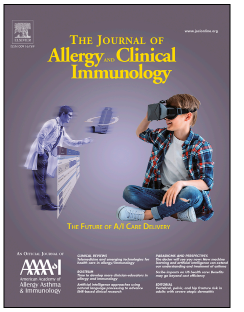

Patterns of Farm Exposure are Associated with Reduced Incidence of Atopic Dermatitis in Early Life

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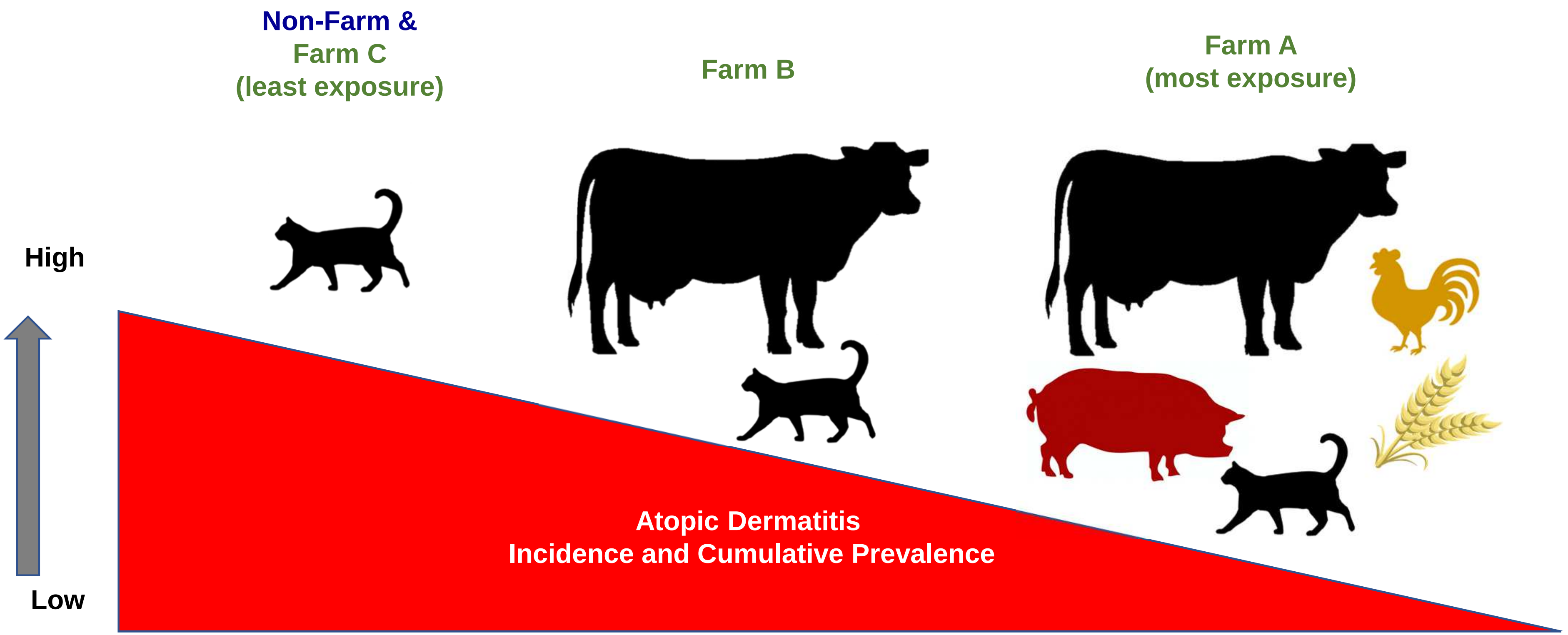
<u>Wisconsin Non-Farm and Farm LCA Exposure Groups</u>



Abbreviation: LCA, latent class analysis

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DECLARATIONS

Ethics Approval and Consent to Participate

All study activities and procedures were approved by the Marshfield Clinic Research Institute (KEI10613) and University of Wisconsin-Madison (2012-1056) Human Subjects Institutional Review Boards.

Consent for Publication

Not applicable.

Availability of Data and Material

The WISC study detailed manual of procedures and specific standard operating procedure source documents are available on reasonable request.

Conflicts of Interest

The authors declare that they have no financial conflicts of interest to report.

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Authors' Contributions

Conception and study design: CGB, CMS, JEG

Data management: BFO

Data analysis: MDE, KEL, MRL, REG

Lead study coordinator: KLB

Drafting and writing of the manuscript: CAS, JEG

All authors read, edited and approved the final manuscript.

Capsule Summary

Wisconsin farm exposures reduce the risk for early onset atopic dermatitis, which is closely associated with subsequent food allergy and asthma. Understanding the mechanisms for this association could lead to prevention of the “atopic march”.

Clinial Implications

Prenatal exposure to a Wisconsin farm environment decreases AD development in offspring, particularly among mothers with diverse exposures to farm animals, feed and bedding.

ABSTRACT

Background

Farm exposures may reduce the risk of atopic dermatitis (AD) in children, but this is controversial and US data are limited.

Objective

This study was conducted to identify patterns of farm exposure in Wisconsin family farms that modify AD incidence and prevalence in early childhood.

Methods

Environmental exposures, health history and clinical outcomes were prospectively recorded for 111 farm families and 129 non-farm families enrolled in the Wisconsin Infant Study Cohort birth cohort study. Exposures from the prenatal and early postnatal (2-month) visits were evaluated together with parental report of AD diagnosis by a healthcare provider through age 24 months. Latent class analysis was performed with prenatal and early postnatal farm-exposure variables to assign farm children to three classes.

Results

Overall, children of farm families had reduced AD incidence (P=0.03). Within farm families, exposures including poultry (3% vs 28%, P=0.003), pig (4% vs 25%, P=0.04), feed grain (13% vs 34%, P=0.02) and number of animal species were inversely associated with AD incidence. Among the latent class groups, children in families with diverse or more intense farm exposures (Classes A and B) had reduced AD incidence, while low-exposure (Class C) infants had AD incidence similar to non-farm children.

Conclusions

Infants in Wisconsin farm families had reduced AD incidence, and patterns of farm exposures further defined AD risk. These findings suggest that exposure to diverse farm animals, feed and bedding during the prenatal period and in early infancy reduce the risk of early-onset AD, a phenotype associated with multiple other atopic diseases.

Key Words: atopic dermatitis, farm effect, children, birth cohort, latent class analysis

Abbreviations

AD: Atopic dermatits

LCA: latent class analysis

MESA: Marshfield Epidemiologic Study Area

PASTURE: Protection Against Allergy Study in Rural Environments European birth cohort

WISC: Wisconsin Infant Study Cohort

Word counts

Abstract – 246

Text – 3639

INTRODUCTION

Atopic dermatitis (AD) is a common chronic and relapsing inflammatory skin disease that is characterized by intense pruritus and recurrent flares.[1] AD impacts more than 10% of children and may have a significant toll on quality of life for the patient and family.[2, 3] AD is often the first clinical manifestation of allergic disease, and early onset and more severe diseases increases the risk for asthma or hay fever.[4-7] The impaired skin barrier in AD allows for transcutaneous food allergen introduction, and can promote the development of food allergy.[8-10] Thus, there are compelling reasons to identify risk factors and potential preventive strategies for AD, which could also have downstream effects to reduce the risk of other atopic diseases.

Early childhood environmental exposures, including pet ownership, sibling contact and skin care practices have been linked to the risk of AD.[11-14] In addition, farm exposures during the prenatal period or during childhood have been inversely associated with to AD in some studies.[14-17] Farm environments can be a rich source of biological and microbial exposures with the potential to promote immune development and thereby reduce the risk of AD. For example, animal and barn-related exposures during pregnancy have been positively related to the number and function of cord blood regulatory T cells and enhanced cord blood cell cytokine responses.[18, 19]

However, several observational studies have reported no effect of the farming environment on AD.[20-27] The conflicting results could be related to variability in local farming characteristics and practices. The quality and intensity of environmental and microbial exposures can vary with characteristics such as the type of farm (e.g. arable [crops], pastoral, mixed), species of animals, and traditional vs. industrial farming practices.[16, 28] In addition, mothers on farms have a range of duties that vary from farm to farm and even over time on the same farm, and the same is likely true for exposures of infants. Finally, while relationships between allergic diseases and traditional European farming exposures have been well studied,[29] it is notable that data from the US are limited. Notably, US farming regions such as Wisconsin still have small family dairy farms where family members are embedded in the farm and its diverse environments. Farm size and characteristics in this region are still variable, however, and range from small family farms to larger mechanized operations. Importantly, dairy farms remain complex environmentally given the presence of crops, animals, and feed.

The Wisconsin Infant Cohort Study (WISC) was started in 2013 to identify environmental exposures that influence immune development, respiratory illnesses and allergic diseases in children from farming families compared to rural families with little or no farm contact.[30] Our hypothesis for this analysis was that there are specific exposures or groups of interrelated exposures on farms in Central Wisconsin that are inversely associated with the incidence of AD. To test this hypothesis, we compared the incidence and cumulative prevalence of AD through 24 months of age for children growing up in farm vs. non-farm families, and for the farm-exposed children, we compared cumulative 2-year AD prevalence by presence and detailed type of farm exposures. Finally, given the diversity of Wisconsin farms and exposures, we used unsupervised clustering techniques to identify patterns of farm exposure and to test for associations between exposure classes and AD incidence through 24 months of age.

METHODS

Study Design

The WISC study is a birth cohort study based in rural Wisconsin with prenatal enrollment of two groups (farm and rural non-farm) of pregnant mothers and their babies to determine how farm exposures influence wheezing illnesses and allergic diseases in early childhood. All families provided written informed consent prior to study enrollment, and all study activities and procedures were approved by the Marshfield Clinic Health System Human Subjects Institutional Review Board. The majority of families enrolled in the WISC study live in the Marshfield Epidemiologic Study Area (MESA). MESA has approximately 85,000 residents, with about 19,000 living in the city of Marshfield and the rest living in rural areas – small towns, villages and countryside. The area has approximately 2,200 farms with a farm population of about 5,400. Farm mothers were defined as living on or within 1/8th mile of a farm, working on a farm, or having a household member who works on a farm. Farm mothers (or household members) were further defined as having regular exposure (≥4 days per week) with cattle (cows, calves, bulls, or steers), goats or pigs. Non-farm mothers were defined as not living on or within 1/8th mile of a farm, working on a farm, or having a household member who works on a farm. Non-farm mothers did not have regular exposure to farms or raise livestock animals as pets (e.g., cattle, goats, pigs, horses or chickens).

Inclusion criteria including meeting criteria listed above and were birth at ≥ 34 weeks gestation. Exclusion criteria included maternal use of antibiotics (except Group B Strep prophylaxis) or corticosteroids in the last trimester of pregnancy, perinatal infections or prolonged rupture of membranes, and the presence of significant respiratory distress after delivery or congenital anomalies. Between April 2013 and May 2018, screening pregnant women in the Marshfield area yielded 612 non-farm and 309 farm woman who met eligibility criteria, and 111 farm families and 129 non-farm families provided informed consent and were enrolled in WISC.[30] Eleven of enrolled families were still awaiting delivery. For this analysis, 104 farm families (94%) and 120 non-farm families (93%) attended the 2-month study visit and were included in the AD and LCA analyses. Withdrawal rates for the farm and non-farm groups were similar (15% and 11%) through May 2018.

Questionnaires to assess environmental and farming exposures was administered prenatally and at postnatal timepoints (2, 9 and 24 months). Additional questionnaires were administered to mothers starting at 2 and 6 months of age and then every 3 months either by phone or in-person to assess child health information and clinical outcomes, including AD.

AD Definition

AD was defined as maternal report by questionnaire of a healthcare provider's diagnosis of AD (collected at 2 and 6 months of age and then every 3 months). Children were labeled as having a healthcare provider's diagnosis of AD if mothers responded positively at least one time to the following question, "Has a healthcare provider told you that your child had eczema (atopic dermatitis) since the last time we talked?". Cumulative prevalence was defined as a "yes" answer to AD diagnosis over the first 2 years (up through the 24 month questionnaire), even if the condition subsequently resolved. The age at which the most recent questionnaire with a completed AD response (either yes or no) was noted, and used as a censoring age for children without a "yes" answer to the AD question.

Statistical Analysis

AD incidence (time in days from birth to date of first AD) was estimated using the Kaplan-Meier method, and was compared between groups using the log-rank test. Longitudinal AD prevalence was estimated using GEE logistic regression with an exchangeable correlation structure and a natural cubic spline term for time. Confidence intervals for prevalence were constructed using a subject-level bootstrap procedure with B=5000 replicates. A proportional hazard model[31] that accounts for the timing of AD development and censoring for incomplete follow-up was used to examine associations between maternal, child, and household characteristics and farm status, and between maternal, prenatal, and early life characteristics and exposures and AD. A chi-square test for trend was used to examine the relationship between animal exposure diversity (number of species) and AD. Latent class analysis (LCA), with manifest variables including 22 specific farm-derived exposures evaluated prenatally and at 2 months of age, was used as a data reduction strategy to identify distinct farm cohort exposure classes. These exposures were evaluated as absent or present, with any unknown response considered absent. The number of latent classes was allowed to vary from 1 to 6, and a 3-class model was selected based on minimizing the Bayesian Information Criterion. All tables show unadjusted p-values, but models were also run with adjustment for the child's sex. Any impact of this adjustment is noted in the text.

RESULTS

Study Population

Of the 104 farm families and 122 non-farm families included in this analysis (Table I), the last completed visit ranged from age 6 months to 48 months with median follow-up of 24 months. This analysis focused on the prenatal and 2 month data for exposures and the AD data up through the 24 month visit (since over half the population is complete up to that age). The farm group had significantly fewer female children (43% vs. 58%, P=0.03). Prenatal dog and cat ownership was lower in non-farm families (52% vs 73%, P=0.0009 and 32% vs 76%, P<0.0001, respectively). Non-farm mothers had a higher proportion of employment outside the home vs farm mothers (78% vs 60%) and lower consumption of raw farm milk during pregnancy (2% vs 15%, P<0.001). Otherwise, sociodemographic characteristics were similar between both groups and there were no group differences in household income, maternal smoking status, mode of delivery, or family history of atopic disease. These relationships persisted after adjustment for sex.

Within the farm cohort, 80% of mothers lived and worked on farms while 16% worked on the farm but lived elsewhere, as previously described.[30] Cows (77%) were the predominant animal kept on farms, followed by bulls and steers (32%), poultry (32%), pigs (19%), and goats (13%). Many farms (43%) reared farm animals of a single species. During pregnancy, two-thirds of farm mothers reported at least weekly, direct contact with cattle (cows, calves, bulls, or steers), followed by 25% with poultry, 10% with pigs, and 7% with goats. Most farms (88%) grew and harvested crops. There were high rates of regular, direct contact between pregnant mothers and hay (76%), feed grain (66%), straw (63%) and silage (58%).

Associations Between Exposures Shared by Farm and Non-Farm Families and AD Development

We first tested whether common prenatal and infancy exposures were associated with AD risk in the farm and non-farm families (Table II). Among children in the farm group, cumulative prevalence of AD was positively related to delivery mode (vaginal delivery 16%, C-section 41%, P=0.01) and inversely related to exclusive breastfeeding (12% vs. 28%, p = 0.05), and there were nonsignificant trends for positive associations with maternal history of AD and asthma. These associations were not present within the non-farm group, and were not altered by adjustment for sex.

Farm Exposures and AD Development

AD incidence was significantly reduced in the farm group compared to the non-farm (P=0.03, Fig. 1A). This relationship was age-dependent with differences evident by the second six months of life. Farm exposure was also associated with reduced cumulative prevalence of AD through age 2 years (P=0.002, Fig. 1B). Both of these relationships persisted after adjustment for sex.

Several specific prenatal and postnatal (2 months) farm-derived exposures were associated with AD risk among children in the farm cohort (Table III). Rates of AD were inversely associated with prenatal exposures to pigs (4% vs 25%, P=0.01), poultry (3% vs 28%, P<0.01) and feed grain (13% vs 34%, P=0.02). Prenatal contact with cattle, goats, pets (dog and cat), forage (hay, straw, and silage), manure, and raw milk consumption were not significantly associated with AD outcomes. Of the farm-derived exposures assessed at age 2 months, only poultry was significantly associated with AD development (0% vs 22%, p=0.03).

We also tested whether maternal prenatal exposure to a diversity of animal species was associated with AD risk. The number of animal species that the pregnant mothers had contact with was inversely associated with rates of AD (0 animals 43%, 1-2 animals 31%, 3-4 animals 16%, 5-6 animals 6%, P=0.01; Fig. 2). These findings suggested an additive effect of farm animal exposures on the risk for AD in children.

Farm Cohort Exposure Classes

Many of the individual farm exposure were interrelated, and we used LCA to group farm families according to patterns of environmental exposures (see Supplemental Fig 1 for selected covariates). The analysis identified 3 distinct exposure classes (Class A, B, and C; descriptive analysis in Supplemental Tables I-II) with high probabilities of class assignment (Fig 3A). Farm Class A included 21% (22/104) of farm families, and was notable for prenatal and early life contact with a variety of farm animal species and a high rate of exposure to both indoor and outdoor dogs. Just over half (54/104) of the farm cohort was classified in Class B, which was remarkable for increased contact with cows or cattle and crops and a high prevalence of cat ownership. Last, Class C included 27% (28/104) of farm families, and had the lowest rate of maternal contact with farm animal species, farm animal feed (silage, feed grain), bedding (hay, straw) and manure (Supplemental Table I), and lower rates of pet ownership (Supplemental Table III).

Maternal history of allergic diseases and asthma, mode of delivery, and day care attendance were not significantly different among the classes (Supplemental Table III). Other than the diversity of animals on the farm, farm characteristics among the classes were similar (Supplemental Table IV), except for small differences in small vegetable farming on home farms (Class A: 14% vs Class B: 0% vs Class C: 7%, P=0.019) and the number of cows milked on the farm (fewest in Class A).

Farm Cohort Exposure Classes and AD Outcome

Farm cohort exposure classes were differentially related to AD incidence (Class C > B > A, P=0.03; Fig 3B), and these differences persisted after adjusting for sex. Notably, the incidence of AD was lowest in children born to mothers with regular contact with multiple animal species and diverse exposures in the barn (Class A). Notably, AD incidence among children with minimal prenatal and postnatal contact with animals (Class C) was similar to that of nonfarm children (Fig 3B).

DISCUSSION

This study was conducted to identify patterns of exposure on Wisconsin farms that are associated with reduced risk of AD. We found that farm exposures both prenatally and in the first year of life were associated with a reduced incidence and cumulative prevalence of AD in farm children when compared to non-farm children. The reduction in AD incidence was evident within the first year of life. Unlike prevalence, incidence is independent of disease duration and remission and may therefore be more valuable in identifying associations with factors related to disease onset. We identified 3 patterns of farm exposures on Wisconsin farms related to maternal and infant exposures: Class A had the most diverse animal and environmental exposures, Class B had less complex animal exposures, and Class C had the least exposures. These patterns differed in their association with AD incidence, and underscore the concept that diverse animal and barn exposures, especially during the prenatal period, reduce the risk of AD. These findings also suggest that the quality and quantity of personal exposure rather than the physical properties of the farm are the most important determinant of AD risk.

AD is similar to asthma in that several natural history phenotypes have been identified with distinct risk factors, and importantly, different prognoses and associations with other diseases. For example, in the Childhood Origins of Asthma birth cohort study, AD that began in the first year of life and persisted was associated with increased numbers of wheezing illnesses and a higher risk of allergic sensitization compared AD that was late onset (after age 3 years) or none/transient.[32] Similarly, in the Protection Against Allergy Study in Rural Environments (PASTURE) European birth cohort, two early onset AD phenotypes (persistent and transient) were associated with increased risk of food allergy, and early persistent AD was also associated with increased risk of asthma compared to children with late onset or no AD.[33] These studies provide evidence that there are different phenotypes of AD, and demonstrate that early onset AD is most strongly linked to subsequent allergic diseases, suggested a possible causal pathway. Thus, understanding mechanisms between farm exposures and reduced rates of early onset AD could also provide insights into the pathogenesis of wheezing illnesses, food allergy and asthma in children.

Notably, studies of farm exposure and AD development have been conflicting, and several previous studies did not demonstrate significant associations between farm exposures and AD development.[20-27] These studies were cross-sectional and AD was ascertained in young adults or in families with school-aged children. Given the age of assessment, AD in these studies was likely of mixed phenotypes that included late onset AD, which we postulate may not be responsive to farming exposures in early life. Furthermore, in some of these studies, the farm environments and maternal/child contact with farm animals were not characterized in detail. In contrast, the two birth cohort studies (PASTURE and WISC [current analysis]) that have assessed early onset AD both have reported inverse relationships between specific farm exposures and AD risk. In the PASTURE/Mechanism of Early Protective Exposures on Allergy Development study, maternal contact with farm animals and cats during pregnancy were inversely related to AD in early life.[14] This study also demonstrated that maternal exposure to multiple farm animal species during pregnancy was inversely related to the probability of AD in children, a relationship that was corroborated by the current study.

Previous studies have identified farm exposures including livestock, animal feed, and the consumption of unprocessed cow's milk that may protect against development of asthma, hay fever, and AD during childhood.[17, 21, 22, 24, 28, 34] Wisconsin farms are quite diverse in terms of size, animals and farming practices, and individual, maternal and child exposures. In our study population we identified multiple animal and barnyard (animal feed, bedding and manure) exposures that were inversely related to AD. Since these factors are interrelated, we used LCA to identify 3 unique exposure classes within the farm cohort. Two of the groups had more barn and farm animal exposures; Group A were more likely to report prenatal and postnatal contacts with a variety of animals, while Group B reported exposures primarily to cows and cattle. Group C reported the least contact with barn and animal exposures and were also least likely to have dogs. Accordingly, the AD risk was lowest in group A and intermediate in group B, while AD risk in Class C (low exposure) closely mirrored that of the non-farm cohort. Notably, patterns of exposure did not link cleanly to physical characteristics of the farm or practices such as milking style. Thus, farm exposures are diverse and are differentially related to AD risk. Notably, an LCA analysis of European farm exposure groups in the GABRIEL Surveys identified three exposure groups (summarized as "no cows", "cows, no cultivation" and "cows and cultivation") and the group with the least exposure to cows had the highest risk for AD.[16] Both studies support the theory that frequent and diverse exposures beginning during the prenatal period have the strongest effect on risk of AD.

In 1989, David Strachan suggested the "hygiene hypothesis" and speculated that infections may protect against allergic disease.[35] More recent iterations of this theory have linked microbial exposures to the risk of inflammatory diseases. Microbial stimuli in early life may help to mold immune development, and dysregulation may initiate and sustain an inflammatory cycle that leads to pathological effects.[36, 37] Traditional farm environments provide rich biological diversity,[28] and in addition to influencing immune development, farm-related microbes could help to inhibit skin colonization with *Staphylococcus aureus*, which has been closely linked to the pathogenesis of AD. Thus, farm-related microbial exposures may account for the lower risk of AD development. Farm-related exposures that could reduce the risk of allergic diseases through mechanisms in addition to those related to microbes, including arabinogalactans (plant-derived polysaccharide), N-glycolylneuraminic acid (Neu5Gc, expressed on mammalian cells) and raw cow milk consumption[28, 38-42] also warrant further investigation with respect to AD.

The strengths of this study include the prospective design and populations of rural children with repeated exposure assessments. Central Wisconsin is a major producer within the U.S. dairy industry and many Wisconsin dairy farms in this region are small- to medium-sized and family owned and operated. Thus, rural Wisconsin is an ideal location to study effects of farm exposures in the US. Study limitations include AD defined by parental report of a healthcare provider's diagnosis of AD. While farm and non-farm families could have different perceptions of medical problems, it is notable that health care utilization as measured by attendance at well child visits was similar between the two cohorts (data not shown). There are also several study limitations to consider. The diagnosis of AD was based on maternal report of diagnosis by a healthcare provider, which could have been affected by recall bias. WISC also has a modest sample size that limits ability to identify single or combinations of specific farm-related exposures related to AD. The prevalence of raw farm milk consumption is considerably lower in Wisconsin compared to Central Europe, which limited our ability to assess associations with AD. In addition, the WISC study is still enrolling, and the analysis of farm exposure patterns will require revision with increased data collection and the addition of more postnatal data.

In conclusion, WISC is the first birth cohort study in the US to study relationships between farm exposure and the risk of AD. Findings in the WISC birth cohort confirm observations from studies in Europe that link prenatal or early-life exposures to barns and diverse animals to reduced risk for AD. In addition, this analysis conducted in the heart of Wisconsin farm country provides new data relating farm-related patterns of exposures in the US to reduced incidence of AD. Finally, we observed that farm exposures are associated with reduced AD incidence beginning in the first year of life. Given the association between early onset AD and allergic outcomes such as food allergy and asthma, identification of farm-related microbiota or other exposures that positively influence early immunobiology and reduce AD development could lead to future preventative strategies for multiple atopic diseases.

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Table I. Maternal, Child and Household Characteristics According to Farm Status

Characteristic	Farm (n=111)	Non-farm (n=129)	P-value
Maternal			
Maternal age (years)			0.12
≥ 40	2%	2%	
35-39	20%	9%	
30-34	40%	44%	
25-29	32%	40%	
18-24	7%	5%	
Education			0.62
High school or less	6%	6%	
Associate degree or some college	29%	28%	
Bachelor's degree	50%	45%	
Graduate degree	11%	18%	
Unknown	4%	3%	
Employed outside the home	60%	78%	0.005
Annual household income			0.24
≥ $100,000	18%	22%	
$25,000-$99,999	63%	68%	
< $25,000	9%	4%	
Unknown	10%	6%	
Marital status			0.37
Married or living with a partner	89%	88%	
Single	5%	8%	
Unknown	6%	4%	
Maternal smoking			
During year prior to pregnancy	9%	15%	0.17
During pregnancy	2%	4%	0.33
Maternal history of AD (ever)	18%	20%	0.67
Maternal history of allergic rhinitis (ever)	11%	18%	0.10
Maternal history of asthma (ever)	16%	21%	0.37
Consumption of raw farm milk during pregnancy	15%	2%	<0.001
Child			
Sex			0.02
Female	43%	58%	

Race/Ethnicity			0.07
White	99%	94%	
Black or African American	1%	2%	
Asian	0%	2%	
Other	0%	2%	
Mode of delivery			
C-section (vs. vaginal)	17%	21%	0.45
Child daycare attendance at least one day per week (age 2 mo)	14%	21%	0.13
Exclusively breastmilk fed (age 2 mo)	50%	47%	0.64
Household			
Number of children in household			0.08
≥ 4	19%	12%	
3	23%	15%	
2	26%	42%	
1	25%	23%	
Unknown	7%	8%	
Dog ownership (prenatal)	73%	52%	<0.001
Dog spends time indoors	47%	52%	0.43
Cat ownership (prenatal)	76%	32%	<0.001
Cat spends time indoors	37%	29%	0.22

*Abbreviations: AD, atopic dermatitis; NS, not significant.

Table II. Cumulative Prevalence of Atopic Dermatitis up to Age 2 Years According to Maternal and Early Life Characteristics in the Farm and Non-farm Groups*

Characteristic	Cumulative Prevalence of Atopic Dermatitis					P-value†
	Farm Group (n=104) Has Characteristic			Non-Farm Group (n=122) Has Characteristic		
	Yes	No	P-value	Yes	No	
Maternal						
History of AD (ever)	33% (6/18)	15% (12/81)	0.10	35% (8/23)	35% (32/94)	0.91
History of AR (ever)	20% (2/10)	18% (16/88)	0.98	43% (9/21)	33% (31/93)	0.38
History of asthma (ever)	6% (1/17)	21% (17/80)	0.08	40% (10/25)	34% (31/92)	0.57
Smoking during pregnancy	0% (0/2)	21% (21/102)	0.38	40% (2/5)	34% (40/117)	0.99
Raw farm milk consumed (prenatal)	7% (1/15)	20% (17/83)	0.21	0% (0/2)	36% (40/116)	0.23
Dog ownership (prenatal)	17% (13/75)	28% (8/29)	0.22	33% (21/64)	36% (21/58)	0.60
Cat ownership (prenatal)	19% (15/78)	23% (6/26)	0.50	26% (10/38)	39% (32/84)	0.29
Child						
Sex (% male)	20% (12/60)	20% (9/44)	0.94	37% (19/51)	32% (23/71)	0.49
Vaginal Delivery	16% (14/87)	41% (7/17)	0.02	36% (35/96)	27% (7/26)	0.35
Daycare	20% (3/15)	20% (18/89)	0.82	37% (10/27)	34% (32/95)	0.55
Exclusively breastfed	12% (5/43)	28% (12/43)	0.05	40% (19/48)	35% (19/55)	0.30
Dog ownership (2 month)	18% (13/73)	26% (8/29)	0.30	31% (19/61)	39% (23/59)	0.34
Cat ownership (2 month)	23% (15/65)	15% (5/36)	0.24	25% (10/40)	41% (32/79)	0.22

*Abbreviations: AD, atopic dermatitis; AR, allergic rhinitis.

† P values were calculated with the likelihood ratio test.

Table III. Associations between Farm-Specific Prenatal and Early Life Exposures with Atopic Dermatitis in the Farm Cohort (n = 104).

Exposure	Atopic Dermatitis (Cumulative Prevalence*)		
	Exposed	Non-Exposed	P-value[†]
Maternal Prenatal Contact			
Hay	16% (13/79)	32% (8/25)	0.14
Straw	18% (12/65)	23% (9/39)	0.56
Feed grain	13% (9/69)	34% (12/35)	0.02
Silage	17% (10/60)	25% (11/44)	0.28
Manure	13% (4/31)	23% (17/73)	0.23
Cows or Cattle	18% (16/91)	38% (5/13)	0.15
Goats	13% (2/16)	22% (19/88)	0.33
Pigs	4% (1/23)	25% (20/81)	0.01
Poultry	3% (1/33)	28% (20/71)	<0.01
Children Contact at 2 months			
Forage	16% (9/55)	24% (12/49)	0.27
Cows or Cattle	17% (12/70)	26% (9/34)	0.24
Pigs	9% (1/11)	22% (20/93)	0.31
Poultry	0% (0/9)	22% (21/95)	0.03
Goats	0% (0/4)	21% (21/100)	0.16
Sheep	33% (1/3)	20% (20/100)	0.67
Horses	0% (0/8)	22% (21/96)	0.10

* Numerators represent the number of children with atopic dermatitis (cumulative prevalence up to age 2 years) and denominators represent the number of mothers or infants who were either exposed (column 2) or not exposed (column 3).

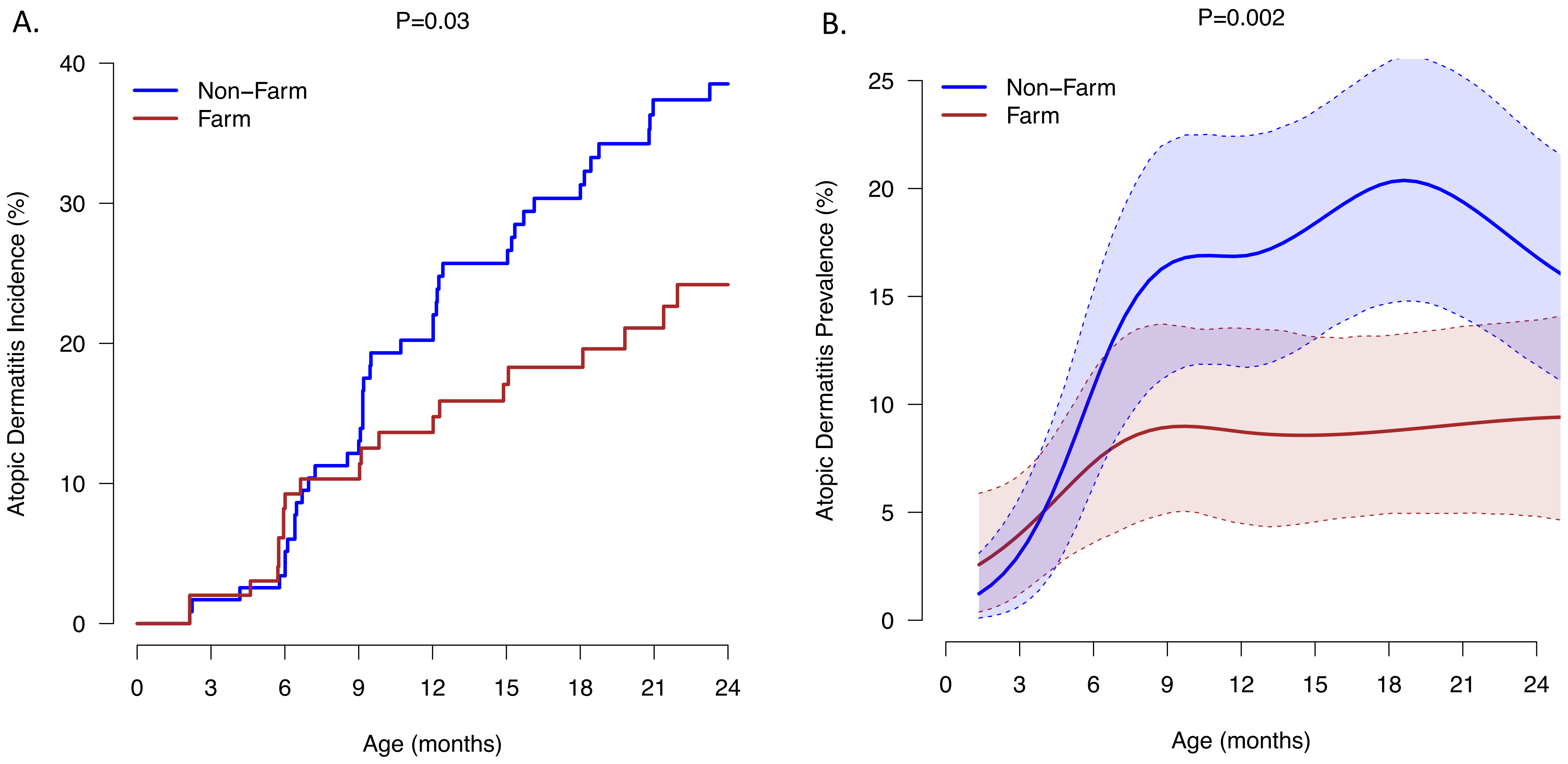
[†] P values were calculated with the likelihood ratio test.

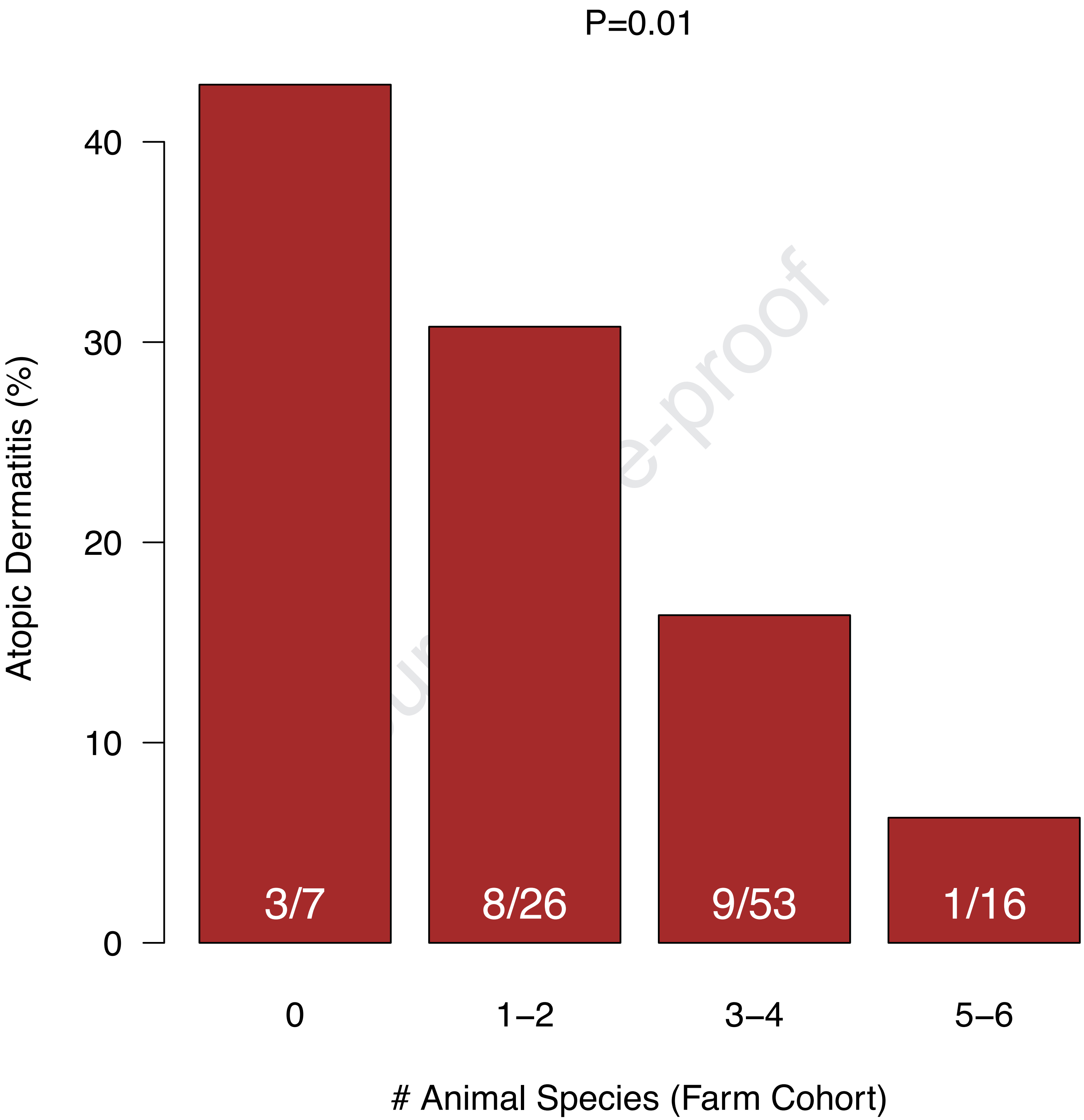
Figure Legends

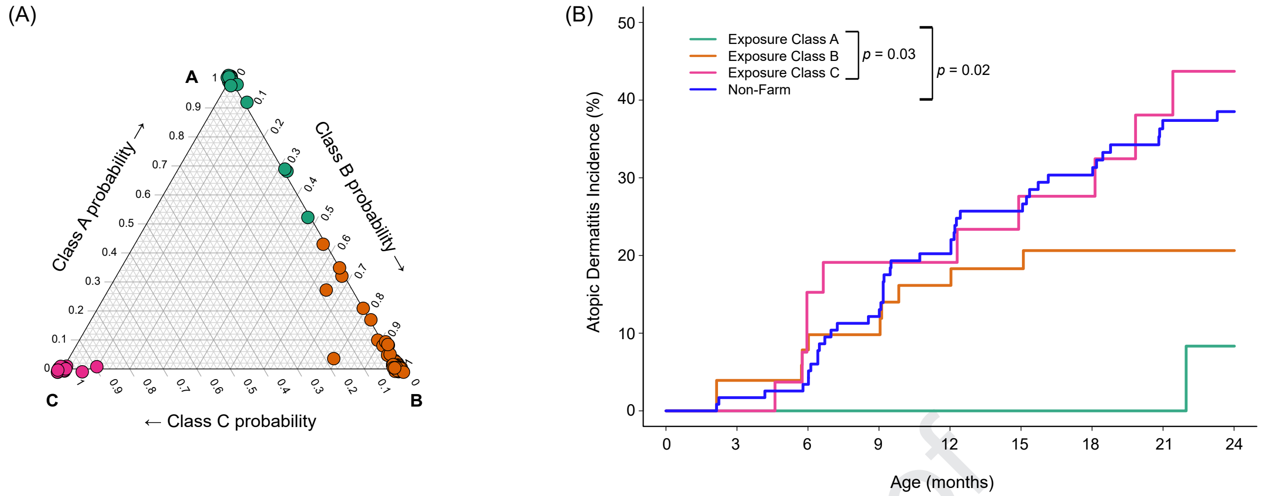
Figure 1: Atopic dermatitis incidence and prevalence by farm status. Exposure to a farm environment was associated with reduced AD incidence (A) and cumulative prevalence (B) during the first two years of life. The lines represent means and shaded areas are 90% (5^{th} – 95^{th} percentile) bootstrap confidence intervals for those means. The duration of follow-up for the 226 children included in the study is as follows: 24 mo, n=135; 21 mo, n=26; 18 mo, n= 17; 15 mo, n = 15; 12 mo, n = 2; 9 mo, n = 8; 6 mo, n = 11; 2 mo, n = 12).

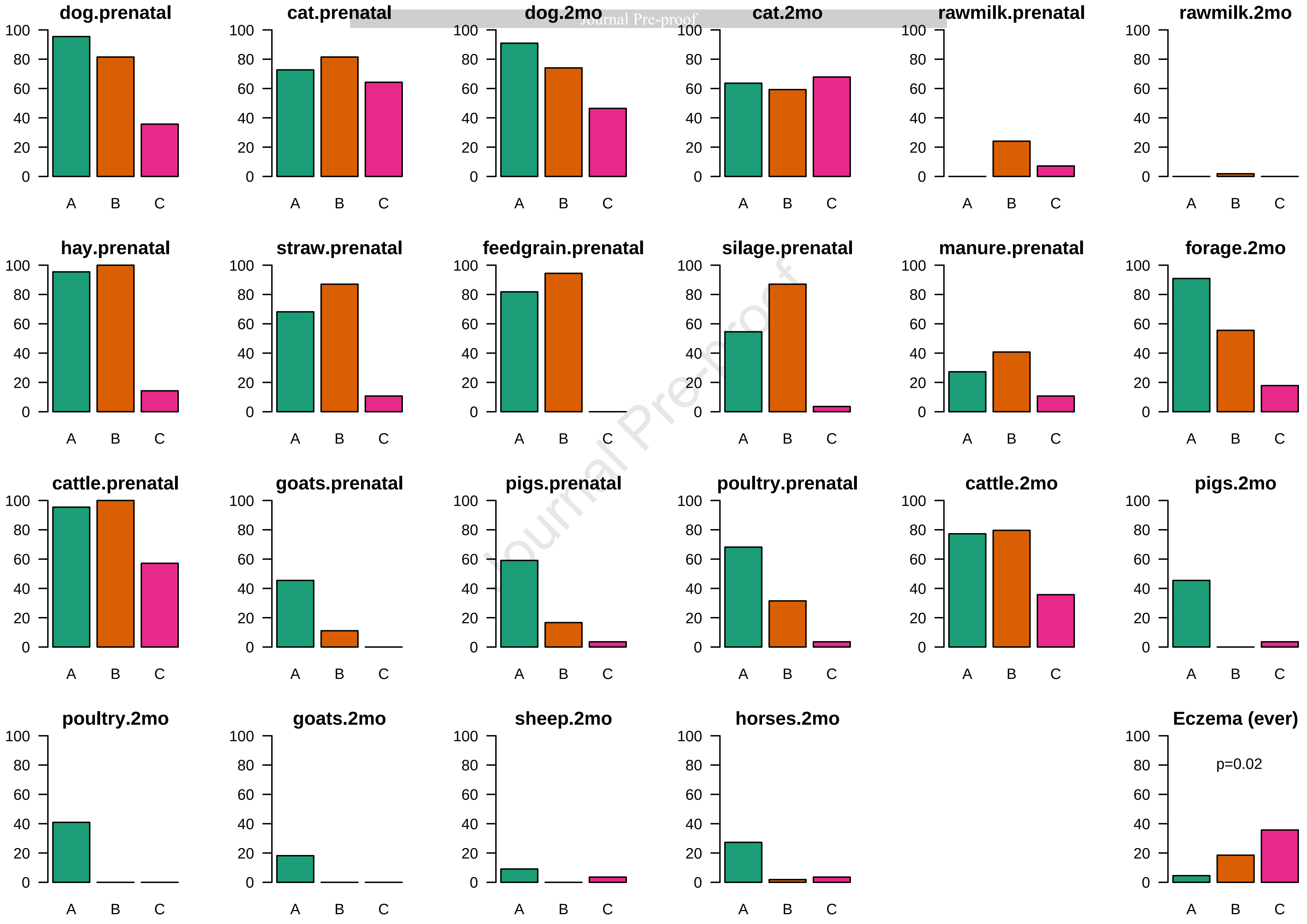
Figure 2: Atopic dermatitis by diversity of animal exposure. Cumulative prevalence of AD up to age 2 years was inversely related to the number of animal species that the mother had contact with during pregnancy.

Figure 3: Atopic dermatitis incidence by farm cohort exposure class. Three distinct patterns of farm exposure were identified by LCA, and the ternary probability plot (A) illustrates the probability of class assignment for each mother and child pair. The three classes of farm exposure were associated with distinct rates of AD incidence in the first two years (B). Group comparisons represent differences in overall AD indicence among the three farm exposure classes (p=0.03) and among the 3 classes considered together with the non-farm children (p=0.02).


A.
P=0.03
Non−Farm
Farm
Atopic Dermatitis Incidence (%)
0
10
20
30
40
0
3
6
9
12
15
18
21
24
Age (months)
B.
P=0.002
Non−Farm
Farm
Atopic Dermatitis Prevalence (%)
0
5
10
15
20
25
0
3
6
9
12
15
18
21
24
Age (months)


P=0.01
Atopic Dermatitis (%)
0
10
20
30
40
3/7
8/26
9/53
1/16
0
1–2
3–4
5–6
Animal Species (Farm Cohort)


(A)
A
C
B
Class A probability →
Class B probability →
← Class C probability
(B)
Exposure Class A
Exposure Class B
Exposure Class C
Non-Farm
p = 0.03
p = 0.02
Atopic Dermatitis Incidence (%)
Age (months)


dog.prenatal
cat.prenatal
dog.2mo
cat.2mo
rawmilk.prenatal
rawmilk.2mo
hay.prenatal
straw.prenatal
feedgrain.prenatal
silage.prenatal
manure.prenatal
forage.2mo
cattle.prenatal
goats.prenatal
pigs.prenatal
poultry.prenatal
cattle.2mo
pigs.2mo
poultry.2mo
goats.2mo
sheep.2mo
horses.2mo
Eczema (ever)
p=0.02
0
20
40
60
80
100
A
B
C

Patterns of Farm Exposure are Associated with Reduced Incidence of Atopic Dermatitis in Early Life

Online Data Supplement

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Methods

Dietary questionnaire. Questionnaires administered prenatally and at postnatal timepoints (2, 9 and 24 months) included questions about consumption of cow milk. Two questions were asked to determine whether the children consumed either “store-bought” or “raw”. The questionnaires were administered by study coordinators, who were instructed to count any kind of farm milk that was not “store-bought” as “raw”.

32 **Supplementary Table I.** Maternal Farm Exposures According to Farm Cohort Exposure Class
33

Exposure	**Class A (n=22)**	**Class B (n=54)**	**Class C (n=28)**
Mother currently works on farm postnatally*	77%	76%	32%
Frequency of postnatal maternal visits to someone else's farm			
Once per week	27%	30%	21%
Less than once per week	68%	67%	75%
Unknown	5%	4%.	4%
Prenatal* direct contact with cattle or cows (at least weekly)			
Milking	32%	69%	7%
Bedding	50%	67%	4%
Manual feeding	68%	80%	11%
Cleaning	55%	74%	7%
Any of above	82%	87%	11%
Postnatal direct contact with cattle or cows (≥ weekly)			
Milking	23%	57%	7%
Bedding	32%	63%	7%
Manual feeding	41%	69%	25%
Cleaning	27%	67%	11%
Any of above	45%	85%	29%
Prenatal direct contact with goats (≥ weekly)			
Milking	0%	4%	0%
Bedding	9%	4%	0%
Manual feeding	18%	6%	0%
Cleaning	5%	0%	0%
Any of above	18%	6%	0%
Postnatal direct contact with goats (≥ weekly)			
Bedding	0%	0%	4%
Manual feeding	14%	2%	4%
Cleaning	0%	0%	0%
Any of above	14%	2%	4%
Prenatal direct contact with pigs (≥ weekly)			
Bedding	14%	4%	0%
Manual feeding	27%	7%	0%
Cleaning	9%	2%	0%
Any of above	27%	7%	0%
Postnatal direct contact with pigs (≥ weekly)			
Bedding	23%	2%	4%
Manual feeding	23%	4%	4%
Cleaning	9%	2%	4%
Any of above	27%	4%	4%

Prenatal direct contact with poultry (≥ weekly)			
Manual feeding	41%	28%	0%
Cleaning	23%	6%	4%
Any of above	45%	30%	4%
Postnatal direct contact with poultry (≥ weekly)			
Manual feeding			
Cleaning	41%	6%	0%
Any of above	9%	2%	0%
	41%	6%	0%
Prenatal direct contact with crops (≥ weekly)			
Hay	95%	100%	14%
Straw	68%	87%	11%
Feed grain	82%	94%	0%
Silage	55%	87%	4%
Manure	27%	41%	11%
Dog in home (prenatal)	95%	81%	36%
Cat in home (prenatal)	73%	81%	64%

* The prenatal exposure data were used in the latent class analysis to identify farm exposure groups and therefore were not subjected to statistical analysis. The maternal postnatal values (which were not used in the latent class analysis) represent maternal postnatal exposure assessed when the child was at age 2 months.

Supplementary Table II. Child Farm Exposures* According to Farm Cohort Exposure Class

Exposure (at least weekly)	**Class A (n=22)**	**Class B (n=54)**	**Class C (n=28)**
Cows or cattle			
Any	77%	80%	36%
At least weekly	50%	59%	18%
Pigs			
Any	45%	0%	4%
At least weekly	14%	0%	4%
Chicken			
Any	41%	0%	0%
At least weekly	27%	0%	0%
Goats			
Any	18%	0%	0%
At least weekly	5%	0%	0%
Sheep			
Any	9%	0%	4%
At least weekly	9%	0%	4%
Horses			
Any	27%	2%	4%
At least weekly	5%	2%	0%
Forage			
Any	91%	56%	18%
At least weekly	59%	41%	14%

*These exposures were assessed at age 2 months, and were used in the latent class analysis of farm exposure classes.

Supplementary Table III. Descriptive Analysis of the Farm Cohort Exposure Classes*

Characteristic	Class A (n=22)	Class B (n=54)	Class C (n=28)	P value
Mother				
Maternal age (years)				0.18
≥ 40	0%	2%	0%	
35-39	23%	15%	25%	
30-34	41%	50%	29%	
25-29	32%	31%	29%	
18-24	5%	2%	18%	
Education				0.04
High school or less	9%	7%	4%	
Associate degree or some college	23%	26%	32%	
Bachelor's degree	45%	57%	39%	
Graduate degree	23%	9%	7%	
Unknown	0%	0%	18%	
Annual household income				0.64
≥ $100,000	27%	19%	14%	
$25,000-$99,999	55%	65%	61%	
< $25,000	14%	7%	7%	
Unknown	5%	9%	18%	
Marital status				<0.001
Married or living with a partner	86%	100%	68%	
Single	14%	0%	11%	
Unknown	0%	0%	21%	
Smoking during pregnancy	0%	2%	4%	1.0
History of AD (ever)	27%	13%	22%	0.26
History of allergic rhinitis (ever)	9%	7%	18%	0.43
History of asthma (ever)	27%	17%	9%	0.28
Children				
Sex (female)	59%	35%	43%	0.17
Race/Ethnicity				1.0
White	100%	98%	100%	
African American	0%	2%	0%	
Vaginal delivery (vs. C-section)	86%	83%	75%	0.34
Daycare attendance ≥ day per week (2 months of age)	18%	15%	11%	0.71
Exclusive breastfeeding	70%	43%	45%	0.14
Household Characteristics				
Number of children in household				0.003
≥ 4	18%	24%	14%	
3	36%	20%	18%	
2	27%	33%	11%	
1	18%	22%	32%	
unknown	0%	0%	25%	

*Values reported as frequencies (% of group total)

49 **Supplementary Table IV.** Farm Characteristics According to Farm Cohort Exposure Class

Characteristic	Class A (n=22)	Class B (n=54)	Class C (n=28)	P-value
Live on a farm	77%	69%	61%	0.46
No. cows milked (work)				0.46
None	14%	14%	25%	
1-25	29%	9%	0%	
26-100	14%	50%	25%	
101-500	29%	14%	38%	
500+	14%	14%	12%	
No. cows milked (home)				0.05
None	50%	10%	27%	
1-25	6%	8%	0%	
26-100	22%	55%	40%	
101-500	17%	25%	27%	
500+	6%	2%	7%	
Milking style (work farm)				
Robotic	0%	0%	7%	0.11
Hand milking	5%	2%	0%	0.45
Step-up/ walkthrough/tie stall	9%	28%	14%	0.13
Other	14%	6%	7%	0.68
Milking style (home farm)				
Robotic	0%	4%	0%	0.72
Hand milking	0%	7%	0%	0.18
Step-up/ walkthrough/tie stall	27%	35%	29%	0.25
Other	14%	22%	7%	0.62
No. household members that work on farm				0.07
0	9%	4%	0%	
1	91%	80%	68%	
2	0%	4%	4%	
3	0%	9%	7%	
4	0%	2%	4%	
Unknown	0%	2%	18%	
Animals on farm (Work)				
Cows/cattle	23%	9%	18%	0.22
Chicken/Poultry	9%	7%	0%	0.29
Horses	5%	6%	0%	0.66
Hogs/Pigs	14%	6%	4%	0.39
Sheep	9%	2%	0%	0.18
Goats	5%	7%	0%	0.41
Other	5%	0%	7%	0.11
Animals on farm (Home)				
Beef cattle	36%	19%	21%	0.26

Chicken/Poultry	59%	24%	4%	<0.001
Horses	27%	7%	0%	0.003
Hogs/Pigs	27%	9%	11%	0.13
Sheep	9%	2%	4%	0.32
Goats	23%	7%	0%	0.02
Other	14%	6%	4%	0.39
Crop grown/harvested on farm (work)	36%	37%	36%	1.00
Crop grown/harvested on farm (home)	73%	72%	61%	0.56
Type of crops grown/ harvested on farm (work)				
Corn	23%	35%	36%	0.55
Hay	32%	35%	29%	0.89
Potatoes	0%	2%	0%	1.00
Small vegetable	0%	0%	7%	0.11
Soybeans	5%	15%	18%	0.42
Other	14%	15%	14%	1.00
Types of crops grown/ harvested on farm (home)				
Corn	55%	61%	57%	0.87
Hay	41%	61%	46%	0.21
Potatoes	0%	2%	0%	1.00
Small vegetable	14%	0%	7%	0.019
Soybeans	41%	41%	36%	0.93
Other	36%	35%	29%	0.85

Figure Legend

Supplementary Figure 1. Variables used to identify farm cohort exposure classes. Exposures to defining farm exposures are illustrated for farm exposure class A, B and C.