with 95%CI.

641 **Figure 4.** Association of consumption of farm milk, consumption of processed milk, and
642 exposure to animal sheds in infancy with gut microbiome richness at month 12.

643 Association of consumption of farm milk (N=624), consumption of processed milk (N=624) and
644 exposure to animal sheds (N=617) with richness at 12 months. Models are adjusted for centers,

645 growing up on a farm, and parental atopy. The forest plot represent the adjusted geometric mean
646 ratios with 95%CI.

647 **Figure 5.** Mediation analysis.

648 Mediation analysis of the protective effect of consumption of farm milk and exposure to animal
649 sheds in infancy on hay fever mediated by gut microbiome richness at 12 months adjusting for
650 centers (N=466). The figure shows the direct (β_1), indirect (β_2) and total (β) effects as well as
651 their respective 95% CI from the path model. The proportion of the mediated (indirect) effect
652 was 18.4%.

653 **Table 1:** Description of the study population

Characteristic	All (N=769)	Hay fever (N=99 (12.9%))	No hay fever (N=670 (87.1%))	<i>P value</i>
	N (%) / Total	N (%) / Total	N (%) / Total	
Farm child (yes)	367 (47.7)/768	31 (31.3)/99	336 (50.2)/670	<i>0.0005</i>
Exposure to cats at age of 2 months (yes)	199 (26.0)/767	19 (19.2)/99	180 (27.0)/668	<i>0.11</i>
Exposure to dogs at age of 2 months (yes)	147 (19.2)/766	17 (17.2)/99	130 (19.5)/667	<i>0.68</i>
Maternal age at pregnancy (years) †	31.2±4.5 (N=769)	31.4±4.4 (N=99)	31.2±4.5 (N=670)	<i>0.52</i>
Maternal smoking (yes)	96 (12.5)/766	16 (16.5)/97	80 (12.0)/669	<i>0.25</i>
Second hand smoking (yes)	33 (4.3)/764	3 (3.1)/98	30 (4.5)/666	<i>0.79</i>
Parental education (yes)				<i>0.13</i>
Low	62 (8.1)/764	3 (3.1)/97	59 (8.9)/667	
Medium	280 (36.7)/764	39 (40.2)/97	241 (36.1)/667	
High	422 (56.7)/764	55 (56.7)/97	367 (55.0)/667	
Use of antibiotics during pregnancy (yes)	204 (27.0)/755	26 (26.5)/98	178 (27.1)/657	<i>1.00</i>

Parental atopy (yes)	416 (54.4)/765	72 (73.5)/98	344 (51.6)/667	<0.0001
Mode of delivery (normal)	624 (81.9)/762	82 (83.7)/98	542 (81.6)/664	0.68
Premature birth (yes)	11 (1.4)/769	1 (1.0)/99	10 (1.5)/670	1.00
Birth weight (kg) †	3.4±0.44 (N=605)	3.4±0.5 (N=82)	3.4±0.4 (N=523)	0.81
Breast feeding 2 months (yes)	711 (92.7)/767	90 (90.9)/99	621 (93.0)/668	0.41
Gender (female)	366 (47.7)/768	42 (42.4)/99	324 (48.4)/669	0.28
Having siblings (yes)	494 (64.2)/769	60 (60.6)/99	434 (64.8)/670	0.43
Use of antibiotics during first year of life (weeks) †	0.03±0.3 (N=746)	0.01±0.1 (N=97)	0.03±0.4 (N=649)	0.86
Doctor's diagnosis of hay fever (yes)	36 (4.7)/769	36 (36.4)/99	NA	NA
Inhalant sensitization (IgE≥0.7 kU/L or SPT≥3mm) at 10.5 years	259 (49.6)/522	66 (86.8)/76*	193 (43.3)/446*	<0.0001
Concomitants				
Asthma (yes)	69 (9.0)/764	28 (28.9)/97	41 (6.2)/667	<0.0001
Eczema (yes)	100 (13.1)/763	36 (36.7)/98	64 (9.6)/665	<0.0001
Food allergy (yes)	41 (5.5)/746	21 (21.7)/97	20 (3.1)/649	<0.0001

654

655 The categorical variables are presented as frequency (percentage) and the continuous variables as mean †: mean±standard deviation. The test for differences

656 between the groups are χ^2 or Fischer's Exact test for categorical variables and Mann Whitney U test for continuous variables.

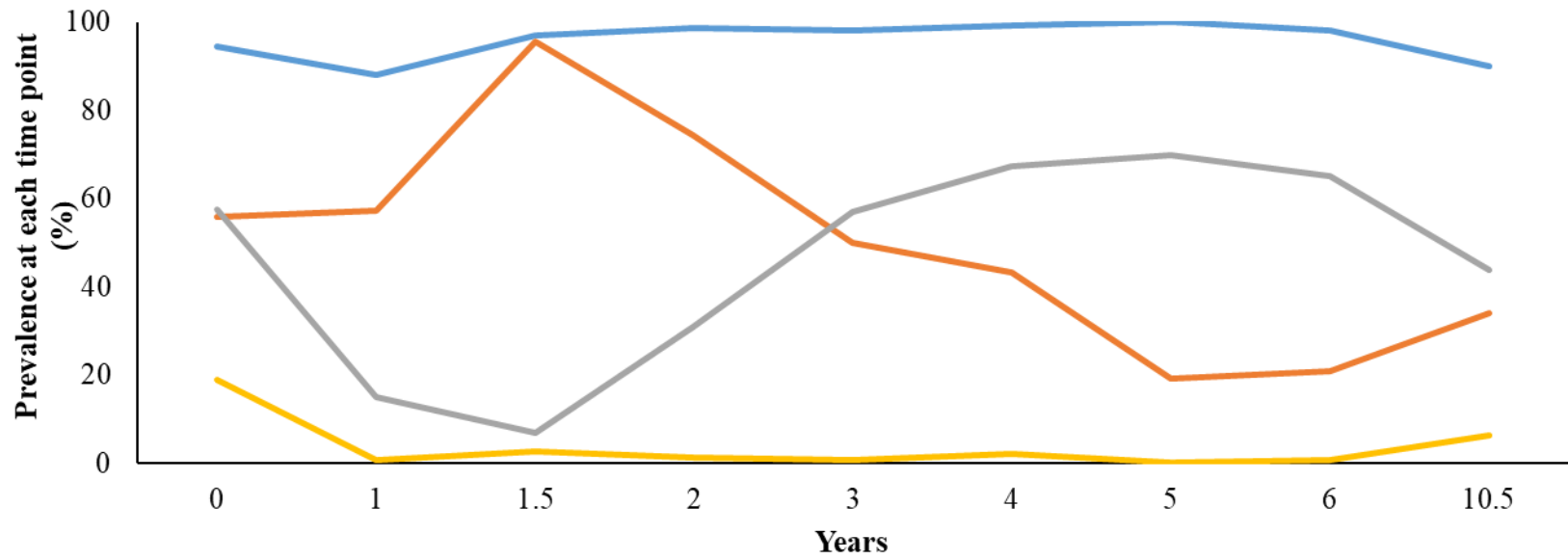
657 Farm child was defined as “Children of mothers living on family-run livestock farms were assigned to the farm group. The non-farm group included children of
658 mothers from the same rural areas but not living on a farm”. Exposure to pets at the age of 2 months (cats and dogs) was defined by asking “if you have cats?”,
659 “if you have dogs?” and “if they stay indoors in the house?”. Maternal smoking during pregnancy was defined using the following questions “Have you in your
660 life smoked more than 5 packs of cigarettes?” Or “Have you quit smoking in the meantime?” and if yes “Was it during this pregnancy?”. Smoking by father,
661 “Have you in your life smoked more than 5 packs of cigarettes?” Or “Do you still smoke?”. Second hand smoking “How many cigarettes are on average per day
662 were smoked in your house by other people?” If greater than 1 then second hand smoking was defined as 1 else 0. Parental education was defined as low (less
663 than 10 years), medium (10 years) and high (greater than 10 years). Parental atopy was defined as doctor’s diagnosis of hay fever, atopic dermatitis, or asthma
664 ever in mother or father. Use of antibiotics during pregnancy was defined by asking “Have you taken antibiotics since the beginning of pregnancy?” Or “Have
665 you taken any antibiotics during this pregnancy?”. Child was defined as premature if the child was born before the completion of 37 weeks of pregnancy. Use of
666 antibiotics by a child during first year of life was defined as “Total No. of weeks with antibiotics ingested”. Breastfeeding at the age of 2 months (yes or no) was
667 defined by asking “if you have ever breastfed?”. SPT: skin prick test. Inhalant sensitization was defined as at least one IgE specific to alder, birch, hazel,
668 plantain, mugwort, alternaria, grass, rye, *Dermatophagoides pteronyssinus*, *Dermatophagoides farina*, cat, dog, or horse at levels $\geq 0.7 \text{IUml}^{-1}$ or SPT (birch,
669 grass, alternaria, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, or dog) $\geq 3 \text{mm}$. Serum specific IgE and SPT was not measured in the
670 Austrian study center, hence only sub-sample N=522 was included.. Asthma was defined as a physician’s diagnosis of asthma or recurrent obstructive bronchitis
671 established until 10.5 years. Eczema and food allergy were defined as physician diagnoses at least once until the age of 10.5 years. NA: not applicable.

672

673

Figure 1.

a)



- Continuous exposure to animal sheds (N (%)=420 (44))
- Only early exposure to animal sheds (N (%)=74 (7.8))
- Only late exposure to animal sheds (N (%)=87 (9.1))
- No exposure to animal sheds (N (%)=373 (39.1))

b)

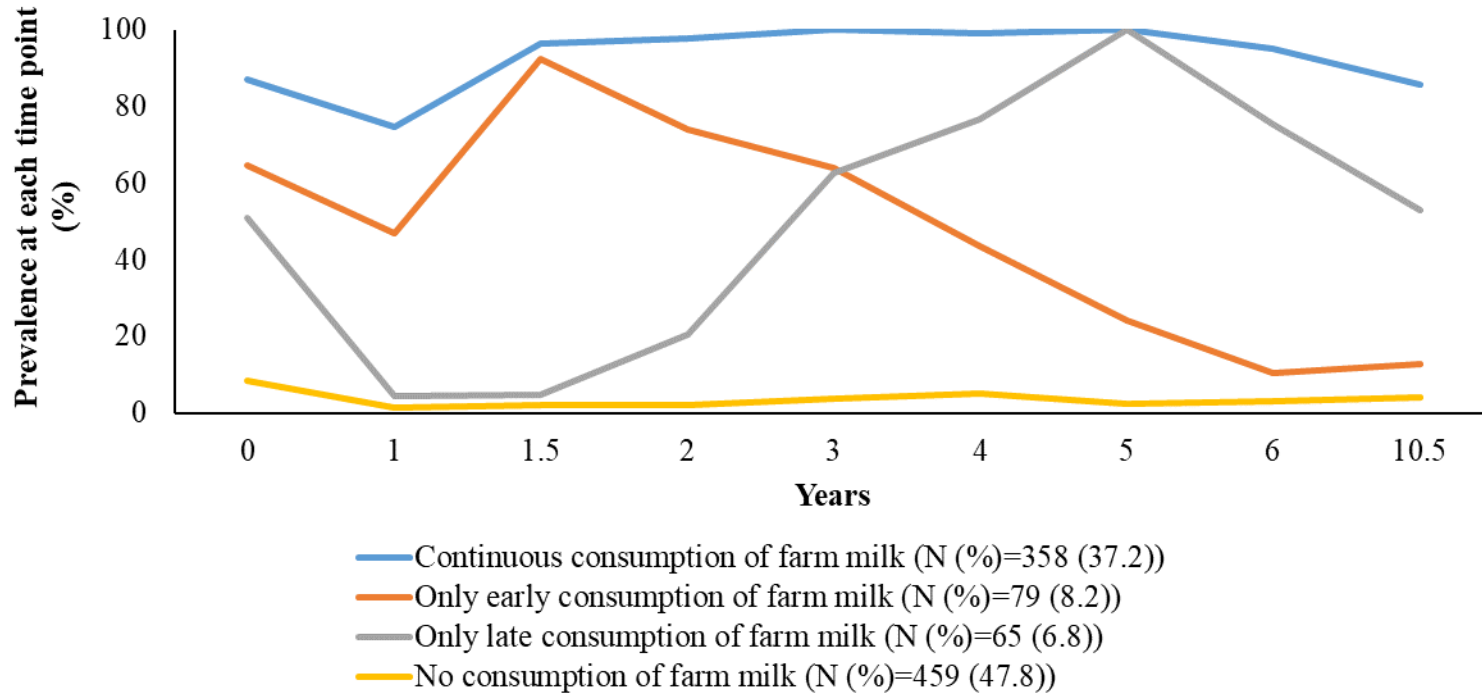


Figure 2

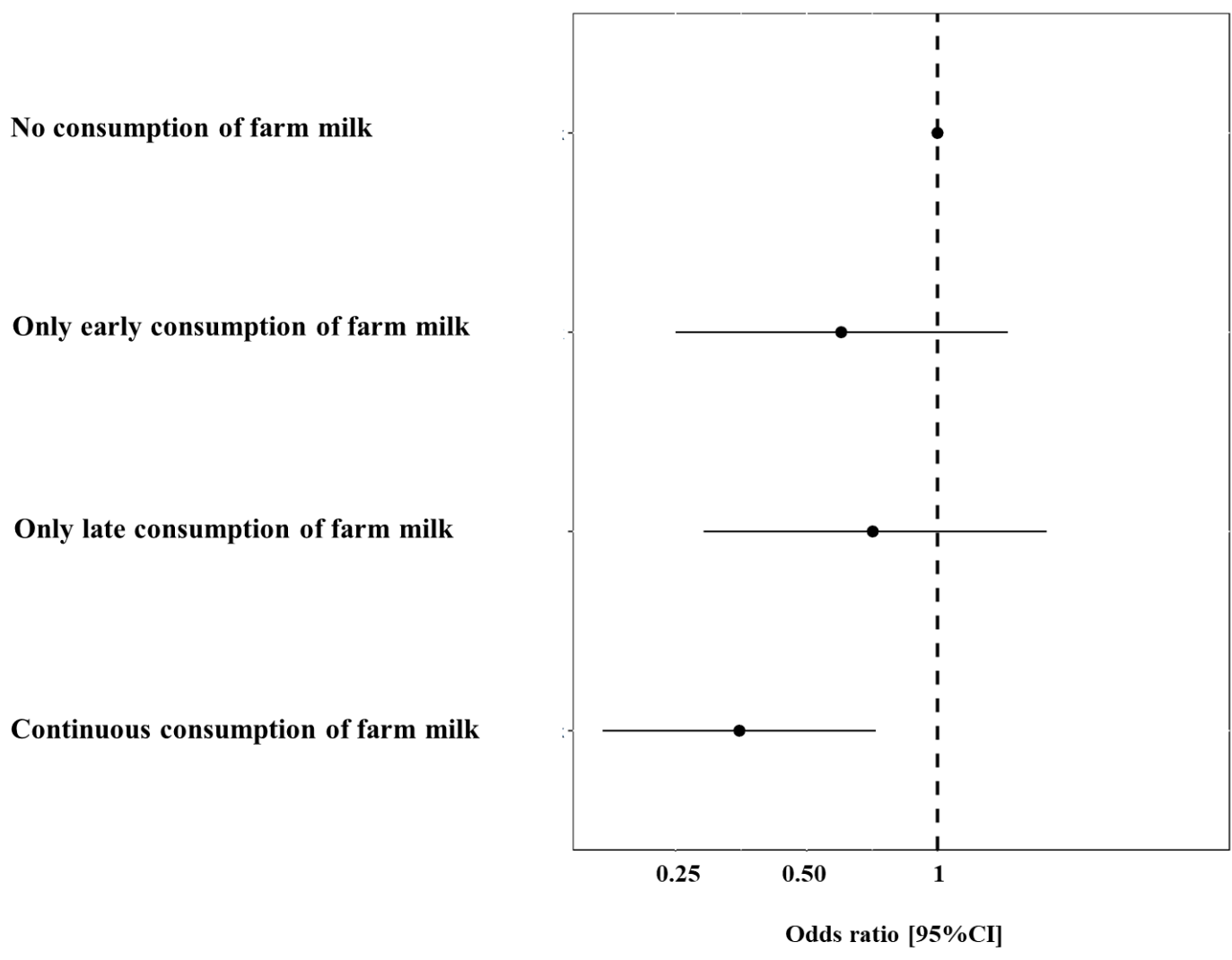


Figure 3

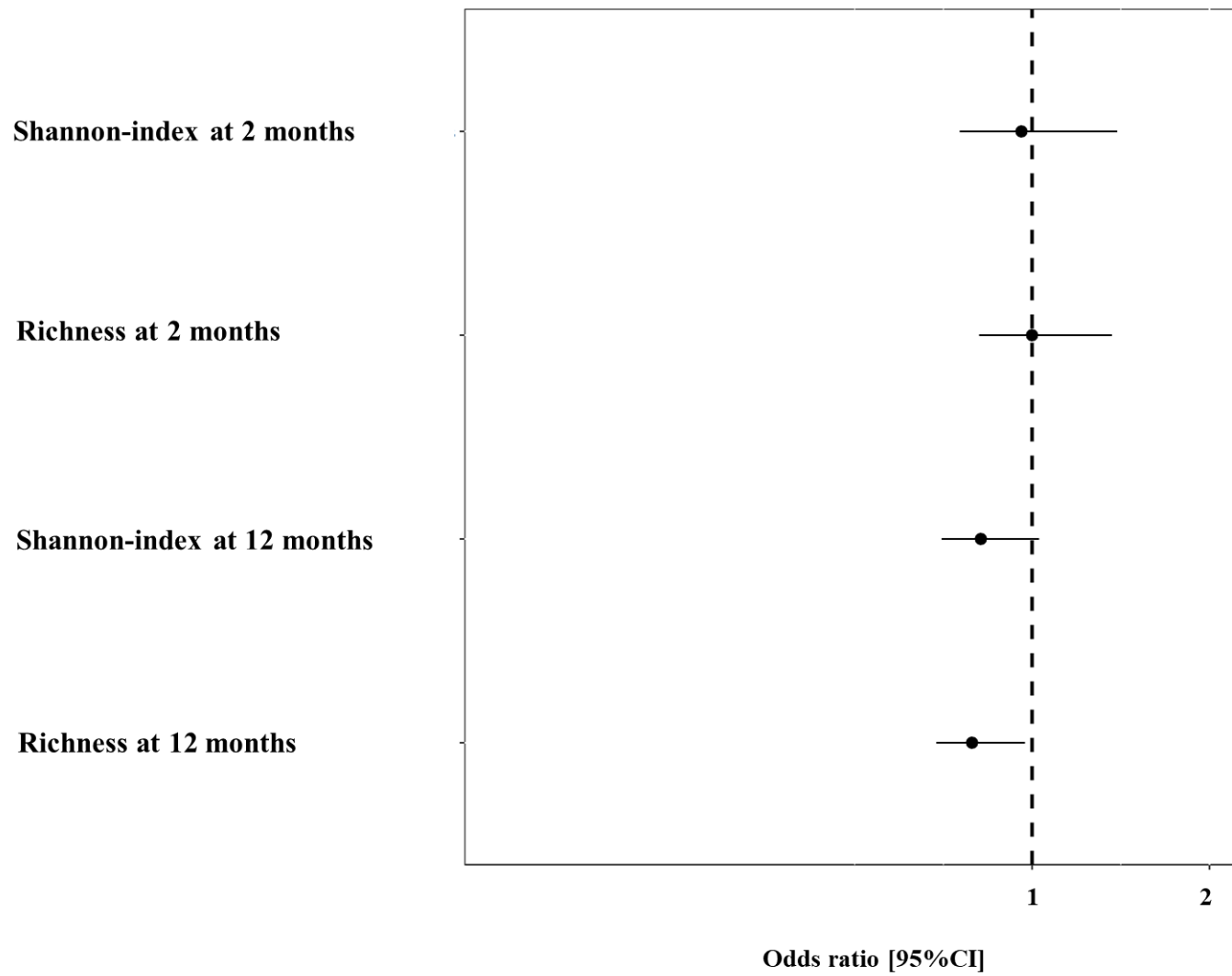


Figure 4.

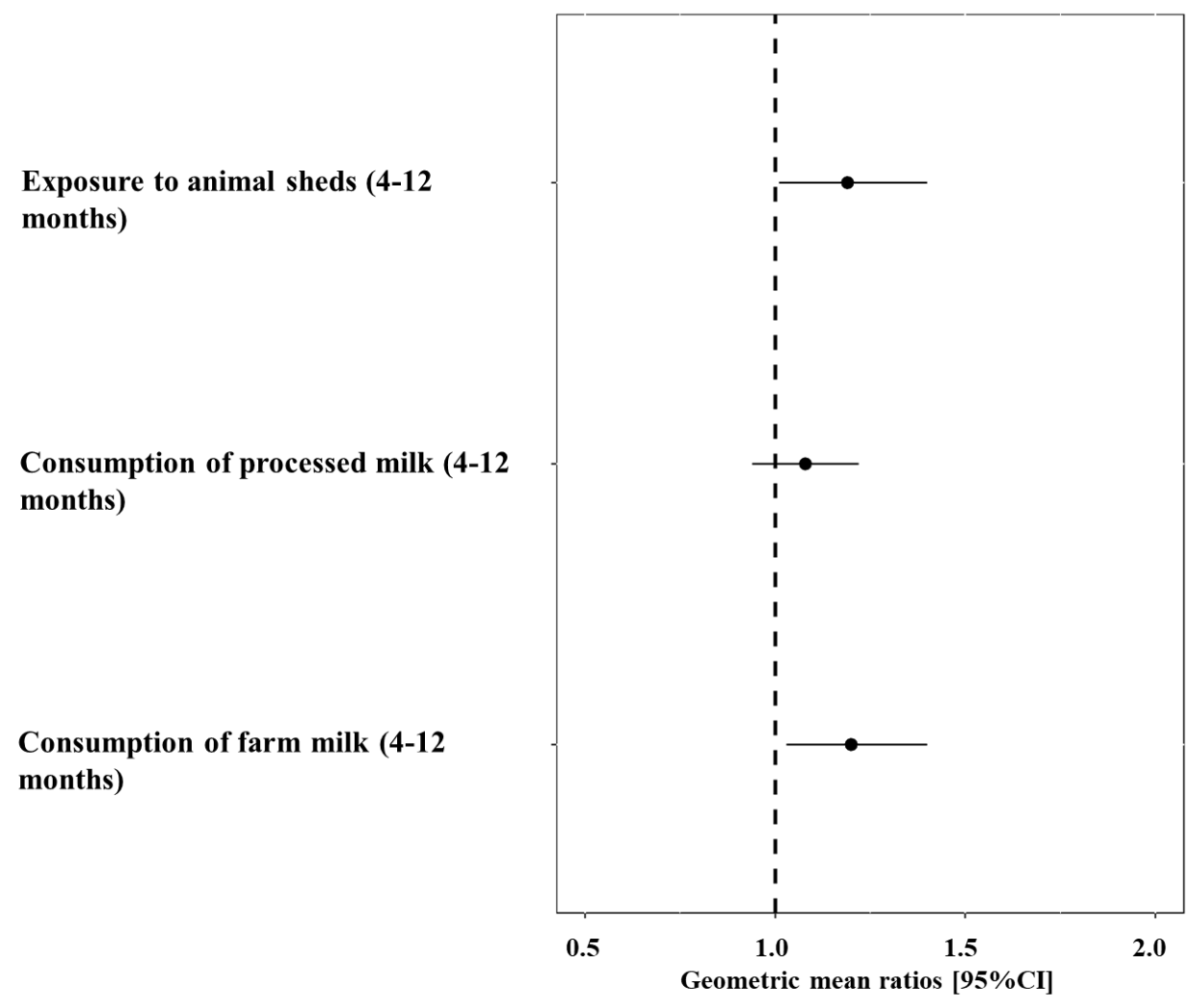
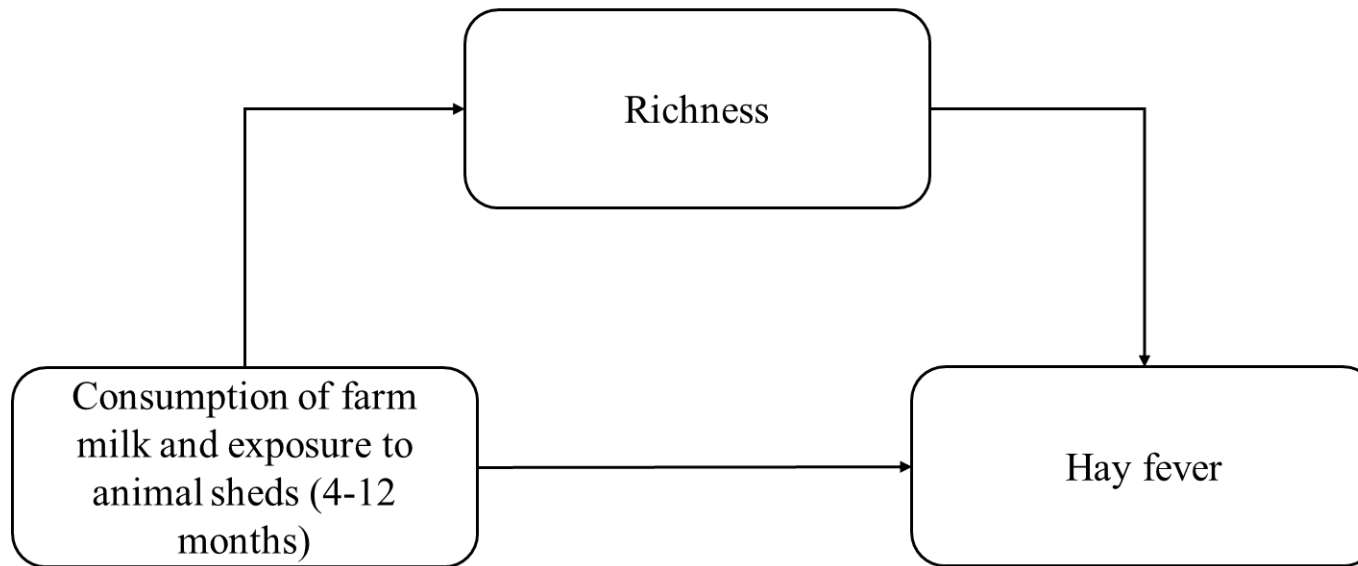


Figure 5.

Total effect, β [95%CI]= -0.98 [-1.88; -0.08]; *P value*=0.03

Indirect effect, β_2 [95%CI]= -0.18 [-0.36; -0.004]; *P value*=0.03



Direct effect, β_1 [95%CI]=-0.80 [-1.70; 0.10]; *P value*=0.08

1 **Online Repository Text**

2 **Continuous rather than solely early farm exposure protect from hay fever development.**

3 Sonali Pechlivanis Ph.D.¹, Martin Depner Ph.D.¹, Pirkka V. Kirjavainen Ph.D.^{2,3}, Caroline
4 Roduit M.D.^{4,5,6}, Martin Täubel Ph.D.², Remo Frei Ph.D.^{4,7}, Chrysanthi Skevaki M.D.^{8,9},
5 Alexander Hose M.A. M.P.H.¹⁰, Cindy Barnig^{11,12}, Elisabeth Schmausser-Hechfellner B.Sc.¹,
6 Markus J. Ege M.D.^{1,9,10}, Bianca Schaub M.D.^{9,10}, Amandine Divaret-Chauveau M.D.^{13,14,15},
7 Roger Lauener M.D.^{4,6}, Anne M. Karvonen Ph.D.², Juha Pekkanen M.D. Ph.D.^{2,16}, Josef
8 Riedler M.D. Ph.D.¹⁷, Sabina Illi Ph.D.¹, Erika von Mutius M.D. M.Sc.^{1,9,10} and the PASTURE
9 Study Group*

10 ¹Institute of Asthma and Allergy Prevention, Helmholtz Zentrum München, German Research
11 Center for Environmental Health, Neuherberg, Germany

12 ²Department of Health Security, Finnish Institute for Health and Welfare, Kuopio, Finland

13 ³Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland

14 ⁴Christine Kühne Center for Allergy Research and Education (CK-CARE), Davos, Switzerland

15 ⁵Children's Hospital, University of Zürich, Zürich, Switzerland

16 ⁶Childrens Hospital of Eastern Switzerland, St. Gallen, Switzerland

17 ⁷Division of Respiratory Medicine, Department of Paediatrics, Inselspital, University of Bern,
18 Bern, Switzerland

19 ⁸Institute of Laboratory Medicine, Universities of Giessen and Marburg Lung Center (UGMLC),
20 Philipps University Marburg, Marburg, Germany

21 ⁹Member of the German Center for Lung Research, DZL, Germany

22 ¹⁰Dr. von Hauner Childrens Hospital, Ludwig Maximilians University Munich, Munich,
23 Germany

24 ¹¹INSERM, EFS BFC, LabEx LipSTIC, UMR1098, Interactions Hôte-Greffon-
25 Tumeur/Ingénierie Cellulaire et Génique, Univ. Bourgogne Franche-Comté, Besançon, France.

26 ¹²Department of Chest Disease, University Hospital of Besançon, Besançon, France.

27 ¹³Pediatric Allergy Department, Children's Hospital, University Hospital of Nancy, Vandoeuvre
28 les Nancy, France

29 ¹⁴UMR6249 Chrono-environment, University of Bourgogne Franche-Comté, France

30 ¹⁵EA3450 DevAH, Faculty of Medicine, University of Lorraine, Vandoeuvre les Nancy, France

31 ¹⁶Department of Public Health, University of Helsinki, Helsinki, Finland

32 ¹⁷Children's Hospital Schwarzach, Schwarzach, Austria

33 * The members of the PASTURE study group are Johanna Theodorou (Dr. von Hauner
34 Children's Hospital, Ludwig Maximilians University Munich, Munich, Germany; Member of the
35 German Center for Lung Research, DZL, Germany), Andreas Böck (Dr. von Hauner Children's
36 Hospital, Ludwig Maximilians University Munich, Munich, Germany), Harald Renz (Institute of
37 Laboratory Medicine, Philipps University of Marburg, Marburg, Germany; Department of

38 Clinical Immunology and Allergology, Laboratory of Immunopathology, Sechenov University,
39 Moscow, Russia), Petra I. Pfefferle (Comprehensive Biobank Marburg CBBM, Fachbereich
40 Medizin der Philipps Universität Marburg, Marburg, Germany), Jon Genuneit (Pediatric
41 Epidemiology, Medical Faculty, Leipzig University, Germany), Michael Kabesch (Department
42 of Pediatric Pneumology and Allergy, University Children's Hospital Regensburg (KUNO) at the
43 Hospital St. Hedwig of the Order of St. John, University of Regensburg, Regensburg, Germany),
44 Marjut Roponen (Department of Environmental and Biological Sciences, University of Eastern
45 Finland, Kuopio, Finland), and Lucie Laurent (University of Besançon, Department of
46 Respiratory Disease, UMR/CNRS6249 Chrono-environment, University Hospital, Besançon,
47 France).

48

49 Corresponding author:

50 Sonali Pechlivanis, PD Dr.

51 Helmholtz Zentrum München

52 German Research Center for Environmental Health

53 Institute of Asthma and Allergy Prevention

54 Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany

55 Telephone: +49 89 3187-43783, Fax: +49 89 4400-54452

56 sonali.pechlivanis@helmholtz-muenchen.de

57

58 Methods:

59 *Questionnaires:*

60 Information were collected through mothers using questionnaires in interviews or self-
61 administered questionnaires within the third trimester of pregnancy and when the children were
62 2, 12, 18 months of age and then at the age of 2, 3, 4, 5, 6, and 10.5 years. Using weekly and
63 monthly diaries and questionnaires from the 8th to 53rd weeks of age, additional information on
64 child's health, nutrition and farm-related exposures were collected (E1, E2).

65 *Definitions of outcome:*

66 Inhalant sensitization at 10.5 years was defined as at least one IgE specific to alder, birch, hazel,
67 plantain, mugwort, alternaria, grass, rye, *Dermatophagoides pteronyssinus*, *Dermatophagoides*
68 *farina*, cat, dog, or horse at levels $\geq 0.7 \text{ IU ml}^{-1}$ or SPT (birch, grass, alternaria, *Dermatophagoides*
69 *pteronyssinus*, *Dermatophagoides farinae*, cat, or dog) $\geq 3 \text{ mm}$. Serum specific IgE and SPT was
70 not measured in the Austrian study center. Serum specific IgE was assessed using the
71 semiquantitative Allergy Screen test panel for atopy (Mediwiss Analytic, Moers; Germany) (E3).
72 As described before, SPTs were performed on the anterior part of the forearm using a
73 Stallerpoint® (Stallergenes, Antony, France) (E4). Incident hay fever at 10.5 years (N=48) was
74 defined by parent reported symptoms (itchy, runny, or blocked nose without a cold accompanied
75 by red itchy eyes) and/or a physician's diagnosis of hay fever in the last 12 months using
76 questionnaires at age 10.5 years and excluding those having hay fever before the age of 10.5
77 years.

78

79 *Assessment of exposures:*

80 Socioeconomic and lifestyle factors, farm-related exposures, health status of women, their
81 husbands and their children were assessed through questionnaires in interviews or self-
82 administered questionnaires to the mothers within the third trimester of pregnancy and when the
83 children were 2, 12, 18 months of age and then at the age of 2, 3, 4, 5, 6, and 10.5 years.
84 Maternal smoking during pregnancy was defined using the following questions “Have you in
85 your life smoked more than 5 packs of cigarettes?” Or “Have you quit smoking in the
86 meantime?” and if yes “Was it during this pregnancy?”. Smoking by father, “Have you in your
87 life smoked more than 5 packs of cigarettes?” Or “Do you still smoke?”. Second hand smoking
88 was defined by asking “How many cigarettes are on average per day were smoked in your house
89 by other people?” If greater than one then second hand smoking was defined as 1 else 0. Parental
90 education was defined as low (less than 10 years), medium (10 years) and high (greater than 10
91 years). Parental atopy (yes or no) was defined as doctor’s diagnosis of hay fever, atopic
92 dermatitis, or asthma ever in mother or father. Use of antibiotics during pregnancy was defined
93 by asking “Have you taken antibiotics since the beginning of pregnancy?” Or “Have you taken
94 any antibiotics during this pregnancy?”. Child was defined as premature if the child was born
95 before the completion of 37 weeks of pregnancy. Use of antibiotics by a child during first year of
96 life was defined as “Total number of weeks with antibiotics ingested”. Further, breastfeeding at
97 age of 2 months (yes or no) was defined by asking “if you have ever breastfed?”, exposure to
98 pets at age of 2 months (cats and dogs) was defined by asking “if you have cats?”, “if you have
99 dogs?” and “if they stay indoors in the house?”, and data on having siblings (yes or no) were also
100 collected. Further, asthma was defined as a physician’s diagnosis of asthma or recurrent

101 obstructive bronchitis established until 10.5 years. Eczema and food allergy were defined as
102 physician diagnoses at least once until the age of 10.5 years.

103 Additionally, the frequency of farm milk consumption at each time point by a child (daily, 1-6
104 times a week, less than once a week or no consumption) was further weighted as follows: weight
105 of 3 was assigned for daily consumption, a weight of 2 for 1-6 times a week, a weight of 1 for
106 consumption less than once a week and 0 for no consumption). The weights over the years were
107 then summed up as farm milk consumption score representing the frequency of farm milk
108 consumed. Since data on frequency of processed milk consumption was available only at age
109 10.5 years, instead of constructing a score it was categorized as daily, 1-6 times a week and no
110 (less than once a week or no) consumption of processed milk.

111 *DNA extraction from fecal samples and sequencing analyses:*

112 Briefly, the fecal samples were frozen within 10 minutes of collection, and stored at -20°C until
113 further processing. Targeted DNA amplifications using primers targeting the V4 region of the
114 16S rRNA gene were performed. The amplicon sequencing was done on Illumina MiSeq
115 instrument producing 250-bp paired end sequences as described previously (E5). Sequencing
116 processing was done using QIIME2-2018.6 (Quantitative Insights Into Microbial Ecology) and
117 reads were denoised using DADA2 (E6, E7). Samples were rarefied at the minimum sequence
118 numbers 1,029. Rarefaction and calculation of richness and Shannon-index was iterated 1,000
119 times and the resulting measures of α -diversity were then averaged (E5). As described
120 previously, SCFA levels were modeled by the relative abundance of bacterial genera in children
121 with available SCFA measurements using the “predict” function of R-package ranger (E5).
122 These prediction models were then applied to predict SCFA production scores (butyrate and
123 propionate) in the entire population.

124 Statistical analyses:

125 *Repeated measure latent classes (exposure classes)*

126 Using farm milk consumption and processed milk consumption exposures together, 3 types of
127 farm and processed milk exposure classes were identified: i) ‘high farm and low processed milk’,
128 ii) ‘mixed consumption of farm and processed milk’, and iii) ‘low farm and high processed milk’
129 (Figure E2(a)). The children were allocated to specific exposure classes by their highest posterior
130 probabilities. The optimal number of exposure classes was then determined according to the
131 Bayesian Information Criterion. Further, the labelling of the exposure classes was based on main
132 features of each class. The analyses were done on children having data at least at 7 of the 8
133 assessed time points for the combined farm and processed milk consumption.

134 The associations between hay fever and farm and shop milk consumption exposure classes was
135 assessed by logistic regression. The above model was adjusted for centers and confounders,
136 (growing up on a farm, and parental asthma and/or atopy). We tested the differences in relative
137 abundance of most common single bacterial genera at 2 and 12 months with hay fever by
138 Wilcoxon test, main associations ($p < 0.05$) were then confirmed in logistic regression models
139 using center-log-ratio-transformed variables. Gut microbiome richness and Shannon-index at 2
140 and 12 months were transformed by dividing the original variable by their respective
141 interquartile range (IQR: IQRrichness_2m: 8.07, IQRShannon-index_2m: 0.75,
142 IQRrichness_12m: 15.9 and IQRShannon-index_12m: 0.75) and the new variables were then
143 included in the regression models (logistic regression to test the association with outcome hay
144 fever at 10.5 years and linear regression to test the associations between consumption of farm

145 milk, consumption of processed milk and exposure to animal sheds in infancy). The association
146 with hay fever is then represented as adjusted odds ratio per IQR of the probability.

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154 sensitization in the first year of life. *J Allergy Clin Immunol.* 2013;131(3):781-8.
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- 161 E6. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME
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- 163 E7. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-
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165 **Figure E1.** Proportion of farm milk consumption and exposure to animal sheds over time in the
166 PASTURE children with data on hay fever at 10.5 years (N=769).

167 **Figure E2.** Association of farm milk consumption exposure classes with the stringent definition
168 of hay fever

169 Models are adjusted for centers, growing up on a farm, and parental atopy. The forest plot
170 represent the aOR with 95% confidence intervals [95% CI].

171 **Figure E3.** Farm and processed milk consumption exposure classes

172 a) Solution for repeated measure latent classes defined by farm and processed milk consumption
173 in the PASTURE children. Numbers in parentheses indicate the total number of children in each
174 class. b) Association of farm and processed milk consumption exposure classes with hay fever.

175 Models are adjusted for centers, growing up on a farm, and parental atopy. The forest plot
176 represent the aOR with 95% CI.

177 **Figure E4.** Association of the frequency of processed milk consumption at the age of 10.5 years
178 with hay fever at 10.5 years in the PASTURE children.

179 Model is adjusted for centers, growing up on a farm, and parental atopy. The forest plot represent
180 the aOR with 95% CI.

181

182 **Table E1.** Description of the included and excluded study population

Characteristic	Included in the study (N=769)	Excluded in the study (N=364)	<i>P value</i>
	N (%) / Total	N (%) / Total	
Farm child (yes)	367 (47.7)/769	164 (45.0)/364	0.41
Exposure to cats at age of 2 months (yes)	199 (26.0)/767	82 (25.9)/323	0.88
Exposure to dogs at age of 2 months (yes)	147 (19.2)/766	48(14.9)/322	0.10
Maternal age at pregnancy (years) †	31.3±4.5 (N=769)	30.2±5.0 (N=364)	<0.003
Maternal smoking (yes)	96 (12.5)/766	62 (17.0)/363	0.04
Second hand smoking (yes)	33 (4.3)/764	16 (5.0)/322	0.63
Parental education (yes)			
Low	62 (8.1)/764	63 (18.1)/349	
Medium	280 (36.7)/764	146 (41.8)/349	
High	422 (55.2)/764	140 (40.1)/349	<0.001
Use of antibiotics during pregnancy (yes)	204 (27.0)/755	85 (26.1)/326	0.77
Parental atopy (yes)	416 (54.4)/765	176 (52.7)/334	0.60

Mode of delivery (normal)	624 (81.9)/762	267 (83.4)/320	0.66
Premature birth (yes)	11 (1.4)/769	50 (13.7)/364	<0.0001
Birth weight (kg) †	3.4±0.4 (N=605)	3.4±0.44 (N=239)	0.31
Breast feeding 2 months (yes)	711 (92.7)/767	294 (91.0)/323	0.27
Gender (female)	366 (47.7)/768	166 (50.9)/326	0.36
Having siblings (yes)	494 (64.2)/769	227 (62.3)/364	0.51
Use of antibiotics during first year of life (weeks) †	0.03±0.3 (N=746)	0.02±0.1 (N=286)	0.50

183 The categorical variables are presented as frequency (percentage) and the continuous variables as †: mean±standard deviation. The test for differences between the
184 groups are χ^2 test for categorical variables and Mann Whitney U test for continuous variables. Farm child was defined as “Children of mothers living on family-
185 run livestock farms were assigned to the farm group. The non-farm group included children of mothers from the same rural areas but not living on a farm”. Exposure
186 to pets at the age of 2 months (cats and dogs) was defined by asking “if you have cats?”, “if you have dogs?” and “if they stay indoors in the house?”. Maternal
187 smoking during pregnancy was defined using the following questions “Have you in your life smoked more than 5 packs of cigarettes?” Or “Have you quit smoking
188 in the meantime?” and if yes “Was it during this pregnancy?”. Smoking by father, “Have you in your life smoked more than 5 packs of cigarettes?” Or “Do you
189 still smoke?”. Second hand smoking “How many cigarettes are on average per day were smoked in your house by other people?” If greater than 1 then second hand
190 smoking was defined as 1 else 0. Parental education was defined as low (less than 10 years), medium (10 years) and high (greater than 10 years). Parental atopy
191 was defined as doctor’s diagnosis of hay fever, atopic dermatitis, or asthma ever in mother or father. Use of antibiotics during pregnancy was defined by asking
192 “Have you taken antibiotics since the beginning of pregnancy?” Or “Have you taken any antibiotics during this pregnancy?”. Child was defined as premature if the
193 child was born before the completion of 37 weeks of pregnancy. Use of antibiotics by a child during first year of life was defined as “Total No. of weeks with
194 antibiotics ingested”. Breastfeeding at the age of 2 months (yes or no) was defined by asking “if you have ever breastfed?”. Asthma was defined as a physician’s
195 diagnosis of asthma or recurrent obstructive bronchitis established until 10.5 years. Eczema and food allergy were defined as physician diagnoses at least once until
196 the age of 10.5 years. NA: not applicable.

197 **Table E2.** Association between animal sheds exposure classes with hay fever at 10.5 years.

	Hay fever/Total	OR [95% CI], <i>P</i> value
Model 1		
No exposure to animal sheds	56 (18.0)/312	1
Only early exposure to animal sheds	2 (3.9)/52	0.26 [0.06; 1.15], 0.08
Only late exposure to animal sheds	9 (12.5)/72	0.88 [0.40; 1.96], 0.76
Continuous exposure to animal sheds	31 (9.4)/329	1.14 [0.50; 2.64], 0.75
Model 2		
No exposure to animal sheds	56 (18.0)/312	1
Only early exposure to animal sheds	2 (3.9)/52	0.32 [0.07; 1.44], 0.14
Only late exposure to animal sheds	9 (12.5)/72	1.04 [0.46; 2.36], 0.92
Continuous exposure to animal sheds	31 (9.4)/329	1.94 [0.79; 4.74], 0.15

198 Model 1: adjusted for centers, growing up on a farm, and parental atopy. Model 2: adjusted for centers, farm milk consumption exposure classes, and parental
 199 atopy. The number of children included in the analyses are different to that shown in Figure 1(a) due to the missing values of hay fever at year 10.5 years.

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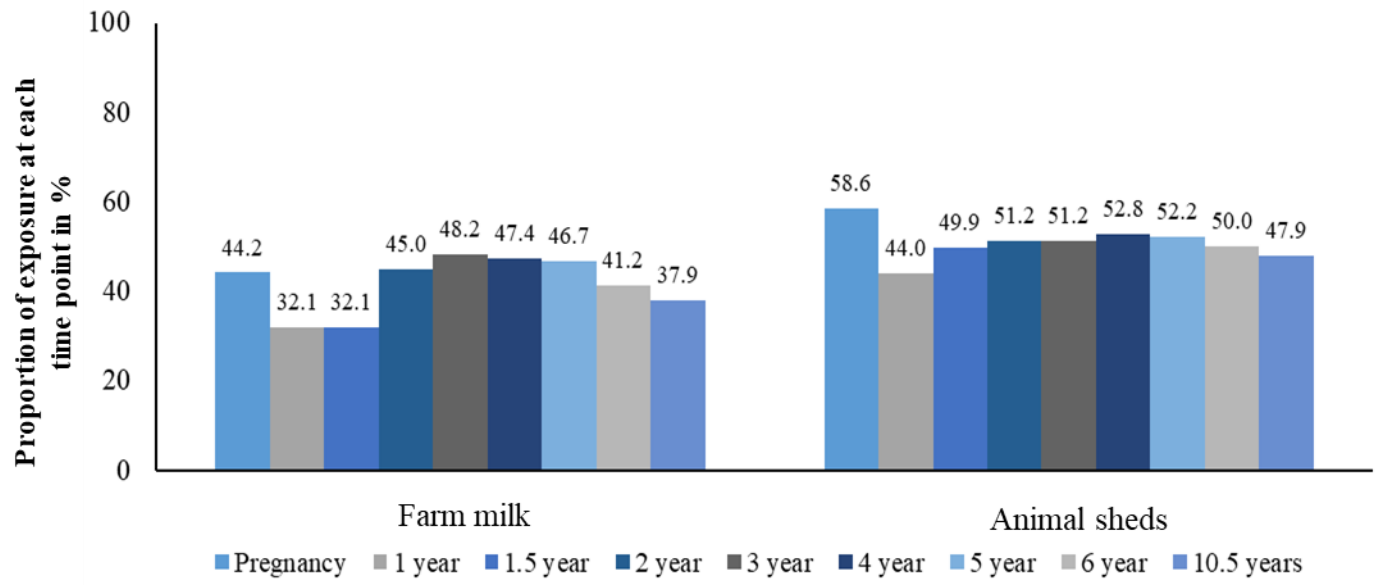
202

203 **Table E3.** Proportion of children in each exposure classes.

Exposure classes	Hay fever (%)/Total
Farm milk	
No consumption of farm milk	70 (18.9)/371
Only early consumption of farm milk	7 (10.9)/64
Only late consumption of farm milk	6 (11.8)/51
Continuous consumption of farm milk	15 (5.4)/279
Farm milk and processed milk	
Low farm milk and high processed milk	49 (16.2)/302
Mixed consumption of farm milk and processed milk	9 (7.1)/127
High farm milk and low processed milk	7 (4.3)/162

204 Numbers in parentheses indicate percent of children with hay fever in each exposure class. The number of children included in the analyses are different to that
 205 shown in Figure 1(b) and Figure E3(a) due to the missing values of hay fever at year 10.5 years.

Figure E1.



No consumption of farm milk

Only early consumption of farm milk

Only late consumption of farm milk

Continuous consumption of farm milk

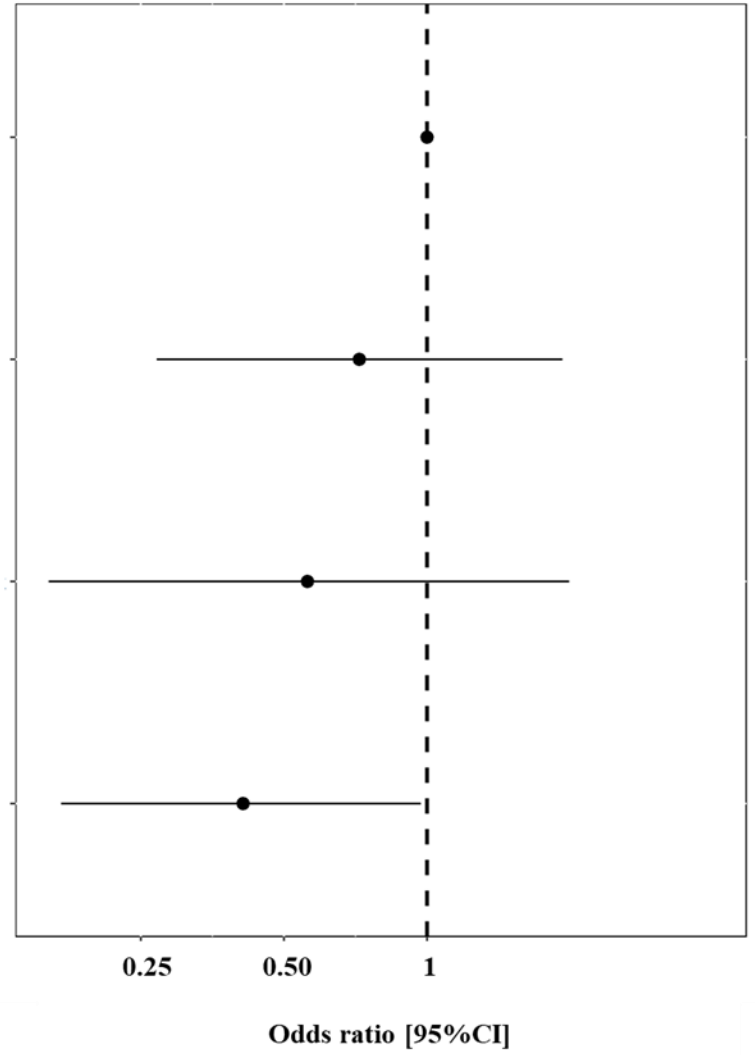
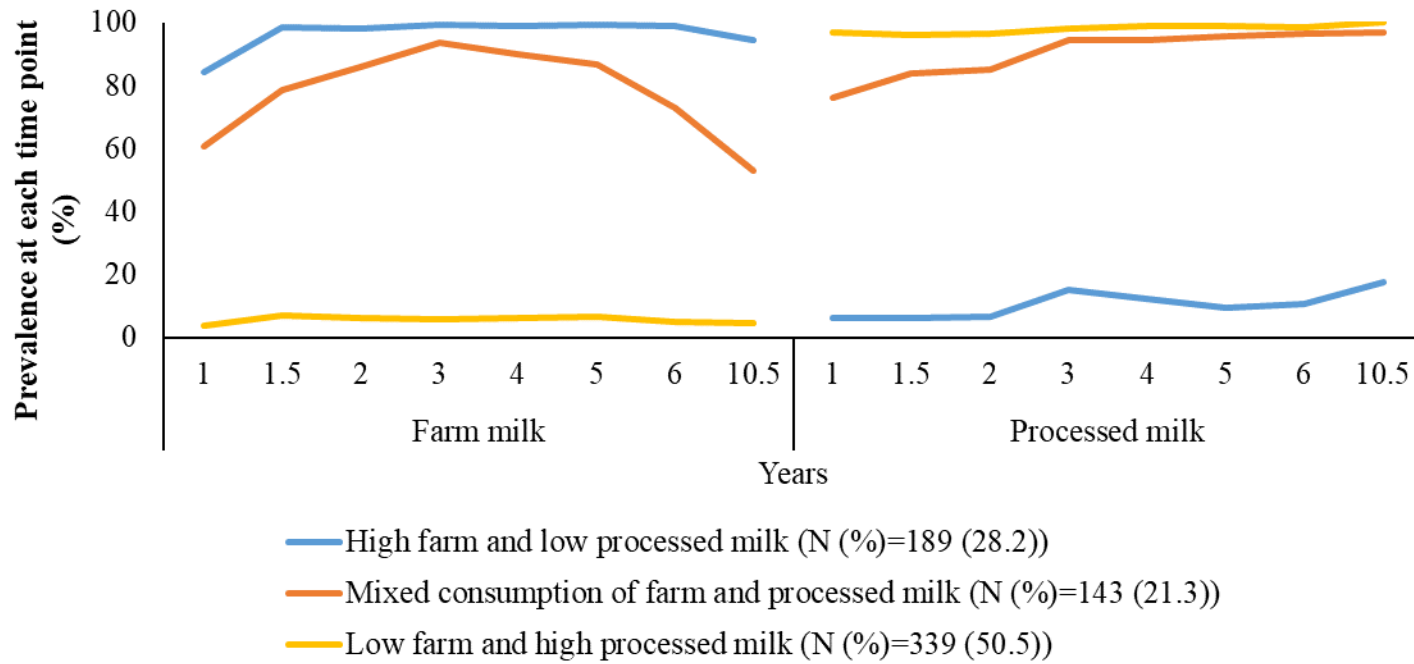


Figure E3.**(a)**

(b)

Low farm and high processed milk

Mixed consumption of farm and processed milk

High farm and low processed milk

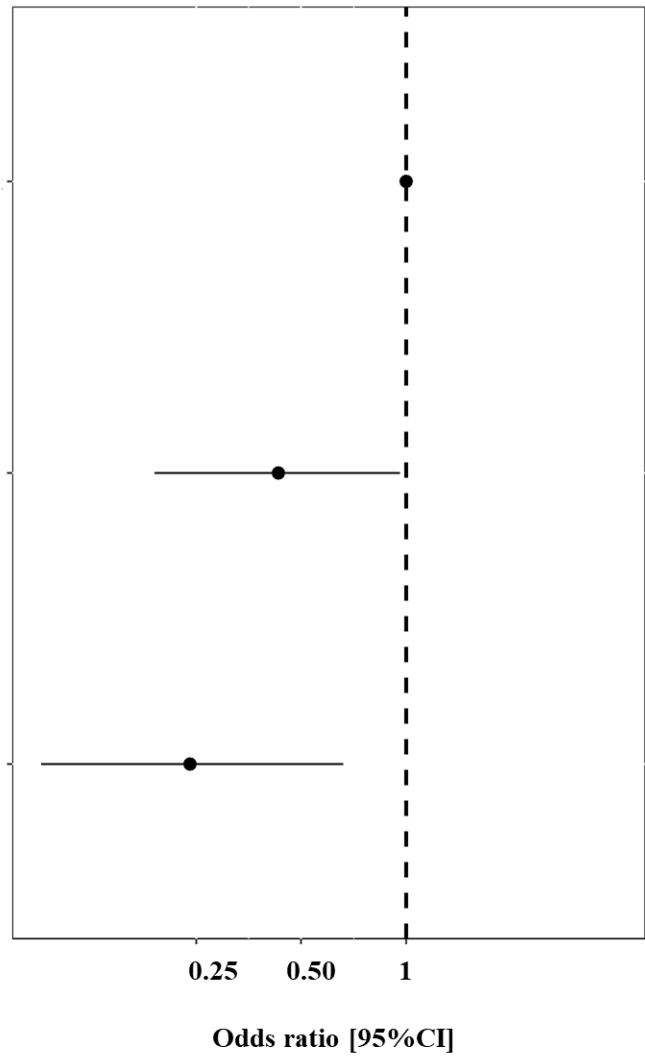
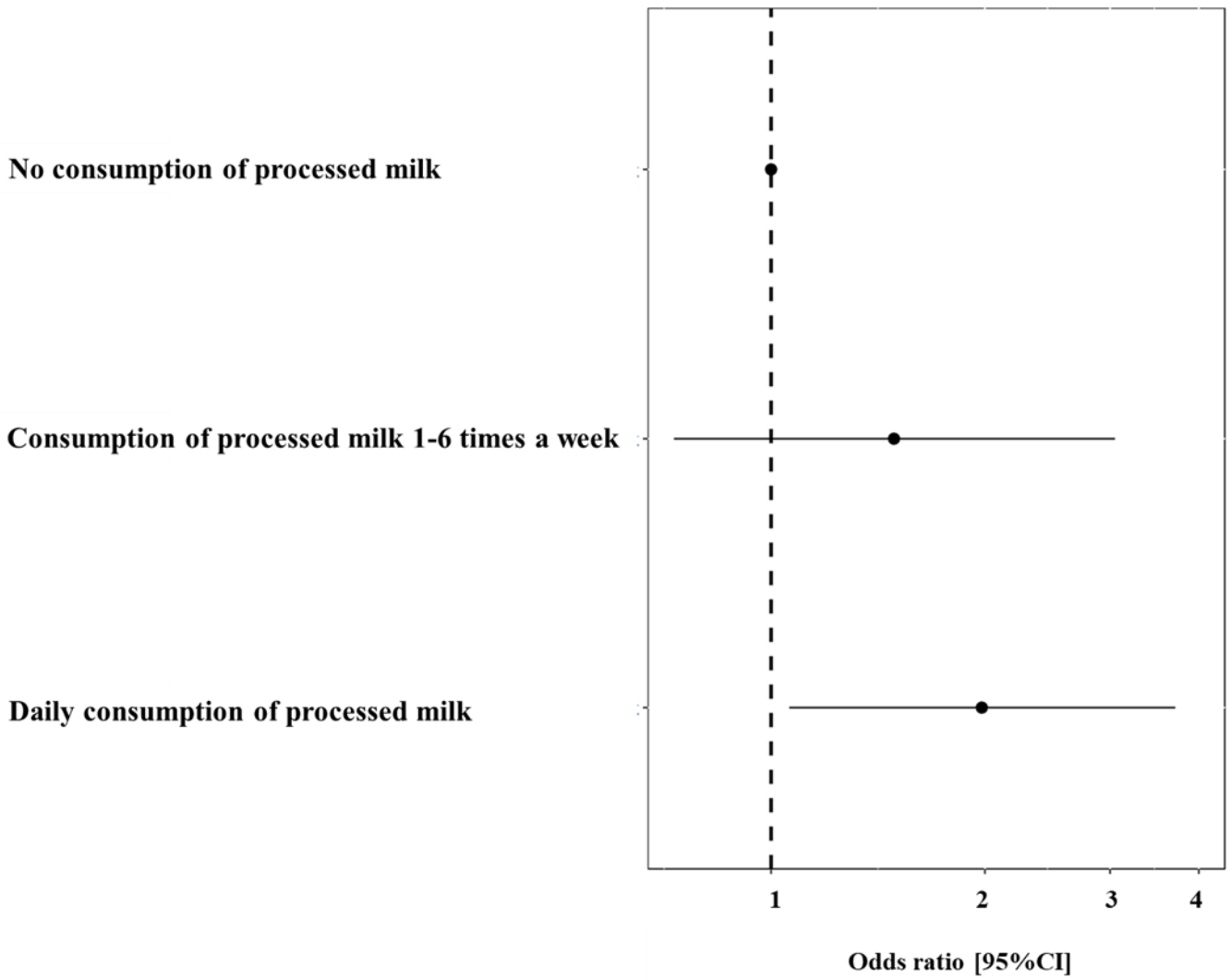


Figure E4.



Continuous consumption of farm milk



Potentially mediated by gut microbiome



Protection of hay fever

ICMJE DISCLOSURE FORM

Date: 30.09.2022

Your Name: Johanna Theodorou

Manuscript Title: **Continuous rather than solely early farm exposure protect from hay fever development.**

Manuscript Number (if known): INPRACTICE-D-22-00656

In the interest of transparency, we ask you to disclose all relationships/activities/interests listed below that are related to the content of your manuscript. "Related" means any relation with for-profit or not-for-profit third parties whose interests may be affected by the content of the manuscript. Disclosure represents a commitment to transparency and does not necessarily indicate a bias. If you are in doubt about whether to list a relationship/activity/interest, it is preferable that you do so.

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Time frame: past 36 months			
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3	Royalties or licenses	X None	

		Name all entities with whom you have this relationship or indicate none (add rows as needed)	Specifications/Comments (e.g., if payments were made to you or to your institution)
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6	Payment for expert testimony	X None	
7	Support for attending meetings and/or travel	X None	
8	Patents planned, issued or pending	X None	
9	Participation on a Data Safety Monitoring Board or Advisory Board	X None	
10	Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid	X None	

		Name all entities with whom you have this relationship or indicate none (add rows as needed)	Specifications/Comments (e.g., if payments were made to you or to your institution)
11	Stock or stock options	X None	
12	Receipt of equipment, materials, drugs, medical writing, gifts or other services	X None	
13	Other financial or non-financial interests	<input checked="" type="checkbox"/> None	

Please place an "X" next to the following statement to indicate your agreement:

X I certify that I have answered every question and have not altered the wording of any of the questions on this form.

ICMJE DISCLOSURE FORM

Date: 9/2/2022

Your Name: Michael Kabesch

Manuscript Title: Continuous rather than solely early farm exposure protect from hay fever development

Manuscript Number (if known): Click or tap here to enter text.

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ICMJJE DISCLOSURE FORM

Date: 9/27/2022

Your Name: Marjut Roponen

Manuscript Title: **Continuous rather than solely early farm exposure protect from hay fever development.**

Manuscript Number (if known): INPRACTICE-D-22-00656

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ICMJE DISCLOSURE FORM

Date: 9/27/2022

Your Name: Jon Genuneit

Manuscript Title: **Continuous rather than solely early farm exposure protect from hay fever development.**

Manuscript Number (if known): INPRACTICE-D-22-00656

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5	Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events	<input type="checkbox"/> None	
		The journal Pediatric Allergy and Immunology (co-owned by Wiley and EAACI)	Personal honorarium for serving as Associate Editor
6	Payment for expert testimony	<input checked="" type="checkbox"/> None	
7	Support for attending meetings and/or travel	<input checked="" type="checkbox"/> None	
8	Patents planned, issued or pending	<input checked="" type="checkbox"/> None	
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ICMJJE DISCLOSURE FORM

Date: 10/4/2022

Your Name: Andreas Böck

Manuscript Title: **Continuous rather than solely early farm exposure protect from hay fever development.**

Manuscript Number (if known): [Click or tap here to enter text.](#)

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12	Receipt of equipment, materials, drugs, medical writing, gifts or other services	<input checked="" type="checkbox"/> None <table border="1" data-bbox="376 560 1490 663"> <tr><td> </td><td> </td></tr> <tr><td> </td><td> </td></tr> <tr><td> </td><td> </td></tr> </table>							
13	Other financial or non-financial interests	<input checked="" type="checkbox"/> None <table border="1" data-bbox="376 768 1490 871"> <tr><td> </td><td> </td></tr> <tr><td> </td><td> </td></tr> <tr><td> </td><td> </td></tr> </table>							

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I certify that I have answered every question and have not altered the wording of any of the questions on this form.

ICMJE DISCLOSURE FORM

Date: 9/27/2022

Your Name: Laurent Lucie

Manuscript Title: **Continuous rather than solely early farm exposure protect from hay fever development.**

Manuscript Number (if known): Click or tap here to enter text.

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The author's relationships/activities/interests should be defined broadly. For example, if your manuscript pertains to the epidemiology of hypertension, you should declare all relationships with manufacturers of antihypertensive medication, even if that medication is not mentioned in the manuscript.

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ICMJE DISCLOSURE FORM

Date: 9/30/2022

Your Name: Petra Ina Pfefferle

Manuscript Title: **Continuous rather than solely early farm exposure protect from hay fever development.**

Manuscript Number (if known): [Click or tap here to enter text.](#)

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Peter J. Apple

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Date: 6.10.2022

Your Name: Harald Renz, MD

Manuscript Title: **Continuous rather than solely early farm exposure protect from hay fever development.**

Manuscript Number (if known):

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