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MARKERS OF MICROBIAL EXPOSURE LOWER THE INCIDENCE OF ATOPIC DERMATITIS

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Short Title

The hygiene hypothesis and atopic dermatitis

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Conflict of interest

The authors declare that they have no conflicts of interest.

Abstract

Background

The hygiene hypothesis proposes that reduced exposure to infectious agents in early life would explain the increase of allergic and autoimmune diseases observed over the past decades in high-income countries.

Methods

We conducted a matched case–control study on incident atopic dermatitis (AD). Cases were 426 outpatient children with a first diagnosed incident AD. Controls were 426 children attending a pediatric/dermatological visit for non-atopic disorders matched to cases (1:1). Particular attention was paid to the time elapsed between the markers of microbial exposure

and disease onset, and we considered for controls the same time-window of exposures from birth as his/her matched case. Odds ratios (ORs) were computed using multivariable conditional logistic regression models, according to center, sex, age and period of enrollment, and including as potential confounders a family history of any allergy in parents, type of delivery, having siblings, keeping pets, age at weaning, and having had ≥4 infections.

Results

The OR of AD first occurrence was 0.35 (*p*-value=0.039) for children who had experienced ≥4 infections compared with those with no infections. A decreasing trend in risk was observed with increasing number of siblings (*p*-value=0.023), the protective effect reaching about 40% for children with 2 or more siblings (OR=0.62; *p*-value=0.048). Pet keeping, in particular daily contact with dogs, was inversely associated with AD risk (OR= 0.40; *p*-value=0.004).

Conclusions

These results support the hygiene hypothesis in its broad sense. Early-life environmental exposures, including pathogens and commensals, act as "microbes contact carriers" influencing immune system balance early in life.

Key Words: Atopic Dermatitis, case-control study, hygiene hypothesis, infections

Abbreviations:

AD: Atopic Dermatitis

OR: Odds ratio

CI: confindence interval

SCORAD: Scoring atopic dermatitis

Introduction

Atopic dermatitis (AD) is the most common relapsing inflammatory skin disease, characterized by intense itching and recurrent eczematous lesions, with onset usually during the first 6 months of life. As for the other atopic diseases, AD has shown over the past three decades a constant increase in prevalence, reaching levels over 20% in most high-income countries (1,2).

As early as mid-‘70s, the Canadian pediatrician John Gerrard and co-workers suggested that allergic disease is the price for relative freedom from diseases caused by viruses, bacteria and helminths (3). In 1989, more than 10 years later, the hygiene hypothesis was first formulated by Strachan based on the observation that a higher frequency of allergic rhinitis and AD was present in first-born children as compared to siblings born in the second, third or fourth position (4). The assumption was that first-borns are less exposed to common infections than their subsequent brothers and sisters, leading to the concept that infections due to pathogens could protect against atopy.

It is assumed that limited exposure to the environmental infectious burden in infancy somehow impairs the normal development of immune regulatory mechanisms, leading to the onset of AD in genetically prone individuals. Initial studies have indicated that exposure in early life to older siblings (4-7) or to children in day-care nurseries (4,7,8) protects against atopy. Concurrently, other studies focusing on rural lifestyles (7,9-12) have further broadened the spectrum of environmental microbial markers to be considered, showing that children living in microbe-rich environments have a lower risk of developing allergic asthma (13) and, to a lesser degree, atopy. Whereas very few studies failed to confirm the protective role of older siblings on AD (14,15), a direct relation between the frequency of common or severe prior infections and that of atopic diseases remains debated (6,7,16,17). This reflects heterogeneous study design (cohort, cross-sectional or case-control studies), difficult data

collection (precise time elapsed between the infectious event and disease onset), and the problem of distinguishing/or not the effect of infection *per se* from that of antibiotic prescription. Recently, the potential role of gut microbiota as modulator of the immune system was suggested; specifically, presymptomatic differences in gut microbiota composition and diversity have been found in the first months of life in children developing atopic dermatitis (18,19).

To our knowledge, this is the first matched case-control study aimed at determining the direct and indirect markers of infection associated with the occurrence of a first medical diagnosis of AD in early childhood, paying particular attention to control, in a precise fashion, the time elapsed between these markers and disease onset.

Methods

Setting and study population

A 1:1 matched case–control study on incident AD was conducted between March 2011 and April 2014 in 10 Italian centers (6 dermatological and 4 pediatrics wards and 1 general practice pediatrician and hospital consultant).

Cases. Cases were outpatient children aged 3-24 months with a first-time doctor-diagnosed AD ascertained during the enrollment visit. Cases were matched to controls (ratio 1:1) for study center, sex, period of interview (±4 months), and age (±1 months for children aged < 11 months and ±2 months for older children), details concerning matching structure are reported in the Extended Methods section of the Supporting Information, Tables S1-S2. Participating dermatologists consensually agreed on a set of diagnostic criteria (itching, eczematous lesions, age-specific affected areas, flexural involvement, exclusion of other diagnosis, e.g., psoriasis and recurrence of AD symptoms within the last 4 weeks reported by parents) at the end of a pilot study on 100 AD cases. To include incident cases only, the

diagnostic criteria included that children should have had the first symptoms no longer than 5 months before the inclusion visit/diagnosis. Thus, children with a previous diagnosis of AD or with first symptoms that appeared more than 5 months before recruitment/diagnosis were excluded from the cases group. During the inclusion visit, the doctor recorded the date of the occurrence of the first symptoms and evaluated the severity of AD by means of the SCORAD index (20,21). In 361 out of the 426 cases included, all criteria were met (for more details, see the Extended Methods section of Supporting Information, Table S3). The study did not include information on sensitization to allergens. In the context of an observational study enrolling very young children, collection of skin prick tests and/or blood samples raises ethical concerns since it is not routinely performed in current Italian clinical practice and is not required by the consensus-based diagnostic criteria for AD defined by clinicians for the study. Data on total IgE performed within a month from study inclusion were retrieved, when available, from medical records.

Controls. Controls were 426 children aged 3-24 months attending a pediatric/dermatological visit, with no history of AD, a recent or ongoing infection and no chronic diseases. Two hundred twenty-seven controls (53%) were recruited during a check-up growth visit, 143 (34%) had a non-atopic dermatologic diagnosis (i.e. 84 angioma or hemangioma, 28 nevi, and 31 other dermatologic conditions), and the remaining 56 children (13%) attended the visit for other care needs (e.g., eye check-up, mandatory vaccinations, minor congenital malformations, traumas).

Less than 3% of both cases and controls approached declined participation.

Ethical issues. All study centers obtained local ethics committee approval. Written consent was obtained from parents for the child, based on the recommendations of the ethics committees of the study hospitals.

Data collection

Trained clinical staff administered an *ad hoc* face-to-face structured questionnaires to children's parents, including information on family socio-economic context, characteristics of the home, maternal pregnancy exposures (e.g., infections and antibiotic use), birth factors (e.g., week of gestation, mode of delivery, child birth weight), child history of any atopic manifestation, childhood diseases from birth, breastfeeding and introduction of solid food, dietary supplementation, and history of atopic diseases in first-degree relatives. Further details regarding data collection are reported in a previous publication (22) and in the Extended Methods section, in the Supplemental Material.

Data on infectious events

A specific section of the questionnaire collected history of child infections from birth. Infections were classified as nasopharyngitis, pharyngotonsillitis, laryngitis, other upper respiratory tract infections, otitis, bronchiolitis/asthmatic bronchitis, catarrhal bronchitis, bronchopneumonia/pneumonia, eye infections, urinary infections, non-parasitic intestinal infections, parasitic infections, cutaneous infections (e.g., mycosis, impetigo) and other types of infections (e.g., sepsis, meningitis, pediatric arthritis). For each infectious episode, parents were asked to report the presence of fever >38°, the use of antibiotics, and when it had occurred. They were also asked if the child had had exanthematous diseases, including chickenpox, sixth disease, measles, mumps and rubella, and time of occurrence. The availability of a detailed schedule of infectious episodes from birth as well as of the date of the occurrence of the first AD symptoms allowed us to identify and exclude from the analyses those infections occurred after symptoms.

Data on siblings, pets and contact with other children

The questionnaire collected information on the number of siblings and their age, and pets keeping, including the type of pets (e.g., dog, cat, rodents, birds), where the pet usually sleeps or spends time, and their daily contact with the child. Parents were also asked where and with whom the child usually spends daytime (e.g., at own home with a parent, at relatives' home, with a baby-sitter, at the nursery school), and if he/she is regularly in contact with other children.

Reproducibility of the data. To assess the reproducibility and reliability of data obtained from the face-to-face interview, a reproducibility study was conducted on 171 subjects. Between December 2011 and November 2012, the same subjects (mothers or fathers) who participated in the first interview were re-contacted by phone by a trained interviewer using a selection of approximately 100 questions from the same questionnaire. The proportion of observed agreement for answers related to direct and indirect markers of contact with microbes was evaluated by Cohen's kappa statistic (K) and the prevalence-adjusted bias-adjusted kappa (PABAK), to adjust for imbalanced situations. For more details and results of this study, see the Extended Methods Section and Table S4-S5, in the Supporting Information.

Statistical analysis

Data were presented as frequency/percent distribution separately for cases and controls. Since we were interested in exposures influencing AD incidence, and AD diagnostic criteria include symptoms recurrence, we adopted a conditional approach in order to consider the same time-window of exposure from birth for a case and its matched control. As an example, for a child aged 6 months at inclusion with AD first symptoms occurring at the age of 4 months (i.e., two months before), we excluded from the analyses those exposures (e.g.,

infectious episodes) occurring between 4 and 6 months of age. Thus, for the matched control, we excluded exposures occurring in the same period (i.e. infectious episodes occurring at 4 months and after). In this way, we ensured that a control had the same time-window to experience an event as the matched case (**Figure 1**).

Odds ratios (ORs), the corresponding 95% confidence intervals (CIs) and p-values, were computed using conditional logistic regression models, matching 1:1 cases and controls. Multivariable models were performed in order to adjust for potential confounding effects (covariates are reported in table footnotes).

Statistical power considerations: The statistical power of a study is function of the frequency of various conditions and exposures measured and of the corresponding ORs. In general, our study had the power to detect a 40% difference in risk for prevalence of exposure of 10-15% or above in controls. Details regarding the statistical analysis are provided in the Extended Methods section in the Supplemental Material.

Results

Demographics

Five hundred sixty-six enrolled children were males (66%). About 52% of children were 3-6 months of age and about 21% were 12 months of age or older. Among cases, 84% of children experienced their first AD symptom not more than 3 months before inclusion. SCORAD in cases ranged from 9.9 to 102.8 (median 49.5; mean=49.7, SD=17.2). **Table 1** details the distribution of cases and controls according to study design and selected children characteristics.

The distribution of cases and controls according to selected potential confounders is reported in Table S6 in the Supporting Information.

Incidence of atopic dermatitis according to markers of infection

The distribution of cases and controls, the ORs and corresponding 95% CIs according to markers of infections are given in Table 2. An inverse association emerged for the total number of infections from birth. Compared to no infection, the adjusted ORs were 0.80 (p=0.311) for 1, 0.66 (p=0.195) for 2-3, and 0.35 (p=0.039) for ≥4 infectious episodes in life, with a significant trend of decreasing risk (p=0.038) when the number of infections was considered as a continuous variable. When infections were distinguished in terms of severity of symptoms and antibiotic use, only those children who experienced infections with fever >38°C, not treated with antibiotics, showed a significant protection when compared to children without infections (OR=0.43, p=0.046). Chickenpox and sixth disease were not significantly associated with AD. Other exanthematous diseases were infrequent in our study sample (2 children had pertussis and 1 child scarlet fever).

Figure 2 shows the association between different infections and AD risk. Single infections were considered separately to get the most reliable measure of the total number of infections. It is interesting to note that, except for skin infections, the ORs were all below unity, with a risk significantly reduced for otitis (OR=0.41).

Incidence of atopic dermatitis according to *siblings, pets and contact with other children*

In Table 3, a significant difference emerged between cases and controls in the number of siblings; 56% of cases and 48% of controls had no siblings, and compared to children with no siblings, the adjusted OR for those with ≥2 siblings was 0.62 (p=0.048), with a significant downward trend (p=0.023). When the age of siblings was analyzed, a significant inverse association with AD occurrence was found (OR=0.54, p=0.014) when children with a sibling with two years of age or less were compared to those with no siblings. Pet keeping was

inversely associated with AD risk, with an OR of 0.73 (p=0.049). When the type of pet was considered, no association emerged for cats, whereas a significantly reduced AD risk was found for having a dog (OR=0.67, p=0.040), in particular for daily contact with a dog (OR=0.40, p=0.004). The association with dog keeping persisted when children with atopic parents were considered separately (OR=0.58, p=0.032). No association was found when the contact with other children in a day-care nursery was analyzed, and no association was as well for age at entry in nursery and time spent in nursery.

Mother's infections and antibiotic use during pregnancy were not found to be significantly related to AD risk in our data (see Table S7 in the Supporting Information).

Discussion

The present study shows that both direct and indirect markers of microbial exposure, the mainstays of the hygiene hypothesis – e.g. high number of infections since birth, having siblings, contact with pets especially dogs – are associated with lower risk of AD first occurrence in young children.

Only a few years ago, according to Strachan's initial postulate, these observations would have been interpreted as resulting from the protective effect of pathogens on AD. However, recent data pointing to the diversity of commensals as modulators of AD occurrence (within the gut microbiota and possibly also the skin microbiota) must be carefully accounted for in the definition of indirect markers of infection (23-26). This has two major consequences. First, an important demand to use robust direct markers for infectious diseases, which in essence do not involve commensals. Secondly, the need for a more in-depth interpretation of indirect markers that may result from the effect of pathogens, commensals or both (27,28).

Herein we have found that a high number of infectious events has a protective effect against AD occurrence. This was more evident in children who had had at least 4 infections since

birth. Importantly, the protection persisted even after taking into account history of allergy in parents, type of delivery, number of siblings, age at weaning, and pet keeping as potential confounders.

Only those children who experienced, as first episode, an infection with fever >38°C not treated with antibiotics showed significant protection against AD occurrence. The paradoxical absence of protection linked to antibiotic prescription may rely on the well-documented impact that their oral delivery has on gut microbiota diversity, leading to increased frequency of atopic diseases (29). Alternatively, one cannot exclude that infections successfully treated with antibiotics have a lower capacity to modulate the immune responses underlying the protective effect on AD.

Our rationale to conduct a case control study has been that such a design offers the possibility to investigate, using a detailed data collection form, a large number of conditions and variables of potential interest, which may not be systematically collected in cohort studies, particularly those based on record linkage follow-up. This is particularly relevant in the context of the hygiene hypothesis which involves a number of direct and indirect markers of infections.

The strengths of our study are the very high rate of response to the questionnaire, having met a clinical consensus-based diagnostic criteria for AD in young children during a pilot study and subsequently adopted by the dermatologists and pediatricians involved in the study. In addition, to our knowledge, this is the first large case-control study on AD in childhood in which incident cases only, rather than prevalent AD cases, were enrolled. Since we were interested on exposures related to AD incidence, and AD diagnostic criteria include symptoms recurrence, we adopted a conditional approach, considering the same time-window of exposure from birth for each case and its matched control.

In a retrospective study, mothers' recall bias may have had an impact. However, in our study we focused on AD in early childhood, with 80% of children being 3-12 months old, 52% of children being first-borns, and most of the others (38%) being second-borns thus mothers are questioned over recent past. To avoid as much as possible underreporting, specific attention was paid to collecting information for each one of the 20 types of infections separately, with particular attention to the time window of occurrence, presence of fever, and antibiotic or antipyretic use. Furthermore, results of the reproducibility study indicated a general substantial agreement between the two interviews for information on different types of infections. The proportions of overall agreement ranged from 79.7% (for upper respiratory tract infections other than nasopharyngitis, pharyngotonsillitis and laryngitis) to 100% (for bronchopneumonia/pneumonia and urinary infections). Results were less favorable for the total number of infections for which the proportion of agreement was 67%, although the agreement coefficient (weighted Kappa/PABAK) of 0.68 indicated substantial agreement (30). This may reflect some difficulties for mothers of older children in precisely recalling the number of infections (as suggested by the results reported in Supporting Information). Nevertheless, this likely affects a modest proportion of our sample since 85% of children were under 1 year of age.

In some epidemiological studies, information bias may, at least in part, explain some negative results on direct markers of infection (5). Still, in one study including very young children and in which particular attention was paid to the time-frame between infectious events and AD onset, the authors were able to show the protective role of infections (16).

Our results, like others (7,8,31), do not confirm a protective role of day-care attendance on AD, even though other data would support the association (32,33). These conflicting findings may reflect the marked difference between countries in the type and intensity of day-care attendance (33). In our study, this lack of effect may be partly due to our focus on very young

children since we studied the incidence rather than the prevalence of AD. Indeed, 50% of our sample was less than 6 months old and only 57 children (7%) had attended day-care nurseries. Of note, few studies have analyzed separately the different types of contact with other children (i.e., day care and siblings). When this was done (34), the protective role of day-care attendance was significant only for children from small families (fewer than 4 members, 78% with no siblings), whereas no protection was evident for children from larger families, as confirmed by our results. Thus, contact with children outside the home appears to be less important as a protective factor than continuous contact with siblings.

Another piece of evidence argues in favor of the influence of commensals on AD occurrence. Namely, in keeping with other studies (35-37), we found that exposure to pets has a favorable effect on the risk of AD occurrence in young children, attributable principally to daily contact with dogs. No association emerged with exposure to cats. The association with dog keeping persisted when children with atopic parents were considered separately. It is known that gut microbial communities differ across mammalian species (38) and in particular that fecal microbiota composition differs between dogs and cats (39). It is also likely that their skin and mucosal microbiomes differ as well, thus explaining why only contact with dogs affected AD occurrence. In addition, it has been suggested that the protection against allergic diseases related to the presence of dogs may be attributable to their influence on household dust microbiome (40,41).

In line with Strachan's initial postulate (4) and with other studies (42,43), we found a protective effect for a higher number of older siblings. An important role emerged for sibling age difference, as the smaller is the difference in age the higher is the protection from AD occurrence. These data suggest that the sibship effect can be partly attributed to the more frequent contact among siblings rather than to a "parity effect" originating *in utero* (7,44). This contact effect ensuing from sibship may be explained in two ways: either transmission

of pathogens or "sharing" of commensals. Transmission of pathogens is suggested by the modest yet present higher number of infections observed in children with older siblings both in AD patients and healthy subjects. Transmission of pathogens cannot however explain the entirety of the contact effect, since the sibship protection persisted even after total number of infections was taken into account as a potential confounder (namely, considering infections as a variable that may have distorted the true relationship between sibship and AD occurrence). This clear-cut result is evidence that not only pathogens, as initially thought, but also more generally environmental microbes within the various microbiota may be the fundamental source of the protective effect of the environment on AD (45). Such a conclusion is well in keeping with recent data showing that the diversity of gut microbiota composition differs according to number of siblings and may play a role in the sibship effect (18,46).

Our study did not consider aspects related to different AD phenotypes and genotypes which would have allowed dissecting the wide spectrum of subsets of patients with AD. Nevertheless, our first aim was to analyse simultaneously all the factors that contribute to hygiene hypothesis postulate considered in epidemiological studies, which have been, as yet, only sporadically addressed all together in a single study.

Beyond this considerations, since AD is commonly considered as the first step in the atopic march, it constitutes a meaningful model for future studies searching to identify key cellular and molecular immune pathways underpinning the protective effect of pathogens and commensals, thus guiding the quest for therapeutic microbial derivatives (28).

Author Contributions

Carlo La Vecchia and Liliane Chatenoud designed the study and supervised the study conduction and analysis.

Carlotta Galeone was responsible for conduction of data collection.

Paola Bertuccio and Federica Turati performed the data analyses.

All authors contributed substantially to interpretation of data.

The paper was written by Liliane Chatenoud , Lucienne Chatenoud , Jean-François Bach and was critically revised by Luigi Naldi and Carlo La Vecchia.

All authors approved the final version of the manuscript before submission.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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Table 1. Distribution of 426 incident cases of atopic dermatitis and 426 matched controls according to study design and children's major characteristics. Italy, 2011-2014.

	Total N=852 N (%)	**Cases N=426 N (%)**	**Controls N=426 N (%)**
Study design characteristics			
Study center			
Milano	314 (36.8)	157 (36.7)	157 (36.7)
Bergamo	296 (34.7)	148 (34.6)	148 (34.6)
Bologna	116 (13.6)	58 (13.6)	58 (13.6)
Other[a]	126 (14.8)	63 (14.8)	63 (14.8)
Distance in months between first symptoms and interview			
0		67 (15.7)	
1		87 (20.4)	
2		110 (25.8)	NA
3		95 (22.3)	
4-5		67 (15.7)	
Child's characteristics			
Sex			
Male	566 (66.4)	283 (66.4)	283 (66.4)
Female	286 (33.6)	143 (33.6)	143 (33.6)
Age (months)			
3	103 (12.1)	48 (11.3)	55 (12.9)
4	128 (15.0)	67 (15.7)	61 (14.3)
5	127 (14.9)	63 (14.8)	64 (15.0)
6	86 (10.1)	42 (9.9)	44 (10.3)
7	65 (7.6)	36 (8.5)	29 (6.8)
8	60 (7.0)	28 (6.6)	32 (7.5)
9	41 (4.8)	19 (4.5)	22 (5.2)
10-11	63 (7.4)	34 (8.0)	29 (6.8)
12-14	63 (7.4)	31 (7.3)	32 (7.5)
15-17	40 (4.7)	21 (4.9)	19 (4.5)
18-19	27 (3.2)	13 (3.1)	14 (3.3)
20-24	49 (5.7)	24 (5.6)	25 (5.9)
SCORAD (classification of severity)			
Mild (9.9-24.9) - *(Median= 20.2)*		31 (7.3)	
Moderate (25-50) - *(Median= 39.7)*		190 (44.6)	NA
Severe (50.1-102.8) - *(Median= 61.9)*		205 (48.1)	
Total IgE (available for 91 cases)			
Pathologic		56 (61.5)	
Normal		35 (38.5)	NA
Mean; Median; Min-Max		*105.1; 20.0; 0-1898*	
Controls diagnosis			
Check-up growth			227 (53.3)
Dermatologic diagnosis[b]			143 (33.6)
Eye check-up[c]			23 (5.4)
Vaccinations		NA	16 (3.8)
Pelvic ultrasound			1 (0.2)
Minor congenital malformation			7 (1.6)
Trauma/Ingestion of foreign body/other			9 (2.1)

NA: Not applicable.
[a]Centers with less than 100 patients (Florence, Garbagnate Milanese, Genova, Padua, Siena-Grosseto, Treviglio, Verona). [b]For dermatologic diagnosis: 84 children had angioma or hemangioma, 28 children had nevi, and 31 other dermatologic conditions. [c]Excluding conjunctivitis.

Table 2. Distribution of 426 incident cases of atopic dermatitis and 426 matched controls according to direct markers of contact with infectious agents. Italy, 2011-2014.

	Cases N=426 N (%)	**Controls N=426 N (%)**	**OR[a] (95% CI)**	**OR[b] (95% CI)**	***p-value (for adjusted OR)***
Child infections since birth					
No[c]	321 (75.4)	307 (71.7)	1[d]	1[d]	
Yes	105 (24.7)	119 (27.9)	0.79 (0.55-1.13)	0.73 (0.50-1.06)	0.098
Total number of infections from birth					
No infection[c]	321 (75.4)	307 (72.1)	1[d]	1[d]	
1	65 (15.2)	68 (16.0)	0.85 (0.57-1.28)	0.80 (0.53-1.23)	0.311
2-3	29 (6.8)	32 (7.5)	0.77 (0.43-1.39)	0.66 (0.35-1.24)	0.195
≥4	11 (2.6)	19 (4.5)	0.41 (0.16-1.05)	0.35 (0.13-0.95)	**0.039**
chi-trend (p-value)			*3.26 (0.071)*	*4.92 (0.027)*	
Continuous			0.87 (0.76-1.00)	0.85 (0.74-0.99)	**0.038**
Infections with fever >38°					
No infection[c]	321 (75.4)	307 (72.1)	1[d]	1[d]	
Only infections without fever	46 (10.8)	41 (9.6)	1.00 (0.63-1.58)	0.87 (0.54-1.41)	0.572
At least one infection with fever	59 (13.9)	78 (18.3)	0.63 (0.40-1.00)	0.61 (0.38-0.98)	**0.043**
Infections with antibiotic use					
No infection[c]	321 (75.4)	307 (72.1)	1[d]	1[d]	
Only infections without antibiotic	33 (7.6)	35 (8.2)	0.85 (0.49-1.46)	0.73 (0.41-1.29)	0.279
At least one infection with antibiotic	72 (16.9)	84 (19.7)	0.76 (0.51-1.15)	0.72 (0.47-1.11)	0.137
Fever >38°/antibiotic at first infection					
No infection[c]	321 (75.4)	307 (72.1)	1[d]	1[d]	
No fever/No antibiotic	27 (6.3)	26 (6.1)	0.87 (0.49-1.56)	0.75 (0.41-1.37)	0.348
No fever/Yes antibiotic[e]	26 (6.1)	23 (5.4)	1.02 (0.58-1.82)	0.95 (0.52-1.74)	0.865
Yes fever/No antibiotic	11 (2.6)	20 (4.7)	0.48 (0.22-1.06)	0.43 (0.18-0.99)	**0.046**
Yes fever/Yes antibiotic	41 (9.6)	50 (11.7)	0.69 (0.41-1.17)	0.67 (0.39-1.17)	0.157
Exanthematous diseases					
Chickenpox					
No	411 (96.5)	410 (96.0)	1[d]	1[d]	
Yes	15 (3.5)	16 (3.8)	0.93 (0.44-1.97)	0.96 (0.44-2.11)	0.912
Sixth disease					
No	410 (96.2)	401 (94.1)	1[d]	1[d]	
Yes	16 (3.8)	25 (5.9)	0.61 (0.31-1.18)	0.75 (0.37-1.51)	0.413

[a] Estimates from logistic regression, conditioned on study center, period of interview, child's age, and sex.
[b] Further adjusted for family history of any allergy in parents, having siblings, pet keeping, age at weaning, and type of delivery.
[c] Including 230 children with only infections after symptoms (125 cases and 105 controls).
[d] Reference category.
[e] Eleven eye infections, 8 bronchiolitis, 7 otitis, 5 bronchiolitis, 5 upper respiratory tract infections other than nasopharyngitis, pharyngotonsillitis and laryngitis, 13 other types of infections.

Table 3. Distribution of 426 incident cases of atopic dermatitis (AD) and 426 matched controls according to number of siblings, contact with pets and contact with other children in nursery, defined as indirect markers of infections. Italy, 2011-2014.

	Cases N=426 N (%)	**Controls N=426 N (%)**	**OR[a] (95% CI)**	**OR[b] (95% CI)**	***p-value (for adjusted OR)***
Number of siblings					
0	238 (55.9)	206 (48.4)	1[c]	1[c]	
1	153 (35.7)	168 (39.4)	0.79 (0.59-1.06)	0.79 (0.58-1.07)	0.120
≥2	35 (8.2)	52 (12.2)	0.60 (0.38-0.95)	0.62 (0.39-0.99)	**0.048**
chi-trend (p-value)			*5.91 (0.015)*	*5.19 (0.023)*	
Youngest siblings' age (years)					
>5	50 (11.7)	50 (11.7)	0.87 (0.56-1.33)	0.85 (0.54-1.33)	0.471
3-5	88 (20.7)	97 (22.8)	0.80 (0.58-1.11)	0.80 (0.57-1.13)	0.204
≤2	50 (11.7)	72 (16.9)	0.53 (0.33-0.85)	0.54 (0.33-0.88)	**0.014**
missing	*0*	*1*	-	-	
chi-trend (p-value)			*6.79 (0.009)*	*5.89 (0.015)*	
Pet keeping since birth[d]					
No	318 (74.6)	293 (68.8)	1[c]	1[c]	
Yes	108 (25.4)	133 (31.2)	0.74 (0.54-1.00)	0.73 (0.53-1.00)	**0.049**
Cat					
No[e]	382 (89.7)	384 (90.1)	1[c]	1[c]	
Yes	44 (10.3)	42 (9.9)	1.05 (0.68-1.64)	1.13 (0.71-1.79)	0.609
Dog					
No[f]	362 (85.0)	340 (79.8)	1[c]	1[c]	
Yes	64 (15.0)	86 (20.2)	0.69 (0.48-0.99)	0.67 (0.46-0.98)	**0.040**
Type of contact with cat only					
No pets	318 (74.7)	293 (68.8)	1[c]	1[c]	
Never contact	7 (1.6)	7 (1.6)	0.85 (0.29-2.45)	1.05 (0.35-3.12)	0.934
Occasional contact	6 (1.4)	8 (1.9)	0.75 (0.26-2.16)	0.75 (0.25-2.25)	0.607
Daily contact	23 (5.4)	14 (3.3)	1.56 (0.76-3.19)	1.47 (0.70-3.07)	0.309
Other pets	72 (16.9)	104 (24.4)	-	-	
Type of contact with dog only					
No pets	318 (74.7)	294 (68.8)	1[c]	1[c]	
Never contact	16 (3.7)	13 (3.1)	1.14 (0.53-2.46)	1.32 (0.60-2.94)	0.493
Occasional contact	22 (5.7)	23 (5.4)	0.87 (0.47-1.62)	0.76 (0.40-1.44)	0.396
Daily contact	18 (4.2)	37 (8.7)	0.44 (0.24-0.80)	0.40 (0.22-0.75)	**0.004**
Other pets	52 (12.2)	60 (14.1)	-	-	

Contact with other children					
No contact	222 (51.9)	194 (45.5)	1[c]	1 [c]	
Only with siblings >5 years old	43 (10.1)	45 (10.7)	0.84 (0.53-1.34)	0.83 (0.51-1.35)	0.449
Only with siblings ≤5 years old	131 (30.8)	160 (37.6)	0.71 (0.52-0.96)	0.72 (0.52-0.99)	**0.044**
In nursery and with siblings	14 (3.3)	14 (3.3)	0.97 (0.39-2.42)	1.15 (0.43-3.09)	0.777
Only in nursery (no siblings)	16 (3.8)	13 (3.1)	1.14 (0.48-2.70)	1.48 (0.58-3.77)	0.414
Age at entry in nursery					
No contact	222 (52.5)	194 (46.0)	1[c]	1 [c]	
Contact with siblings only	174 (41.1)	205 (48.6)	0.75 (0.56-0.99)	0.75 (0.56-1.00)	**0.054**
Entry at age 4-9 months old	15 (3.6)	11 (2.6)	1.00 (0.40-2.50)	1.12 (0.41-3.07)	0.825
Entry at age 10-19 months old	12 (2.8)	12 (2.8)	0.90 (0.33-2.45)	1.13 (0.38-3.32)	0.829
missing	*3*	*4*	-	-	
Time spent in nursery					
No contact	222 (52.5)	194 (46.0)	1[c]	1 [c]	
Contact with siblings only	174 (41.1)	205 (48.6)	0.75 (0.56-0.99)	0.75 (0.56-1.01)	**0.056**
Time spent: 1-4 months	11 (2.6)	12 (2.8)	0.75 (0.29-1.92)	0.87 (0.32-2.40)	0.789
Time spent: 5-16 months	16 (3.8)	11 (2.6)	1.29 (0.45-3.68)	1.59 (0.49-5.09)	0.439
missing	*3*	*4*	-	-	

[a] Estimates from logistic regression, conditioned on study center, period of interview, child's age, and sex.
[b] Further adjusted for family history of any allergy in parents, age at weaning, type of delivery, having had 4 or more infections, and, when appropriate, having siblings and pets keeping.
[c] Reference category.
[d] All pets are in contact with children since birth and therefore before symptoms.
[e] Among the 766 children without cats, there were 129 with dogs and 26 with other animals (mainly fish, bird and rabbit).
[f] Among the 702 children without dogs, there were 65 children with cats and 26 with other animals (mainly fish, bird and rabbit).