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Childhood acute lymphoblastic leukaemia and indicators of early immune stimulation: the Estelle study (SFCE)

R Ajrouche[1,2], J Rudant[1,3,4], L Orsi[1,3], A Petit[5,6], A Baruchel[7,8], A Lambilliotte[9], M Gambart[10], G Michel[11], Y Bertrand[12], S Ducassou[13], V Gandemer[14], C Paillard[15], L Saumet[16], N Blin[17], D Hémon[1,3] and J Clavel*[,1,3,4]

[1]Epidemiology of childhood and adolescent cancers, CRESS, INSERM U1153, Villejuif, France; [2]Paris-Sud University, Le Kremlin Bicêtre, France; [3]Paris-Descartes University, Paris, France; [4]RNHE—National Registry of Childhood Hematopoietic Malignancies, Villejuif, France; [5]AP-HP, Hôpital Armand Trousseau, Paris, France; [6]Université Paris 6 Pierre et Marie Curie, Paris, France; [7]AP-HP, Hôpital Robert Debré, Paris, France; [8]Université Paris 7, Paris, France; [9]Hôpital Jeanne de Flandre, CHRU, Lille, France; [10]Hôpital des Enfants, Toulouse, France; [11]AP-HM, Hôpital la Timone, Marseille, France; [12]Institut d'Hématologie et d'Oncologie Pédiatrique, Lyon, France; [13]Hôpital Pellegrin Tripode, Bordeaux, France; [14]CHU Hôpital Sud, Rennes, France; [15]Hôpital de Hautepierre, Strasbourg, France; [16]Hôpital Arnaud de Villeneuve, Montpellier, France and [17]Hôpital Mère-Enfant, CHU-Nantes, Nantes, France

Background: Factors related to early stimulation of the immune system (breastfeeding, proxies for exposure to infectious agents, normal delivery, and exposure to animals in early life) have been suggested to decrease the risk of childhood acute lymphoblastic leukaemia (ALL).

Methods: The national registry-based case–control study, ESTELLE, was carried out in France in 2010–2011. Population controls were frequency matched with cases on age and gender. The participation rates were 93% for cases and 86% for controls. Data were obtained from structured telephone questionnaires administered to mothers. Odds ratios (OR) were estimated using unconditional regression models adjusted for age, gender, and potential confounders.

Results: In all, 617 ALL and 1225 controls aged $\geqslant$1 year were included. Inverse associations between ALL and early common infections (OR = 0.8, 95% confidence interval (CI): 0.6, 1.0), non-first born ($\geqslant$3 *vs* 1; OR = 0.7, 95% CI: 0.5, 1.0), attendance of a day-care centre before age 1 year (OR = 0.7, 95% CI: 0.5, 1.0), breastfeeding (OR = 0.8, 95% CI: 0.7, 1.0), and regular contact with pets (OR = 0.8, 95% CI: 0.7, 1.0) in infancy were observed.

Conclusions: The results support the hypothesis that conditions promoting the maturation of the immune system in infancy have a protective role with respect to ALL.

Childhood acute leukaemia (CL) is the most common cancer in children, with ~450 new cases per year in France (Lacour *et al*, 2010a). In developed countries, the incidence of childhood acute lymphoblastic leukaemia (ALL), accounting for 80% of CL, markedly peaks at age 2–5 years. This particular age pattern has led to the hypothesis that environmental agents acting in early childhood may be involved.

One area that has been the subject of much interest, but remains controversial, is the role of the immune system and exposure to infections in early life in relation to the aetiology of ALL. The illness could be promoted indirectly by an abnormal or deregulated immune response to one or more common infections (Greaves, 1988; Ford *et al*, 2009; Kinlen, 2012). In this context, minimum previous exposure to infectious agents during infancy and

*Correspondence: Dr J Clavel; E-mail: jacqueline.clavel@inserm.fr

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inappropriate immune system modulation may modulate the risk of ALL later in childhood in the event of infection (Greaves, 2006). Associations with several proxies of exposure to infectious agents in infancy have been reported. The proxies include day-care attendance (Petridou *et al*, 1993, 1997; Infante-Rivard *et al*, 2000; Neglia *et al*, 2000; Rosenbaum *et al*, 2000; Chan *et al*, 2002; Perrillat *et al*, 2002; Jourdan-Da Silva *et al*, 2004; Gilham *et al*, 2005; Ma *et al*, 2005; Kamper-Jorgensen *et al*, 2008; Urayama *et al*, 2008; Rudant *et al*, 2010; Urayama *et al*, 2010; Rudant, 2014) and birth order(Dockerty *et al*, 1999; Little, 1999; McKinney *et al*, 1999; Schuz *et al*, 1999; Infante-Rivard *et al*, 2000; Neglia *et al*, 2000; Rosenbaum *et al*, 2000; Petridou *et al*, 2001; Murray *et al*, 2002; Naumburg *et al*, 2002; Perrillat *et al*, 2002; Reynolds *et al*, 2002; Hjalgrim *et al*, 2004; Jourdan-Da Silva *et al*, 2004; Gilham *et al*, 2005; Ma *et al*, 2005; Altieri *et al*, 2006; Wong and Dockerty, 2006; Simpson *et al*, 2007; Kamper-Jorgensen *et al*, 2008; MacArthur *et al*, 2008; Rudant *et al*, 2010, 2014), while the association with a history of early infection is less consistent (van Steensel-Moll *et al*, 1986; Dockerty *et al*, 1999; Neglia *et al*, 2000; Chan *et al*, 2002; Perrillat *et al*, 2002; Jourdan-Da Silva *et al*, 2004; Ma *et al*, 2005; Rosenbaum *et al*, 2005; Roman *et al*, 2007; Cardwell *et al*, 2008; Ma *et al*, 2009; Rudant *et al*, 2010; Urayama *et al*, 2011; Chang *et al*, 2012; Vestergaard *et al*, 2013). However, because children who develop ALL may have a deregulated immune response from birth and present with more symptomatic events in the event of infection (Chang *et al*, 2011, 2012; Crouch *et al*, 2012), the overall direction of the association between ALL and infections may be less predictable, and may depend on the intensity of the symptoms considered.

Other factors, like breastfeeding, mode of delivery, or early exposure to animals, are also related to early immune stimulation and may influence the risk of CL. Meta-analyses suggest that prolonged breastfeeding has a protective effect with respect to CL (Kwan *et al*, 2005; Martin *et al*, 2005; Rudant, 2014). Indeed, mounting evidence suggests a key role of the microbiome in human health, especially the induction of immune tolerance and adaptive and innate function (Round and Mazmanian, 2009; Lee and Mazmanian, 2010). Mode of delivery and breastfeeding have a profound impact on the composition of the microbiome (Siggers *et al*, 2008; Fernandez *et al*, 2013), which is a determinant of early immune programming and subsequent response to infections (Madan *et al*, 2012). Only one previous study, conducted by the authors' group, suggested that exposure to animals during infancy, which may also stimulate maturation of the naive immune system, protected against leukaemia (Rudant *et al*, 2010).

The aim of the present study was to investigate the links between ALL (immunological and cytogenetic subtypes) and various indicators of early immune modulation, such as breastfeeding, mode of delivery, proxies for exposure to infectious agents (history of common infections in infancy, day-care attendance, and birth order), and exposure to animals in early life, on the basis of the ESTELLE study.

MATERIALS AND METHODS

The ESTELLE study was conducted to investigate the role of infectious, environmental, and genetic factors in CL, lymphoma, neuroblastoma, and brain tumour. This paper focuses on CL.

Case and control ascertainment. The design of the ESTELLE study has been reported elsewhere (Ajrouche *et al*, 2014).The cases were directly identified by the investigators of the National Registries of Childhood Haematopoietic Malignancies (NRCH) and Solid Tumours (NRST) in all the paediatric oncology units in France (Lacour *et al*, 2010b). The eligible CL cases consisted children newly diagnosed with CL between 1 January 2010 and 31 December 2011 who were <15 years old and residing in mainland France at the time of diagnosis. Cases were not eligible if they had been adopted ($n=5$), or if their biological mother had died ($n=4$), was absent ($n=1$), did not speak French ($n=31$), or had a serious social problem ($n=7$) or psychiatric disorder ($n=5$), or if the child was in palliative care or had died ($n=8$). Information on the leukaemia subtype was obtained subsequently from the NRCH.

The population controls were children free from cancer selected between 2010 and 2011 in France using a quota sampling method. One million telephone numbers were randomly generated, 312 022 of which were allocated numbers. The numbers were distributed in 40 successive sets over the 2-year subject-recruitment period. Of the 312 022 numbers that were dialled, 34 983 resulted in a contact with a household. Up to 15 call attempts were made for each number and up to 12 ring tones for each call. Quotas were used to obtain, overall, at least one control per case for each year of age, gender, and type of cancer based on the numbers expected on the basis of the national registries. Controls aged <1 year were overrepresented in order to increase power in that age category. The quotas also ensured that the control group had the same distribution as the overall population for the number of children aged <15 years living in the household, conditional on age. Like the cases, children who had been adopted, or whose biological mother had died or did not speak French were not eligible as controls. In all, 747 of the 803 eligible CL cases (93%) including 714 cases of ALL and 1421 of the 1662 eligible controls (86%) participated in the ESTELLE study.

Data collection. The same trained interviewers carried out the interviews with the cases' and controls' mothers using structured questionnaires with computer-assisted telephone interviewing). The cases' mothers were interviewed on average 6 months after diagnosis. The questionnaire elicited information on demographic and socioeconomic characteristics, childhood environment and lifestyle, family and personal medical history, maternal reproductive history and child-care history as determined by maternal statements, the mother's reading of her child's health-care record, or both sources.

In particular, the interviews included questions on mode of delivery and motivation for caesarean section; breastfeeding and duration of breastfeeding; history of common childhood infections during the first year of life (tonsillitis, otitis, upper respiratory tract infections, gastroenteritis, bronchiolitis and other lower respiratory tract infections, and urinary tract infections) with the frequency of episodes for each type of infection; ear, nose, and throat (ENT) surgery for repeated common infections (adenoidectomy, tonsillectomy, paracentesis) before the age of 4 years; neonatal infection; history of paediatric infections (measles, rubella, chicken pox, mumps, whooping cough, scarlet fever, hand, foot and mouth disease, meningitis, mononucleosis); and history of hospitalisation for infections and other causes. Detailed information was collected on the type of childcare (day-care centre 'crèche', nanny), the age when care started, and the magnitude of child-care attendance (duration of stay, mean hours per week, total number of children attending the centre). Cumulative exposure to a type of childcare was calculated for each child (number of months attending child care $\times$ mean hours per week at that child care $\times$ number of other children at childcare multiplied by 4.35 (number of weeks per month)). Data on the number of other children in the household and birth order were also collected. Mothers were also asked how often the child had visited a farm and whether the child had been in regular contact, at least once a week, with animals (cats, dogs, domestic birds, domestic rodents, poultry, rabbits, pigs, cows, sheep, goats, horses, and other animals).

Statistical analysis. The analyses were restricted to children aged 1 year or older: first, to ensure that all the cases and controls had had a complete opportunity to encounter the exposures of interest,

which take place in the first year of life; and second, because common infections occurring before 1 year of age may have been related to a pre-diagnostic phase of the disease in the ALL cases aged <1 year at diagnosis.

Odds ratios (ORs) and their 95% confidence intervals (CIs) were estimated using unconditional logistic regression models, including the stratification variables age and gender used for quota sampling. Systematic adjustment was conducted for parental professional category, maternal age and urban/rural status of the place of residence, and for animal exposure, housing (apartment or house) during the first year of life. The stability of the results was tested after additional adjustments for parental education, paternal smoking, and after exclusion of cases and controls with chromosomal abnormalities. Analyses were carried out by CL subtype and by ALL cytogenetic and immunologic subtype. The SAS software package (version 9, Cary, NC, USA) was used for all the analyses.

RESULTS

A total of 617 ALL cases, including 485 B-cell precursor ALL (BCP-ALL), 94T-cell ALL and 26 Burkitt ALL, and 1225 controls aged 1 year or older were included in the analyses (Supplementary Table 1).

Case–control comparability. The controls were similar to the whole case group with respect to age and gender (Ajrouche *et al*, 2014) but not to the leukaemia cases considered alone because of overrepresentation of the age group 2–4 years. Nevertheless, all quota sampling strata included at least one control per ALL case (Supplementary Table 2). Overall, educational level and professional category were similar for the cases' and controls' parents. The case mothers were significantly more often <25 years old at the time of the index child's birth than the control mothers. The residences of the cases were slightly more often in urban areas than those of the controls (Table 1).

Description of the exposure of interest for the controls. The controls whose parents had a higher social–professional category or a higher education degree attended a day-care centre in their first year of life more often and were breastfed for at least 6 months more often than the other controls.

The non-firstborn children had attended a day-care centre in the first year of life less often (13.5% *vs* 17.2%; OR = 0.63, 95% CI: 0.47, 0.85) and were breastfed for 6 months or more slightly more often (19.7% *vs* 14.2%; OR = 1.18, 95% CI: 0.88, 1.58) than first-born children. Children delivered by caesarean section were breast fed slightly less often than those born by normal delivery (55.6% *vs* 60.2%; OR = 0.81, 95% CI: 0.59, 1.10).

Four or more common infections in the first year of life were reported more often for the children who had attended a day-care centre in their first year of life, and reported slightly more frequently for non-firstborn and less frequently for children breastfed for 6 months or more. In addition, the second-born children had visited a farm more than first-born children.

Early common infections, birth order, day-care attendance, breastfeeding, and mode of delivery. Overall, a history of any common infections before 1 year old was significantly negatively associated with ALL (OR = 0.75, 95% CI: 0.57, 0.99); the ORs decreased with increasing frequency of episodes (P trend = 0.02) and were also significant for ENT surgery before age 4 years (OR = 0.63, 95% CI: 0.40, 0.99), whereas hospitalisation for common infection was positively, but not significantly, associated with ALL (OR = 1.48, 95% CI: 0.91, 2.41) (Table 2). When the analysis was restricted to mothers to whom the child's health record was available at the time of interview, the results were not substantially changed (Supplementary Table 3). Regarding the specific site of infection, ALL was negatively and significantly associated only with early conjunctivitis (OR = 0.76, 95% CI: 0.58, 0.99) and gastroenteritis (OR = 0.71, 95% CI: 0.56, 0.89) (Supplementary Table 4).

Firstborn status was associated with ALL (Table 3), and the ORs decreased significantly with increasing birth order (P trend = 0.03).

Table 1. Sociodemographic characteristics of the cases of ALL and controls – children aged 1–14 years (Estelle study, France, 2010–2011)

	Controls (N = 1225)		ALL (N = 617)			
	n	%	*N*	%	OR	95% CI
Maternal educational						
<Secondary diploma	356	(29%)	186	(30%)	1.15	0.91–1.45
Secondary diploma	268	(22%)	133	(22%)	1.02	0.79–1.31
>Secondary diploma	600	(49%)	297	(48%)	1.00	Ref.
Missing	1		1			
Parental professional category (higher of the two parents)						
Intellectual/scientific jobs, managers and intermediate professions	813	(66%)	388	(63%)	1.00	Ref.
Administrative and sales workers	255	(21%)	138	(22%)	1.14	0.90–1.46
Service workers and factory workers	157	(13%)	91	(15%)	1.21	0.91–1.61
Maternal age at child's birth (years)						
<25	123	(10%)	96	(16%)	1.72	1.24–2.38
25–29	392	(32%)	213	(35%)	1.20	0.95–1.53
30–34	444	(36%)	205	(33%)	1.00	Ref.
35 or more	266	(22%)	103	(17%)	0.77	0.58–1.02
					P trend <0.001	
Place of residence at diagnosis						
Rural (<5000 people)	511	(42%)	243	(39%)	1.00	Ref.
Intermediate (5000–100 000 people)	273	(22%)	128	(21%)	1.03	0.79–1.34
Urban (>100 000 people)	435	(36%)	244	(40%)	1.20	0.96–1.50
Missing	6		2			
Mother's country of birth						
France	1114	(91%)	550	(89%)	1.00	Ref.
Other countries	111	(9%)	67	(11%)	1.26	0.91–1.75

Abbreviations: ALL = acute lymphoblastic leukaemia; CI = confidence interval; OR = odds ratio; Ref. = reference category. ORs and 95% CI estimated by unconditional logistic regression adjusted for age and gender.

Table 2. Associations between common infections in the first year of life and ALL – children aged 1–14 years (Estelle study, France, 2010–2011)

	Controls ($N=1225$)		ALL ($N=617$)			
	n	%	*N*	%	OR[a]	95% CI
Any common infections before 1 year[b]						
None	160	(13%)	99	(16%)	1.00	Ref.
At least one	1065	(87%)	518	(84%)	0.75	0.57–0.99
1	128	(87%)	71	(84%)	0.87	0.59–1.29
2	186	(15%)	92	(15%)	0.81	0.57–1.17
3	151	(12%)	71	(12%)	0.71	0.48–1.05
4+	600	(49%)	284	(46%)	0.72	0.54–0.97
					P trend = 0.02	
Hospitalisation for common infection before age 1 year						
No	1185	(97%)	585	(95%)	1.00	Ref.
Yes	40	(3%)	32	(5%)	1.48	0.91–2.41
Neonatal infection						
No	1203	(98%)	609	(99%)	1.00	Ref.
Yes	22	(2%)	8	(1%)	0.68	0.29–1.58
Specific infection before age 1 year[c]						
No	1164	(95%)	586	(95%)	1.00	Ref.
Yes	61	(5%)	31	(5%)	0.97	0.62–1.53
ENT surgery for common infections before age 4 years[d]						
No	1141	(93%)	589	(95%)	1.00	Ref.
Yes	84	(7%)	28	(5%)	0.63	0.40–0.99

Abbreviations: ALL = acute lymphoblastic leukaemia; CI = confidence interval; ENT = ear, nose, and throat; OR = odds ratio; Ref. = reference category.
[a]ORs and 95% CI estimated by unconditional logistic regression adjusted for age and gender, paternal professional category, and degree of urbanisation.
[b]Tonsilitis, otitis, rhinopharyngitis, laryngitis, conjonctivitis, bronchiolitis, pulmonary infection, gastroenteritis, or urinary tract infection.
[c]Three measles, 1 rubella, 58 chicken pox, 1 mumps, 1 whooping cough, 4 scarlet fever, 1 meningitis, 1 mononucleosis among controls and 5 measles, 0 rubella, 25 chicken pox, 1 mumps, 2 whooping cough, 2 scarlet fever, 1 meningitis, 0 mononucleosis among cases.
[d]Ear, nose, and throat surgery (tonsillectomy, adenoidectomy, paracenteses).

Day-care centre attendance in infancy was negatively associated with ALL (OR = 0.71, 95% CI: 0.53, 0.96). The results were similar for part-time (OR = 0.67, 95% CI: 0.42, 1.07) and full-time (OR = 0.73, 95% CI: 0.51, 1.04) attendance. With regard to the other characteristics of day-care attendance, ALL was significantly and negatively associated with attending day-care before 6 months. No association with the other types of childcare (parents, nanny), with child-hours or with the number of other children at the nanny's was observed.

Breastfeeding was significantly associated with ALL (OR = 0.80, 95% CI: 0.66, 0.99). There was no significant difference between the cases and controls regarding caesarean section (Table 3), whatever its motivation (Supplementary Table 5). The OR was slightly greater than the unit (OR = 1.28, 95% CI: 0.88, 1.87) when the analysis was restricted to first-borns.

Contacts with pets and farm animals, farm visits. Out of 1225 control children, 539 (44%) have had contact with a dog and 432 (35%) with a cat at home before the age of 1 year. Regular contacts with pets in the first year of life were negatively associated with ALL (OR = 0.84, 95% CI: 0.68, 1.04) and the relationship was significant for regular contact with cats (OR = 0.75, 95% CI: 0.59, 0.96). Regular contacts with farm animals in the first year of life tended to be negatively associated with ALL (OR = 0.93, 95% CI: 0.65, 1.32), but not significantly (Table 4). The proportion of children who visited a farm at least once a month before age 1 year was lower for the ALL cases than for the controls, but without any dose–response relationship.

Subtype analysis. The ORs for BCP-ALL were similar to those for all ALL (Table 5). There was no significant heterogeneity of BCP-ALL with respect to hyperdiploidy or ETV6-RUNX1 status.

Adjustments and sensitivity analysis. The associations were very similar after adjustment for education, maternal age at birth, and paternal smoking, and after exclusion of the children with Down's syndrome (six cases of ALL and no control). Stratified analyses showed that the relationships with the main exposures of interest were stable across 5-year age groups. There was no interaction with age. The results were not substantially changed when all of the variables of interest were adjusted for each other (Supplementary Table 6). The association between ALL and early common infections was stronger for non-breastfed children (OR = 0.61, 95% CI: 0.38, 0.98) than for breastfed children (OR = 0.79, 95% CI: 0.55, 1.12), but there was no significant interaction. Further, the association between ALL and day-care attendance tended to decrease, although not significantly, with increasing birth order (first-born children: 0.59 (0.40–0.87), second-born children: 0.75 (0.44–1.25); third-born children 1.00 (0.52–1.92)). In addition, the associations between common infection, breastfeeding, day-care attendance, birth order, and ALL were unchanged when the children delivered by caesarean section were excluded from the analysis.

DISCUSSION

In the present study, ALL was negatively associated with several factors generally considered to stimulate the immune system in infancy: having an older sibling, attending a day-care centre before age 1 year, being breastfed, early common infections, and regular contacts with animals. One of the main strengths is that the study concomitantly investigated those indicators of early immune stimulation with respect to ALL. The size of the study generated sufficient statistical power for most of the associations under study, but the power was limited for analyses by subtype.

The cases were identified in all the paediatric cancer units in mainland France by the data collection system of the French

Table 3. Birth order, type of childcare, and breastfeeding in the first year of life, mode of delivery, and ALL – children aged 1–14 years (Estelle study, France, 2010–2011)

	Controls ($N=1225$)		ALL ($N=617$)			
	n	%	*N*	%	OR[a]	95% CI
Birth order						
1	508	(41%)	303	(49%)	1.00	Ref.
2	430	(35%)	206	(33%)	0.84	0.67–1.06
⩾ 3	287	(23%)	108	(18%)	0.72	0.54–0.97
					P trend = 0.03	
Type of childcare						
Home care (parents only)	586	(48%)	313	(51%)	1.00	Ref.
Nanny only	417	(34%)	215	(35%)	0.97	0.77–1.22
Day-care	222	(18%)	89	(14%)	0.71	0.53–0.96
Characteristics of day-care attendance[b]						
Frequency of day-care attendance						
Home care	586	(48%)	313	(51%)	1.00	Ref.
Part-time attendance (1–2 days per week)	78	(6%)	29	(5%)	0.67	0.42–1.07
Full-time attendance (3–5 days per week)	144	(12%)	60	(10%)	0.73	0.51–1.04
Age at start of day-care attendance						
Home care	586	(48%)	313	(51%)	1.00	Ref.
<6 months	102	(8%)	38	(6%)	0.66	0.43–1.00
⩾6 months	120	(10%)	51	(8%)	0.75	0.52–1.08
Characteristics of childcare by nanny[c]						
Cumulative duration						
Home care	586	(48%)	313	(51%)	1.00	Ref.
1st tertile (childhood hours<1566)	139	(11%)	78	(13%)	1.02	0.74–1.42
2nd tertile (1566 ⩽childhood hours<3132)	148	(12%)	71	(12%)	0.92	0.66–1.28
3rd tertile (childhood hours⩾3132)	127	(10%)	65	(11%)	0.96	0.68–1.36
Missing	3		1			
Number of other children at nanny's						
Home care	586	(48%)	313	(51%)	1.00	Ref.
None	29	(2%)	19	(3%)	1.34	0.74–2.49
1 or 2	264	(22%)	129	(21%)	0.91	0.66–1.28
>2	121	(10%)	66	(11%)	1.02	0.68–1.36
Missing	3		1			
Breastfeeding						
No	497	(41%)	278	(45%)	1.00	Ref.
Yes	728	(59%)	339	(55%)	0.80	0.66–0.99
<6 months	488	(40%)	233	(38%)	0.81	0.65–1.02
⩾6 months	240	(20%)	106	(17%)	0.78	0.59–1.04
Caesarian section						
No	1011	(83%)	501	(81%)	1.00	Ref.
Yes	214	(17%)	116	(19%)	1.11	0.85–1.43

Abbreviations: ALL = acute lymphoblastic leukaemia; CI = confidence interval; OR = odds ratio; Ref. = reference category.
[a]ORs and 95% CI estimated by unconditional logistic regression adjusted for age, gender, parental professional category, maternal age, and degree of urbanisation.
[b]Children with childcare by nanny were excluded from the analyses.
[c]Children with childcare by the day-care centre were excluded from the analyses.

NRCH, which has a high degree of completeness. Thus, very few diagnosed cases are likely to have escaped inclusion because of non-identification. Moreover, the participation of the eligible case mothers was very high (93%). Control selection was based on random generation of listed and unlisted telephone numbers. This procedure may have induced bias because households with no landline number were not accessible for control selection, whereas the cases with a cell phone only (10% of the cases) were included. Compared with the cases' mothers, the controls' mothers were aged <25 years less often, which may be related to the fact that the households with cell phones only could not be accessed by the selection procedure. However, having a cell phone only was not associated with the exposures of interest for the cases and the analyses excluding the cases with no landline number led to similar results. In addition, the analyses were adjusted for maternal age. Bias could also have occurred if greater participation of eligible controls with higher socioeconomic status led to overrepresentation of day-care attendance among participating controls, and then to overestimation of the inverse associations with ALL. Actually, in this study the participation rates among the eligible controls and cases were high and adjustments for educational or professional social category, which were made in all the analyses, had no impact on the estimates. In fact, a Danish registry-based study, based on 176 ALL cases and 1571 controls with complete child-care registration and thus free from participation bias, also showed an inverse association between ALL and child-care attendance during the first 2 years of life (Kamper-Jorgensen *et al*, 2008). In addition, the distribution of the responding controls by birth order and breastfeeding was similar to that of the French national perinatal surveys (15 000 births each) carried out in 1998, 2003, and 2010 (Blondel *et al*, 2012). On average, 43.3% of the newborns in those surveys were firstborn, 34.1% were second born, and 24.5% were third born or more, compared with 41.0%, 35.0%, and 23.9%, respectively, of the included controls born between 1998 and 2010. Similarly, 61.2% of the newborns in the surveys were breastfed from birth compared with 60.9% of the study controls.

Table 4. Contacts with animals and visits to farms in the first year of life and ALL – children aged 1–14 years (Estelle study, France, 2010–2011)

	Controls (*N* = 1225)		ALL (*N* = 617)			
	n	%	*N*	%	OR[a]	95% CI
Contact at least once a week with any animals before age 1 year						
No	494	(40%)	280	(45%)	1.00	Ref.
Yes	731	(60%)	337	(55%)	0.84	0.68–1.04
With pets						
No	502	(41%)	289	(47%)	1.00	Ref.
Yes	721	(59%)	328	(53%)	0.81	0.66–1.00
Cats	432	(35%)	179	(29%)	0.75	0.59–0.96
Dogs	539	(44%)	244	(40%)	0.84	0.67–1.05
Rodents	56	(5%)	17	(3%)	0.56	0.31–1.01
Birds	58	(5%)	20	(3%)	0.60	0.34–1.03
With farm animals						
No	1105	(90%)	562	(91%)	1.00	Ref.
Yes	118	(10%)	54	(9%)	0.93	0.65–1.32
Cows	27	(2%)	3	(0%)	0.24	0.07–0.81
Sheep, goats	23	(2%)	6	(1%)	0.55	0.22–1.40
Pigs	7	(1%)	1	(0%)	0.26	0.03–2.47
Rabbits	71	(6%)	30	(5%)	0.87	0.55–1.36
Horses, ponies, donkies	36	(3%)	15	(2%)	0.83	0.44–1.59
Poultry	57	(5%)	21	(3%)	0.78	0.46–1.33
Farm visit at least once before age 1 year						
No	780	(64%)	439	(71%)	1.00	Ref.
Yes	440	(36%)	175	(28%)	0.70	0.56–0.87
Several days per year	306	(25%)	115	(19%)	0.65	0.50–0.83
Several days per month	80	(7%)	41	(7%)	0.98	0.67–1.47
Several days per week	54	(4%)	19	(3%)	0.61	0.34–1.08

Abbreviations: ALL = acute lymphoblastic leukaemia; CI = confidence interval; OR = odds ratio; Ref. = reference category.
[a]ORs and 95% CI estimated by unconditional logistic regression adjusted for age, gender, parental category, degree of urbanisation, housing.

Table 5. Relationship between factors related to immune stimulation and acute ALL by subtype –children aged 1–14 years (Estelle study, France, 2010–2011)

	BCP-ALL (*N* = 485)															
	Numerical abnormalities						ETV6-RUNX1									
	None (*N* = 179)		47–50 chromosomes (*N* = 70)		>50 chromosomes (*N* = 161)		Negative (*N* = 335)		Positive (*N* = 119)		Total BCP-ALL		Burkitt's ALL (*N* = 26)		T-cell ALL (*N* = 94)	
	OR[a]	95% CI	OR[a]	95% CI	OR[a]	95% CI	OR[a]	95% CI	OR[a]	95% CI	OR[a]	95% CI	OR[a]	95% CI	OR[a]	95% CI
Non-firstborn	0.65	0.47–0.89	0.52	0.31–0.85	0.73	0.52–1.03	0.71	0.56–0.92	0.65	0.44–0.95	0.72	0.57–0.89	0.32	0.14–0.74	0.73	0.47–1.12
Caesarian section	1.23	0.83–1.82	0.94	0.49–1.78	0.76	0.47–1.21	0.99	0.72–1.37	0.98	0.59–1.61	1.05	0.79–1.38	0.88	0.29–2.64	0.93	0.53–1.64
Breastfeeding	1.01	0.73–1.41	0.96	0.58–1.58	0.60	0.43–0.84	0.82	0.61–1.03	0.84	0.56–1.25	0.82	0.66–1.02	0.55	0.24–1.23	0.78	0.51–1.21
Day-care	0.93	0.61–1.43	0.78	0.40–1.54	0.74	0.46–1.20	0.83	0.59–1.17	0.62	0.36–1.10	0.77	0.57–1.04	0.59	0.17–2.06	0.47	0.24–0.95
Any common infections before age 1 year	0.77	0.50–1.21	0.73	0.37–1.44	0.89	0.55–1.43	0.74	0.53–1.04	0.78	0.45–1.34	0.74	0.55–0.99	0.83	0.27–2.48	0.84	0.46–1.53
ENT surgery for common infections before 4 years[b]	0.57	0.25–1.26	0.42	0.10–1.77	0.49	0.19–1.26	0.66	0.37–1.17	0.37	0.17–1.21	0.54	0.32–0.92	0.43	0.06–3.33	1.09	0.52–2.29
Contact with animal before age 1 year	0.72	0.52–1.00	0.73	0.44–1.21	0.82	0.58–1.16	0.84	0.65–1.08	0.69	0.46–1.03	0.78	0.62–0.98	2.01	0.81–4.97	0.86	0.56–1.34

Abbreviations: AL = acute leukaemia; ALL = acute lymphoblastic leukaemia; BCP = B-cell precursor ; CI = confidence interval; ENT = Ear, nose, and throat; OR = odds ratio.
[a]ORs and 95% CI estimated by unconditional logistic regression adjusted for age, gender, parental category, degree of urbanisation.
[b]Ear, nose, and throat surgery (tonsillectomy, adenoidectomy, paracenteses).

Misclassification of infections is likely to have occurred as they were described retrospectively and were reported by maternal interview. However, in the present study 94% of the control mothers *vs* 84% of the case mothers had their child's health records available during the interview to help them recall medical events. In the UK Childhood Cancer Study (Simpson *et al*, 2007) medical information contemporaneously recorded in medical records differed from maternal recall of infections confirmed by a general practitioner: an episode of any infection recorded by the general practitioner was slightly more underreported by the cases' mothers (66%) than by the controls' mothers (72%). Owing to the difficulty of retrospectively measuring the occurrence of childhood infectious diseases, indicators of the opportunities for exposure to infection through physical and social contacts, such as birth order and type of childcare, were used. These variables are easier to obtain, less prone to recall bias, and probably track contacts with infectious

agents that result in symptomatic or asymptomatic infections. The findings of a validation study also suggest that young adults reliably report contact with pets for children aged 0–6 years (Nicholas *et al*, 2009).

The present study showed an inverse association between maternally reported common infections and ALL, but the association between hospitalisation for common infection and ALL was positive (based on a small number). This finding supports the hypothesis that infections may be more symptomatic in children who will subsequently develop ALL if a deregulated immune response already exists in infancy (Wiemels, 2012), and that the link may depend on the intensity of the symptoms considered.

The variables considered as proxy measures of the opportunities for exposure to infectious agents are less subject to misclassifications. Among them, being firstborn was positively associated with ALL in several studies (van Steensel-Moll *et al*, 1986; McKinney *et al*, 1999; Schuz *et al*, 1999; Infante-Rivard *et al*, 2000; Dockerty *et al*, 2001; Hjalgrim *et al*, 2004; Ma *et al*, 2005; Urayama *et al*, 2011), but not in other studies (Petridou *et al*, 1993; Dockerty *et al*, 1999; Neglia *et al*, 2000; Rosenbaum *et al*, 2000; Murray *et al*, 2002; Okcu *et al*, 2002; Perrillat *et al*, 2002; Reynolds *et al*, 2002; Oksuzyan *et al*, 2012), and even negatively associated in two studies (Ou *et al*, 2002; Jourdan-Da Silva *et al*, 2004).

As day-care centre attendance greatly increases the likelihood of being exposed to infectious agents in infancy (Lu *et al*, 2004; Nesti and Goldbaum, 2007; Kamper-Jorgensen *et al*, 2011; Enserink *et al*, 2012), day-care in early life has also been considered a good surrogate for investigation of the role of early immune system stimulation. The results of this study were consistent with those of the meta-analysis (Urayama *et al*, 2010) and pooled analysis (Rudant, 2014) that reported negative associations between CL and day-care centre attendance. In the present study, a significant and negative association with day-care centre attendance before age 6 months was also observed, and is consistent with the inverse trend reported in the CLIC study. The association with day-care attendance was not observed for third-born children, which is compatible with the assumption that older siblings may be a source of exposure to infectious agents. Interestingly, another strength of the study is that it provides detailed information on the type of childcare. However it showed no association with other types of childcare (nanny). Thus, based on the present data reverse causality is not a likely explanation for the inverse relationship between ALL and day-care centre attendance. Indeed, the cases were not reported to have had more infections than the controls. In addition, in France, registration at a day-care centre usually takes place before birth because the number of places is limited. This makes it unlikely that hospitalisation for infection could have prevented attendance of a day-care centre.

With regard to breastfeeding, the present study and the literature point to an inverse association with prolonged breastfeeding (Kwan *et al*, 2004; Martin *et al*, 2005; Rudant, 2014). In fact, breast milk protects the child against infections, boosts the immune system, and contributes to its modulation either by factors with anti-infective, hormonal, enzymatic, tropic or biological activity, or through a modulating effect on the neonatal immune system exerted by cells, cytokines, and other immune agents in human milk (Chirico *et al*, 2008), or by affecting the infant's gut microflora (Wold and Adlerberth, 2000).

Perinatal exposures to microorganisms is also determined by the mode of delivery, which results in significant differences in the composition of the gut microflora during the first 6–12 months of extrauterine life (Gronlund *et al*, 1999). This period is critical in adaptive immune development. However, to date, there is no clear evidence for an association between ALL and caesarean section. This association was not observed in the present study. Only two studies found a positive and significant association between caesarean section and ALL(Kaye *et al*, 1991; Francis *et al*, 2014), whereas three other studies found no statistically significant association (Reynolds *et al*, 2002; Podvin *et al*, 2006; Johnson *et al*, 2008).

Regular contact with animals during infancy may also be considered a factor stimulating the immune system. It has been suggested that keeping pets, especially dogs, may increase exposure to bacterial compounds such as endotoxins, which may enhance type 1 lymphocyte (T-helper 1) development in children (Campo *et al*, 2006). In the past, a few studies have reported either positive (Bross and Gibson, 1970; Nishi and Miyake, 1989; Petridou *et al*, 1997) or null (Buckley *et al*, 1994; Dockerty *et al*, 1999; Swensen *et al*, 2001) associations between CL and contacts with pets during childhood. However, none of those studies specifically addressed exposure in infancy, which is the critical period in that context. Two of the studies also reported results for exposure to animals other than cats and dogs during childhood and showed significant positive associations (Buckley *et al*, 1994; Swensen *et al*, 2001), although not specifically for exposure in early life. At last, one paper reported a negative association with living on a farm during childhood (Dockerty *et al*, 1999). The possibility of residual confounding also remains a possible issue. Farm-related exposures may include a multitude of potentially carcinogenic factors such as fungi, microbes, and pesticides, which are suspected of being associated with childhood cancer in general (Infante-Rivard and Weichenthal, 2007). The authors' previous study – Escale (2003–2004) – similarly evidenced the protective role of being in contact with animals in the first year of life and the influence of early mixing with others in day-care nurseries.

The underlying mechanism for this observation and the microorganism exposures that might be the root cause need further investigation. In addition, genes involved in the immune system, such as those coding for human lymphocyte antigen polymorphisms, have been found to be associated with CL (Taylor *et al*, 2009), and investigation of the way in which the polymorphisms of indentified genes in GWAS, such as (*ARID5B, IKZF1, GATA3, CEBPE*, etc), and other immunity genes interact with early common infections, breastfeeding, and other factors linked to early stimulation of the immune system also constitutes a promising prospect.

Overall, the present study does not support the hypothesis that caesarean section is related to ALL, but has generated further evidence that breastfeeding, day-care attendance, common infections, and regular contacts with animals in infancy, all of which are conditions promoting the maturation of the immune system, may have a protective role with respect to ALL.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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