

Atopic dermatitis and the hygiene hypothesis: a case-control study

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Background The notion that lack of exposure to infection in early life leads to development of atopic disease has come to be known as the hygiene hypothesis. It has arisen from observations of the rapidly rising prevalence of atopic diseases in recent decades and the lower prevalence of atopy with rising birth order. Direct evidence for the hypothesis to date is inconsistent.

Methods A case-control study set in Norfolk, UK of 602 children aged 1–5 years. Cases and controls were defined using the UK Diagnostic Criteria for atopic dermatitis (AD) and a range of direct and indirect methods were used to measure exposure to infection during infancy. Odds ratios (OR) for the effect of these measures were calculated using logistic regression with adjustment for possible biological and social confounding factors.

Results Reduced odds of AD were associated with rising birth order (OR for one older sibling 0.59, 95% CI: 0.42, 0.84 and for ≥ 2 older siblings 0.49, 95% CI: 0.31, 0.77). None of the measures of infection reduced the odds of AD significantly, either in the unadjusted or adjusted analyses. None of the measures of infection explained the protective effect of older siblings.

Conclusions Increased exposure to infection does not explain the reduced risk of AD in second and subsequent siblings. More generally, these data cast doubt on the hygiene hypothesis as a causal explanation for AD in young children.

Keywords Atopic dermatitis, eczema, hygiene hypothesis, infection

Atopic dermatitis (AD) is a chronic, disabling disease of the skin that currently affects about 15% of school-age children in industrialized societies.^{1,2} There is compelling evidence that the true prevalence of AD in these populations has increased 2- to 3-fold in the last four decades.^{3–6} This rate of change is too rapid to be explained by genetic factors. Environmental changes must therefore be responsible but despite a plethora of hypotheses and some tantalizing clues this puzzle remains largely unsolved.^{7,8}

After initial observations in large UK cohorts on the effect of family size,^{9,10} a number of studies have shown an inverse

relationship between birth order and the chances of developing symptomatic atopic disease.¹¹ In addition, a stepwise increase in the prevalence of AD with rising socioeconomic status was observed in a cohort of UK children born in 1958¹² and similar findings have been reported elsewhere.^{5,13,14} These observations, together with evidence of a bias towards Th2 immune responses in atopy, resulted in what has come to be known as the hygiene hypothesis.¹⁰ Expressed simply, this suggests that a reduced exposure to microbial pathogens in early life, especially those that result in a Th1 immune response, increases the chances of the expression of clinical atopic disease. Epidemiological evidence supporting the hygiene hypothesis to date has been inconsistent¹⁵ and some clinical and biological studies have suggested a more complex interaction between immunological challenges in early life with programming of the immune system.^{16–19} Despite this there is still a widely held view that exposure to common infections in infancy protects against the manifestations of atopic disease in later childhood and adulthood.

This case-control study was designed to rigorously test the hypothesis with respect to AD, a disease that is perhaps more 'purely atopic' than either asthma or hay fever, but that has

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received less attention in epidemiological studies of this type. Our aim was to measure exposure to infection with validated methods in a number of different ways and to analyse the effects of exposure on the risk of properly defined AD in pre-school children, controlling carefully for potential confounding variables.

Methods

The study was approved by the local research ethics committee and parents gave informed consent which included access to their child's general practice notes.

Sample size calculations suggested that 270 cases and controls each would be required to show (with 90% power to a significance level of 5%) that a 30% exposure rate to infection would reduce the odds ratio (OR) of developing AD to 0.5. The exposure rate of 30% was derived from a local seroprevalence study of antibodies to Epstein–Barr virus and cytomegalovirus in the 1–4 years age group and the reduction in OR based on a review of the literature. We increased the sample size by around 10% to account for missing data, so aimed to recruit around 300 cases and 300 controls.

Subjects were selected from patient lists of 12 general practices in and around Norwich, UK. Potential cases and controls were identified according to whether or not topical steroids had been prescribed. Roughly equal numbers of cases and controls at each age group between 1 and 4 years were selected randomly from these lists using a computerized random number generator. Families were invited to join by mail, with a telephone reminder. Willing families were then visited. Children were only included as cases if they fulfilled all of the UK diagnostic criteria for AD²⁰ including evidence of current flexural dermatitis. Children were not included as controls if they met any of the UK diagnostic criteria.

Exposure measures were collected from three sources:

Parent questionnaire

During home visits a questionnaire was administered to a parent (usually the mother) asking about common infectious symptoms in infancy and of exposure to a series of specific infections such as measles, chickenpox, pneumonia etc.

General practitioners' (GP) records

GP records of subjects during infancy were examined and all consultations coded using a predefined algorithm to classify the reason for the consultation. Definite or likely infectious episodes were recorded according to the type of infection. Particular care was taken to exclude possible atopic conditions, so, for example, when the total number of infections were calculated, infective skin conditions were excluded because these included a number of cases of infected eczema, and symptoms of lower respiratory infections were excluded to avoid confusion with infantile asthma. The number of antibiotic prescriptions was also recorded.

Salivary antibodies

Samples of saliva were collected from cases and controls using 'Oracol' devices (Malvern Medical Developments, Worcester, UK) and processed in the Central Public Health Laboratory, Colindale, UK.²¹ IgG antibodies to Epstein–Barr virus and Varicella Zoster

virus were measured by capture ELISA and an indirect ELISA method respectively.^{22,23} Positivity was determined by a comparison of optical densities using mixture model analysis.²⁴

These measures were chosen to provide a wide variety of markers of exposure to infections in infancy and early childhood. The measures from parental recall were comprehensive but would suffer from subjective interpretation and memory bias, the measures from general practice were more valid medically and timed exposure to infancy but might be biased by parental healthcare usage. The viruses were chosen as common infections in infancy and early childhood which would act as objective markers for more general exposure to contagious infection. We hypothesized that the findings would be strongest if there was a consistent pattern of results across all these different types of measure.

Possible confounding and sociodemographic factors were collected from the initial parent questionnaire. These data included family structure, numbers of older and younger siblings, family history of atopic conditions, method of infant feeding, income, attendance at child care and preschool groups, number of house moves, crowding and room sharing in infancy, and a range of socioeconomic factors both at the present and when the child was an infant. The computerized district immunization records of each child were examined to provide information on age of uptake of routine childhood immunizations.

Logistic regression was used with the presence of AD as the dependent variable. Unadjusted OR were calculated for the effect of the various possible explanatory variables. From these results possible confounding variables were identified and adjusted OR for the effect of the various measures of exposure to infection were then calculated.

Role of the funding sources

Referees for the Eastern National Health Service executive made comments on the initial protocol which resulted in small changes, otherwise neither of the funders had any further active involvement in study design, data collection, analysis, or writing of the paper.

Results

In all, 307 cases and 295 controls were recruited. Recruitment was lower among patients from the more deprived urban practices but there were no obvious differences in the pattern of recruitment between cases and controls. Table 1 shows the distribution of demographic, biological, and socioeconomic variables between cases and controls. Cases and controls are well matched on most background factors. There appears to be a higher proportion of parental atopy among controls than expected.

We have calculated unadjusted OR for the effect of these variables on the occurrence of AD and, where appropriate, we also show OR adjusted for the effect of numbers of older siblings and the number of parents with a history of an atopic condition. The strongest effect is seen for the number of parents with an atopic condition and the number of older siblings. There are inconsistent trends of effects of the socioeconomic variables. Some of the measures of the socioeconomic environment in infancy such as room sharing and crowding during the first year of life show a possible protective effect which is reduced to insignificance when adjusted for the number of older siblings,

Table 1 Distribution of biological, social, and family factors between cases and controls

	No. (%) of cases n = 307	No. (%) of controls n = 295	Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Biological and genetic factors				
Mother has an atopic condition	190 (62)	121 (41)	2.34 (1.68, 3.24)	n/a
Father has an atopic condition	154 (50)	110 (37)	1.69 (1.22, 2.34)	n/a
No. of atopic parents				
Neither	56 (18)	108 (37)		
1	158 (51)	143 (48)	2.13 (1.44, 3.16)	n/a
Both	93 (30)	44 (15)	4.08 (2.51, 6.62)	n/a
No. of older sibs				
None	159 (52)	111 (38)		
1	107 (35)	126 (43)	0.59 (0.42, 0.84)	n/a
≥2	41 (13)	58 (20)	0.49 (0.31, 0.77)	n/a
Breastfed				
Never	72 (23)	69 (23)		
<4 weeks	48 (16)	48 (16)	0.96 (0.57, 1.61)	0.87 (0.51, 1.50)
≥4 weeks	187 (61)	178 (60)	1.01 (0.68, 1.49)	1.02 (0.68, 1.53)
Mother smoked during pregnancy	51 (17)	44 (15)	1.14 (0.73, 1.76)	1.15 (0.72, 1.82)
Mother smoked in infants first year	64 (21)	51 (17)	1.26 (0.84, 1.90)	1.32 (0.86, 2.03)
≥1 younger sibs (ref.—none)	82 (27)	65 (22)	1.29 (0.89, 1.87)	0.96 (0.63, 1.47)
Social, crowding, and child care				
No. of people living at home (continuous variable)	n/a	n/a	0.86 (0.72, 1.04)	1.16 (0.89, 1.52)
Room sharing in year 1	33 (11)	58 (20)	0.49 (0.31, 0.78)	0.63 (0.38, 1.03)
Crowding in year 1				
≤0.75 person per room	206 (68)	177 (60)		
>0.75–1 person per room	82 (27)	106 (36)	0.66 (0.47, 0.94)	0.90 (0.60, 1.36)
>1 person per room	16 (5)	12 (4)	1.15 (0.53, 2.49)	1.57 (0.69, 3.56)
Crowding in current house				
≤0.75 person per room	208 (68)	191 (65)		
0.75–1 person per room	92 (30)	98 (33)	0.86 (0.61, 1.22)	1.03 (0.71, 1.51)
>1 person per room	7 (2)	6 (2)	1.07 (0.35, 3.25)	1.50 (0.46, 4.86)
Day care nursery	37 (12)	40 (14)	0.87 (0.54, 1.41)	0.80 (0.48, 1.31)
Mother and toddler group	175 (57)	161 (55)	1.10 (0.80, 1.52)	1.04 (0.75, 1.46)
Playgroup	128 (42)	124 (42)	0.99 (0.71, 1.36)	0.99 (0.71, 1.39)
Nursery school	64 (21)	60 (20)	1.03 (0.70, 1.53)	1.06 (0.70, 1.60)
Childminder	33 (11)	32 (11)	0.99 (0.59, 1.66)	0.92 (0.54, 1.57)
Other children's group	64 (21)	63 (21)	0.97 (0.66, 1.44)	0.90 (0.60, 1.35)
Lone parenthood in first year	12 (4)	18 (6)	0.63 (0.30, 1.32)	0.58 (0.27, 1.26)
Age (years) father left full time education				
≤16	176 (58)	169 (58)		
17–18	73 (24)	46 (16)	1.52 (0.99, 2.33)	1.48 (0.95, 2.30)
>18	57 (19)	76 (26)	0.72 (0.48, 1.08)	0.65 (0.42, 0.98)
Age (years) mother left full time education				
≤16	155 (50)	140 (47)		
17–18	85 (28)	75 (25)	1.02 (0.70, 1.51)	0.93 (0.62, 1.39)
18	67 (22)	80 (27)	0.76 (0.51, 1.12)	0.69 (0.46, 1.04)
Fathers social class in first year				
Non-manual	151 (49)	156 (53)		
Manual	139 (45)	117 (40)	1.23 (0.88, 1.71)	1.27 (0.90, 1.80)
Other	17 (6)	22 (7)	0.80 (0.41, 1.56)	1.01 (0.50, 2.02)
Mother's social class in first year				
Non-manual	107 (35)	107 (36)		
Manual	41 (13)	40 (14)	1.02 (0.61, 1.71)	1.06 (0.62, 1.80)
Other	159 (52)	148 (50)	1.07 (0.76, 1.52)	1.15 (0.80, 1.67)
Father's current social class				
Non-manual	159 (52)	158 (54)		
Manual	139 (45)	124 (42)	1.11 (0.80, 1.55)	1.19 (0.85, 1.68)
Other	9 (3)	13 (4)	0.69 (0.29, 1.66)	0.81 (0.33, 2.01)
Mother's current social class				
Non-manual	206 (67)	192 (65)		
Manual	96 (31)	95 (32)	0.94 (0.67, 1.33)	1.01 (0.71, 1.50)
Other	5 (2)	8 (3)	0.58 (0.19, 1.81)	0.75 (0.23, 2.47)
Non-owner-occupied home	64 (21)	72 (24)	0.82 (0.56, 1.20)	0.86 (0.58, 1.28)
House moves in first year				
None	127 (41)	104 (35)		
1	117 (38)	117 (40)	0.82 (0.57, 1.18)	0.80 (0.55, 1.17)
≥2	63 (21)	74 (25)	0.70 (0.46, 1.07)	0.63 (0.40, 0.98)
No. of cars used by family				
None	20 (7)	16 (5)		
1	129 (42)	148 (50)	0.70 (0.35, 1.40)	0.71 (0.34, 1.46)
≥2	158 (51)	131 (44)	0.97 (0.48, 1.94)	0.99 (0.48, 2.06)
Receipt of benefits	54 (18)	63 (21)	0.79 (0.53, 1.19)	0.84 (0.55, 1.29)
Weekly income				
<£200	39 (13)	36 (12)		
£200–£399	157 (52)	140 (48)	1.04 (0.62, 1.72)	1.02 (0.60, 1.73)
>£400	108 (36)	116 (40)	0.86 (0.51, 1.45)	0.83 (0.48, 1.43)

Note: Odds ratios adjusted for number of parents with atopy and number of older siblings in the right hand columns.

while the number of house moves in the first year shows a significant protective association after adjustment.

Table 2 shows the effect of the various measures of infection on the odds of AD. The first columns show the unadjusted ratios and the second show the OR adjusted for number of older siblings, number of parents with an atopic history, receipt of welfare benefits, room sharing in the first year, number of house moves in the first year, and the number of cars owned by the family. The latter four adjustment variables were chosen either because they had an effect on the outcome or because they identified extremes of the social distribution that might potentially mask an effect of the

infection variables. As an intermediate step we adjusted only for parental atopy and number of older siblings. These results were no different to the fully adjusted model and so are not shown.

None of the variables measuring exposure to infection showed a significant protective effect, either in the unadjusted or in the adjusted analyses. The only measure suggesting a possible trend in this direction is positive virology for Epstein–Barr virus but the effect is small with an OR of around 0.7 in both adjusted and unadjusted analyses. In all other measures of infection exposure the OR are either close to 1, or show a non-significant or barely significant trend for exposure

Table 2 Effects of different measures of exposure to infections on the odds of atopic dermatitis

	Exposure variable		No. of cases	No. of controls	Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Salivary antibody measures	VZV ^a salivary IgG	Negative	149	151		
		Positive	120	113	1.08 (0.76, 1.52)	1.30 (0.90, 1.89)
	EBV ^b salivary IgG	Negative	245	224		
		Positive	51	64	0.73 (0.48, 1.10)	0.71 (0.46, 1.09)
GP ^c records from infancy	All definite and likely infections by 1 year	0–1	114	135		
		2–3	100	90	1.32 (0.90, 1.92)	1.22 (0.82, 1.83)
		≥4	93	69	1.59 (1.07, 2.38)	1.44 (0.94, 2.19)
	Non-atopic infections by 1 year ^d	0–1	142	148		
		2–3	95	89	1.11 (0.77, 1.61)	1.08 (0.73, 1.60)
		≥4	66	51	1.35 (0.88, 2.08)	1.30 (0.82, 2.06)
	Gastrointestinal infections by 1 year	0	253	250		
		≥1	50	38	1.30 (0.82, 2.05)	1.29 (0.80, 2.09)
	All definite and likely infections by 3 months	0	178	180		
		1	78	79	1.00 (0.69, 1.45)	0.96 (0.65, 1.43)
		≥2	51	35	1.47 (0.91, 2.38)	1.48 (0.90, 2.46)
	Antibiotic prescriptions in infancy	Total antibiotic prescriptions by 1 year	0	154	149	
1–2			113	116	0.94 (0.67, 1.31)	0.99 (0.69, 1.43)
≥3			33	22	1.45 (0.81, 2.60)	1.36 (0.74, 2.52)
Prescriptions of antibiotics without concurrent antiatopic medications by 1 year		0/162	153			
		1–2	115	117	0.93 (0.66, 1.30)	0.95 (0.67, 1.36)
	≥3	23	17	1.28 (0.66, 2.48)	1.30 (0.64, 2.62)	
Immunization status	Pertussis immunization by 5 months	Incomplete	31	37		
		Complete	271	251	1.29 (0.78, 2.14)	1.22 (0.71, 2.10)
	MMR ^e immunization by 15 months	Incomplete	50	47		
		Complete	238	234	0.96 (0.62, 1.48)	0.94 (0.59, 1.49)
	MMR immunization ever (age >18 months)	Yes	278	274		
	No	10	7	1.41 (0.53, 3.75)	1.29 (0.47, 3.56)	
Infectious symptoms in infancy from parental questionnaire	Frequency of minor infections compared with ‘other children’	Less than	89	109		
		Same as	156	151	1.27 (0.88, 1.81)	1.33 (0.91, 1.93)
		More than	61	35	2.13 (1.29, 3.52)	2.44 (1.43, 4.15)
	Frequency of diarrhoea and vomiting illness	None	162	154		
		1–2	120	125	0.91 (0.65, 1.27)	0.93 (0.65, 1.32)
		≥3	22	14	1.49 (0.74, 3.02)	1.70 (0.80, 3.62)
	Frequency of ear infections	None	207	214		
		1–2	69	57	1.25 (0.84, 1.87)	1.27 (0.83, 1.93)
		≥3	22	18	1.26 (0.66, 2.42)	1.51 (0.75, 3.04)
	Frequency of coughs and colds	≤2	142	169		
≥3		163	125	1.55 (1.12, 2.14)	1.48 (1.05, 2.08)	
Specific infections from parental questionnaire	Bronchiolitis		73	55	1.36 (0.92, 2.02)	1.47 (0.97, 2.22)
	Chickenpox		136	129	1.04 (0.76, 1.44)	1.23 (0.86, 1.74)
	Pneumonia		6	3	1.94 (0.48, 7.83)	2.52 (0.59, 10.8)

^a Varicella Zoster virus.

^b Epstein–Barr virus.

^c General Practitioner.

^d All definite infections apart from skin plus all likely infections apart from skin, lower respiratory, and unspecified respiratory.

^e Measles, mumps, rubella.

In the right hand columns odds ratios are adjusted for parental atopy, elder siblings, receipt of benefits, room sharing in first year, number of house moves in first year, number of cars owned by the family.

to infection being associated with increased odds of AD. Adjustment makes little difference to any of the OR.

In general, measures of exposure to infection that showed the largest positive association with AD were subjective, such as parental report of minor infections and coughs and colds, and could have included possible early manifestations of AD or other atopic conditions. Excluding possible atopic episodes from the infective episode category (as described in Methods) did not alter this positive association, and it persisted even for conditions that have no connection with atopy such as diarrhoea and vomiting.

We then looked to see whether any of the infection variables accounted for the protective effect of older siblings. Table 3 shows the distribution of the infection measures according to the number of older siblings among control children only. In order to account plausibly for the protective effect of older

siblings, at the very least we need to show a large increase in the proportion of infection among control children with greater numbers of older siblings. The only measures which meet this test are exposure to chickenpox, either serologically or by parental report, and 'frequency of minor infections' by parental report.

To test whether these, or any of the other infection measures, account for all or part of the protective effect of older siblings we carried out a series of regression analyses in which the unadjusted OR for the effect of older siblings was sequentially adjusted for the various measures of infection. If the effect of older siblings is mediated through increased exposure to infection, we would expect to see the unadjusted odds of AD being reduced when the infection variables are introduced into the model. The results of these are shown in Table 4. In none of these cases were the unadjusted OR for the effect of older siblings on AD reduced by the measure of exposure to infection.

Table 3 Measures of exposure to infection in control subjects only according to number of older siblings

		No older siblings		One older sibling		≥2 older siblings	
		No.		No.	Odds ratio (95% CI)	No.	Odds ratio (95% CI)
Varicella Zoster virus Ig G	Negative	72		49		30	
	Positive	31		61	2.89 (1.64, 5.09)	21	1.63 (0.81, 3.27)
Epstein-Barr IgG	Negative	86		95		43	
	Positive	22		29	1.19 (0.64, 2.23)	13	1.18 (0.54, 2.57)
^a All definite and likely infections by 1 year	−1	51		55		29	
	≥2	60		70	1.08 (0.65, 1.81)	29	0.85 (0.45, 1.61)
^a Non-atopic infections by 1 year	0–1	57		59		32	
	≥2	50		64	1.24 (0.74, 2.08)	26	0.93 (0.49, 1.76)
Gastrointestinal infections by 1 year	0	93		103		54	
	≥1	14		20	1.29 (0.62, 2.70)	4	0.49 (0.15, 1.57)
^a All definite and likely infections by 3 months	0	62		69		49	
	≥1	49		56	1.03 (0.61, 1.72)	9	0.23 (0.10, 0.52)
Total antibiotic prescriptions by 1 year	0	57		64		28	
	≥1	49		59	1.07 (0.64, 1.81)	30	1.25 (0.66, 2.37)
Prescriptions of antibiotics without antiatopics by 1 year	0	58		66		29	
	≥1	48		57	1.04 (0.62, 1.76)	29	1.21 (0.64, 2.29)
Pertussis immunization by 5 months	Incomplete	9		16		12	
	Complete	100		108	0.61 (0.26, 1.44)	43	0.32 (0.13, 0.82)
MMR ^b immunization by 15 months	Incomplete	13		22		12	
	Complete	93		100	0.64 (0.30, 1.33)	41	0.48 (0.20, 1.14)
MMR immunization ever	Yes	103		120		51	
	No	3		2	0.57 (0.09, 3.49)	2	1.35 (0.22, 8.31)
^a Frequency of minor infections	Less than or same	102		107		51	
	More than	9		19	2.01 (0.87, 4.65)	7	1.56 (0.55, 4.42)
^a Frequency of diarrhoea and vomiting	None	61		62		31	
	≥1	49		63	1.27 (0.76, 2.12)	27	1.08 (0.57, 2.05)
^a Frequency of ear infections	None	78		94		42	
	≥1	30		29	0.80 (0.44, 1.45)	16	0.99 (0.49, 2.02)
Frequency of coughs and colds	≤2	64		70		35	
	≥3	47		55	1.07 (0.64, 1.79)	23	0.90 (0.47, 1.71)
Bronchiolitis	No	90		94		48	
	Yes	19		29	1.46 (0.77, 2.79)	7	0.69 (0.27, 1.76)
Chickenpox	No	79		56		31	
	Yes	32		70	3.09 (1.80, 5.29)	27	2.15 (1.11, 4.15)
Pneumonia	No	110		125		57	
	Yes	1		1	n/a	1	n/a

^a These measures have been dichotomized for convenience.

^b Measles, mumps, rubella.

Table 4 Odds ratios for the effect of older siblings on atopic dermatitis risk, adjusted for a range of possible infective factors

Adjustment variables	One older sibling		≥2 older siblings	
	Odds ratio	95% CI	Odds ratio	95% CI
Unadjusted	0.59	0.42, 0.84	0.49	0.31, 0.79
Varicella Zoster virus salivary IgG +ve	0.58	0.40, 0.85	0.51	0.31, 0.83
Epstein–Barr virus salivary IgG +ve	0.59	0.41, 0.84	0.49	0.31, 0.80
All infectious symptoms in infancy ^a	0.59	0.41, 0.84	0.49	0.31, 0.78
Non-atopic infections in infancy ^a	0.57	0.40, 0.82	0.44	0.27, 0.70
Gastrointestinal infections in infancy ^a	0.58	0.41, 0.83	0.44	0.28, 0.71
All infectious symptoms by 3 months ^a	0.60	0.42, 0.85	0.50	0.31, 0.79
Total antibiotic prescriptions in infancy	0.58	0.41, 0.83	0.42	0.26, 0.68
Prescriptions of antibiotics without antiatopics in infancy	0.58	0.41, 0.83	0.42	0.26, 0.68
Pertussis immunization complete by 5 months	0.58	0.41, 0.83	0.51	0.32, 0.83
MMR ^b immunization complete by 15 months	0.57	0.39, 0.82	0.53	0.33, 0.86
No MMR immunization	0.57	0.40, 0.82	0.53	0.33, 0.86
Minor infections in infancy (parent ques.)	0.56	0.39, 0.81	0.46	0.28, 0.74
Diarrhoea and vomiting in infancy (parent ques.)	0.58	0.41, 0.83	0.47	0.30, 0.76
Ear infection in infancy (parent ques.)	0.59	0.41, 0.84	0.45	0.28, 0.72
Coughs and colds in infancy (parent ques.)	0.60	0.42, 0.85	0.48	0.30, 0.77
Bronchiolitis (parent ques.)	0.59	0.41, 0.84	0.48	0.30, 0.77
Chickenpox (parent ques.)	0.57	0.40, 0.82	0.48	0.30, 0.76
Pneumonia (parent ques.)	0.59	0.41, 0.84	0.48	0.30, 0.77

^a From GP records.^b Measles, mumps, rubella.

In particular, no effect was shown with Epstein–Barr virus or Varicella Zoster virus antibody status nor with chickenpox infection or frequency of minor infections by parental report. Thus there is no evidence that exposure to any of the infections we measured contributes to the protective effect of having older siblings.

Discussion

This study was designed to test the hygiene hypothesis as an explanation for the onset of AD in children. In line with other studies we have found an apparent protective effect of larger sibship sizes and have confirmed that this is due to the number of older siblings.¹¹ No protective effect of exposure to infection was demonstrated. Furthermore, adjusting for the various infection variables did not reduce the effect of the number of older siblings on the odds of AD.

The strengths of this study were the clear and validated definition for both cases and controls and the diverse measures of previous exposure to infection. Lack of clear case definition is a weakness of previous studies of this type that have relied on parental report or historical diagnoses. The UK diagnostic criteria require both a convincing history and current clinical evidence of active AD. Any child not meeting these exacting criteria were excluded as cases regardless of history or previous medical records, while any child who had a previous history suggestive of AD was excluded as a control. We did not actively exclude children with other atopic conditions but because of the

method of selection, few control children had asthma or hay fever (3.4% and 2.7% respectively).

When considering previous exposure our intent was not to identify the effect of any specific infectious agent but rather to include a wide range of indicators of exposure to infections in general. These ranged from the objective measurement of salivary antibodies to two viruses, the GP records which enabled timing of infectious symptoms to be restricted to infancy, and the more subjective information from parental recall. All these methods have drawbacks, but our prior argument was that the findings would be strengthened by a consistent pattern of results across all the measures and indeed this was the case.

The validity of the salivary antibody tests have been reported.^{22,23} In addition, the salivary antibody test for Varicella Zoster showed a high level of internal consistency with parental report of chickenpox (213 of 244 children with a positive history tested positive, while 270 of 298 children with a negative history tested negative). Data from GP medical records has been used to measure exposure to infections in previous studies,^{25–27} while Bruijnzeels and colleagues found greater consistency in data from medical records than either diaries or interview.²⁸ In order to minimize bias, an independent researcher unaware of the case or control status of the children, and only vaguely aware of the hypothesis of the study, scrutinized and coded the GP records. Different consulting patterns of parents for first children and subsequent siblings may have introduced a bias, for example there was a small but significant correlation between number of visits for non-atopic, non-infective conditions and

number of older siblings ($r = -0.12$, $P = 0.003$). Table 3 shows that children with ≥ 2 older siblings presented less frequently to the GP with infectious symptoms, but this was not the case for children with one older sibling. Thus, any possible bias from parents' consulting behaviour is likely to have been small and restricted to the results from children with ≥ 2 older siblings. We made no attempt to validate the parent questionnaire, although the wording of some of the infection questions followed the format developed for the Warwick Child Health and Morbidity Profile^{29,30} and there were positive associations between the parents' replies and data from the GP records (e.g. ear infections, $r = 0.43$, $P < 0.001$, diarrhoea and vomiting, $r = 0.20$, $P < 0.001$). Although the results from the parent questionnaire were considered to provide the least robust evidence, such data can certainly be accurate, particularly in young children.³¹

Our sample sizes were large enough to reliably identify a reduction of the odds of AD by a half, which is the size of effect reported in other studies.^{32–35} We are therefore confident that we are not missing a protective effect due to low power or residual confounding.

The consistent findings in the literature are the rising prevalence over the past 30 years and the reduced risk of atopy in second and subsequent siblings. The hygiene hypothesis arose from these observations but one of the subsequent challenges has been to identify any infectious, microbial, or toxic exposure that varies markedly with birth order.

Support for the hypothesis that early infection protects against the subsequent development of atopic disorders is largely indirect. Protective effects have been found for overcrowding,³⁶ attendance at day care nurseries,^{37,38} and an anthroposophic lifestyle,³⁹ while an increased risk has been shown for consumption of antibiotics in infancy.^{27,40,41} Our results also show a possible effect of the social environment in infancy, for example the protective effect of room sharing, moderate crowding, and frequency of house moves, but this is not explained by early infection, and we found no effect of antibiotic prescriptions.

Studies using more direct measures of infection have shown inconsistent results, some appearing to demonstrate the protective role of tuberculosis,³³ BCG,⁴² measles,³⁴ Epstein–Barr virus,⁴³ hepatitis A,^{32,44} *Toxoplasma*,⁴⁴ and respiratory infections,³⁵ and others showing no effect, (or even a potentiating

effect) from BCG,^{45,46} rubella and pertussis,⁴⁷ measles, mumps, rubella, and Varicella Zoster,^{44,47,48} all infections in the first months of life,⁴⁹ and early life respiratory infections.^{50,51}

We have only examined AD, but our results are in line with those studies that found no protective effect on atopy of infection in early life. The consistent finding of protective effects of older siblings suggests that programming of the immune system to be susceptible to allergic sensitization occurs in childhood and our finding that the sibling effect is seen among very young children suggests it occurs very early in life. Two possible explanations are the immunomodulatory effects of different patterns of neonatal gut colonization^{17,19} or factors acting on the immune response before birth.^{52,53} Our results would be consistent with either of these explanations.

In conclusion, this study has found no relationship between the manifestation of AD in young children and infection in early life. The hygiene hypothesis may explain the rise in prevalence of AD over the previous 30 years, but it does not explain why children born during the 1990s in a developed country manifest the disease. There are now a number of well-designed studies that show no protective effect or a small positive correlation between early life infection and subsequent atopic symptoms. Taken together these cast doubt on the hygiene hypothesis as an explanation for AD.

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KEY MESSAGES

- The hygiene hypothesis was based on reduced prevalence of atopic disease in children with large numbers of siblings and proposes that exposure to infections in infancy protects against the subsequent manifestation of atopic disease.
- This large case-control study of preschool children with atopic dermatitis has measured exposure to infection in infancy using a variety of approaches.
- No measure of infection showed a protective effect for atopic dermatitis.
- The protective effect of older siblings was confirmed.
- Exposure to common infections in infancy does not protect against the manifestation of atopic dermatitis, nor does it explain the sibling effect.

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