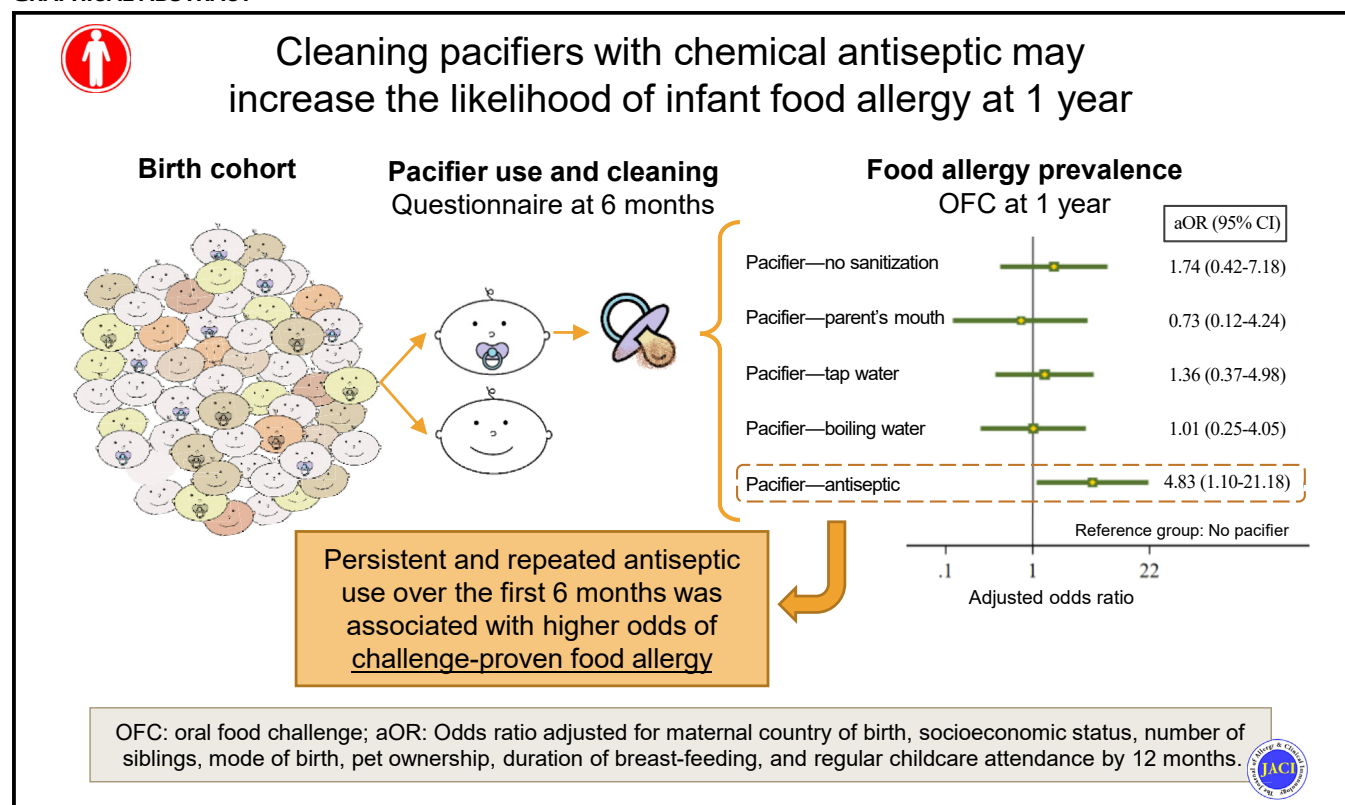


Infant pacifier sanitization and risk of challenge-proven food allergy: A cohort study

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GRAPHICAL ABSTRACT



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Background: Environmental microbial exposure plays a role in immune system development and susceptibility to food allergy. **Objective:** We sought to investigate whether infant pacifier use during the first postnatal year, with further consideration of sanitization, alters the risk of food allergy by age 1 year.

Methods: The birth cohort recruited pregnant mothers at under 28 weeks' gestation in southeast Australia, with 894 families followed up when infants turned 1 year. Infants were excluded if born under 32 weeks, with a serious illness, major congenital malformation, or genetic disease. Questionnaire data, collected at recruitment and infant ages 1, 6, and 12 months, included pacifier use and pacifier sanitization (defined as the joint exposure of a pacifier and cleaning methods). Challenge-proven food allergy was assessed at 12 months.

Results: Any pacifier use at 6 months was associated with food allergy (adjusted odds ratio, 1.94; 95% CI, 1.04-3.61), but not pacifier use at other ages. This overall association was driven by the joint exposure of pacifier-antiseptic use (adjusted odds ratio, 4.83; 95% CI, 1.10-21.18) compared with no pacifier use. Using pacifiers without antiseptic at 6 months was not associated with food allergy. Among pacifier users, antiseptic cleaning was still associated with food allergy (adjusted odds ratio, 3.56; 95% CI, 1.18-10.77) compared with no antiseptic use. Furthermore, persistent and repeated antiseptic use over the first 6 months was associated with higher food allergy risk ($P = .029$).

Conclusions: This is the first report of a pacifier-antiseptic combination being associated with a higher risk of subsequent food allergy. Future work should investigate underlying biological pathways. (J Allergy Clin Immunol 2021;■■■:■■■-■■■.)

Key words: Pacifier, dummy, food allergy, birth cohort, microbial exposure, sanitization, antiseptic

Food allergy (FA) is a serious disease that can cause life-threatening anaphylaxis due to an immune-mediated reaction to food proteins. FAs commence in infancy, but prevention methods are still not well understood. Maternal and infant gut microbiome have been repeatedly studied in relation to immune regulation and susceptibility to allergic disease. Prenatal pet exposure has been shown to influence differentiation of regulatory T immune cells *in utero*.^{1,2} *Prevotella copri* in maternal gut microbiota during pregnancy is associated with decreased likelihood of allergic disease in the infant.³ Early-life gut microbiota have been reported to suppress IgE production and, in mouse models, linked to resistance to FA.^{4,5} Various microbial exposures, including vaginal births, siblings, dog ownership, and infant antibiotics, have been associated with a decreased risk of FA.⁶⁻⁸ Little is known about the relationship between oral microbial exposures, local immune mechanisms of tolerance induction, and IgE-mediated FA.

Pacifiers (also known as dummies or soothers), commonly used in infancy and recommended in the United States,^{9,10} are another source of microbial exposure in early life.⁹⁻¹¹ A Swedish birth cohort found potential benefits of shared microbial exposure as infants whose parents sucked their pacifier (ie, used their own mouth to clean it) had reduced food sensitization, asthma, and eczema at 18 months compared with infants whose parents used other cleaning methods.¹² Another cohort study identified lower total IgE levels in children from 10 to 18 months if mothers reported sucking the infants' pacifier ($n = 9$ of 74) at 6 months compared with not sucking their pacifier.¹³ Despite most infant

Abbreviations used

aOR: Adjusted odds ratio
BIS: Barwon Infant Study
FA: Food allergy
OR: Odds ratio

pacifiers being cleaned in some way, no previous work has finely classified pacifier use in the context of the associated cleaning method (sanitization), nor has challenge-proven FA been examined. Here, we define "pacifier sanitization" as the joint exposure of a pacifier and a specific cleaning method (eg, tap water and chemical antiseptic). The Barwon Infant Study (BIS) is a population-based cohort study of 1074 infants that measured FA outcomes.^{3,14,15} We aimed to investigate combinations of infant pacifier sanitization in the first year of life in association with FA prevalence at age 1 year.

METHODS

Study design

The BIS is a population-based birth cohort study of 1074 infants. Women were recruited antenatally from 2 main hospitals in the Barwon region in southeast Australia. The cohort has been described elsewhere.¹⁵ Briefly, pregnant mothers older than 18 years and at less than 28 weeks' gestation were recruited, with infants excluded if born before 32 weeks, with a serious illness, major congenital malformation, or genetic disease. Participating mothers were required to give informed consent, complete questionnaires without assistance of an interpreter, and be an Australian permanent resident. Ninety percent of mothers approached were eligible, and 33% of those invited participated in the study. A total of 1074 mother-infant pairs were recruited at baseline, with 894 followed up when infants turned 1 year of age.

This study was approved by the Barwon Health Human Research and Ethics Committee (HREC 10/24). Parents or guardians provided written informed consent.

Data collection

Participants data was collected at the 28-week antenatal, baseline appointment and followed up when the infant was 1, 6, and 12 months old. Parents were asked to provide information on demographic measures and lifestyle choices (for further details, see this article's **Methods** section in the Online Repository at www.jacionline.org and **Box E1** in this article's Online Repository at www.jacionline.org). FA outcomes were assessed at the 12-month appointment.

Definitions. Pacifier use alone: Parents were asked, "Does your baby use a dummy/pacifier?"

Pacifier sanitization: Parents were asked, "When your baby's dummy/pacifier drops to the floor, do you usually (ie, more than half the time)..." and to select one of the options: (a) Give it back to your baby without washing; (b) Put in own mouth before giving back to baby; (c) Wash it under tap water; (d) Wash in boiling water; (e) Wash/sterilize with antiseptic agent.

Here, we define "pacifier sanitization" as the joint exposure of a pacifier and a specific cleaning method (eg, tap water or antiseptic). We also grouped the above options (a) to (d) into "pacifier-no-antiseptic" versus only option (e), "pacifier-antiseptic."

Socioeconomic status: Index of relative socioeconomic disadvantage by statistical area 1 from the socioeconomic indexes for areas by the Australian Bureau of Statistics 2011.

Biological samples. Fecal samples were collected from mothers at 36 weeks' gestation and infants at 6 and 12 months. The 16S rRNA V4 region of *P copri* was analyzed from fecal samples, with methods previously described.³

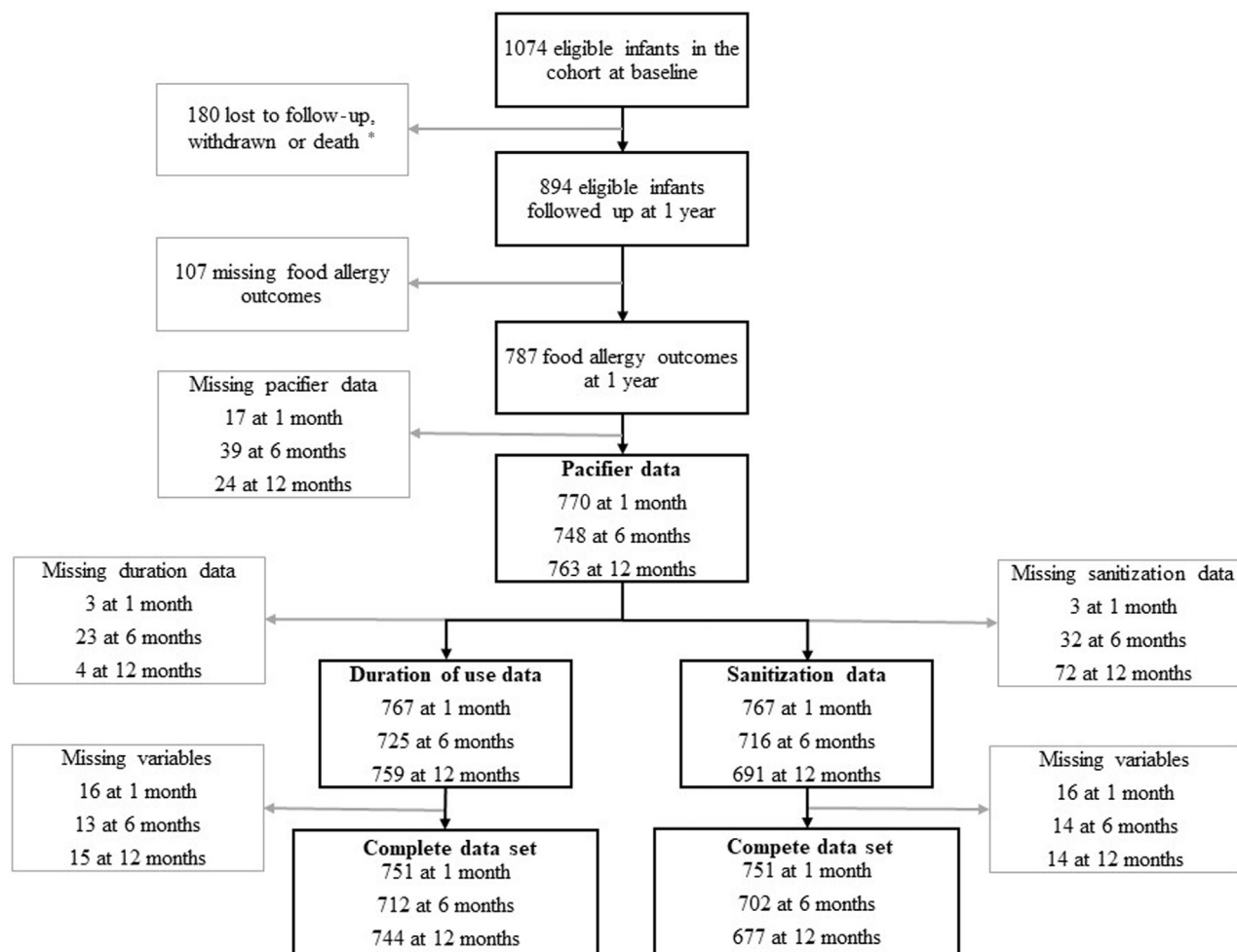


FIG 1. Flowchart of participation by exposure and outcome variables when the infant is 1, 6, and 12 months old. Complete data set indicates our sample of participants with complete data on exposures, outcomes, and potential confounders. Variables refer to the minimal confounder set. *Details previously reported in the study cohort profile.¹⁵

Outcome measure: FA at 1 year. FA was tested by oral food challenge at 1 year ($n = 894$ followed up at 1 year) with methods previously described.¹⁴ Briefly, children underwent skin prick tests to negative and positive controls (saline and histamine) and common allergenic foods: cow's milk, egg, peanut, cashew, and/or sesame. FA was defined as a positive skin prick test result with wheals of 1 mm or greater than those achieved with the negative control, confirmed by a positive oral food challenge, unless there was a clear recent history of an immediate-type reaction to the food.¹⁵ Three infants were classified as food allergic on the basis of a history of IgE-mediated reaction in the 2 preceding months.¹⁴ Oral food challenges were undertaken on those with a detectable skin prick test wheal size according to standardized, hospital-based challenge protocols, with predetermined stopping criteria established by the HealthNuts study.¹⁶⁻¹⁸ Challenges involved giving the child increasing doses of the food until reaching the maximum dose on day 1 in the hospital, with continued ingestion of the maximum dose at home until day 7.

Statistical analysis

A longitudinal regression model was used to assess the association between exposure to pacifier use at 1, 6, and 12 months, including sanitization methods, and the risk of FA at 12 months, including all exposure time points in the model.¹⁹ Missing data were not treated in any way, and only participants with complete records were included in analyses; however, only a few variables

were missing for a few participants in the analyses. Logistic regression models were adjusted for a minimum set of confounders, chosen a priori and informed by a directed acyclic graph¹⁹ (DAGitty, version 2.3; see Fig E1 in this article's Online Repository at www.jacionline.org): socioeconomic status, regular childcare attendance by 12 months, duration of breast-feeding, mode of birth, maternal country of birth, pet ownership, and number of siblings.²⁰ Regression outputs were presented as odds ratios (ORs) or adjusted odds ratios (aORs). Based on the disjunctive causal criteria for confounding,²⁰ additional confounding variables were examined as sensitivity analyses, particularly those already identified to be FA predictors in this cohort.^{3,14,21,22} The following confounders were individually added to the multivariable model and were retained if the aOR changed by greater than 10% as per the change-in-estimates method.²³ These factors were father's country of birth, age of peanut and egg introduction, doctor-diagnosed eczema by age 6 months, dog ownership, parental sucking of child utensils, use of unboiled water, home disinfectant use or home chlorine use during pregnancy or the child's first year of life, and presence of *P. copri* in the maternal microbiome during pregnancy or the child microbiome in the first year of life. We evaluated the area under the receiver-operating characteristic curve and Hosmer-Lemeshow goodness-of-fit for all the primary logistic regression models.

We performed multiple sensitivity analyses by performing logistic regression analyses, adjusted to the minimum set of confounders as described above, to restricted populations or exposure groups. To examine whether associations were due to any pacifier use alone or the added antiseptic, we

examined the effect of sanitization among pacifier users only. Among persistent pacifier users, we used a multivariable logistic regression model to assess the frequency of pacifier-antiseptic use over the first 6 months as an ordinal variable, adjusting for the same covariates indicated previously. This ordinal variable was treated first as categorical (results per-level), then as continuous (trend result). Two-sample comparisons of categorical variables were assessed using the chi-square test for contingency tables. All analyses were conducted with the statistical software package Stata (release 15; StataCorp, College Station, Tex).

RESULTS

Sample characteristics

The BIS cohort began with 1074 infants at baseline, 894 (83.2%) of whom were followed up at 1 year (Fig 1). FA outcomes were measured in 787 infants (88.0%), with 7.7% ($n = 61$ of 787) diagnosed as food allergic at 1 year.¹⁴ Most infants were reported allergic to egg (68.5%), with only a few allergic to peanut, cashew, sesame, and cow's milk ($n = 6, 1, 1,$ and 2 , respectively).¹⁴ Although this cohort was set up before the development of this analysis, we have 80% power to detect a 10.3% increase in FA prevalence, from 5.5% to 15.8% with a sample size of 787 participants, of whom 61 are food allergic ($\alpha = 0.05$). Missing data are presented in Figure 1. All the final, primary models corresponded to receiver-operating characteristic curves, with an area under the curve of more than 73% and high P values in goodness-of-fit when evaluated with Hosmer-Lemeshow tests, indicating our models were good fits (see Table E1 in this article's Online Repository at www.jacionline.org).¹⁵ Infants in this sample (with FA outcomes at 1 year and complete demographic data) were representative of the entire baseline cohort, with 51.0% males, and 91.1% of mothers born in Australia (Table I). Additional characteristics related to microbial and chemical exposures by 6 months showed that more pacifier-antiseptic users owned dogs, but had similar postnatal hygiene (see Table E2 in this article's Online Repository at www.jacionline.org). Pacifier-antiseptic users were also more likely to be breast-fed for less than 6 months and attend childcare before 12 months. Pacifier use decreased with age, and, among those using pacifiers, most parents reported sanitizing the pacifiers in some way (Fig 2; 96.5%, 80.7%, and 56.6% sanitized at 1, 6, and 12 months, respectively).

Entire cohort: The joint exposure of pacifier and cleaning method and FA

Any pacifier use at 6 months was associated with FA at 12 months (aOR, 1.94; 95% CI, 1.04-3.61; $P = .037$), but not pacifier use at 1 or 12 months (data not shown).

The joint exposure of pacifier and cleaning method versus no pacifier use appeared to be key, highlighting the importance of considering combined exposure. We found that the association between any pacifier use at 6 months and FA was driven by the pacifier-antiseptic exposure. Other categories, including pacifier-own mouth and pacifier-boiling water, were not associated with risk of FA (Fig 3). In fact, among all the categories and time points, a higher risk of FA was only evident for pacifier-antiseptic use at age 6 months (aOR, 4.83; 95% CI, 1.10-21.18; Fig 3) compared with no pacifier (for other time points and unadjusted OR, see Tables E3-E6 in this article's Online Repository at www.jacionline.org). Associations persisted after adjusting for additional potential confounders.

TABLE I. Characteristics of participants compared with the whole baseline cohort

Demographic	Participants	Baseline Cohort
No.	787	1074
Family characteristics		
Socioeconomic disadvantage 2011,* quintile (N=1061)		
Low (most disadvantaged)	132 (17.0)	215 (20.3)
2	160 (20.6)	211 (19.9)
3	157 (20.2)	211 (19.9)
4	163 (21.0)	215 (20.3)
High (most advantaged)	165 (21.2)	209 (19.7)
Mother's education level (N = 1068)		
No university degree	350 (44.6)	520 (48.7)
University degree	434 (55.4)	548 (51.3)
Mother's country of birth (N = 1072)		
Australia	717 (91.1)	967 (90.2)
Asia or Middle East	13 (1.7)	23 (2.1)
Other	57 (7.2)	82 (7.6)
Father's country of birth (N = 1035)		
Australia	685 (90.1)	924 (89.3)
Asia or Middle East	9 (1.2)	18 (1.7)
Other	66 (8.7)	93 (9.0)
Mother's age at conception (y) (N = 1074)		
Mean $\pm$ SD	31.9 $\pm$ 4.5	31.3 $\pm$ 4.8
Siblings (N = 1071)		
No siblings	321 (40.8)	450 (42.0)
One sibling	280 (35.6)	382 (35.7)
Two siblings	146 (18.6)	184 (17.2)
Three or more siblings	40 (5.1)	55 (5.1)
Child characteristics		
Sex		
Female	386 (49.0)	519 (48.3)
Male	401 (51.0)	555 (51.7)
Gestation (wk) (N = 1074)		
32-36	36 (4.6)	47 (4.4)
37-42	751 (95.4)	1027 (95.6)
Birthweight (g) (N = 1073)		
Mean $\pm$ SD	3534.5 $\pm$ 527.2	3527.6 $\pm$ 519.3
Method of birth (N = 1072)		
Vaginal	533 (67.7)	739 (68.9)
Unplanned cesarean section	110 (14.0)	155 (14.5)
Planned cesarean section	144 (18.3)	178 (16.6)
Infant ancestry (N = 841)		
British/Irish/European	575 (73.6)	620 (73.7)
Mixed	181 (23.2)	191 (22.7)
Non-British/Irish/European	9 (1.2)	12 (1.4)
Aboriginal/Torres Strait Islander	16 (2.0)	18 (2.1)

Value are n (%) unless otherwise indicated.

*Index of relative socioeconomic disadvantage by statistical area 1 from the Socio-Economic Indexes for Areas by the Australian Bureau of Statistics 2011.

Among pacifier users: Antiseptics and FA

Among the pacifier user subgroup, antiseptic compared with no antiseptic use was also associated with an increased risk of FA (aOR, 3.56; 95% CI, 1.18-10.77; $P = .025$), ruling out the association at 6 months being due to pacifier use alone. Associations persisted after adjusting for additional potential confounders, as well as daily duration of use. Among pacifier users, use of own mouth was inversely associated with FA compared with nonantiseptic cleaning methods (own mouth, tap water, boiling water, or none); however, the 95% CI was very wide and crossed the null (aOR, 0.49; 95% CI, 0.14-1.75) (see Table E7 in this article's Online Repository at www.jacionline.org).

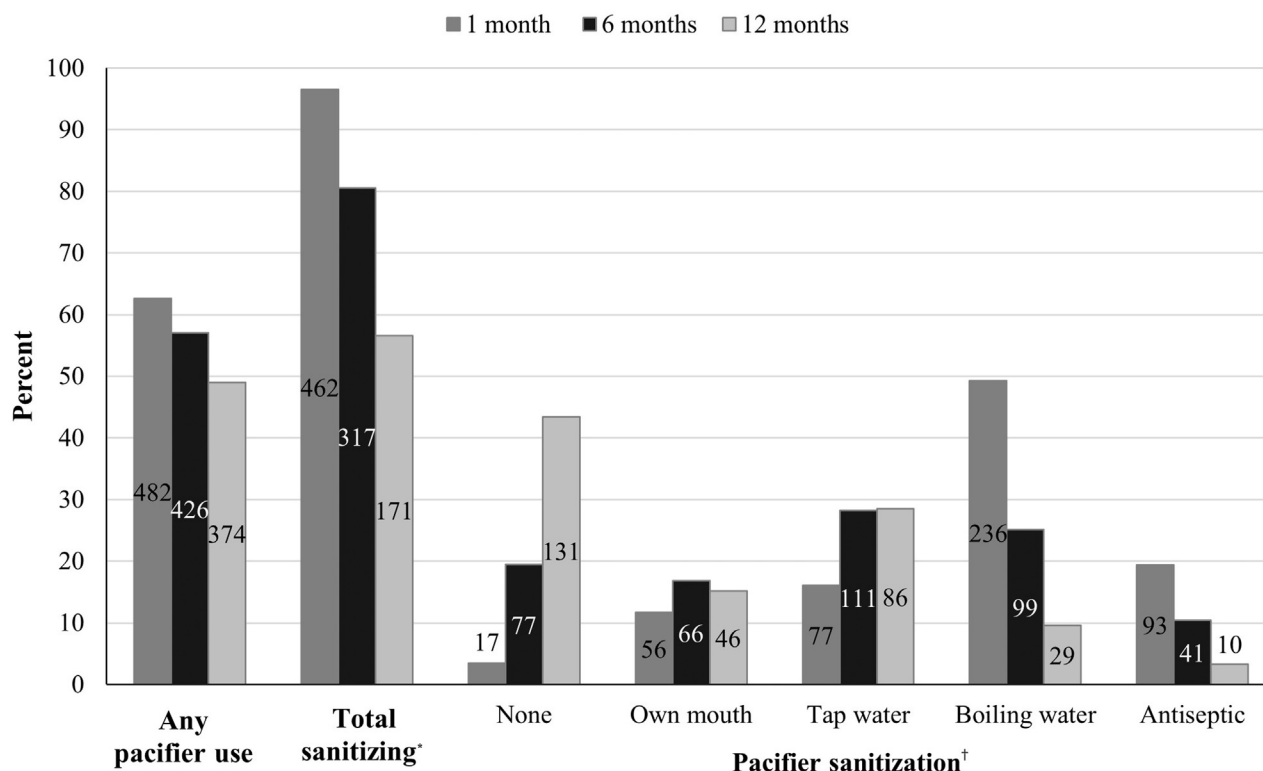


FIG 2. Pacifier use and sanitization at 1, 6, and 12 months (n = 770, 770, 763, respectively). *Percent sanitizing among pacifier users. †Percent among participants sanitizing.

Among persistent pacifier users: Length of antiseptic use

There was high persistence of pacifier use in the first 6 months of life, with most infants (77.3%; 95% CI, 73.6%-80.7%; n = 432 of 559) who used a pacifier at 1 month still using it at 6 months (defined persistent pacifier users). Among these persistent pacifier users, we observed a trend across categories ($P = .029$; Table II), with the highest aOR evident for those with persistent antiseptic use (aOR, 5.66; 95% CI, 1.56-20.50).

Additional analyses in the birth cohort

We examined pacifier sanitization among infants with both FA and eczema, commonly co-occurring allergic outcomes, and found that 22.2% (6 of 27) had been given chemically sanitized pacifiers. Only 5.1% (35 of 686) of children without co-occurring FA and eczema had been given chemically sanitized pacifiers ($P < .001$).

There was also no correlation between pacifier-antiseptic use at 6 months and the presence of *P copri* microbiota in maternal or infant stool (data not shown), though the sample sizes for prenatal, 6-month, and 12-month *P copri* in stool here were very small (n/N = 53/293, 16/338, and 47/292, respectively).³

DISCUSSION

We observed that half of our cohort was still using pacifiers by age 12 months. Pacifier sanitization decreased with age, with 1- and 6-month-old infants more likely to have their pacifier

sanitized than those at 12 months. Using a pacifier increased the likelihood of FA only if used at 6 months. This increased likelihood of FA with any pacifier use at 6 months was driven by the joint exposure of pacifier-antiseptic use, the only joint pacifier sanitization exposure associated with FA. Other pacifier sanitization methods were not found to be associated to FA. Even among pacifier users at 6 months, antiseptic predicted FA. We also observed a trend of increased FA with continued use of antiseptics over the first 6 months of life.

The findings here have some causal features.²⁴ The magnitude of the prospective association is high. Dose-response trends are evident regarding increasing daily use in hours and increasing length of use over the first 6 months. There is also plausible biological evidence that oral and gut microbiota play a role in early-life development of the immune system and susceptibility to FA, particularly at 6 months.^{3,6,25} An infant's gut microbiome matures throughout their first year of life, with critical changes occurring as infants are weaned onto solid foods around 6 months.^{26,27} Some microbial species promote or protect against inflammatory environments via the development of T_H2 response producing IgE or regulatory T-cell response suppressing IgE production and this balance shapes the mucosal immune system.⁴ Age of exposure has been important for other allergy risk factors, such as the protective effect of introduction of allergenic foods before 12 months, so it is plausible that the effect of exposure to antiseptics on pacifiers on allergic disease would be age-dependent.^{28,29}

The association identified between pacifier-antiseptic and an increased likelihood of FA prompts consideration of the underlying mechanism. We were unable to replicate the results of a

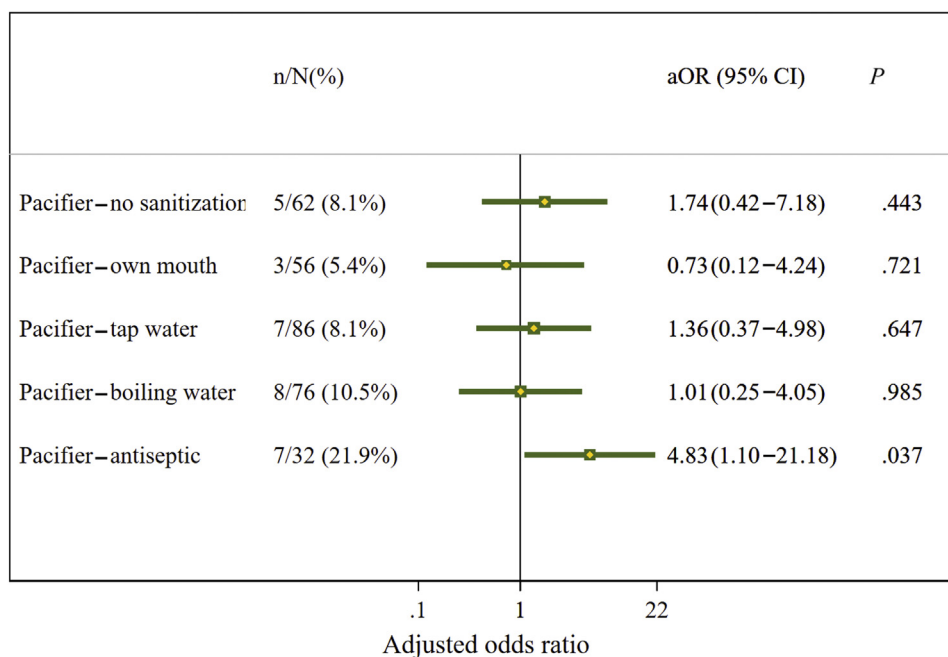


FIG 3. Joint pacifier sanitization categories at 6 months and risk of FA (n = 625). Comparator group is no pacifier use (n = 18 of 313 [5.8%]). OR adjusted for socioeconomic status, childcare, breast-feeding, mode of birth, maternal country of birth, pets, and siblings.

TABLE II. Pacifier-antiseptic use in the first 6 mo and FA risk among persistent pacifier users (n = 353)

Pacifier with antiseptic	No. (% food allergic)	aOR*	95% CI	P value
Never	18/244 (7.4%)	Reference group		
Once (1 or 6 mo)	7/88 (8.0%)	1.21	0.46-3.22	.699
Twice (1 and 6 mo)	5/21 (23.8%)	5.66	1.56-20.50	.008
Trend†				.029

*OR adjusted for socioeconomic status, childcare, breast-feeding, mode of birth, maternal country of birth, pets, and siblings.

†This ordinal variable was treated first as categorical (results per-level), then as continuous (trend result).

study by Hesselmar et al¹² showing that shared microbes via parental pacifier sucking (termed “own mouth” in this study) was protective against allergic disease at 18 months (eczema OR, 0.37, 95% CI, 0.15-0.91; asthma OR, 0.12, 95% CI, 0.01-0.99; sensitization OR, 0.37, 95% CI, 0.10-1.27, n = 184).¹² We did find a similar protective estimate against FA, although our estimate was imprecise due to small numbers, with the aOR crossing the null value of 1. Pacifier-antiseptic use would introduce the least microbes into the infant’s mouth compared with all other sanitization methods, with the exception of pacifier-boiling water; however, we did not observe similar results for the 2 methods, as we had expected. The disparate result with use of boiling water to clean pacifiers suggests that the mechanism may not be the lack of microbial introduction, because both cleaning methods should sterilize the pacifier. It is possible that some parents who reported “boiling” used previously boiled water to simply rinse the pacifier with, rather than submerging the pacifier continuously in boiling water for the 3 to 5 minutes required to kill all bacteria, introducing nondifferential misclassification. Alternatively, the chemicals in the antiseptic may be the component causing the higher FA risk, rather than the lack of sterilization.

The chemicals in the antiseptic could alter the oral microbial flora, because a different microbial pattern has been demonstrated for in

18-month-old infants with pacifiers cleaned by antiseptics compared with parental sucking.¹² We have not yet analyzed the oral microbiome in pacifier-antiseptic users; however, there was no association between pacifier-antiseptic use and *P copri* in maternal gut in pregnancy (previously found to be protective of FA), suggesting the antiseptic use may not be affecting *P copri*.³ Another possibility could be pacifier-antiseptic increases plasticizers leaching from the pacifier and, therefore, increases infant exposure to plasticizers, substances previously linked to allergic disease.³⁰

The strength of this study is the use of a birth cohort and longitudinal design that collected prospective data on pacifier sanitization and a broad range of other oral microbial and chemical exposures with low loss to follow-up considering how intensive the study was. We also performed objective oral food challenges to obtain FA outcomes. We have a large sample size, but FA is a rare outcome, so some analyses were restricted by sample size. We are limited by the pacifier sanitization question’s wording, which asked about the method used “more than 50% of the time,” rather than “ever,” restricting the application of our results. We also lack details on the type of antiseptic agents used to sanitize the pacifiers. It should be noted that pacifier-antiseptic use may be a marker for general high usage of chemicals, or high hygiene standards, in the home. We lack evidence to fully explain the mechanism between use of pacifiers with antiseptic and FA

and believe this study should be used as hypothesis generating or proof-of-concept that can be used to plan future studies.

In Australia, pacifier use is not actively encouraged or discouraged; rather, it is suggested each family does what suits it best; however, its use is recommended in the United States from 1 month onward to prevent sudden infant death syndrome.^{10,11,31,32} Importantly, pacifier use has not been shown to affect breast-feeding duration.³³ Although maintaining appropriate sterilization of pacifiers (technique unspecified) is recommended by Raising Children's Network in Australia until 6 months to protect infants from infection,³² we believe guidelines on pacifier use should consider the evidence on associated sterilization techniques, including the findings from this report. Further studies should focus on early-life oral exposure to antiseptic agents and how they affect the development of infant allergy and the infant oral and gut microbiome.

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Key messages

- Pacifiers sanitized with antiseptic agents at 6 months were a risk factor for subsequent challenge-proven FA in 1-year-old infants.
- Among pacifier users, antiseptic cleaning at 6 months was positively associated with FA, compared with no antiseptic use. Furthermore, persistent and repeated use of antiseptic cleaning over the first 6 months was associated with higher FA risk ($P = .029$).

REFERENCES

- Aichhbaumik N, Zoratti EM, Strickler R, Wegienka G, Ownby DR, Havstad S, et al. Prenatal exposure to household pets influences fetal immunoglobulin E production. *Clin Exp Allergy* 2008;38:1787-94.
- Fujimura KE, Sitarik AR, Havstad S, Lin DL, Levan S, Fadrosch D, et al. Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. *Nat Med* 2016;22:1187-91.
- Vuillermin PJ, O'Hely M, Collier F, Allen KJ, Tang MLK, Harrison LC, et al. Maternal carriage of *Prevotella* during pregnancy associates with protection against food allergy in the offspring. *Nat Commun* 2020;11:1452.
- Berin MC, Sampson HA. Mucosal immunology of food allergy. *Curr Biol* 2013;23:R389-400.
- Bunyavanich S, Berin MC. Food allergy and the microbiome: current understandings and future directions. *J Allergy Clin Immunol* 2019;144:1468-77.
- Marrs T, Bruce KD, Logan K, Rivett DW, Perkin MR, Lack G, et al. Is there an association between microbial exposure and food allergy? A systematic review. *Pediatr Allergy Immunol* 2013;24:311-20.e8.
- Martin PE, Eckert JK, Koplin JJ, Lowe AJ, Gurrin LC, Dharmage SC, et al. Which infants with eczema are at risk of food allergy? Results from a population-based cohort. *Clin Exp Allergy* 2015;45:255-64.
- Gupta RS, Singh AM, Walkner M, Caruso D, Bryce PJ, Wang X, et al. Hygiene factors associated with childhood food allergy and asthma. *Allergy Asthma Proc* 2016;37:e140-6.
- Comina E, Marion K, Renaud FN, Dore J, Bergeron E, Freney J. Pacifiers: a microbial reservoir. *Nurs Health Sci* 2006;8:216-23.
- Sexton S, Natale R. Risks and benefits of pacifiers. *Am Fam Phys* 2009;79:681-5.
- Moon RY. Task Force on Sudden Infant Death Syndrome. SIDS and other sleep-related infant deaths: expansion of recommendations for a safe infant sleeping environment. *Pediatrics* 2011;128:e1341-67.
- Hesselmar B, Sjöberg F, Saalman R, Aberg N, Adlerberth I, Wold AE. Pacifier cleaning practices and risk of allergy development. *Pediatrics* 2013;131:e1829-37.
- Abou-Jaoude E, Sitarik A, Havstad S, Ownby D, Jones K, Kim H, et al. Association between pacifier cleaning methods and child total IgE. *Ann Allergy Asthma Immunol* 2018;121:S47.
- Molloy J, Koplin JJ, Allen KJ, Tang MLK, Collier F, Carlin JB, et al. Vitamin D insufficiency in the first 6 months of infancy and challenge-proven IgE-mediated food allergy at 1 year of age: a case-cohort study. *Allergy* 2017;72:1222-31.
- Vuillermin P, Saffery R, Allen KJ, Carlin JB, Tang ML, Ranganathan S, et al. Cohort profile: the Barwon Infant Study. *Int J Epidemiol* 2015;44:1148-60.
- Koplin JJ, Tang MLK, Martin PE, Osborne NJ, Lowe AJ, Ponsonby A-L, et al. Pre-determined challenge eligibility and cessation criteria for oral food challenges in the HealthNuts population-based study of infants. *J Allergy Clin Immunol* 2012;129:1145-7.
- Osborne NJ, Koplin JJ, Martin PE, Gurrin LC, Thiele L, Tang ML, et al. The HealthNuts population-based study of paediatric food allergy: validity, safety and acceptability. *Clin Exp Allergy* 2010;40:1516-22.
- Koplin JJ, Osborne NJ, Wake M, Martin PE, Gurrin LC, Robinson MN, et al. Can early introduction of egg prevent egg allergy in infants? *J Allergy Clin Immunol* 2010;126:807-13.
- Welten M, de Kroon MLA, Renders CM, Steyerberg EW, Raat H, Twisk JWR, et al. Repeatedly measured predictors: a comparison of methods for prediction modeling. *Diagn Progn Res* 2018;2:5.
- Textor J, van der Zander B, Gilthorpe MS, Liškiewicz M, Ellison GT. Robust causal inference using directed acyclic graphs: the R package 'dagitty'. *Int J Epidemiol* 2016;45:1887-94.
- Moreno-Betancur M, Koplin JJ, Ponsonby A-L, Lynch J, Carlin JB. Measuring the difference in risk factor distributions on cross-population differences in disease occurrence: a causal approach. *Int J Epidemiol* 2018;47:217-25.
- VanderWeele TJ. Principles of confounder selection. *Eur J Epidemiol* 2019;34:211-9.
- Rothman KJ, Greenland S, Lash TL. Modern epidemiology. 3rd ed. Philadelphia, Pa: Lippincott Williams & Wilkins; 2008.
- Hill AB. The environment and disease: association or causation? *Proc R Soc Med* 1965;58:295-300.
- Johnston LK, Chien KB, Bryce PJ. The immunology of food allergy. *J Immunol* 2014;192:2529-34.
- Stark PL, Lee A. The microbial ecology of the large bowel of breastfed and formula-fed infants during the first year of life. *J Med Microbiol* 1982;15:189-203.
- Valles Y, Artacho A, Pascual-Garcia A, Ferrus ML, Gosalbes MJ, Abellan JJ, et al. Microbial succession in the gut: directional trends of taxonomic and functional change in a birth cohort of Spanish infants. *PLOS Genet* 2014;10:e1004406.
- Ierodiakonou D, Garcia-Larsen V, Logan A, Groome A, Cunha S, Chivinge J, et al. Timing of allergenic food introduction to the infant diet and risk of allergic or autoimmune disease: a systematic review and meta-analysis. *JAMA* 2016;316:1181-92.
- Larson K, McLaughlin J, Stonehouse M, Young B, Haglund K. Introducing allergenic food into infants' diets: systematic review. *MCN Am J Matern Child Nurs* 2017;42:72-80.
- Jahreis S, Trump S, Bauer M, Bauer T, Thurmman L, Feltsen R, et al. Maternal phthalate exposure promotes allergic airway inflammation over 2 generations through epigenetic modifications. *J Allergy Clin Immunol* 2018;141:741-53.
- Mitchell EA, Freemantle J, Young J, Byard RW. Scientific consensus forum to review the evidence underpinning the recommendations of the Australian SIDS and Kids Safe Sleeping Health Promotion Programme—October 2010. *J Paediatr Child Health* 2012;48:626-33.
- Raising Children's Network. Dummies: advantages and disadvantages. Australia: Raising Children's Network; 2017 [updated Aug 2017]. Available from: <https://raisingchildren.net.au/newborns/sleep/settling-routines/dummies#looking-after-the-dummy-nav-title>.
- Jaafar SH, Ho JJ, Jahanfar S, Angolkar M. Effect of restricted pacifier use in breastfeeding term infants for increasing duration of breastfeeding. *Cochrane Database Syst Rev* 2016;(8):CD007202.

METHODS

Data collection

The postnatal hygiene score, modified from Sherriff and Golding,^{E1} is a cumulative sum of 3 variables, each categorized from 1 to 4: maternal handwashing habits after diaper change (don't clean, wipe or rinse with water only, wash hands with soap, use a hand sanitizer), daily baby handwashing frequency (never, 1-2, 3-4, 5+ times), and baby bathing frequency ($\leq$ 1/wk, several times/wk, 1/d, >1/d). The score ranges from 3 (least hygienic) to 12 (most

hygienic). A random subcohort of participants was asked questions regarding sharing of utensils ($n = 133$) and use of unboiled water for drinking or formula preparation ($n = 138$) at 6 months, categorized as binary yes or never answers.

REFERENCE

- E1. Sherriff A, Golding J. Hygiene levels in a contemporary population cohort are associated with wheezing and atopic eczema in preschool infants. *Arch Dis Child* 2002;87:26-9.

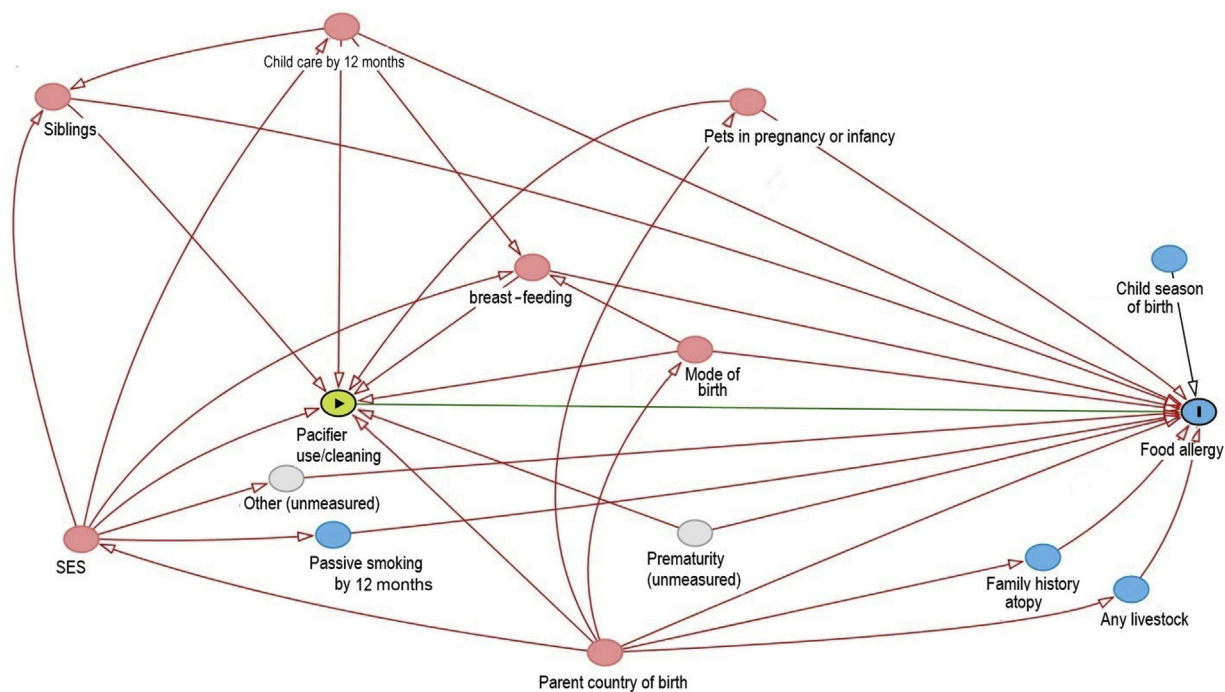


FIG E1. Directed acyclic graph for the primary logistic regression model, constructed a priori with DAGitty. Pink circles, ancestor variable of exposure (pacifier use/cleaning) and outcome (FA) requiring adjustment. Blue circles, ancestor variable of outcome only. Gray circles, unmeasured variables.

Box E1. Exact wording of exposure questions

Does your baby use a dummy/pacifier? Yes/No
If yes, how many hours per day on average does your baby use a dummy/pacifier? (number of hours per day on average)
When your baby's dummy/pacifier drops to the floor do you usually (ie, more than half the time)...? (choose one)

- Give it back to your baby without washing
- Put in own mouth before giving back to baby
- Wash it under tap water
- Wash in boiling water
- Wash/sterilize with antiseptic agent

TABLE E1. Area under the ROC curve of the primary logistic regression models used, both unadjusted and adjusted

Result	Model	n	GOF <i>P</i> value	AUC (95% CI)
Table E4	Pacifier sanitization and FA, unadjusted	637	.635	63.8% (55.3-72.3)
Figure 3	Pacifier sanitization and FA, adjusted	625	.840	73.8% (66.5-81.0)
Table II	Persistent pacifier use from 1 to 6 mo, adjusted	353	.914	77.5% (69.8-85.3)

AUC, Area under the ROC curve; *GOF*, Hosmer-Lemeshow goodness-of-fit test; *ROC*, receiver-operating characteristic.

TABLE E2. Analysis of factors related to using antiseptic on pacifiers in the first 6 mo

Factor	Antiseptic ever used		Total
	No (N = 473)	Yes (N = 113)	
Family characteristics			
Family history of asthma, eczema, or hay fever (N = 577)			
No	77 (16.6)	15 (13.4)	92 (15.9)
Yes	388 (83.4)	97 (86.6)	485 (84.1)
Any passive smoking around child in first year (N = 586)			
No	441 (93.2)	107 (94.7)	548 (93.5)
Yes	32 (6.8)	6 (5.3)	38 (6.5)
Pet ownership (N = 584)			
No	130 (27.5)	31 (27.7)	161 (27.6)
Yes	342 (72.5)	81 (72.3)	423 (72.4)
Dog ownership (N = 569)			
No	226 (49.0)	42 (38.9)	268 (47.1)
Yes	235 (51.0)	66 (61.1)	301 (52.9)
Hygiene score at 4 wk (N = 583)			
Median (IQR)	7.0 (6.0-8.0)	7.0 (7.0-8.0)	7.0 (6.0-8.0)
Hygiene score at 6 mo (N = 573)			
Median (IQR)	8.0 (7.0-9.0)	9.0 (8.0-9.0)	8.0 (7.0-9.0)
Disinfectant use at trimester 3 (N = 396)			
No	66 (20.3)	12 (16.9)	78 (19.7)
Yes	259 (79.7)	59 (83.1)	318 (80.3)
Disinfectant use at 4 wk (N = 584)			
No	164 (34.8)	37 (32.7)	201 (34.4)
Yes	307 (65.2)	76 (67.3)	383 (65.6)
Disinfectant use at 6 mo (N = 580)			
No	122 (25.8)	18 (16.7)	140 (24.1)
Yes	350 (74.2)	90 (83.3)	440 (75.9)
Disinfectant use at 12 mo (N = 579)			
No	112 (23.9)	28 (25.5)	140 (24.2)
Yes	357 (76.1)	82 (74.5)	439 (75.8)
Chlorine use at 4 wk (N = 585)			
No	297 (62.9)	63 (55.8)	360 (61.5)
Yes	175 (37.1)	50 (44.2)	225 (38.5)
Chlorine use at 6 mo (N = 580)			
No	230 (48.6)	53 (49.5)	283 (48.8)
Yes	243 (51.4)	54 (50.5)	297 (51.2)
Chlorine use at 12 mo (N = 579)			
No	200 (42.6)	46 (41.8)	246 (42.5)
Yes	269 (57.4)	64 (58.2)	333 (57.5)
Chlorinated pool at 6 mo (N = 579)			
No	263 (55.8)	60 (55.6)	323 (55.8)
Yes	208 (44.2)	48 (44.4)	256 (44.2)
Child characteristics			
Doctor-diagnosed eczema by 6 mo (N = 580)			
No	403 (85.4)	83 (76.9)	486 (83.8)
Yes	69 (14.6)	25 (23.1)	94 (16.2)
Duration of breast-feeding (N = 585)			
No breast milk	6 (1.3)	3 (2.7)	9 (1.5)
<4 wk	36 (7.6)	19 (16.8)	55 (9.4)
1-6 mo	95 (20.1)	49 (43.4)	144 (24.6)
6-12 mo	112 (23.7)	18 (15.9)	130 (22.2)
>12 mo	223 (47.2)	24 (21.2)	247 (42.2)
Age of solids introduction (N = 560)			
<3 mo	10 (2.2)	3 (2.8)	13 (2.3)
4-6 mo	417 (91.9)	100 (94.3)	517 (92.3)
7-9 mo	12 (2.6)	1 (0.9)	13 (2.3)
10-12 mo	15 (3.3)	2 (1.9)	17 (3.0)
Age of peanut introduction (N = 586)			
<12 mo	150 (31.7)	40 (35.4)	190 (32.4)
≥12 mo	323 (68.3)	73 (64.6)	396 (67.6)
Age of egg introduction (N = 496)			
<10 mo	355 (89.0)	89 (91.8)	444 (89.5)

(Continued)

TABLE E2. (*Continued*)

Factor	Antiseptic ever used		Total
	No (N = 473)	Yes (N = 113)	
10-11 mo	28 (7.0)	5 (5.2)	33 (6.7)
≥12 mo	16 (4.0)	3 (3.1)	19 (3.8)
Childcare attendance by 12 mo (N = 585)			
No	265 (56.0)	56 (50.0)	321 (54.9)
Yes	208 (44.0)	56 (50.0)	264 (45.1)
Any siblings (N = 361)			
No	111 (44.8)	60 (53.1)	171 (47.4)
Yes	137 (55.2)	53 (46.9)	190 (52.6)

IQR, Interquartile range.

Values are n (%) unless otherwise indicated.

TABLE E3. Pacifier sanitization and daily use at 1 mo and FA outcomes at 1 y

Exposure	n/N (% food allergic)	aOR*	95% CI	P value
Pacifier sanitization (n = 625)				
No pacifier	20 of 269 (7.4)	Reference group		
Pacifier—not cleaned	1 of 11 (9.1)	1.84	0.19-17.72	.600
Pacifier—own mouth	3 of 42 (7.1)	1.77	0.36-8.75	.482
Pacifier—tap water	4 of 55 (7.3)	0.90	0.25-3.21	.867
Pacifier—boiling water	13 of 179 (7.3)	0.90	0.38-2.15	.817
Pacifier—antiseptic	7 of 69 (10.1)	0.93	0.29-2.96	.896
Daily use (h/d) (n = 699)				
No pacifier	21 of 270 (7.8)	Reference group		
0.5-1 h	15 of 214 (7.0)	0.76	0.33-1.71	.501
2-7 h	15 of 190 (7.9)	0.82	0.34-1.97	.656
≥8 h	2 of 25 (8.0)	0.78	0.14-4.20	.772

*OR adjusted for socioeconomic status, childcare, breast-feeding, mode of birth, maternal country of birth, pets, and siblings.

TABLE E4. Pacifier sanitization and daily use at 12 mo on FA outcomes at 1 y

Exposure	n/N (% food allergic)	aOR*	95% CI	P value
Sanitization method (n = 625)				
No pacifier	22 of 362 (6.1)	Reference group		
Pacifier—not cleaned	7 of 113 (6.2)	0.82	0.24-2.84	.758
Pacifier—own mouth	3 of 40 (7.5)	0.95	0.18-4.92	.949
Pacifier—tap water	9 of 72 (12.5)	2.37	0.71-7.90	.160
Pacifier—boiling water	5 of 29 (17.2)	3.33	0.77-14.48	.109
Pacifier—antiseptic	2 of 9 (22.2)	1.86	0.24-14.28	.551
Daily use (h/d) (n = 699)				
No pacifier	23 of 366 (6.3)	Reference group		
0.5-1 h	5 of 35 (14.3)	2.50	0.68-9.21	.169
2-7 h	15 of 198 (7.6)	0.93	0.31-2.75	.893
≥8 h	10 of 100 (10.0)	1.02	0.29-3.63	.970

*OR adjusted for socioeconomic status, childcare, breast-feeding, mode of birth, maternal country of birth, pets, and siblings.

TABLE E5. Unadjusted pacifier sanitization at each exposure time on FA outcomes at 1 y

Exposure	n/N (% food allergic)	OR	95% CI	P value
1 mo				
No pacifier	22 of 288 (7.6)	Reference group		
Pacifier—not cleaned	1 of 17 (5.9)	0.76	0.10-5.97	.790
Pacifier—own mouth	3 of 56 (5.4)	0.68	0.20-2.37	.549
Pacifier—tap water	8 of 77 (10.4)	1.40	0.60-3.28	.437
Pacifier—boiling water	19 of 236 (8.1)	1.06	0.56-2.01	.861
Pacifier—antiseptic	8 of 93 (8.6)	1.14	0.49-2.65	.764
6 mo				
No pacifier	18 of 322 (5.6)	Reference group		
Pacifier—not cleaned	5 of 77 (6.5)	1.17	0.42-3.26	.760
Pacifier—own mouth	3 of 66 (4.5)	0.80	0.23-2.81	.733
Pacifier—tap water	10 of 111 (9.0)	1.67	0.75-3.74	.211
Pacifier—boiling water	9 of 99 (9.1)	1.69	0.73-3.89	.218
Pacifier—antiseptic	9 of 41 (22.0)	4.75	1.97-11.44	.001
12 mo				
No pacifier	27 of 389 (6.9)	Reference group		
Pacifier—not cleaned	7 of 131 (5.3)	0.76	0.32-1.78	.524
Pacifier—own mouth	4 of 46 (8.7)	1.28	0.43-3.83	.662
Pacifier—tap water	10 of 86 (11.6)	1.76	0.82-3.80	.147
Pacifier—boiling water	5 of 29 (17.2)	2.79	0.99-7.90	.053
Pacifier—antiseptic	2 of 10 (20.0)	3.35	0.68-16.57	.138

Univariate logistic regression model. No adjustments have been made.

TABLE E6. Unadjusted daily pacifier use at each exposure time on FA outcomes at 1 y

Exposure	n/N (% food allergic)	OR	95% CI	P value
1 mo				
No pacifier	22 of 284 (7.75)	Reference group		
0.5-1 h	20 of 243 (8.23)	1.07	0.57-2.01	.838
2-7 h	17 of 211 (8.06)	1.04	0.54-2.02	.899
≥8 h	2 of 29 (6.90)	0.88	0.20-3.96	.870
6 mo				
No pacifier	18 of 315 (5.71)	Reference group		
0.5-1 h	8 of 92 (8.70)	1.57	0.66-3.74	.307
2-7 h	16 of 213 (7.51)	1.34	0.67-2.69	.410
≥8 h	13 of 105 (12.38)	2.33	1.10-4.94	.027
12 mo				
No pacifier	27 of 389 (6.94)	Reference group		
0.5-1 h	6 of 40 (15.00)	2.37	0.91-6.13	.076
2-7 h	15 of 218 (6.88)	0.99	0.52-1.91	.978
≥8 h	11 of 112 (9.82)	1.46	0.70-3.05	.313

Univariate logistic regression model. No adjustments have been made.

TABLE E7. Sensitivity analysis, among pacifier users at 6 mo, and FA outcomes at 1 y (n = 387)

Sanitization method	n/N (% food allergic)	aOR*	95% CI	P value
Other method†	24 of 282 (8.5)	Reference group		
Own mouth	3 of 65 (4.6)	0.49	0.14-1.73	.267
Antiseptic agent	9 of 40 (22.5)	3.61	1.41-9.25	.007

*OR adjusted for socioeconomic status, childcare, breast-feeding, mode of birth, maternal country of birth, pets, and siblings.

†Other methods include sanitization with tap water, boiling water, or none.