



Review article

Atopy risk among school-aged children in relation to early exposures to a farm environment: A systematic review

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ABSTRACT

Background and objectives: Childhood atopy is a complex condition with both a genetic and an environmental component. This systematic review will explore the current understanding of the importance of early life exposures to a farm in the development of atopy measured by objective markers of skin prick testing, and specific IgE measurements in school age children.

Methods: A systematic review was performed.

Results: Among 7285 references identified, 14 studies met the inclusion criteria (13 cross-sectional studies and 1 case-control study). The results were fairly consistent in that early farm-related exposures can protect children from becoming atopic at school age. In general, there was heterogeneity in the assessment of outcomes and exposures.

Conclusions: Early-life farm exposures are associated with a protective effect on childhood atopy as assessed by objective markers. Future work should focus on understanding specific farm exposures that may important in these associations between atopy and farm exposures in children.

1. Introduction

Atopy, the tendency to produce an exaggerated immunoglobulin E (IgE) immune response to a common allergen, usually measured by serum IgE and/or skin prick tests, and atopic diseases (atopic dermatitis, asthma, and hay fever) have become major public health issues, particularly in affluent societies [1], including Canada. Atopic diseases diminish a patient's quality of life, have considerable socio-economic costs, cause patient morbidity, and lead to high healthcare utilization [2–5]. Atopic individuals are at increased risk of developing symptoms of asthma, atopic dermatitis, and atopic rhinoconjunctivitis [6,7]. Atopy and atopic diseases have become a major public health issue, particularly in affluent societies [1], including Canada. Atopic diseases diminish a patient's quality of life, have considerable socio-economic costs, cause patient morbidity, and lead to high healthcare utilization [2–5]. Atopic individuals are at increased risk of developing symptoms of asthma, atopic dermatitis, and atopic rhinoconjunctivitis [6,7].

The prevalence of atopy and atopic diseases has risen in recent

decades in many nations around the world, predominantly in countries with a westernized lifestyle [1,8]. Atopy is likely caused by interactions between genetic and environmental factors. Genetic factors are clearly important, but cannot independently explain the increased prevalence of atopic diseases in recent decades [9,10]. Recent evidence links multiple changes in modern dietary intakes [11,12], lifestyle factors [13,14] and surrounding environments [15,16] to adverse influences on the developing immune system and increasing prevalence of early childhood inflammatory disease including atopic disease [17].

Atopic related diseases, which can include atopic dermatitis, atopic rhinitis, allergies and asthma, are some of the most common chronic childhood diseases. The clinical definition of atopy is an increased Immunoglobulin E (IgE) antibody response to an allergen and confirmation of sensitization with positive skin prick test (SPT) or presence of allergen-specific IgE antibodies identified by blood tests [18]. Atopy is characterized by an imbalance in the production of T-helper 1 (Th1) and T-helper 2 (Th2) cytokines through a Th-2 polarization and related IgE-mediated reactions [19]. Typically, individuals with atopy have

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excess production of IgE and Th2 cytokines [20].

Early childhood, including the prenatal period, has been highlighted as a period of great risk for developing atopy. A growing body of evidence indicates that atopy and atopic disease has fetal origins and the fetal time period is a critical window of immune system development [21,22]. Earlier studies report immune dysregulation at birth suggesting that prenatal exposures may influence the programming of the neonatal immune response [23]. It is possible that maternal exposure may affect the developing immune response through direct exposure of the fetus to antigens that cross the placental barrier [24,25]. Alternatively, maternal response to an environmental exposure may influence the *in-utero* environment for the developing fetus [26]. Maternal sensitization to allergens has been shown to be associated with an elevated production of the Th2-related cytokine interleukin (IL)-13 in the infant [27]. Fetal programming may occur through imprinting of the infant gut microbiota [28,29] or epigenetic regulation of immune function that begins *in utero* [30]. Recent reports indicate that the gut microbiota composition during the first months of life influences atopy development [31,32]. It has been suggested that factors influencing the early maturation of the immune system play an important role in the presence of atopic disease [33].

The farming environment is rich in microbes. It has been suggested that this microbial exposure may be protective against atopy in children and partially explain the observed lower prevalence of atopy and asthma in rural areas. Animal sheds, especially cow sheds, are found to contain a large variety of bacteria, fungi and their compounds [34–36]. Because of this high level of exposure, a farming environment offers an opportunity to evaluate the importance of microbial exposures to maturation of the immune system and children's respiratory health and atopy [37,38]. Research has shown that early-life microbial exposure may be important in reducing the risk of atopy and hay fever in farm children [39]. It has been suggested that during a critical inception phase of lung development within the first 3 years of life, children are more likely to develop asthma [40,41]. Prenatal factors related to placental function including exposures to respiratory viruses, bacteria, and allergens have been suggested to play a role [41,42].

While there have been reviews looking at the association between farm exposure and asthma [43] or atopy [44,45], none have looked specifically at the relationship between early life farm exposures (pregnancy and the first year of life) with atopy/atopic sensitization measured by objective markers (skin prick testing, and serum specific IgE measurements) in children at school age (6–12 years old). The aim of this systematic review was to determine if farm-related exposures in early life protect children at school age from atopy as measured by objective markers.

2. Methods

This review was conducted following the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guideline for meta-analysis of observational studies [46]. Despite the guideline also describing details specific to meta-analysis, we only applied criteria applicable to a systematic review from these guidelines because we did not conduct any meta-analysis techniques in this paper.

A systematic search was performed using search engines for medical literature including Medline through the PubMed interface, Embase, the Web of Science and the reference lists from studies. The literature was searched with the free text search terms such as the following query string: *child* or childhood or early life or postnatal or prenatal or antenatal or neonatal or pregnant or pregnancy or in utero or infan* or maternal exposure or newborn) AND (farm* or agricultur* or dairy or grain or livestock or endotoxin or beta-glucan or lipopolysaccharide or mold) AND (atop* or Skin prick test* or Immunoglobulin E or IgE or atopic sensitization or hypersensitivity or allerg*)*.

Electronic and manual searching was carried out in November 2019. The search was restricted to English language articles. Lists of studies

identified from searching electronic databases were combined and duplicate abstracts removed. Papers were screened for relevance to the review based on the information contained in the title and abstract. Non-human studies were excluded based on the abstracts and not counted as search hits. Reference lists of all publications containing relevant content including reviews, editorials, commentaries, or letters were compared with the already retrieved hits. A publication was selected for further evaluation if it met inclusion criterion (below).

2.1. Inclusion/exclusion criteria

All retrieved abstracts were read, and a given publication was deemed relevant if (A) they captured exposures to a farm environment in early life (prenatal, and or the first year of life); (B) outcomes included objective markers of atopic sensitization defined in one of two ways: a positive skin prick test reaction to at least one specific allergen; or elevated specific IgE to at least one specific allergen and outcomes were measured in school-age children (C) the study design is either cross-sectional, case-control, or cohort; (D) the selected articles were peer-reviewed full-text; and (E) the publication date was after January 1, 2000. Publications that described only adult or occupational exposures to a farm were excluded. Original articles that did not describe data on atopy measured by objective markers in relation to exposures to a farm environment in early life were also excluded. Searches were restricted to all infant and non-food atopy report. Two reviewers (LC&JL) compared the search findings and discussed article inclusion.

2.2. Data extractions and assessment of quality

Data regarding study design, characteristics of the study population, measurements of exposures and outcome(s) together with 95% confidence intervals (95% CI) as well as the type of point estimate (e.g. odds ratio [OR]) were extracted and presented in a table format. Quality assessment of included papers was carried out using the "Effective public health practice project (EPHPP) quality assessment tool for quantitative studies" [47]. The EPHPP tool previously had the validity and inter-rater reliability evaluated [48,49]. The inter-rater reliability of the individual domains of the EPHPP tool is considered fair agreement among raters (the intra-class correlation coefficient (ICC) = 0.60) [48, 49]. This tool was used in a previous systematic review related to asthma and environmental exposures [50]. The quality assessment score used in this evaluation tool included: selection bias, study design, confounders, blinding, data collection methods, withdrawals and drop-outs. We assessed validity based on the original tool as well as in a modified version. In the modified version, we did not include the "blinding" criteria due to the nature of included articles (observational where blinding is typically not mentioned). A global rating for each paper was then scored and categorized into one of the three categories (strong, moderate, or weak). Two of the authors (LC & JL) reviewed the articles for validity assessment then came to consensus when there were discrepancies. The quality of the included articles was based on the consensus decision and had a global rating score applied. An article was considered "strong" if there was no "weak" rating, while a "moderate" article had one "weak" rating, and a "weak" article had two or more "weak" ratings.

As part of our analysis, we also summarized the results of the studies based on sensitivity analyses around the methods used to assess atopy (Skin test vs. IgE measurements), study designs, and timing of exposures (during pregnancy vs. the first year of life).

Publication bias was evaluated by visualization of funnel plots.

3. Results

There were 7285 references identified from electronic databases. Initial screening produced 21 potentially relevant references and 14 studies met the inclusion criteria after further screening of full-text

articles. Search results are summarized in Fig. 1 based on the Quality of Reporting of Meta-analyses QUORUM flow diagram. The summary of studies is located in Table 1. There were 13 cross-sectional studies and 1 case-control study. Most studies (9 of 14) were conducted in European countries. Most of the cross-sectional studies had a large sample size (sample size ranged between 293 and -8334). In contrast, the only case-control study had considerably fewer study subjects ($n = 103$) (see Table 2).

In general, there was heterogeneity in the assessment of outcomes and exposures of interest. Out of 14 studies, nine based atopy on skin testing; four used blood tests with specific IgE measurements; and one used both methods (Table 1). The choice of specific allergen extracts for skin prick testing varied widely between studies and was completed for a range of 6–15 allergens. A positive test reaction was defined similarly across SPT studies (the cut-off of ≥ 3 mm wheal). The threshold levels used to define a positive response using specific-IgE levels, however, were different between studies. These cut-offs included 0.35 kU/L, 0.7 kU/L or 3.5 kU/L (Table 1).

As shown in Table 1, the strengths of associations between early-life farm exposures and childhood atopy varied between studies. Fig. 2 shows the strengths of associations among the included articles. In Figs. 2 and 9 out of 13 studies (1 study did not report any OR) showed statistically significant protective effects, while 3 showed a non-statistically significant protective effect, and 1 study showed a non-statistically significant risk effect.

There was variation in definitions of type and timing of exposure [e.g. exposures to stables in the first year of life, farming in infancy, main

line of production in infancy (dairy, livestock, plant, etc.), maternal exposure to stables during pregnancy, regular contact with farm animals ever, the child growing up on a farm, farmer's children identified by initial survey, early farm animal exposure, barn or stable exposure, parental farming at less than first year of age].

Of the 14 studies included, 13 were cross-sectional in design. Among these, ten studies showed a statistically significant protective effect, one showed mixed results, one showed a non-statistically significant protective effect, and one did not report the strength of association (e.g. OR). Overall, the strength of associations ranged from 0.18 to 1.3. There were three articles from a large multi-nations project (the Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community – the GABRIEL and GABRIEL Advanced surveys) [51–53].

Only one case-control study was found. Martikainen et al. [54] (2015) conducted of French and Finnish children, including 65 children with asthma (26 farming and 39 non-farming) and 103 children without asthma (56 farming and 47 non-farming). The authors found no statistically significant association between early life exposures (e.g. staying in stable or barn) and the outcome of atopy-measured by specific IgE levels-at school age (at age 6).

3.1. SPT vs. IgE measurement methods

The use of specific IgE measurements showed more consistent results compared to the skin prick test method (Fig. 3). Five out of five specific IgE-based studies showed a protective effect of early life farm exposures

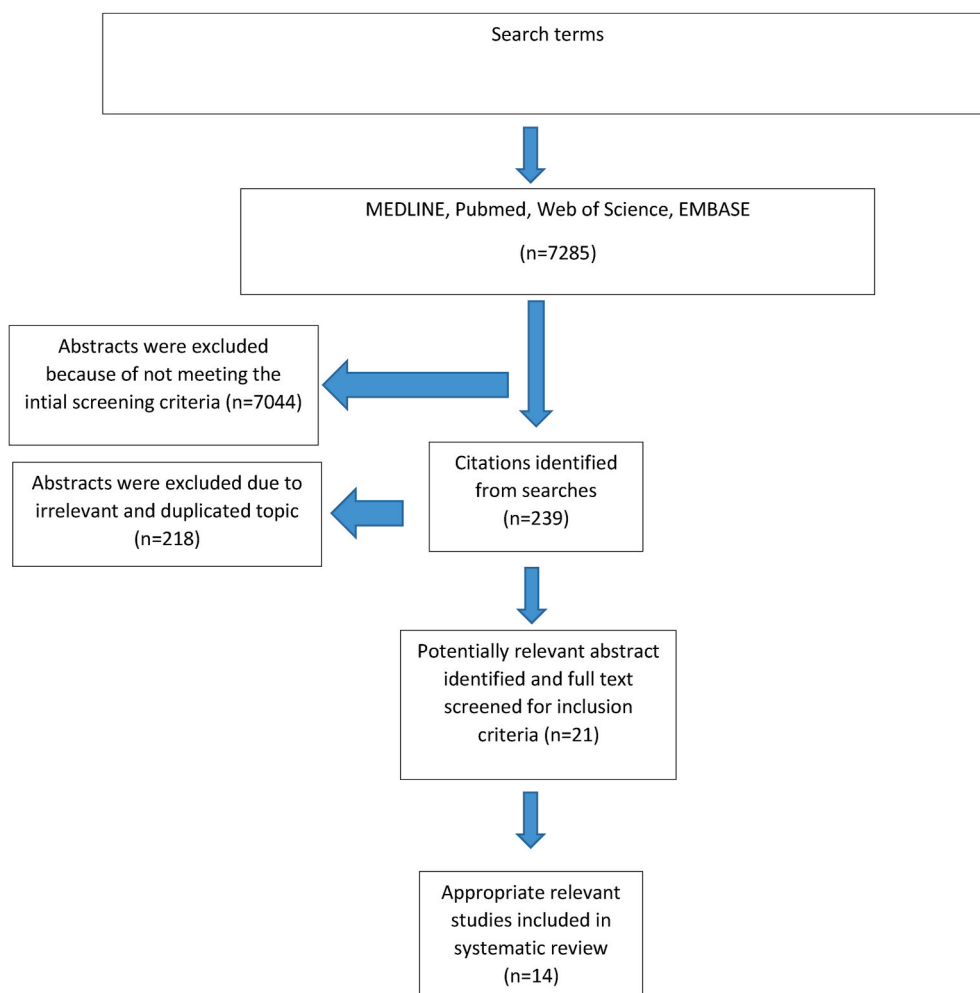


Fig. 1. QUORUM flow chart to select articles to the systematic review.

Table 1

Associations between early-life farm exposures and atopic sensitization/atopy defined by specific IgE and/or skin prick test measured in children.

First author reference # (Year published) Location	Study Design	Study population (Sample size)	Type & timing of exposure	Outcome assessment	Outcome & strength of association
Cross-sectional studies					
Riedler et al. [55] (2001) Rural areas of Austria, Germany, and Switzerland.	Cross-sectional	N = 812; 6–13 years old	Exposure to stables in the first year of life, farm milk consumption	Atopic sensitization was defined as at least one positive specific IgE test result of 3.5 kU/L or greater for the six aeroallergens (house dust mite, storage mite, grass and birch pollen, cat dander, and cow epithelium).	-Exposure to stables and farm milk in the 1st year of life (n = 218): [aOR = 0.32 (0.17–0.62)] -Exposure to stables, but not farm milk in the 1st year of life: [aOR = 0.56 (0.25–1.27)] -Farm milk but no stables [aOR = 0.43 (0.24–0.77)] -Pregnant mother active daily on farm vs. pregnant mother not active daily on farm and child not exposed to stable and farm milk in 1st year [aOR = 0.18 (0.06–0.56)] -Having above average exposure (more than 20 min in the stables) in the 1st year of life (n = 133) [aOR = 0.28 (0.12–0.66)] -Having above median aged <5 years (n = 122) [aOR = 0.23 (0.10–0.53)]
Klintberg et al. [81] (2001) The island of Gotland, in the Baltic Sea	Cross-sectional	N = 650 children born in 1989 (356 boys and 351 girls) was divided into two groups of exposures, all either living in a farming environment or never having lived in such an environment. -range 7–8 years old	Living in a farming environment during the first year of life	The skin reactivity to standardized allergen extracts: pollen (birch, timothy, mugwort), animal dander (cat, dog, horse), <i>Alternaria alternata</i> and food allergens (egg, hazelnut, peanut), house dust mites (Dermatophagoides pteronyssinus, Dermatophagoides farinae) and storage mites (Lepidoglyphus destructor, Tyrophagus putrescentiae and Acarus siro). Atopy was defined as a mean wheal size ≥ 3 mm.	–32% of the children had at least one positive test. -Sensitization to any allergen panel (Birch, Timothy, Cat, A. alternata, Dermatophagoides pteronyssinus, Dermatophagoides farinae): [aRR = 0.94 (0.70–1.25)]
Braun-Fahrlander et al. [63] (2002) Rural areas of Germany, Austria, or Switzerland	Cross-sectional	N = 812; 6–13 years old	Exposure to farming during the first year of life	Atopy was defined by at least one positive test for specific IgE indicating a titer of at least 3.5 kU per liter for one or more of the six airborne allergens (house dust mites, storage mites, grass pollen, birch pollen, cat dander, and cow epithelium).	-Exposure to farming in the first year of life showed a strong inverse association with atopy [aOR = 0.45 (0.30–0.68)]
Wickens et al. [82], (2002) New Zealand	Cross-sectional	N = 293; 7–10 years old	-Farm exposures in the first year of life	Skin prick tests to common allergens (Dermatophagoides farinae, D. pteronyssinus, mould mix, ockroach, rye grass, timothy grass, cat, dog). A positive SPT reaction (SPT +) was defined according to the presence of a mean wheal diameter of 3 mm or more to at least one of the allergens.	-Exposure in the first year of life: +Farm abode: [aOR = 1.3 (0.5–3.6)] +Regular poultry: [aOR = 1.1 (0.4–3.5)] +Regular pig: [aOR = 0.2 (0.1–0.9)]
Remes et al. [79] (2003) Finland	Cross-sectional	N = 714; 6–13 years old	-Mother farming in infancy, main line of production in infancy (dairy, livestock, plant,..)	Any reaction (SPT ≥ 3 mm) to one or more of allergens: birch, timothy grass and mygwort pollens, cat and dog epithelial danders, and house dust mite (Dermatophagoides pteronyssinus).	-Farming in infancy: [aOR = 0.68 (0.48–0.95)] -Main line of production in infancy: +Dairy farming (ref.: no farming): [aOR = 0.68 (0.46–1.00)] +Other livestock (ref.: no farming): [aOR = 0.51 (0.28–0.93)] +Plant farming (ref.: no farming): [aOR = 0.74 (0.42–1.32)] +Other or nor defined: [aOR = 0.46 (0.09–2.38)]
Zekveld et al. [83] (2005) rural Crete	Cross-sectional		Children's exposure to and contact with farm animals	Skin-prick tests involving a series of 10 common aeroallergens: grass	Children with regular animal contact, at any stage of life, were

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Table 1 (continued)

First author reference # (Year published) Location	Study Design	Study population (Sample size)	Type & timing of exposure	Outcome assessment	Outcome & strength of association
		N = 797 Schoolchildren aged 7–18 yrs	or pets and consumption of farm milk at three different stages of life: infancy, at 5 years of age, and during the past year.	pollen (Mediterranean mix), Parietaria, olive blossom, cat fur, Dermatophagoides pteronyssinus and D. farinae, goat epithelium, Cladosporium, cockroach, and poultry. The negative and positive controls were saline and histamine respectively. Atopy was defined as a mean wheal size ≥ 3 mm	equally likely to be atopic or have current wheeze than children with less frequent or no contact. For example, the prevalence of atopy among sheep/goats exposures at infancy (daily, weekly, never) were 33%, 20%, 23% (p-trend = 0.49)
Ege et al. [56] (2006) Multi-country European study (Austria, Germany, the Netherlands, Sweden, and Switzerland)	Cross- sectional	N = 2086; 5–13 years old	-Maternal exposure to stables during pregnancy	Allergen-specific IgE for common inhalant. Atopy was defined as IgE values of 3.5 kU/L or greater	-Maternal exposure to stables during pregnancy [aOR = 0.58 (0.39–0.86)]
Perkin et al. [57] (2006) UK	Cross- sectional	N = 879; mean age 8.11 years	-Early farm animal exposure or barn or stable exposure	Skin prick testing: a panel of allergens (dog hair, cat hair, horse hair, cow hair, 6-grass mix, house dust mite (Dermatophagoides pteronyssinus), Acarus siro, Lepidoglyphus destructor, Tyrophagus putrescentiae) and positive and negative controls. A positive response was a 3-mm or larger mean wheal diameter was defined as atopy.	-Early farm animal exposure aOR = 0.53 (0.29–0.99)
von Hertzen et al. [58] (2006) Finland & Russia	Cross- sectional	N = 732; 7–16 years old	Parental farming at less than first year of age	A standard set of 9 airborne and food allergens (birch timothy grass, mugwort, cat, dog, horse, cow, Dermatophagoides pteronyssinus, and fish). Atopy was defined as a mean wheal size ≥ 3 mm.	Parental farming at less than first year of age vs. currently: - In Finland: [aOR = 0.45 (0.22–0.91); - In Russia: [aOR = 0.47 (0.22–1.03) - Both 2 countries: [aOR = 0.50 (0.31–0.81)]
Waser et al. [84] (2006) Austria, Germany, the Netherlands, Sweden, and Switzerland.	Cross- sectional	N = 3979 Children aged 5–13 years	Farm milk consumption in the first year of life	-Allergen-specific IgE measurements -A mix of common inhalant allergens (birch, timothy, mugwort, Dermatophagoides pteronyssinus and farinae, cat, dog and horse epithelium and Cladosporium herbarum) Atopic sensitization was defined as allergen-specific IgE ≥ 0.35 kU/L. In addition, a cut-off value of 3.5kU/L was also considered for the analyses. Pollen sensitization was defined as positive grass pollen mix and/or positive tree pollen mix.	-Using a cut-off level of 0.35 kU/ L, a significant inversed association was found for a sensitization to horse dander 0.50 (0.28–0.87). -Using a cut-off level of ≥ 3.5 kU/ L, the negative association with farm milk consumption for pollen sensitization [aOR = 0.67 (0.47–0.96)]
Loss et al. [52] (2011) rural regions of Germany, Austria, and Switzerland	Cross- sectional	N = 8334 6- to 12-year-old school children	Farm milk consumption by questionnaire	Atopy was defined as positive test results for specific IgE antibodies against Dermatophagoides pteronyssinus, cat, or birch (cutoff, 0.7 kU/L) or against a grass mix (cutoff, 0.35 kU/L)	-First unboiled farm milk <1 y OR = 0.72 (0.61–0.85) -First unboiled farm milk >1 y OR = 0.78 (0.63–0.97)
Illi et al. [51] (2012) Rural areas of southern Germany (Bavaria and Baden-Wuerttemberg Switzerland Austria and Poland)	Cross- sectional	N = 7682 Mean age: 8.7 $\pm$ 1.4 years	Farm exposures (pregnancy to age 3 years)	Serum IgE antibodies against inhalant (Dermatophagoides pteronyssinus, cat, grass mix [sweet vernal grass, rye grass, timothy grass, cultivated rye, and velvet grass], birch, and mugwort) and food (egg white, cow's milk, fish, wheat, peanut, and soybean) allergens. Atopic sensitization was defined as specific IgE antibodies of at least 0.7 kU/L against D pteronyssinus, cat, or birch or a positive reaction (0.35 kU/L) to the grass mix.	Farm exposures (pregnancy to age 3 years) -Contact with animals +Cow: [aOR = 0.75 (0.65–0.88)] +Poultry: [aOR = 0.76 (0.64–0.91)] +Horses: [aOR = 0.79 (0.63–0.99)] -Stay in cow sheds: [aOR = 0.78 (0.67–0.92)] -Contact with animal feed: +Hay: [aOR = 0.74 (0.63–0.86)] +Grain: [aOR = 0.72(0.61–0.86)] +Corn: [aOR = 0.78(0.64–0.95)] +Corn silage: [aOR = 0.79 (0.65–0.95)] -Stay in barn: [aOR = 0.70 (0.59–0.82)]

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Table 1 (continued)

First author reference # (Year published) Location	Study Design	Study population (Sample size)	Type & timing of exposure	Outcome assessment	Outcome & strength of association
Macneil et al. [53] (2013) Poland	Cross-sectional	N = 2586 6- to 12-year-old school children	Early exposures (pregnancy to age 3 years) to a farm or farm-related	-Specific serum immunoglobulin E (IgE) antibodies measured to: <i>D. pteronysinus</i> , cat dander, grass mix and common silver birch. Atopy was defined by specific IgE $\geq$ 0.7 kU/l against <i>D. pteronyssinus</i> , cat or birch or a positive reaction (0.35 kU/l) to the grass mix. -Skin prick testing (SPT) was performed using extracts from <i>D. pteronyssinus</i> , <i>D. farinae</i> , mixed grasses, birch and cat epithelia with histamine and saline controls (ALK-Abelló, Hungerford, UK). Atopy was defined as a mean wheal size $\geq$ 3 mm.	-Stay in fodder storage room: aOR = [0.79 (0.64–0.98)] - Early-life contact with grain was inversely related to the risk of atopy measured by IgE [aOR = 0.66 (0.47–0.92)] -Early-life contact with poultry was inversely related to the risk of atopy measured by SPT [aOR = 0.54 (0.38–0.77)]
Case-control study Martikainen et al. [54] (2015) French and Finnish children from the PASTURE study	Case-control	65 asthmatic (26 farming and 39 nonfarming) and 103 nonasthmatic (56 farming and 47 nonfarming) children.	Prenatal maternal consumption of farm milk, prenatal exposures to the stable and barn.	Specific IgE levels in serum against 6 food (hen's egg, cow's milk, peanut, hazelnut, carrot, and wheat flour) and 13 inhalant (Dermatophagoides pteronyssinus; Dermatophagoides farinae; cat; horse; dog; Alternaria species; mugwort, plantain, alder, birch, hazel, and rye pollen; and grass pollen mix) allergens were assessed at age 6 by using the Allergy Screen test panel for atopy. Positive sensitization against perennial, seasonal, or food allergens was defined by using a cutoff for specific IgE of 0.70 IU/ml or greater.	Farmer during pregnancy [aOR = 0.81 (0.38–1.72)]. Stay in stable [aOR = 1.05 (0.48–2.26)]; Stay in barn [aOR = 0.9 (0.42–1.93)]

and atopy at school age. Among nine SPT-based studies, 1 showed a non-statistically significant increased risk, 6 showed statistically significant protective effects and 2 studies found no associations with early farm exposures.

3.2. Exposure timing (pregnancy vs first years) between farm-related exposures and atopy

The type and timing of exposure were not similar between studies. Some studies considered “early-life” farm exposures as parental farming in infancy or having been born and raised on a farm [55,56]. Others considered “early-life” farm exposures as exposure during the first years [57,58].

3.3. Validity assessment

Using the original evaluation tool (with the “blinding” criteria) all of the included studies were classified as “weak”. This was primarily due to the cross-sectional study design and not having blinding. Only two studies mentioned the act of blinding. Using the modified evaluation tool (without the “blinding” criterion), most (10 out of 14) of the included studies were in a “moderate” level, while 3 out of 14 studies were categorized in “weak” level, and one study was in the “strong” level.

For the specific validity criteria, there was some variability. For “Selection bias”, 1 study was considered “weak”, while 8 were considered “moderate” and 5 were considered “strong”. For “Study design”, 13 studies were considered “weak”, while 1 was considered “moderate” and 0 was considered “strong”. For “Confounders”, 1 study was considered

“weak”, while 2 were considered “moderate” and 11 were considered “strong”. For “Data Collection Methods”, 13 studies were considered “strong”, while 1 was considered “moderate”, and 0 was considered “weak”. For “Withdrawals and Drop-outs”, 4 studies were considered “strong”, while 8 were considered “moderate”, and 2 were considered “weak” (see Table 2).

3.4. Publication bias assessment

Figs. 4 and 5 show the funnel plots for articles included. Fig. 4 depicted the odds ratios in relation to sample sizes of included articles with main exposures, while Fig. 5 depicted all estimates (Odds ratios) related to farm exposures and other related exposures mentioned in the included articles. Visually, both figures showed only minor asymmetry, suggesting a lower risk of publication bias.

4. Discussion

The aim of this systematic review was to determine if farm-related exposures in early life protect children at school age from atopy as measured by objective markers. Fourteen studies were selected in this systematic review. The results are fairly consistent in that early farm-related exposures can protect school-age children from being atopic.

A similarly focused review was completed by Campbell et al. [44] although there were some notable differences. Campbell et al. conducted a systematic review and meta-analysis of the research investigating associations between farm exposure and objective measures of atopy, that is serum IgE or skin prick tests results, at any age, including into adulthood while our current systematic review focuses explicitly on

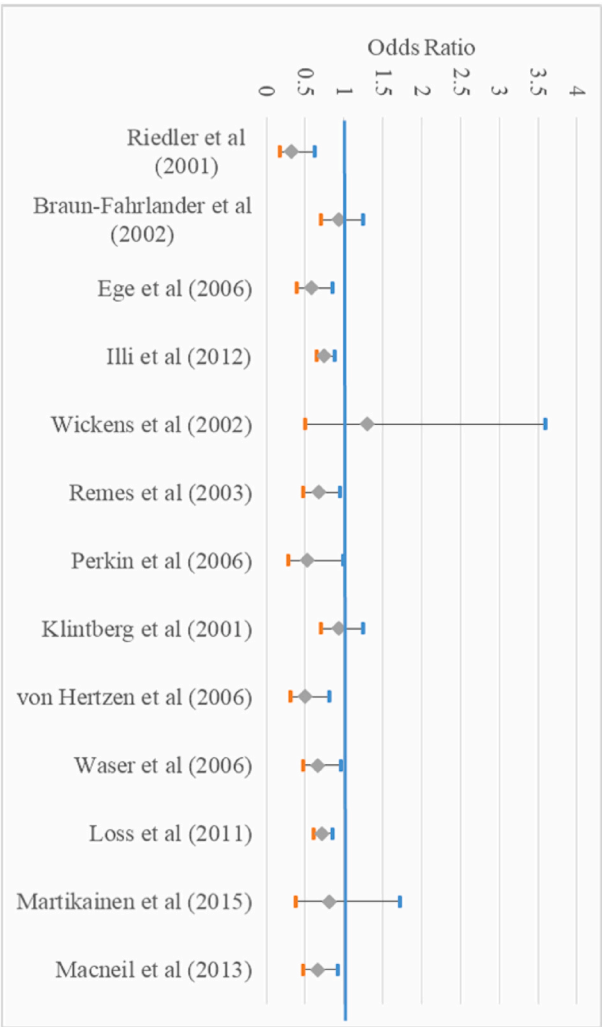


Fig. 2. Summary of strengths of associations among included articles.

the farm exposures during the first year of life and a specific outcome which is atopy presence at school-age measured by serum IgE and/or skin prick testing. Campell et al. concluded that exposure to farming in early childhood was associated with a protective effect on objective markers of atopy, while inconsistent findings were observed in adults with early life exposures. Our results extended that work by showing that early life exposures to a farm protect children from atopy at school-age specifically.

The microbial environment of the farm with high exposures to gram positive bacteria and gram negative bacteria during pregnancy and early childhood has been shown to be associated with a protection against atopy [56,59–61]. Of the farm-related factors, early-life and consistent contact to stables and the consumption of unpasteurized milk have been most strongly associated with decreased risk of IgE-sensitization [62]. In addition, prenatal exposure to animal sheds and hay has been reported to be inversely associated with cord blood IgE levels against seasonal allergens [59]. A variety of microbial products such as endotoxin, muramic acid, and [beta]-glucans [63–65] may play a major role in protecting from asthma and atopy. Endotoxin levels are found to be higher in rural areas and a farming environment compared to the urban areas [66–68]. Maternal contact with farm animal species and barns and consumption of farm-produced butter during pregnancy enhanced the production of pro-inflammatory cord blood cytokines [69]. Most notably, maternal exposure to a higher number of farm animal species substantially enhanced the expression of the T-regulatory cell marker glucocorticoid-induced tumor necrosis factor (TNF) receptor and the

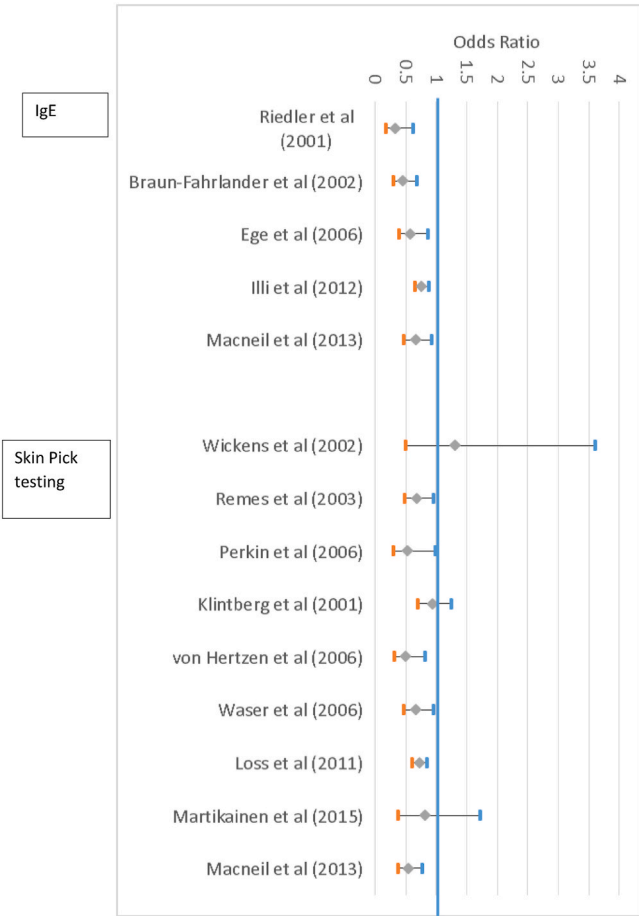


Fig. 3. SPT vs. specific IgE measurement methods.

Table 2
Summary of results from validity assessment of studied included in the systematic review.

Item	Classification		
	Weak	Moderate	Strong
Selection bias	1	8	5
Study design	13	1	0
Confounders	1	2	11
Blinding	0	1	1
Data Collection methods	0	1	13
Withdrawals and Drop-outs	2	8	4

secretion of interferon-gamma (IFN-γ) by cord blood cells in response to atogen and peptidoglycan [70], which is associated with the protection against atopy in children.

There is an ongoing debate about whether atopic sensitization can occur prenatally [71–73] and whether maternal antibodies can influence cord blood IgE levels [74]. Epidemiological studies (including our findings in the current systematic review) indicate that mothers exposed to a farm environment during pregnancy protect against atopic sensitization in their children, whereas exposures during infancy alone or during the first years of life have weaker or no effect [55,59]. However, the mechanisms of prenatal atopy are complicated and not well-understood.

Recently, some studies have applied indicators of microbial exposure, such as a microbial diversity score to predict atopy and asthma and this may be a useful to describe microbial exposure [75]. In our systematic review, the definitions of farm exposures were limited to the “label” of exposures such as exposures to farm dwelling, poultry,

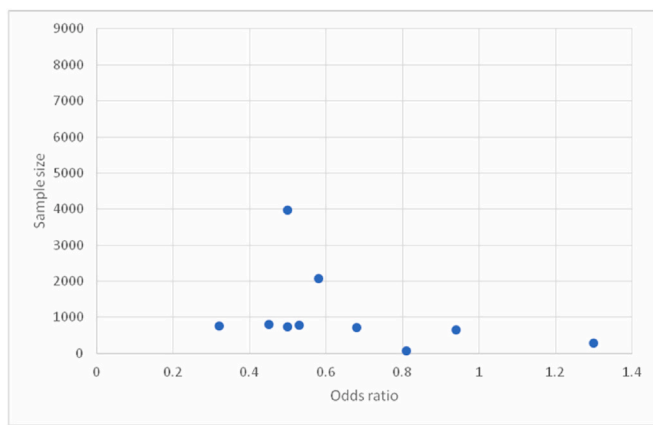


Fig. 4. Funnel plots of included articles (with main exposures).

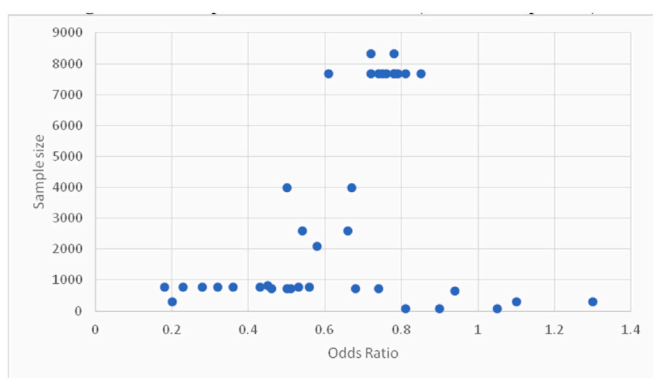


Fig. 5. Funnel plots of included articles (with main exposures and other related exposures).

livestock, farm milk, and stables, and so on. Future studies should look into “farm” exposures with a reliable and objective measurement to derive a better measure of what constitutes a farm exposures or how these should be grouped.

We found that the association between early life farm exposures and atopy at school-age was more consistent using the specific IgE tests compared to the skin prick testing. Blood tests detect IgE in the blood, while skin tests detect IgE response in the skin. There does not exist a so-called gold standard to detect the presence of atopy. Both specific IgE and skin prick testing (SPT) have advantages and disadvantages. While SPT is cheap, quick, and sensitive, sIgE assays are more specific, but more expensive and results are not immediate. The comparison between the two methods has been evaluated and results have shown that SPTs are more sensitive than the IgE test, whereas the IgE test is more specific [76,77]. Moreover, the threshold to determine the presence of atopy in each method is different and can also differ between studies. While most studies use the cut-off of ≥ 3 mm wheal size using the skin prick tests, thresholds used in IgE tests may vary more often, ranging from 0.35 kU/l to 3.5 kU/l (as shown in our current systematic review). While the cut-offs and methods used are varied (either skin test or blood test), atopic status was used as a label and such a label has been commonly used to compare the outcomes of the two tests.

The majority of data included in this review were drawn from cross-sectional studies, and therefore the cause-effect relationship of the observed phenomena may be questioned. However, we looked exclusively at studies that used objective markers of atopy which can help minimize the misclassification of outcomes. Also, in our systematic review, we examined “quasi-longitudinal” exposures as the exposures in question, which were based on recall of their occurrence at early ages. This will help us limit issues around reverse-causation. Differential

recall bias is not likely to occur because the outcomes (IgE levels and SPT results) were laboratory parameters. Furthermore, this systematic review is potentially influenced by submission bias (where some investigators do not submit papers that find no statistically significant associations), potentially resulting in some publication bias. However, we found that publication bias was not likely to occur based on our funnel plots.

In our current systematic review, most studies used questionnaire report to collect data on exposures. These detailed questionnaires not only assessed the type of exposure but also its time period. In a previous study [51], when analyzing the association between timing and outcomes, the effects of exposures early in life (from pregnancy up to age 3 years) showed much stronger effects than current exposure at the time of outcome assessment [51]. This correlates with findings from other studies that observed an effect of farm exposure in pregnancy on specific IgE levels and cytokine responses in cord blood, indicating a protective farm effect as early as *in utero* [59,69,70].

Until now, there has been no review on the association between early life farm exposures and atopy measured by objective markers such as IgE measurements and skin prick testing at school age according to systematic search criteria. We performed a reasonable and replicable systematic search using the electronic database PubMed, Embase, and other sources. However in this report we included only studies assessing the influence of early farm exposures on objective markers of atopic sensitization, and not the studies assessing a particular atopic disease (asthma, atopic eczema, hay fever) as an outcome. This approach had permitted us to assess the influence of early life farm exposures on the immune system more objectively with particular respect to the early mechanisms of atopic immune response.

One of the main limitations of the original studies’ outcome definitions was the difference in the quantity and type of allergen extracts as well as cut-off used to identify positive IgE and skin prick test results. In reality, it is difficult to have complete uniformity in the use of exact procedures and panels of allergens. Therefore, cautions are needed when interpreting results as well as comparing findings between studies. Another limitation is the definition of type and timing of farm exposures. As shown in Table 1, early life farm exposures were considered differently in studies such as parental farming in infancy or having been born and raised on a farm [55,78] or farm-related exposures up to one year of age [58,79]. Most of the included results are generalizable within a European context because most of the studies were from Europe, many studies were population-based, and they were conducted in a variety of countries with a diverse range of farming practices. However, not all countries or continents were represented by the included studies. Also, there were three studies in this systematic review originating from a large project: GABRIEL and GABRIEL Advanced surveys [51–53]. The generalization at a global perspective may be limited because not all countries or continents were represented by the included studies. Furthermore, the validity of articles included in this systematic review was moderate. We used an evaluation tool which previously had the validity and inter-rater reliability evaluated [48,49]. The scores for the included articles in our current systematic review were low due to the use of cross-sectional studies. Ideally, a meta-analysis would have been performed. However, it is recommended not to conduct a meta-analysis when there is considerable variation in results and inconsistency in the direction of effect, potentially misleading the overall effect of interest [80]. In our current systematic review, since there was a great deal of heterogeneity in the definitions of both exposures and outcomes, a meta-analysis was not performed.

In conclusion, we found a consistent association between early farm-related exposures and a protective effect against atopy in school age children. Future work should focus on higher quality studies that report larger samples, prospective prenatal cohort designs with clear and specific definitions of farm exposures along with increased use of objective markers for these exposures and disease outcomes. This will help investigators to isolate key factors in the prevention of atopic diseases; and

better indicators of the total quantity and diversity of microbial exposures on a farm.

Declaration of competing interest

The authors have no conflict of interest related to this study to disclose.

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