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ORIGINAL ARTICLE

Exposure to farm environment and its correlations with total IgE, IL-13, and IL-33 serum levels in patients with atopy and asthma

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Abstract
Background: The aim of the study was to evaluate total immunoglobulin E (IgE), IL-13, and IL-33 serum level in people with bronchial asthma and atopy, and in healthy control group depending on their exposure to farm animals currently and in the first year of life.
Methods: The study included 174 individuals living in rural areas and in a small town. Standardized questions from the International Study of Asthma and Allergy in Childhood and The European Community Respiratory Health Survey (ECRHS) questionnaires were used to define asthma. Atopic status was verified by skin prick tests. Rural exposure including contact with livestock was verified by adequate questionnaire. Total serum IgE, IL-13, and IL-33 levels were assessed by ELISA (enzyme-linked immunosorbent assay) tests.
Results: Participants with atopy and bronchial asthma were characterized by high level of immunoglobulin E. Tendency to lower serum IgE level was observed among people reporting present contact with farm animals. Also, among those having contact with livestock in their first year of life, the analogous tendency was noticed. No difference in serum IL-13 levels in participants with asthma and atopy, and controls was observed, and there was no effect of exposure on farm animals on the concentration of IL-13. The highest IL-33 level was found in the atopic group, and the lowest in the control group. Participants currently exposed to farm animals were predisposed to have lower IL-33 serum level.
Conclusion: Exposure of farm animals currently and in first year of life may result in a lower level of total IgE. Correlation between IL-13 and IL-33 serum levels and contact with livestock was not confirmed.

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Introduction

The increase in the prevalence of allergic diseases, including asthma, was well documented in many epidemiological studies over the past years.[1] Genetics is a strong determinant of allergy development, but such rapid increase in disease prevalence is most likely explained by environmental factor changes like household environment, infectious agents, medications, and allergen exposure.[2] It was found that growing up on the farm could be a protective factor in the development of asthma and other allergic diseases.[3] The significant reduction in the rates of asthma and atopic diseases in farm residents has been shown in European (ALEX, GABRIELA, and PASTURE surveys) and American studies.[4,5] Consumption of unpasteurized farm milk, exposure to livestock and stables, and bacterial diversity in home dust contributes to this protective effect.[4-6] Regular exposure to microbes and endotoxins modulate the human immune system preventing hypersensitivity.[7]

However, the biomarkers associated with protection from allergy development are not fully understood. Early life immunoprogramming and typical cytokine pattern of immune cells may be different in people exposed and not exposed to livestock.[4] IL-33 acts as an epithelial and endothelial alarmin in allergic and inflammatory diseases being rapidly released into the airway after allergen exposure. This cytokine activates group 2 innate lymphoid cells (ILC2) to produce Th2-type cytokines like IL-4, IL-5, IL-9, and IL-13. IL-13 is critical for IgE synthesis, eosinophil tissue accumulation, goblet cells hyperplasia, mucus secretion, and it additionally supports dendritic cells migration (favoring antigen presentation to naive Th cells) and macrophages polarization toward M2 leading to fibrosis.[8,9] Despite such a wide range of action in allergy and atopy, the role of these two cytokines is not well understood in protection against the diseases with allergic background and even less is known about their action in rural populations. This is why we decided to have a closer look at IL-13 and IL_33 in our study.

This study was designed to identify the associations of farm exposures (currently and in the first year of life) with total IgE and cytokine serum levels (IL-13 and IL-33) in the groups of asthmatics and atopics.

Patients and Methods

Patients

Hundred and seventy four participants, all above 5 years of age, were recruited from citizens of a small town and nearby villages in lower Silesia, south-west Poland based on the previous cross-sectional epidemiological study described elsewhere.[10] All of them after providing written informed consent responded to the questionnaire and underwent a skin prick test. Blood samples were also obtained and serum portions were frozen after centrifugation.

Study subjects included 58 asthmatics (22 with atopic asthma and 36 with non-atopic asthma), 58 patients with atopy, and 58 healthy controls. All three groups were similar not only in terms of sociodemographic characteristics (sex and age) but also the place of residence (village-city) and the frequency of contacts with farm animals. The subgroups according to sex, age (under and above 57), and dwelling were also created. The mean age of the whole population was 44.3 ± 19.1 years (range 9–75 years) and males and females were 74 and 100, respectively.

The characteristic of study participants has been presented in Tables 1 and 2. In the group of asthmatics 14 people (24.1%), in the group of atopics 15 people (25.9%), and in the control group 18 people (31%) had current contact with farm animals. In the first year of life such contact had 40 asthmatics (67%), 36 atopics (62.1%) and 45 health controls (77.6%). Unfortunately, only few of the study participants had contact with cows, sheep, or horses. A larger number of respondents reported poultry handling and egg collection.

The study was approved by the Bioethics Committee at the Medical University in Wrocław (No. KB 105/2019/2019).

Diagnosis of asthma and atopy

The questionnaire used in this study was based on the International Study of Asthma and Allergy in Childhood (ISAAC) questionnaire.[10] Qualified nurses assisted in filling

Table 1 Characteristics of surveyed population at the time of collecting data.

	All	Asthmatics	Atopics	Control group	ANOVA *p* value
Female sex, no. (%)	100 (57.5)	33 (56.9)	33 (56.9)	34 (58.6)	0.977
Age (y), median (range)	45 (9–75)	48 (10–74)	37 (9–74)	49 (10–75)	0.233
Place of residence—village, no. (%)	101 (58.0)	33 (56.9)	34 (58.6)	34 (58.6)	0.977
Live on a farm?, no. (%)	50 (28.7)	14 (24.1)	17 (29.3)	19 (32.8)	0.587
Contact with cows	4 (2.3)	2 (3.4)	1 (1.7)	1 (1.7)	0.404
Contact with pigs	17 (9.8)	7 (12.1)	6 (10.3)	4 (6.9)	0.909
Contact with poultry	39 (22.4)	11 (19)	15 (25.9)	13 (22.4)	0.472
Contact with sheep or goat	4 (2.3)	1 (1.7)	2 (3.4)	1 (1.7)	0.404
Contact with horses	1 (0.6)	0 (0.0)	0 (0.0)	1 (1.7)	0.734
Milking cows	3 (1.7)	1 (1.7)	1 (1.7)	1 (1.7)	0.734
Cleaning barns or stables	15 (8.6)	7 (12.1)	5 (8.6)	3 (5.2)	0.361
Collecting eggs	32 (18.4)	10 (17.2)	10 (17.2)	12 (20.7)	0.398
Any contact with farm animals	47 (27)	14 (24.1)	15 (25.9)	18 (31)	-

Table 2 Characteristics of surveyed population in the first year of life.

Live on a farm?, no. (%)	90 (51.7)	29 (50.0)	24 (41.4)	37 (63.8)	0.051
Contact with cows	84 (48.3)	31 (53.4)	22 (37.9)	31 (53.4)	0.155
Contact with pigs	92 (52.9)	32 (55.2)	24 (41.4)	36 (62.1)	0.076
Contact with poultry	97 (55.7)	33 (56.9)	25 (43.1)	39 (67.2)	0.032
Contact with sheep or goats	35 (20.1)	17 (29.3)	6 (10.3)	12 (20.7)	0.039
Contact with horses	51 (29.3)	15 (25.9)	12 (20.7)	24 (41.4)	0.039
Any contact with farm animals	121 (69.5)	40 (67)	36 (62.1)	45 (77.6)	-

Table 3 Medians and lower and upper quartiles of immunological parameters' concentration.

	All, median $[Q_1; Q_3]$	Asthmatics, median $[Q_1; Q_3]$	Atopics, median $[Q_1; Q_3]$	Control group, median $[Q_1; Q_3]$	P value, median $[Q_1; Q_3]$
IgE (ng/mL)	67 [34; 111]	108 [48; 136]	88 [66; 123]	28 [18; 53]	<0.001
IL-13 (pg/mL)	10 [4; 19]	11 [6; 23]	11 [3; 19]	7 [3; 15]	0.297
IL-33 (pg/mL)	29 [16; 63]	32 [16;76]	34 [17; 83]	22 [14; 44]	0.049

the form and in the case of children under 15 the answers were provided by their mothers.

Bronchial asthma was diagnosed based on the question: *Has a doctor ever told you (about your child) that you (your child) have bronchial asthma?*

Atopy was diagnosed in participants with positive skin prick test results for at least one out of four tested allergens (house dust mites, cat, a mixture of grass pollen, a mixture of tree pollen) chosen according to the local epidemiological data. The wheel diameter at least 3 mm greater than that of the negative control was considered as a positive result.

Measurement and analysis of serum IgE and cytokine levels

Sera were stored at a monitored temperature of −20 degrees Celcius. Levels of IgE, IL-13, and IL-33 were measured by enzyme-linked immunosorbent assay. Total serum IgE level was determined in all subjects by the Elabscience QuickKey Human IgE (Immunoglobulin E) ELISA Kit, catalog No: E-TSEL-H0016.

LEGEND MAX Human IL-13 ELISA Kit with Pre-coated Plates (catalog No: 435207) and LEGEND MAX Human IL-33 ELISA Kit with Pre-coated Plates (catalog No: 435907) were used to assay serum cytokines. Standards and samples were incubated sequentially with the antibody coated on strip well plate, bioinylated detection antibody, Avidin-HRP B Solution, and with Substrate Solution F. The reaction was stopped with the addition of Stop Solution and absorbance at the 450 nm wavelength was read. A logarithmic curve was plotted showing IL-13 or IL-33 concentration on the *y* axis and absorbance on the *x* axis.

Statistical analysis

The data were statistically analyzed using Statistica v.13 (TIBCO Software Inc.) and the EXCEL spreadsheet (MicroSoft). Pearson's chi-squared test of independence was used to assess the strength of the relationship between two qualitative variables. For quantitative features, a non-parametric U Mann-Whitney test was used. Comparisons were performed with ANOVA and post hoc tests. A P-value less than 0.05 was considered statistically significant in all analyses.

Results

Participants with asthma and atopy had higher IgE concentrations than the control group regardless of gender, age, and place of residence ($p < 0.001$) (Table 3). There were no differences of total IgE within the group of atopics, asthmatics, and control in terms of sex, gender, and place of living. We were not able to show significant associations between farming and IgE levels but a tendency for lower IgE levels in farm residents with asthma than in asthmatics living out of the farm was observed. Exposure to farm animals was connected with lower serum IgE levels both in the entire study population (57 in farmers vs. 74 ng/mL in non-farmers, $p = 0.151$) and among asthmatics and atopics (80.6 vs 110.7 ng/mL, $p = 0.241$ and 73.4 vs 92.5 ng/mL, $p = 0.172$, respectively). There was no corresponding trend in the control group (Table 4). In the entire study sample, people reporting contact with farm animals in their first year of life had lower total IgE concentration than in participants without such exposure (64 vs. 90 ng/mL, $p = 0.053$). The difference was less expressed in the group of only atopics (81.6 vs 93.1 ng/mL, $p = 0.181$). In asthmatics and in the control group, there were no such differences (107.7 vs 106.6 ng/mL, $p = 0.373$ and 30.1 vs 23.8 ng/mL, $p = 0.305$, respectively) (Table 5). The levels of IgE were independent of different contact frequencies with particular species of animals: cows, pigs, poultry, sheep, and horses, and performing specific tasks on the farm like milking cows, cleaning stables, or collecting eggs. Subjects declaring contacts with poultry or

Table 4 Medians of immunological parameters' concentration depending on contact with farm animals at the time of collecting data.

	All			Asthmatics			Atopics			Control group		
Contact with farm animals	Yes	No	P value	Yes	No	P value	Yes	No	P value	Yes	No	P value
IgE (ng/mL)	57	74	0.151	80.6	110.7	0.241	73.4	92.5	0.172	34.8	25.2	0.410
IL-13 (pg/mL)	11	10	0.910	8.6	9	1.0	11.1	8.1	0.702	4.5	7.5	0.275
IL-33 (pg/mL)	25	32	0.094	31.6	34.1	0.507	29.1	38.9	0.303	15.2	22.6	0.275

Table 5 Medians of immunological parameters' concentration depending on contact with farm animals in the first year of life.

	All			Asthmatics			Atopics			Control group		
Contact in with farm animals	Yes	No	P value	Yes	No	P value	Yes	No	P value	Yes	No	P value
IgE (ng/mL)	64	90	0.053	107.7	106.6	0.373	81.6	93.1	0.181	30.1	23.8	0.305
IL-13 (pg/mL)	9	11	0.681	7.7	14.5	0.204	8.6	7.9	0.890	7.5	5.5	0.400
IL-33 (pg/mL)	31	27	0.871	32.1	38	0.687	42.4	31.3	0.860	23.8	18.7	0.222

Table 6 Medians and lower and upper quartiles of immunological parameters' concentration within asthmatics with and without atopy.

Asthmatics	Atopy, median [Q_1; Q_3]	No atopy, median [Q_1; Q_3]	P value
IgE (ng/mL)	118.0 [97.7; 158.0]	76.1 [41.6; 123.5]	0.018
IL-13 (pg/mL)	16.6 [7.2; 26.2]	8.6 [6.3; 14.2]	0.092
IL-33 (pg/mL)	39.1 [16.1; 87.0]	31.8 [15.3; 73.9]	0.432

harvesting eggs (sometimes or often) had a tendency to lower IgE level.

Serum IL-13 levels were comparable between groups of asthmatics, atopics, and controls (Table 3). When subgroups were separated, the levels were lower in the control group than in atopics and asthmatics among males, people younger than 57, and those living outside the farm (p = 0.081, p = 0.062 and p = 0.168, respectively). The diagnostic value of IL-13 concentration as a test for the presence of asthma and atopy was unsatisfactory. For IL-13 concentration ≥ 7.98 pg/mL, the sensitivity of the test was 64.0%, specificity 55.7%, and the likelihood ratio LR + = 1.43. The concentration of IL-13 in the serum of participants with atopic asthma was higher than in patients with non-atopic asthma (16.6 vs 8.6 pg/mL; p = 0.092) (Table 6). In the entire study group, IL-13 levels were not related to present contact with farm animals as well as in the first year of life (11 vs. 10 pg/mL; p = 0.910 and 9 vs. 11 pg/mL; p = 0.681). In the atopic group, there was no correlation between serum IL-13 levels and livestock contacts both in the first year of life and at present (8.6 vs 7.9 pg/mL, p = 0.890 and 11.1 vs 8.1 pg/mL, p = 0.702). Also healthy controls had comparable IL-13 concentrations regardless of exposition on livestock both in the first year of life and at present (7.5 vs 5.5 pg/mL, p = 0.400 and 4.5 vs 7.5 pg/mL, p = 0.275). Among patients with asthma, the level of IL-13 was lower in people reporting a contact with farm animals in the first year of life (7.7 vs 14.5 pg/mL, p = 0.204) (Tables 4 and 5).

Serum IL-33 level was higher in people with atopy or asthma than in the control group (p = 0.049) independently of sex and place of residence (Table 3). The correlation was the strongest in patients under 57 years of age (p = 0.006). We have observed IL-33 levels above 46.5 pg/mL to be a significant test for the presence of atopy. For IL-33 concentration ≥46.5 pg/mL, the test sensitivity is 41.4%, specificity 81.0%, and the likelihood ratio LR + = 2.18. Comparable serum IL-33 levels were detected in atopic and non-atopic asthmatics (Table 6). Responders living on a farm had decreased concentration of IL-33. In the entire study population, exposure to livestock was associated with lower serum IL-33 levels (25 vs. 32 pg/mL; p = 0.094), whereas exposure to livestock in the first year of life did not affect serum IL-33 levels (31 vs 27 pg/mL; p = 0.871). No significant associations were seen separately in groups of asthmatics, atopics, and healthy controls according their contacts with farm animals in the first year of life—serum IL-33 levels were comparable regardless of exposition (32.1 vs 38 pg/mL; p = 0.687; 42.4 vs 31.3 pg/mL; p = 0.860 and 23.8 vs 18.7 pg/mL; p = 0.222, respectively). We also did not observe correlations between serum IL-33 levels in this groups and present contacts with animals living on the farm (31.6 vs 34.1 pg/mL, p = 0.507; 29.1 vs 38.9 pg/mL, p = 0.303 and 15.2 vs 22.6 pg/mL, p = 0.275, respectively) (Tables 4 and 5). Among people reporting contacts with particular animals (cows, horses, sheep, pigs, poultry) and farm activities, there was also no significant difference in the concentration of IL-33.

Age up to 60 years, IgE level ≥ 44.8 ng/mL and IL-33 ≥ 22.7 pg/mL were found to be independent predictors of atopy (Table 7). The lack of contact with animals in childhood increased the incidence of atopy (41.5% vs. 29.8%, p = 0.180). IgE level ≥ 93.56 ng/mL was the only risk factor for asthma (Table 8).

Table 7 Adjusted odds ratios and 95% CIs for associations between atopy and potential risk factors.

	$OR_{adj.}$	95% CI
Lack of current contact with farm animals	0.98	0.47-2.04
Lack of contact with farm animals in the first year of life	1.31	0.65-2.66
Age up to 60 years	**2.63**	**1.20-5.77**
Male sex	0.92	0.48-1.77
Living in the village	0.98	0.51-1.88
Living on the farm	1.08	0.53-2.20
IgE ≥ 44.79 ng/mL	**9.01**	**3.34-24.3**
IL-33 ≥ 22.7 pg/mL	**2.55**	**1.27-5.12**
IL-13 ≥ 11.12 pg/mL	1.42	0.70-2.91

Table 8 Odds ratios and 95% CIs for associations between asthma and potential risk factors.

	$OR_{adj.}$	95% CI
Lack of current contact with farm animals	0.91	0.42-1.96
Lack of contact with farm animals in the first year of life	0.92	0.34-2.49
Age above 60 years	1.29	0.56-2.99
Male sex	1.07	0.56-2.07
Living in the town	0.83	0.38-1.81
Living on the farm	0.67	0.25-1.80
IgE ≥ 93.56 ng/mL	**5.90**	**2.72-12.8**
IL-33 ≥ 30.81 pg/mL	1.30	0.60-2.81
IL-13 ≥ 5.4 pg/mL	1.75	0.71-4.31

Discussion

The protective effect of farm exposure in the development of atopic diseases has been proved in several studies. However, the respective immunological mechanisms still remains unclear.[4] In this study, we demonstrated differences in serum IgE and interleukin 33 levels in patients with asthma, atopy, and in healthy controls. Accordingly, IgE and IL-33 levels turned out to be independent predictors of atopy. We also found that early exposition to livestock influenced serum IgE levels and present contacts with farm animals were related to serum IL-33 levels. No clear association of interleukin 13 with livestock contact was found.

We have shown that serum IgE level is higher in people with asthma and atopy, which is consistent with the observations of other authors like Burrows et al. and Momen et al.[11,12] It was demonstrated that the concentration of total serum IgE was lower in people with a farming lifestyle. Similarly, participants of a Dutch study living close to farms (<327 meters) were less likely to achieve serum IgE levels above 100 IU/mL compared to those living more than 527 meters from the nearest farm. Also, the density of farms close to home has been shown to be associated with atopy.[13]

We have analyzed the concentration of IgE in individuals differing in the frequency of contacts with farm animals and farm activities and we did not observe any significant associations. Quite remarkably, few people had contact with cows, sheep, or horses. Only handling poultry and collecting eggs were reported by a larger number of respondents. When we considered only these groups, there was a tendency toward higher serum total IgE levels in people who did not report contact with poultry and collecting eggs. Gautam et al. studied the immune response in people working on chicken farms. Chicken breeders had a threefold higher serum IgE level, but statistical significance was not reached.[14]

Farming lifestyle in the first year of life was associated with decreased serum IgE concentration. Other study assessing total IgE levels in the serum of Danish agricultural school students showed significantly lower levels of this immunoglobulin in those who spent their childhood on a farm, both compared to farm residents growing up out of farm and those from the countryside who never lived on a farm.[15] There were also similar observations involving residents of Western Europe[16] and North America[17] but the direct comparison of studies is challenging due to specific—not total—IgE measurements (to airborne allergens) in former studies. von Hertzen et al. studying the populations of Finland and Russia based on two generations, showed that the parents' farming lifestyle in the first year of child's life has a protective effect on the development of atopy.[18] In parallel, Ojwang et al. have not shown associations between exposure to livestock or visits to farm buildings during the first year of life and the risk of asthma or allergies.[19] Early life exposure protection can last a lifetime and affect the course of allergic diseases.[20]

IL-13 plays a pivotal role in the evolution and maintenance of allergic inflammation by promoting IgE production, secretion of leukotrienes, bronchial hyper-reactivity, overproduction of mucus, and fibrosis. IL-13 is involved in the process of macrophages and monocytes adhesion and directly affects eosinophils by activating them, directing to the inflamed area, and prolonging their survival. Lung tissue fibroblasts are regulated by IL-13, which induces remodeling and fibrosis.[21]

Patients with asthma have significantly higher sputum IL-13 levels[22] and reveal an increased IL-13 mRNA expression in bronchoalveolar lavage (BAL) cells.[23] Moreover, the skin upregulation of IL-13 (and IL-4) is a key feature in atopic dermatitis. Increased serum concentrations were found in AD versus healthy individuals, and they correlated with SCORAD and were reduced with treatment.[24] Tralokinumab, a human IL-13 neutralizing IgG4 monoclonal antibody, is an approved drug for the treatment of moderate-to-severe atopic dermatitis[25] and showed great improvement in FEV1 in a study with patients with uncontrolled moderate-to-severe asthma.[26]

In our study, there was no difference in serum IL-13 levels in asthmatics, atopics, and the control group. Davoodi et al. reached similar conclusions, also not finding a statistically significant difference between the control group and asthmatics.[27] On the other hand, Dimitrova et al. observed positive association between bronchial asthma and elevated serum IL-13 levels.[28] We can assume that differences in the results are related to different patients' qualification criteria. Dimitrowa et al. recruited people with severe and moderate asthma only, excluding those with mild asthma.

Among patients with atopic asthma, serum IL-13 concentration was higher comparing to those with non-atopic asthma. Similar results are found in work of Hussein et al.[29]

In our population, contact with farm animals was not related to the serum IL-13 level. Interestingly, a trend for lower IL-13 concentrations was observed in people reporting contact with poultry or harvesting eggs. It has also been found that frequent contact predisposes to lower IL-13 concentrations than occasional contact. Gautam et al. measured interleukin 13 secreted by peripheral blood mononuclear cells. The spontaneous production of this cytokine did not differ between the group of farmers and office workers. After stimulation, a greater increase was observed in the nonfarmer group.[14] The results of this study are only partially consistent with ours; it seems that the main problem is the small size of study groups.

Also, farming lifestyle in the first year of life does not correlate with serum IL-13 levels. Identical conclusions were drawn by Yu et al., who studied the cytokines of mononuclear cells, although blood did not come from infants in the first year of life, but was obtained from the umbilical cord during childbirth, and the exposure concerned pregnant women. There were no significant differences in the secretion of IL-13.[30]

IL-33 receptor complex is present on a range of innate and adaptive immune cells. IL-33 was shown to act as an epithelial and endothelial alarmin in allergic and inflammatory diseases produced in response to infectious or environmental factors. Inhibiting antiviral immunity and the cytotoxic reaction and affecting dendritic and epithelial cells IL-33 amplifies hyperresponsiveness and airway inflammation.[31]

A genome-wide association study (GWAS) proved the existence of multiple single nucleotide polymorphisms (SNPs) in the IL-33 gene that are involved in susceptibility to asthma.[32] In a mouse model, it was shown that increased IL-33 levels remain in the lung tissue in a state of lasting inflammation and progressive remodeling.[33] IL-33 levels have been found to be elevated in induced sputum, bronchial biopsies, and serum of patients with asthma compared with nonasthmatic controls[9,34,35] and the increase of IL-33 could be related to their allergic status.[38] The monoclonal antibody targeting IL-33—Itepekimab improved asthma control and quality of life, as compared to placebo in patients with moderate-to-severe asthma.[36]

In our study, serum IL-33 levels were significantly higher in atopics than in asthmatics and healthy volunteers. IL-33 levels above 46.5 pg/mL proved to be a significant test for the presence of atopy. Voloshyna et al. reached similar conclusions obtaining higher concentrations of IL-33 in the serum of atopics than in the control group.[37] However, some studies have proved that asthmatics are characterized by increased serum IL-33 levels[12]; in our study, it was just a trend.

We have observed that farming lifestyle was inversely related to serum IL-33 levels ($p = 0.094$). There was no difference in IL-33 concentration in people reporting different frequencies of contacts with particular animals (cows, horses, sheep, pigs, poultry) and farm activities ($p > 0.05$). Contacts with livestock during the first year of life did not affect serum IL-33 levels.

A few important aspects of the study require consideration. Although testing the serum interleukins 13 and 33 levels is widely used and analyzed in various diseases, it seems difficult to take into consideration all variables that may influence these levels. When analyzing the available literature, it can be concluded that it would be valuable (especially for the IL-13 evaluation) referring the measurements to the degree of advancement and activity of the disease. Unfortunately, we did not have such patients' data. It should also be noted that testing the concentration of these cytokines in the serum is only one of the methods of assessing their production in the body. The available publications often indicate that it is worth paying attention to their level, for example, in BAL or the lung tissue. The fact that our study group was relatively small and reported frequent contacts only with poultry (contacts with other animals were rare) limited our ability to show clearer correlations between exposures and allergy outcomes. The precision of laboratory measurements may be also significant, the sensitivity and specificity of available tests are sometimes questionable.[38]

The strengths of the study include access to a well-defined atopy and asthma group with well-characterized rural exposures in the first year of life and currently. According to the author's knowledge, it is the first study of its kind in the Polish population.

Conclusions

Lower serum IgE levels in individuals exposed to farm animals may confirm relation between farm exposure and its protective effect on the development of asthma and atopy. The correlation was even stronger when contacts occurred in the first year of life. Our data also support the hypotheses that IL-33 is involved in the pathogenesis of asthma and atopy—serum concentrations of this cytokine were higher in both patients with atopy and asthma in comparison to healthy controls. Lower serum IL-33 levels in people exposed to livestock is an interesting observation, but its meaning should be further studied. The described research did not confirm differences in serum IL-13 levels depending on asthma or atopy and also did not confirm the importance of rural exposure on the production of interleukin 13 in the studied population. It can be assumed that information about the severity and activity of the disease and the parallel study of cytokines in other body fluids and tissues could contribute to more precise conclusions. Furthermore, larger-scale studies are needed to better explore the impact of livestock contacts on IL-13 and IL-33 production.

Conflict of Interest

The authors have no conflict of interest to declare.

References

1. Asher MI, Montefort S, Bjorksten B, Lai CK, Strachan DP, Weiland SK, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema

in childhood: ISAAC Phases One and Three repeat multi-country cross-sectional surveys. Lancet. 2006;368:733-743. https://doi.org/10.1016/S0140-6736(06)69283-0
2. Kim H, Sitarik AR, Woodcroft K, Johnson CC, Zoratti E. Birth mode, breastfeeding, pet exposure, and antibiotic use: associations with the gut microbiome and sensitization in children. Curr Allergy Asthma Rep. 2019;19:22. https://doi.org/10.1007/s11882-019-0851-9
3. Riedler J, Braun-Fahrländer C, Eder W, Schreuer M, Waser M, Maisch S, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. Lancet. 2001;358:1129-1133. https://doi.org/10.1016/S0140-6736(01)06252-3
4. von Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. Nat Rev Immunol. 2010;10:861-868. https://doi.org/10.1038/nri2871
5. Stein MM, Hrusch CL, Gozdz J, Igartua C, Pivniouk V, Murray SE, et al. Innate immunity and asthma risk in amish and hutterite farm children. N Engl J Med. 2016;375:411-421. https://doi.org/10.1056/NEJMoa1508749
6. Ege MJ, Mayer M, Normand AC, Genuneit J, Cookson WO, Braun-Fahrlander C, et al. Exposure to environmental microorganisms and childhood asthma. N Engl J Med. 2011;364:701-709. https://doi.org/10.1056/NEJMoa1007302
7. Kirjavainen PV, Karvonen AM, Adams RI, Täubel M, Roponen M, Tuoresmäki P, et al. Farm-like indoor microbiota in non-farm homes protects children from asthma development. Nat Med. 2019;25:1089-1095. https://doi.org/10.1038/s41591-019-0469-4
8. Peebles RS Jr, Aronica MA. Proinflammatory pathways in the pathogenesis of asthma. Clin Chest Med. 2019;40:29-50. https://doi.org/10.1016/j.ccm.2018.10.014
9. Hamzaoui A, Berraies A, Kaabachi W, Haifa M, Ammar J, Kamel H. Induced sputum levels of IL-33 and soluble ST2 in young asthmatic children. J Asthma. 2013;50:803-809. https://doi.org/10.3109/02770903.2013.816317
10. Sozańska B, Błaszczyk M, Pearce N, Cullinan P. Atopy and allergic respiratory disease in rural Poland before and after accession to the European Union. J Allergy Clin Immunol. 2014 May;133(5):1347-1353. https://doi.org/10.1016/j.jaci.2013.10.035
11. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE le- vels and skin-test reactivity to allergens. N Engl J Med. 1989;320:271-277. https://doi.org/10.1056/NEJM198902023200502
12. Momen T, Ahanchian H, Reisi M, Shamsdin SA, Shahsanai A, Keivanfar M. Comparison of interleukin-33 serum levels in asthmatic patients with a control group and relation with the severity of the disease. Int J Prev Med. 2017;8:65. https://doi.org/10.4103/ijpvm.IJPVM_179_16
13. Borlée F, Yzermans CJ, Krop EJM, Maassen CBM, Schellevis FG, Heederik DJJ, et al. Residential proximity to livestock farms is associated with a lower prevalence of atopy. Occup Environ Med. 2018;75:453-460. https://doi.org/10.1136/oemed-2017-104769
14. Gautam R, Heo Y, Lim G, Song E, Roque K, Lee J, et al. Altered immune responses in broiler chicken husbandry workers and their association with endotoxin exposure. Ind Health. 2018;56:10-19. https://doi.org/10.2486/indhealth.2017-0049
15. Portengen L, Sigsgaard T, Omland O, Hjort C, Heederik D, Doekes G. Low prevalence of atopy in young Danish farmers and farming students born and raised on a farm. Clin Exp Allergy. 2002;32:247-253. https://doi.org/10.1046/j.1365-2222.2002.01310.x
16. Illi S, Depner M, Genuneit J, Horak E, Loss G, Strunz-Lehner C, et al. Protection from childhood asthma and allergy in Alpine farm environments—the GABRIEL advanced studies. J Allergy Clin Immunol. 2012;129:1470-1477. https://doi.org/10.1016/j.jaci.2012.03.013
17. House JS, Wyss AB, Hoppin JA, Richards M, Long S, Umbach DM, et al. Early-life farm exposures and adult asthma and atopy in the agricultural lung health study. J Allergy Clin Immunol. 2017;140:249-256. https://doi.org/10.1016/j.jaci.2016.09.036
18. von Hertzen L, Mäkelä MJ, Petäys T, Jousilahti P, Kosunen TU, Laatikainen T, et al. Growing disparities in atopy between the Finns and the Russians: a comparison of 2 generations. J Allergy Clin Immunol. 2006;117:151-157. https://doi.org/10.1016/j.jaci.2005.07.028
19. Ojwang V, Near BI, Takkinen HM, Kaila M, Niemelä O, Haapala AM, et al. Early exposure to cats, dogs and farm animals and the risk of childhood asthma and allergy. Pediatr Allergy Immunol. 2020;31:265-272. https://doi.org/10.1111/pai.13186
20. Leynaert B, Neukirch C, Jarvis D, Chinn S, Burney P, Neukirch F. Does living on a farm during childhood protect against asthma, allergic rhinitis, and atopy in adulthood? Am J Respir Crit Care Med. 2001;164:1829-1834. https://doi.org/10.1164/ajrccm.164.10.2103137
21. Wills-Karp M. Interleukin-13 in asthma pathogenesis. Immunol Rev. 2004;202:175-190. https://doi.org/10.1111/j.0105-2896.2004.00215.x
22. Berry MA, Parker D, Neale N, Woodman L, Morgan A, Monk P, et al. Sputum and bronchial submucosal IL13 expression in asthma and eosinophilic bronchitis. J Allergy Clin Immunol. 2004;114:1106-1109. https://doi.org/10.1016/j.jaci.2004.08.032
23. Prieto J, Lensmar C, Roquet A, van der Ploeg I, Gigliotti D, Eklund A, et al. Increased interleukin-13 mRNA expression in bronchoalveolar lavage cells of atopic patients with mild asthma after repeated low-dose allergen provocations. Respir Med. 2000;94:806-814. https://doi.org/10.1053/rmed.2000.0826
24. Ungar B, Garcet S, Gonzalez J, Dhingra N, Correa da Rosa J, Shemer A, et al. An integrated model of atopic dermatitis biomarkers highlights the systemic nature of the disease. J Invest Dermatol. 2017;137:603-613. https://doi.org/10.1016/j.jid.2016.09.037
25. Duggan S. Tralokinumab: first approval. Drugs. 2021;81:1657-1663. https://doi.org/10.1007/s40265-021-01583-1
26. Piper E, Brightling C, Niven R, Oh C, Faggioni R, Poon K, et al. A phase II placebo-controlled study of tralokinumab in moderate-to-severe asthma. Eur Respir J. 2013;41:330-338. https://doi.org/10.1183/09031936.00223411
27. Davoodi P, Mahesh PA, Holla AD, Vijayakumar GS, Jayaraj BS, Chandrashekara S, et al. Serum levels of interleukin-13 and interferon- gamma from adult patients with asthma in Mysore. Cytokine. 2012;60:431-437. https://doi.org/10.1016/j.cyto.2012.05.012
28. Dimitrova D, Youroukova V, Ivanova-Todorova E, Tumangelova-Yuzeir K, Velikova T. Serum levels of IL-5, IL-6, IL-8, IL-13 and IL-17A in pre-defined groups of adult patients with moderate and severe bronchial asthma. Resp Med. 2019;154:144-154. https://doi.org/10.1016/j.rmed.2019.06.024
29. Hussein YM, El-Tarhouny SA, Shalaby SM, Mohamed RH, Hassan TH, El-Sherbeny HM, et al. Interleukin-13 receptor A1 gene polymorphism and IL-13 serum level in atopic and non-atopic Egyptian children. Immunol Invest. 2011;40:523-534. https://doi.org/10.3109/08820139.2011.565106
30. Yu J, Liu X, Li Y, Meng S, Wu F, Yan B, et al. Maternal exposure to farming environment protects offspring against allergic diseases by modulating the neonatal TLR-Tregs-Th axis. Clin Transl Allergy. 2018;8:34. https://doi.org/10.1186/s13601-018-0220-0
31. Ravanetti L, Dijkhuis A, Dekker T, Sabogal Pineros YS, Ravi A, Dierdorp BS, et al. IL-33 drives influenza-induced asthma exacerbations by halting innate and adaptive anti-viral immunity.

J Allergy Clin Immunol. 2019;143:1355–1370. https://doi.org/10.1016/j.jaci.2018.08.051

32. Mofatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, et al. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med. 2010;363:1211–1221. https://doi.org/10.1056/NEJMoa0906312
33. Allinne J, Scott G, Lim WK, Birchard D, Erjefält JS, Sandén C, et al. IL-33 blockade impacts mediators of persistence and exacerbation in a model of chronic airway inflammation. J Allergy Clin Immunol. 2019;144:1624–1637. https://doi.org/10.1016/j.jaci.2019.08.039
34. Gasiuniene E, Janulaityte I, Zemeckiene Z, Barkauskiene D, Sitkauskiene B. Elevated levels of interleukin-33 are associated with allergic and eosinophilic asthma. Scand J Immunol. 2019;89:e12724. https://doi.org/10.1111/sji.12724
35. Wang W, Li Y, Lv Z, Chen Y, Li Y, Huang K, et al. Bronchial allergen challenge of patients with atopic asthma triggers an alarmin (IL-33, TSLP, and IL-25) response in the airways epithelium and submucosa. J Immunol. 2018;201:2221–2231. https://doi.org/10.4049/jimmunol.1800709
36. Wechsler ME, Ruddy MK, Pavord ID, Israel E, Rabe KF, Ford LB, et al. Efficacy and safety of itepekimab in patients with moderate-to-severe asthma. N Engl J Med. 2021;385:1656–1668. https://doi.org/10.1056/NEJMoa2024257
37. Voloshyna I, Mucci T, Sher J, Fonacier LS, Littlefield MJ, Carsons S, et al. Plasma IL-33 in atopic patients correlates with pro-inflammatory cytokines and changes cholesterol transport protein expression: a surprising neutral overall impact on atherogenicity. Clin Exp Allergy. 2015;45:1554–1565. https://doi.org/10.1111/cea.12516
38. Ketelaar ME, Nawijn MC, Shaw DE, Koppelman GH, Sayers I. The challenge of measuring IL-33 in serum using commercial ELISA: lessons from asthma. Clin Exp Allergy. 2016;46:884–887. https://doi.org/10.1111/cea.12718