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Pacifier Cleaning Practices and Risk of Allergy Development

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KEY WORDS

allergy, asthma, child, eczema, infant, microbiota, pacifiers, sensitization

ABBREVIATIONS

CI—confidence interval

IgE—immunoglobulin E

OR—odds ratio

PCR—polymerase chain reaction

T-RFLP—terminal restriction fragment length polymorphism

Mr Hesselmar was involved in the planning and design of the study and interpretation of the data, and was responsible for the design of the protocols used, construction and validation of clinical databases and diagnoses, writing of the manuscript, and data/statistical analyses, with the exception of the analyses of the saliva samples. Mr Hesselmar, Mr Saalman, and Mr Åberg examined the children at all follow-ups. Ms Sjöberg was responsible for data analysis, analyses of the saliva samples, and data interpretation and writing concerning the terminal restriction fragment length polymorphism analysis. Mr Saalman was involved in the planning and design of the study, interpretation of the data, writing of the manuscript, and also examined children regarding allergic manifestations related to food allergy. Mr Åberg was involved in the planning and design of the study and in the design of the protocols used, and was responsible for the collection of basic data regarding the children, and participated in discussions related to the production of the manuscript. Ms Adlerberth was involved in the planning and in the design of the study and in the interpretation of the data, and was chiefly responsible for the microbial analyses and is a cosupervisor of Ms Sjöberg. Ms Adlerberth and Ms Wold initiated the AllergyFlora cohort on which the current study is based. Ms Wold was involved in the planning and design of the study, interpretation of the data, writing of the manuscript, and in the design of figures. She was the primary investigator of the AllergyFlora cohort, and is the main supervisor of Ms Sjöberg. All authors had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

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WHAT'S KNOWN ON THIS SUBJECT: Infants with a diverse gut microbial flora are less likely to develop eczema and allergy.



WHAT THIS STUDY ADDS: Parental sucking of their infant's pacifier is associated with a reduced risk of allergy development and an altered oral flora in their child. Transfer of oral microbes from parent to infant via the pacifier might be used in primary prevention.

abstract

FREE

OBJECTIVE: Immune stimulation through exposure to commensal microbes may protect against allergy development. Oral microbes may be transferred from parents to infants via pacifiers. We investigated whether pacifier cleaning practices affected the risk of allergy development.

METHODS: A birth-cohort of 184 infants was examined for clinical allergy and sensitization to airborne and food allergens at 18 and 36 months of age and, in addition, promptly on occurrence of symptoms. Pacifier use and pacifier cleaning practices were recorded during interviews with the parents when the children were 6 months old. The oral microbiota of the infants was characterized by analysis of saliva samples collected at 4 months of age.

RESULTS: Children whose parents “cleaned” their pacifier by sucking it ($n = 65$) were less likely to have asthma (odds ratio [OR] 0.12; 95% confidence interval [CI] 0.01–0.99), eczema (OR 0.37; 95% CI 0.15–0.91), and sensitization (OR 0.37; 95% CI 0.10–1.27) at 18 months of age than children whose parents did not use this cleaning technique ($n = 58$). Protection against eczema remained at age 36 months (hazard ratio 0.51; $P = .04$). Vaginal delivery and parental pacifier sucking yielded independent and additive protective effects against eczema development. The salivary microbiota differed between children whose parents cleaned their pacifier by sucking it and children whose parents did not use this practice.

CONCLUSIONS: Parental sucking of their infant's pacifier may reduce the risk of allergy development, possibly via immune stimulation by microbes transferred to the infant via the parent's saliva. *Pediatrics* 2013;131:1–9

The prevalence of immunoglobulin E (IgE)-mediated (atopic) allergy increased strongly during the 20th century, and today 1 in 3 children in affluent countries are affected.¹ Allergy results from the failure to develop immune tolerance to harmless inhaled or ingested proteins, termed allergens. Instead, an immune response occurs (sensitization), which may lead to symptoms (clinical allergy) on renewed contact with the allergen(s). The cause of the increased prevalence of allergy is unknown, although circumstantial evidence points to reduced exposure of infants and young children to microbes as a risk factor, observations that were summarized as “the hygiene hypothesis.”² Hence, poverty, crowded housing, large families, early contact with pets or farm animals, and early exposure to foodborne microbes are associated with a reduced risk of allergy development.^{2–5} Furthermore, acquisition of commensal gut bacteria is delayed in Western infants⁶ and a gut microbiota of low complexity during the neonatal period is a risk factor for allergy development.^{7–9} Vaginal delivery, which leads to neonatal exposure to a complex maternal microbiota, is associated with a reduced risk of allergy development compared with cesarean delivery.^{10–12}

The oral cavity contains a complex mixture of aerobic and anaerobic bacteria with more than 600 prevalent taxa.¹³ Infants may be exposed to parents’ oral microbes, transferred in saliva via kissing. Saliva is also transferred if the parent puts the infant’s feeding spoon or pacifier (dummy/soother) into his or her own mouth before giving it back to the infant. Whether early exposure to oral microbes is protective against allergy development has, to the best of our knowledge, not yet been studied. The aim of the study was to examine whether the mode by which parents clean their infant’s pacifier affects the risk of allergy development in the infant.

METHODS

Inclusion and Follow-up of AllergyFlora Cohort

Pregnant women were recruited into the AllergyFlora study at Mölndal Hospital, Greater Gothenburg, Sweden, and their infants were included in the birth cohort 1 to 3 days after delivery. Exclusion criteria were preterm birth (<38 weeks’ gestation) and neonatal intensive care. We mainly approached families with at least 1 allergic parent so that the birth cohort would include a high proportion of children with allergic disease. All parents provided written informed consent for their children to participate in the study. The study was approved by the Ethics Committee of the University of Gothenburg, Sweden (R 448-97 and Ö 446-00).

On inclusion, a structured interview was conducted focusing on the pregnancy, delivery, and family structure and housing conditions. Diaries covering the infant’s first and second 6 months of life were kept by the parents, who were asked to record food introduction, weaning, diseases and medications, and other significant events. This information was reported during structured telephone interviews with the parents when the children were 6 months old. During this interview the parents were also asked: “Does the child use a pacifier?” and “Is it cleaned by boiling, rinsing in tap water, or by the parents sucking on it?” (>1 option could be selected).

Sensitization and Clinical Allergy Diagnoses

A pediatric allergist examined the children, reviewed their medical charts, and performed a structured interview with the parents when the children were 18 and 36 months old, and whenever symptoms suggesting the commencement of allergy occurred. Venous blood was analyzed for eosinophilic granulocyte counts, which increase in patients with allergies,

and for allergen-specific IgE (see Sensitization, later in this article).

For clinical diagnosis, the examining pediatrician used the following criteria.

Eczema

Diagnosed according to Williams’ criteria for atopic dermatitis.¹⁴ Eczema at 18 months denoted diagnosis at any time before or at 18 months; eczema at 36 months required symptoms after 24 months of age.

Asthma

Persistent wheezing for ≥ 4 weeks or ≥ 3 episodes of wheezing combined with ≥ 1 minor criterion (symptoms between colds, eczema, allergic rhinoconjunctivitis, or food allergy). For asthma at 36 months, ≥ 1 wheezing episode should have occurred after 24 months of age, and response to inhaled glucocorticoids or leukotriene antagonists was included among the minor criteria.

Sensitization

Presence of specific IgE against inhalant allergens (birch, timothy grass, mugwort, cat, dog, horse, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and mold; Phadiatop, Phadia AB, Uppsala, Sweden) or against food antigens (milk, egg, soy, fish, wheat, and peanut; ImmunoCAP food-mix test, Phadia AB). A positive reaction was defined as a sum of allergen-specific IgEs ≥ 0.35 kU/L in either assay.

Parental History of Allergy

A doctor’s diagnosis of asthma, allergic rhinoconjunctivitis, or eczema, as reported at interviews.

Analysis of Infants’ Salivary Microbiota

For infants included after February 2001, saliva samples were collected at 4 months of age. The samples were stored at -20°C until microbiota fingerprinting

was performed by using terminal restriction fragment length polymorphism (T-RFLP). Bacterial DNA was extracted from 150 μL saliva (QIAamp DNA Stool Mini Kit; Qiagen, Hilden, Germany) according to the manufacturer's instructions, but included shaking (1200 rpm) with 2-mm glass beads (10–12 beads/sample) for 30 minutes at 5°C after addition of the ASL buffer. Extracted DNA, 50 ng as measured by spectrophotometry (version 3.3; NanoDrop Technologies, Wilmington, DE), was mixed with 0.3 μM of the 16S rRNA universal primers ENV1 (5'-6-FAM-AGA GTT TGA TII TGG CTC AG -3'; *Escherichia coli* 16S rRNA gene sequence nucleotide positions 8–27) and ENV 2 (5'-CGG ITA CCT TGT TAC GAC TT-3'; *E coli* 16S rRNA gene sequence nucleotide positions 1511–1492), 25 μL Hot Start Taq Master Mix (2.5 U Taq DNA polymerase, 1.5 mM MgCl_2 , 200 μM dNTP) (Qiagen), 1 mM MgCl_2 , and water to a final volume of 50 μL . The ENV1 primer was labeled with 6-FAM fluorescent dye. The polymerase chain reaction (PCR) program was run as follows: an initial activation of Taq polymerase at 95°C for 15 minutes; 25 cycles of 94°C for 1 minute (DNA denaturation), 50°C for 45 seconds (primer annealing), and 72°C for 2 minutes (primer extension), with a final extension at 72°C for 7 minutes. PCR amplification products were examined by agarose gel electrophoresis. Triplicate samples were pooled and purified (QIAquick PCR purification kit, Qiagen), and 100 ng was digested by using 16 U of *MspI* endonuclease (New England Biolabs, Ipswich, MA) at 37°C for 5 hours in a final volume of 5 μL . A mixture of 1 μL of digested DNA, 9 μL of formamide, and 0.5 μL of DNA size standard (LIZ 1200; Applied Biosystems, Life Technologies Corporation, Carlsbad, CA) was denatured at 95°C for 3 minutes, placed on ice, and separated for 80 minutes at 15 kV on an ABI PRISM 310 genetic analyzer with POP-4 gel matrix and 5-second

injection time (Applied Biosystems). Fragment lengths were analyzed by using GeneMapper (version 4; Applied Biosystems) and the local institution's Southern blot method. "True" peaks were separated from "noise" by statistical analysis using Perl and R software (www.ibest.edu/tools/trflp_stats/index.php).¹⁵ Peaks with a relative area >3 SDs from the mean were identified as true signals and removed. This process was repeated until no more true peaks were identified. Saliva samples were analyzed in triplicate; only peaks appearing in all runs were included (terminal restriction fragments were considered to be identical if they differed by no more than ± 1 bp in different runs), and the peak areas were averaged.

Statistical Analyses

Statistical analyses were performed by using SPSS software (version 15.0.1.1; IBM SPSS Statistics, IBM Corporation, Chicago, IL). Fisher's exact and χ^2 tests were used to compare proportions; the χ^2 test was used for trend analysis; the Mann-Whitney test and analysis of variance (ANOVA) were used to compare continuous data and binary logistic regression to adjust for confounding variables. Kaplan-Meier analyses were used to monitor allergy development over time, with the use of Cox regression to adjust for confounders. Orthogonal projection to latent structures was used to analyze the salivary microbiota in relation to pacifier cleaning practices (SIMCA-P+ software, version 12.0; Umetrics AB, Umeå, Sweden).

RESULTS

Between 1998 and 2003, 206 pregnant women were recruited into the study; 187 infants met the criteria for inclusion in the AllergyFlora birth cohort. A total of 184 children were followed until the child was 18 months old and 174 until the age of 36 months. Eighty percent of

the children had at least 1 allergic parent and 74% used a pacifier during the first 6 months of life (Table 1). As seen in Table 2, almost all parents cleaned the pacifier by rinsing it in tap water, but approximately half of them also boiled it and almost half reported cleaning the pacifier by sucking it before giving it back to the infant.

Pacifier Cleaning Practices and Allergy at 18 Months of Age

By the age of 18 months, 25% of the children had developed eczema and 5% asthma. Sensitization to food antigens occurred in 15%, whereas sensitization to inhaled antigens was uncommon (Table 3).

We examined whether the presence of eczema, asthma, or atopy at 18 months was related to pacifier cleaning practices during the child's first 6 months of life. Figure 1 shows the odds ratio (OR) of allergy and sensitization in relation to pacifier use and pacifier cleaning practices. As seen in the figure, pacifier use per se was not significantly associated with clinical allergy or sensitization (Fig 1). In pacifier-using children, a cleaning method was compared with those not using that specific cleaning method. Boiling the pacifier was associated with an increased prevalence of asthma, but the effect was not significant.

TABLE 1 Characteristics of the Infant Cohort

Characteristic	n (%) ^a
Male	93 (50)
At least 1 parent with a history of allergy ^b	148 (80)
Mother with a history of allergy	91 (49)
Father with a history of allergy	75 (41)
Cesarean delivery	25 (14)
Siblings at time of birth	90 (49)
Exclusively breastfed for >4 mo ^c	65 (35)
Use of pacifier in the first 6 mo of life ^c	136 (74)

Percentages are based on the 184 children who remained at the 18-mo follow-up.

^a Reported in telephone interview at 6 mo.

^b Reported in questionnaire at inclusion: a history of doctor-diagnosed asthma, allergic rhinoconjunctivitis, or eczema.

^c Noted by parents in a diary and reported during a telephone interview at 6 mo of age.

TABLE 2 Pacifier Cleaning Practices Among the 136 Infants Using a Pacifier During the First 6 mo of Life

Technique	No. of children	%
Rinsing in tap water	113	83
Boiling	74	54
Parental sucking of the pacifier	65	48
Not reported	13	10
Total	136	100

Techniques for cleaning the pacifier were reported in a telephone interview when the infant was 6 mo old.

TABLE 3 Allergic Diseases and Sensitization at 18 and 36 mo of Age

Disease	No. of children (%)			
	At 18 mo (n = 184)		At 36 mo (n = 174)	
Eczema	46	(25)	40	(23)
Asthma	10	(5)	14	(8)
Sensitization	25	(15) ^c	26	(15) ^d
Phadiatop ^a	3	(2) ^c	3	(2) ^d
Food-mix ^d	25	(15) ^c	26	(15) ^d

^a Immunoglobulin E antibodies to birch, timothy grass, mugwort, cat, dog, horse, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, and mold.

^b Immunoglobulin E antibodies to cow's milk, egg, fish, wheat, soy, and peanut.

^c n = 169.

^d n = 171.

However, parental cleaning of the pacifier by sucking it was strongly associated with the risk of allergy development. Both eczema (OR 0.37; 95% confidence interval [CI] 0.15–0.91; $P = .02$) and asthma (OR 0.12; 95% CI 0.01–0.99; $P = .03$) were strongly reduced in children whose parents had this habit. There was also a trend toward lower risk of

sensitization in this group (OR 0.37; 95% CI 0.10–1.27; $P = .08$) (Fig 1). Furthermore, the blood eosinophil count at 18 months of age was also lower in children whose parents sucked on their pacifier than in other pacifier-using children (Fig 2).

Parental Pacifier Sucking and Respiratory Infections in the Child

Respiratory infections in the child's first 6 months were recorded by parents and reported at the 6-month telephone interview. On average, 1.5 respiratory infections per child were noted by the parents (CI 1.3–1.7). This frequency did not differ between children whose parents sucked their pacifier (1.4) and other pacifier-using children (1.5; ANOVA, $P = .75$).

Analysis of Confounding Factors

We analyzed the relationship between parental pacifier sucking and possible confounding factors. Parents of vaginally delivered infants were significantly more likely to suck the child's pacifier than parents of infants delivered by cesarean (Fisher's exact test, $P = .02$; Table 4). Conversely, mothers with a university-level degree tended to be less likely to suck the child's pacifier, although this association was not significant (Table 4). After logistic regression to adjust for delivery mode and mother's education, the protective

effect of parental pacifier sucking on eczema development during the child's first 18 months of life remained (OR 0.27, 95% CI 0.086–0.819; $P = .02$). The protective effect on asthma could not be analyzed in this way because of the low number of cases.

We speculated that delivery via the vaginal route, which exposes the infant to the maternal vaginal microbiota, and parent/infant pacifier sharing, which leads to exposure to parental oral microbiota, may confer additive protective effects against allergy development by exposure to a variety of commensal microbes. We thus stratified the cohort into 3 groups: (1) vaginally delivered infants with pacifier-sucking parents; (2) cesarean-delivered infants whose parents did not suck their pacifiers; and (3) infants who were either vaginally delivered or exposed to parents' oral microbiota by pacifier sharing. We then compared the prevalence of eczema in infants at 18 months of age in these 3 groups (Fig 3). The group exposed to both maternal vaginal and parental oral microbiota had the lowest prevalence of eczema (20%), whereas infants exposed to neither maternal vaginal or parental oral microbiota had the highest prevalence (54%). The group of children who were either vaginally delivered or whose parents sucked their pacifiers had an intermediate eczema prevalence (31%) (Fig 3).

Parental Pacifier Sucking and Allergy During the Child's First 36 Months of Life

The children in the cohort were followed up at 36 months of age. Kaplan-Meier curves were calculated for eczema, asthma, and sensitization (Fig 4). Development of eczema up to 36 months of age was significantly less likely in infants whose parents sucked on their pacifiers during their first 6 months of life, as compared with other pacifier-using children ($P = .04$). The protective effect of parental pacifier sucking on

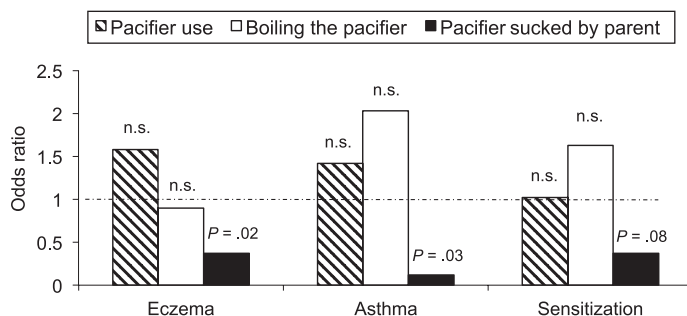


FIGURE 1

The relationship between atopy or allergy in children at 18 months of age and in relation to pacifier use and different pacifier cleaning practices. ORs are given for pacifier versus no pacifier use. For children using a pacifier, the way of cleaning the pacifier was compared with those not using that specific cleaning method. Ns, not significant.

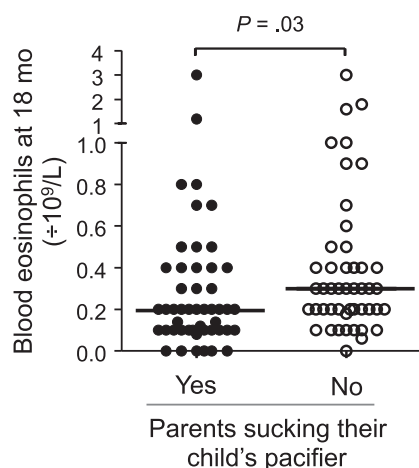


FIGURE 2

Blood eosinophil counts at 18 months of age for pacifier-using children whose parents cleaned their pacifier by sucking it compared with those whose parents did not suck the pacifier

asthma and sensitization was not statistically significant.

Parental Pacifier Sucking and Child's Salivary Microbiota at 4 Months Old

If parents' salivary microbes are transferred to the infant via the pacifier, it may affect development of the infant's

salivary microbiota. This hypothesis was examined by fingerprinting bacterial DNA in infants' saliva, which had been sampled from 64 infants at 4 months of age. We excluded cesarean-delivered infants in this analysis, because their oral microbiota has been shown to differ from that of vaginally delivered infants,¹⁶ and because mode of delivery was associated with parental sucking of the infant's pacifier. After exclusion of cesarean-delivered infants and nonpacifier users, 33 infants remained, 21 of them had parents who sucked on their pacifier.

T-RFLP analysis of bacterial DNA from infants' saliva was used to identify different bacterial groups and orthogonal projection onto latent structures, a regression variety of principal component analysis, to reveal whether infants whose parents cleaned their pacifier by sucking it could be separated from those whose parents did not have this habit, based on microbiota composition. Figure 5 shows that these 2 groups could be distinguished based on the pattern of microbes in their

saliva. One infant (outlier bottom left) had a microbiota clearly deviant from the others.

DISCUSSION

Early acquisition of a complex intestinal microbiota has been identified to provide protection against allergy development, suggesting that commensal bacteria may provide the developing immune system with crucial signals for its proper maturation.⁷⁻⁹ Here, we demonstrate that a common parental practice, sucking on the infant's pacifier before it is given back to the infant, is associated with protection against early eczema development and asthma symptoms. Furthermore, the blood eosinophil counts were lower at 18 months of age in children whose parents sucked on their pacifiers, compared with other pacifier-using children whose parents did not have this habit.

It is possible that the observed effect of parental pacifier sucking is due to the transfer of oral bacteria from the parents, via the pacifier, to their infant. By no doubt, this habit allows for a close oral contact between parents and child, facilitating bacterial transfer at a very young age, before the child starts to use spoons and so forth. Using T-RFLP as a fingerprinting method to characterize the infant's salivary bacteria, we have obtained suggestive evidence that this practice influenced the infant's oral microbiota composition. T-RFLP is a suitable method for depicting complex bacterial communities; cleaving of total bacterial DNA generates a series of peaks, each of which represents a bacterial group (usually a genus). As identification of T-RFLP peaks requires a substantial amount of additional work, we do not, at present, know the identity of the microbes that are more or less prevalent in the saliva of the 2 groups of infants.

We examined whether selection bias could have caused the association. The

TABLE 4 Analysis of Factors Associated With Parental Sucking of the Child's Pacifier

Factor	Factor Present	Parents Sucking the Child's Pacifier		P Value ^a
		Yes, n (%)	No, n (%)	
Mother with a history of allergy ^b	Yes	35 (55)	27 (47)	.47
	No	29 (45)	31 (53)	
Father with a history of allergy ^b	Yes	25 (39)	23 (40)	1.00
	No	39 (61)	35 (60)	
Mother smoking at time of birth	Yes	5 (8)	5 (9)	1.00
	No	58 (92)	53 (91)	
Father smoking at time of birth	Yes	6 (9)	3 (5)	.50
	No	58 (91)	55 (95)	
Siblings at time of birth	Yes	30 (47)	33 (57)	.28
	No	34 (53)	25 (43)	
Delivered by caesarean section	Yes	5 (8)	13 (23)	.02
	No	59 (92)	43 (77)	
Exclusively breastfed for >4 mo	Yes	22 (34)	19 (33)	1.00
	No	43 (66)	39 (67)	
Cat or dog in home at time of birth	Yes	9 (14)	6 (11)	.78
	No	55 (86)	50 (89)	
Mother with University degree ^c	Yes	16 (44)	20 (65)	.14
	No	20 (56)	11 (35)	
Father with University degree ^c	Yes	14 (41)	16 (57)	.31
	No	20 (59)	12 (43)	

^a Exact test.

^b A history of doctor-diagnosed asthma, allergic rhinoconjunctivitis, or eczema.

^c University degree or equivalent.

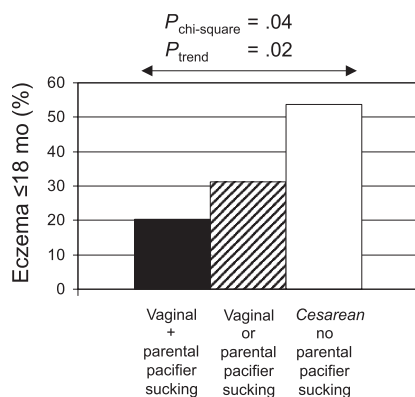


FIGURE 3

The prevalence of eczema at 18 months in the cohort when stratified as (1) vaginally delivered infants with pacifier-sucking parents (*black bar*; $n = 59$); (2) infants who were either vaginally delivered or exposed to parents' oral microbiota by pacifier sharing (*hatched bar*; $n = 48$); and (3) cesarean-delivered infants whose parents did not suck on their pacifier (*open bar*; $n = 13$)

parental practice of cleaning the child's pacifier by sucking it was neither associated with parental allergy, duration of breastfeeding, smoking, or pet keeping. However, it was significantly positively associated with the infant

having been born vaginally. The basis for this association can only be speculated on. During a vaginal delivery, the infant is exposed to bodily fluids and sometimes soiled with feces; after a normal delivery, the mother and infant will be speedily discharged from the hospital. In contrast, cesarean delivery is a strictly sterile surgical procedure followed by hospital stay and substantial medical attention. Theoretically, the "sterility culture" of the hospital may be transmitted to the parents and influence their subsequent care of their infant, even when they return home. It cannot be excluded that parental pacifier sucking is a marker of a common general behavior of close oral contact, allowing for bacterial transfer from parent to child by other oral routes, such as spoons, feeding bottles, kisses, and so forth. As a consequence, parents who do not suck their infant's pacifier might be generally more cautious against bacterial transfer.

Importantly, however, the protective effect against eczema of parental pacifier sucking remained after controlling for delivery mode by multiple regression analysis. Furthermore, vaginal delivery and parental pacifier sucking conferred additive and independent protective effects against allergy development in the infant. Hence, the prevalence of eczema was ~2.5 times lower at 18 months of age in vaginally delivered children whose parents sucked their pacifiers than in cesarean-delivered children whose parents did not have this habit (20% vs 54%); an intermediate protective effect was seen in children exposed to either vaginal delivery or parental pacifier sucking. Thus, vaginal delivery, which is a source for transfer of a complex microbiota from mother to infant¹⁷ and parent and infant sharing of a pacifier might both lead to microbial stimulation, with beneficial effects on allergy development. Infants delivered by cesarean have an increased risk of asthma^{10–12} and sensitization¹¹ and may benefit the most from the increased bacterial exposure linked to parental pacifier sucking. This is in line with the suggestion that "children delivered by caesarean section could be targeted for future preventive interventions, such as the use of probiotics."¹¹ Respiratory pathogens might be transferred by parental pacifier sucking, but we found no increased respiratory infection rate in their children. Another concern is that cariogenic bacteria may be transferred.¹⁸ However, caries seems to be unrelated to pacifier use¹⁹ and may even be negatively associated with "close" salivary contact between infant and parent.²⁰ Transfer of a complex microbiota from parent to infant may help build up resistance to colonization with more pathogenic bacteria.

The relatively small scale of the current study may be a potential weakness. Negative findings could be a result of

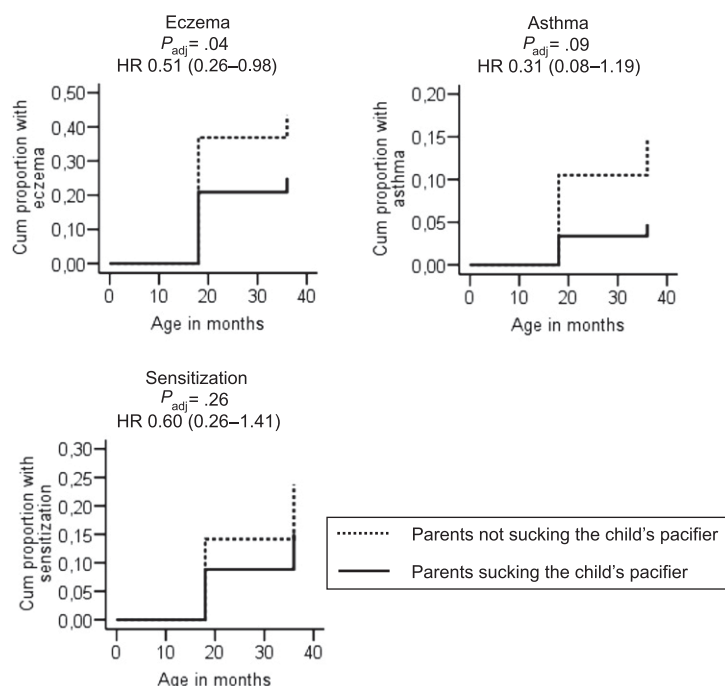
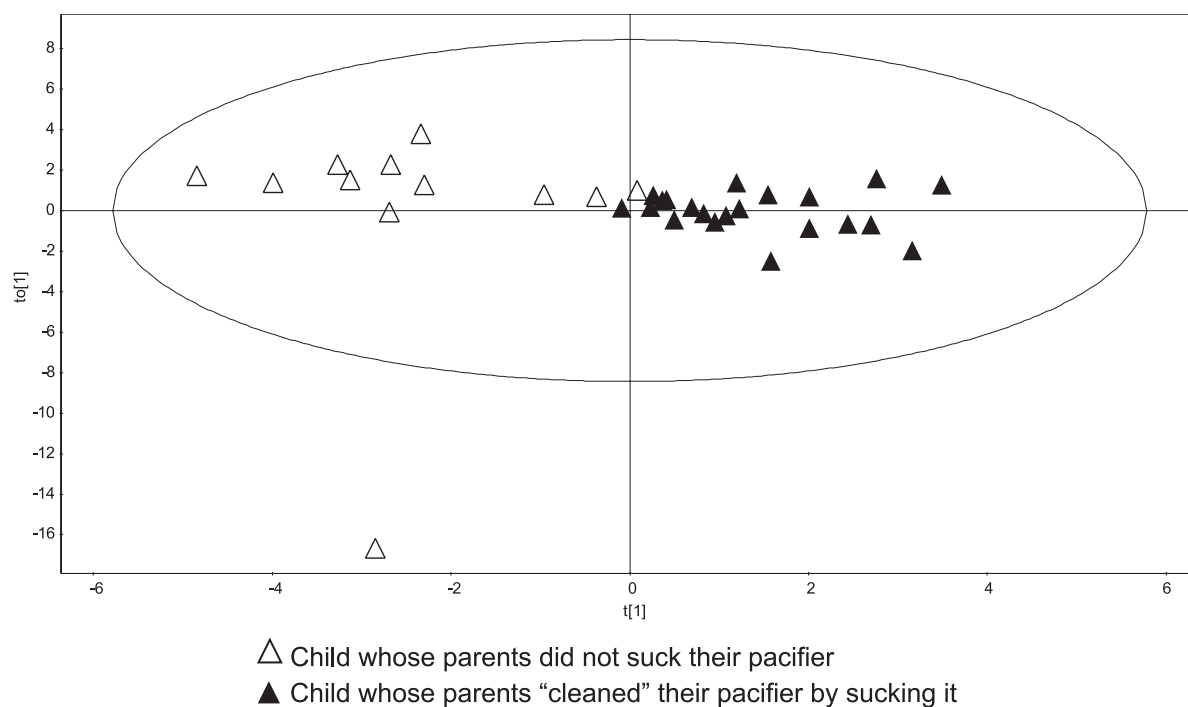


FIGURE 4

Kaplan-Meier curves for the development of eczema, asthma, and sensitization during the child's first 36 months. Hazard Ratios (HR, 95% confidence limits) and P values were adjusted by Cox regression for mode of delivery (vaginal versus cesarean).

**FIGURE 5**

The oral microbiota of children at 4 months of age in relation to parents' habit of cleaning the pacifier. Each symbol represents 1 infant and its position is based on the totality of bacterial taxa (genus/species) in saliva as generated by T-RFLP (digestion of total bacterial DNA and separation of the fragments). The data were analyzed by orthogonal projection to latent structures, a variety of principal component regression analysis, with parental pacifier sucking as the Y-variable, and the totality of bacterial taxa as X-variables. The first component, representing the largest variations among the X-variables, is represented along the horizontal axis. The second component, depicted along the vertical axis, represents the second largest variations among the X-variables.

the small sample size. However, the size of the study is also its strength, as it was possible to use a very detailed and structured follow-up. Diaries were used by the parents and structured interviews were conducted every 6 months. Children with signs and symptoms suggestive of allergic disease were examined by a study pediatrician within a few days, and diagnoses were all based on strict criteria. For eczema, we used the validated criteria of Williams.¹⁴ Asthma in early childhood is notoriously difficult to distinguish from viral wheeze, which is unlikely to progress to true asthma.²¹ Our criteria for an asthma diagnosis are based on previous studies demonstrating that atopy and/or symptoms between episodes^{22,23} and eczema²⁴ are significant indicators of asthma in young infants with wheezing, especially persistent asthma with onset in early childhood.²⁵ Even though

this is far from the strict asthma criteria used in older children and adults, the criteria used are probably the best available without using infant lung function tests and invasive methods. With these limitations in mind, it is important to emphasize the need to re-evaluate the findings from the study in larger studies, and in older children with a better-defined asthma and an established atopic profile. A follow-up of the cohort is ongoing, in which both lung function and airway reactivity are tested.

Oral tolerance is the normal physiologic response to harmless proteins, and it has been known for decades that the presence of commensal microbiota is a prerequisite for the development of normal tolerance against harmless protein antigens.²⁶ Hitherto, attention has almost exclusively focused on the small intestine as the inductive site for

oral tolerance and the influence of gut microbiota on handling of dietary antigens. However, the oral cavity is actually the first site of encounter between foreign antigens and the lymphoid system and allows the lymphoid to sample antigens that have not been denatured by acid and cut by digestive enzymes. The oropharynx is surrounded by dense lymphoid tissue (ie, the adenoids) and palatine and pharyngeal tonsils. Similar to the gut-associated lymphoid tissues, these are covered by M-cells that are specialized in antigen uptake and delivery to antigen-presenting cells and lymphocytes beneath the epithelium.²⁷ Furthermore, dendritic cells capture antigens in the oral mucosa and migrate to cervico-mandibular lymph nodes,²⁸ similar to the way dietary antigens are carried to the mesenteric lymph nodes. The tonsils are rich in T cells with a regulatory phenotype,²⁹

and applications of contact allergens on the oral mucosa leads to active tolerance induction.³⁰ Thus, there is no reason to believe that active oral tolerance could not be induced already in the oral cavity. Exposure of the infant to parental saliva might accelerate development of a complex oral/pharyngeal microbiota that, similar to a complex gut microbiota,^{7–9} might beneficially affect tolerogenic handling of antigens by

the oral/pharyngeal lymphoid tissues. Moreover, oral bacteria are swallowed and hence also affect the composition of the microbiota in the small intestine, which may in turn regulate tolerance development in the gut. Further studies are now required to establish if parental pacifier sucking could be a simple and safe method to reduce allergy development in infants and young children, as our study suggests.

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Pacifier Cleaning Practices and Risk of Allergy Development

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