SAÚDE E AMBIENTE
V.10 • N.1 • 2025 - Fluxo Contínuo

ISSN Digital: **2316-3798**
ISSN Impresso: **2316-3313**
DOI: **10.17564/2316-3798.2025v10n1p298-316**

INTERFACES CIENTÍFICAS

OCCUPATIONAL EXPOSITION TO PESTICIDES INCREASES THE RISK OF AUTOIMMUNITY AND AUTOIMMUNE DISEASES: A SYSTEMATIC REVIEW AND META-ANALYSIS

A EXPOSIÇÃO OCUPACIONAL A AGROTÓXICOS AUMENTA O RISCO DE AUTOIMUNIDADE E DOENÇAS AUTOIMUNES: UMA REVISÃO SISTEMÁTICA E META-ANÁLISE

LA EXPOSICIÓN OCUPACIONAL A PESTICIDAS AUMENTA EL RIESGO DE AUTOINMUNIDAD Y ENFERMEDADES AUTOINMUNES: UNA REVISIÓN SISTEMÁTICA Y METANÁLISIS

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ABSTRACT

Studies have suggested the existence of a relationship between exposure to pesticides and autoimmunity as well as autoimmune diseases. Parallel to this, the increase in exposure to pesticides in recent decades and in the identification of autoimmunity such as autoimmune diseases throughout the world is a reality. Given this scenario, more in-depth investigations are increasingly necessary to determine this relationship. Thus, we performed a comprehensive meta-analysis from published studies to answer the question: “Is there a higher risk for autoimmunity in farmers/rural workers exposed to pesticides?”. The databases: Pubmed, Embase, Lilacs, Scopus, CAF-e and BVS were searched to identify qualified literatures. The meta-analysis was based on the calculation via Multivariate/Multilevel Linear (Mixed-Effects) Model and a total of 259 parameters in nine articles were included in the study. The results showed that the random effect for all analyses was 0.36 (0.33- 0.39; p<.0001) and the meta-analysis showed a significant heterogeneity among the studies. All together, these results are a consequence of the variation in the immune parameters evaluated by each selected study and in the different groups of individuals evaluated among the included studies. The bias analysis was complemented by the sensibility analysis, which showed that withdrawing one study, the overall effect remains positive, and the heterogeneity does not change significantly. These findings indicated that exposure to pesticides might increase the risk of autoimmunity in farmers and rural workers. Further studies are needed to better understand the underlying mechanisms of immune alterations compatible with autoimmunity due to occupational exposure to pesticides.

KEYWORDS

Pesticides; farmers; rural workers; autoimmunity.

RESUMO

Estudos têm sugerido a existência de uma relação entre a exposição a agrotóxicos e o desenvolvimento de autoimunidade e doenças autoimunes. Paralelo a isto, o aumento expressivo na utilização de agrotóxicos nas últimas décadas e o aumento dos casos de autoimunidade e de doenças autoimunes em todo o mundo são uma realidade. Diante desse cenário, torna-se cada vez mais necessário o aprofundamento das investigações para averiguar tal relação. Assim, realizamos uma meta-análise abrangente de estudos publicados nesta temática para responder à pergunta: “Existe um risco maior de autoimunidade em agricultores/trabalhadores rurais expostos a agrotóxicos?”. As bases de dados: Pubmed, Embase, Lilacs, Scopus, CAF-e e BVS foram consultadas para identificar literaturas qualificadas. A meta-análise foi baseada no cálculo via Modelo Linear Multivariado/Multinível (Efeitos Mistos) e um total de 259 parâmetros em nove artigos foram incluídos no estudo. Os resultados mostraram que o efeito aleatório para todas as análises foi de 0,36 (0,33- 0,39; p<,0001) e a meta-análise mostrou uma heterogeneidade significativa entre os estudos. Em conjunto, esses resultados são uma consequência da variação nos parâmetros imunológicos avaliados por cada estudo selecionado e nos diferentes grupos de indivíduos avaliados entre os estudos. A análise de viés foi complementada pela análise de sensibilidade, que mostrou que o efeito geral permaneceu positivo e a heterogeneidade não mudou significativamente caso algum estudo fosse removido das análises. Ao analisar conjuntamente os dados dos artigos selecionados, fica evidente que a exposição a agrotóxicos aumenta o risco de autoimunidade em agricultores e trabalhadores rurais. Mais estudos são necessários para entender melhor os mecanismos subjacentes às alterações imunológicas compatíveis com a autoimunidade devido à exposição ocupacional a agrotóxicos.

PALAVRAS-CHAVE

Pesticidas; agricultores; trabalhadores rurais; autoimunidade.

RESUMEN

Los estudios han sugerido la existencia de una relación entre la exposición a pesticidas y la autoinmunidad, así como las enfermedades autoinmunes. Paralelamente a esto, el incremento en la exposición a pesticidas en las últimas décadas y en la identificación de autoinmunidad tal como las enfermedades autoinmunes en todo el mundo es una realidad. Ante este escenario, cada vez se hacen necesarias investigaciones más profundas para determinar esta relación. Por lo tanto, realizamos un metanálisis exhaustivo de estudios publicados para responder a la pregunta: "¿Existe un mayor riesgo de autoinmunidad en agricultores/ trabajadores rurales expuestos a pesticidas?". Se realizaron búsquedas en las bases de datos: Pubmed, Embase, Lilacs, Scopus, CAF-e y BVS para identificar literatura calificada. El metanálisis se basó en el cálculo a través del modelo lineal multivariado / multinivel (efectos mixtos) y se incluyeron en el estudio un total de 259 parámetros en nueve artículos. Los resultados mostraron que el efecto aleatorio para todos los análisis fue de 0,36 (0,33- 0,39; p<,0001) y el metanálisis mostró una heterogeneidad significativa entre los estudios. En conjunto, estos resultados son consecuencia de la variación en los parámetros inmunológicos evaluados por cada estudio seleccionado y en los diferentes grupos de individuos evaluados entre los estudios incluidos. El análisis de sesgo se complementó con un análisis de sensibilidad, que mostró que el efecto general siguió siendo positivo y la heterogeneidad no cambió significativamente si se eliminaba algún estudio de los análisis. Al analizar conjuntamente los datos de los artículos seleccionados, es evidente que la exposición a pesticidas aumenta el riesgo de autoinmunidad en agricultores y trabajadores rurales. Se necesitan más estudios para comprender mejor los mecanismos subyacentes a los cambios inmunológicos compatibles con la autoinmunidad debido a la exposición ocupacional a pesticidas.

PALABRAS CLAVE

Pesticidas; agricultores; trabajadores rurales; autoinmunidad.

1 INTRODUCTION

Autoimmunity is a process constituted by the failure of central and peripheral tolerance which leads to both humoral and cellular self-reactive immune responses. This condition may evolute to autoimmune diseases, which comprises more than 70 different diseases which have distinct patterns of injury, target tissues, onset, and treatment protocols (LLEO *et al.*, 2010; PISETSKY, 2023).

Despite the variability of manifestations, a shared feature of autoimmune diseases is the contribution of a complex interaction between genetic and environmental factors, most of which have not been identified (LLEO *et al.*, 2010; MILLER, 2023; PISETSKY, 2023).

Concerning the environmental factors, occupational exposure to pesticides has been investigated for the involvement in the trigger of the autoimmune process. Pesticide immunotoxicity, resulting in immunosuppression or immunostimulation, has been shown to possibly impact the occurrence of autoimmunity specially in farmers and pesticide-exposed rural workers (STEERENBERG *et al.*, 2008; JACOBSEN-PEREIRA *et al.*, 2020).

It is important to reinforce that, although pesticide exposure occurs predominantly through an occupational way of exposure, mainly in farmers and rural workers, different ways of exposure are also possible, through contact with contaminated soil, water and food. Based on the exposed, some speculation about the association between these relations of these different types of exposures and the development of several diseases already reported in the literature, such as brain diseases, thyroid disorders, reproductive diseases, infections, tumors, and even autoimmune diseases, have been raised (SPIEWAK; STOJEK, 2003; DALVIE; LONDON, 2006; STEERENBERG *et al.*, 2008; FREIRE *et al.*, 2013).

Based on the likely relation between pesticide exposure and both autoimmunity and autoimmune diseases, reinforced by the fact that pesticides exposition has increased in the last decades, and by the fact that autoimmunity identification as well as autoimmune diseases are dramatically increasing worldwide, this field of investigation needs more understanding (MAKSYMIV, 2015; MILLER, 2023).

Therefore, the goal of this study was to better understand and evidence the relationship between occupational exposure to pesticides and immune alterations compatible with autoimmunity by a systematic literature review and meta-analysis.

Meta-analyses are useful tools for understanding the clinical processes being investigated since they allow us to investigate sources of variation and different effects between subgroups of studies (LEE, 2018). This study is pioneer since there are no published meta-analytic studies concerning this topic and the obtained results may be helpful for providing reliable answers by aggregating different studies on the same topic with an accurate estimate of the effect size, with considerably increased statistical power, which is important when the power of the primary studies are limited due to the small sample size.

Furthermore, a better understanding of the impact of pesticides towards the immune system and other related organs and systems, searching for markers of early exposure as well as which kind of alteration happens in these markers, can help prevent the development of diseases due to pesticide exposure. This could encourage the establishment of preventive measures by healthcare organizations for the precise management in anticipation of irreversible immune alterations associated with autoimmune diseases.

2. MATERIAL AND METHODS

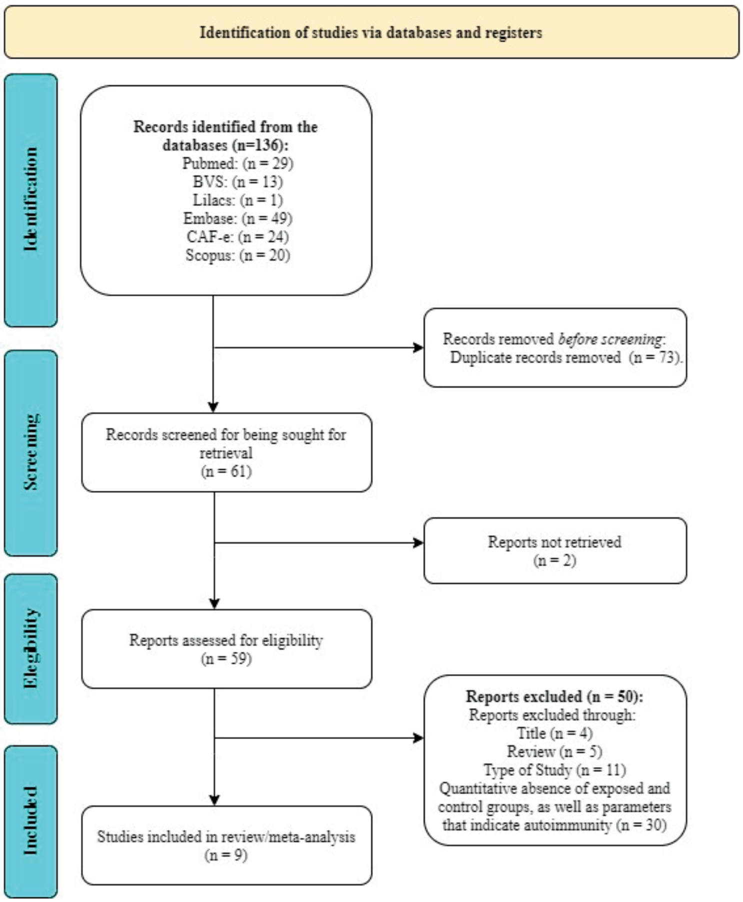
2.1 SEARCH STRATEGY

This systematic review and meta-analysis were conducted by the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidance protocol (PAGE *et al.*, 2021).

The research question proposed was: “Is there a higher risk for autoimmunity in farmers/rural workers exposed to pesticides?”. The criteria included in the study were determined using the Population, Exposure, Comparator, and Outcomes (PECO) framework. “P” refers to farmers and rural workers, while “E” refers to pesticides (insecticides, herbicides, and antifungal agents). “C” refers to farmers and rural workers who are not exposed to pesticides. “O” refers to the increased risk of autoimmunity phenomena. (MORGAN *et al.*, 2018).

The search was made through the databases: Pubmed, Embase, Lilacs, Scopus, CAF-e and BVS, up to June and July 2023. The following keywords/MeSH Terms were used for the inclusion of papers: ("Insecticides, Organophosphate" OR "Pesticides" OR "Agrochemicals" OR "Insecticides" OR "Herbicides" OR "Antifungal Agents") AND ("Pesticide Exposure" OR "Occupational Exposure") AND ("Rural Workers" OR "Rural Population" OR "Farmers") AND ("Autoimmunity" OR "Autoimmune Diseases" OR "Autoantibodies"). The detailed search process can be found in Figure 1.

Figure 1 - Flowchart of the study selection process for the systematic review and meta-analysis, according to the PRISMA protocol by using the PECO model (Population, Exposure, Comparator, and Outcome). The search was carried out in the Pubmed, Embase, Lilacs, Scopus, CAF-e, and BVS databases until June and July 2023, using descriptors related to pesticides, occupational exposure, rural workers, and autoimmune diseases. A total of 136 records were identified, of which 73 duplicates were excluded. After screening and eligibility assessment, a total of 9 studies were included in this study.



2.2 ELIGIBILITY CRITERIA

To fit the eligibility criteria, the selected studies should fulfill the following requirements: (1) Available full length epidemiological studies involving human beings and exploring the relationship between occupational pesticide exposure and immunological alterations related to the autoimmune process. (2) Studies presenting measurable immunological parameters, whether quantitative or qualitative. (3) Exposed versus unexposed case-control, cross sectional, or cohort studies (4) Studies reporting effect estimates (RR, OR, HR, PC) or data that could allow calculation of the size effect. It is important to mention that not reviews, meta-analyses, case reports as well as conference papers were not included.

2.3 STATISTICAL ANALYSIS

All data analyses were realized by R software program (R core Team 2021®) and *metafor* package (VIECHTBAUER, 2005). The meta-analysis was based on the calculation via Multivariate/Multilevel Linear (Mixed-Effects) Model, thus obtaining the effect by the restricted maximum likelihood "REML" (VIECHTBAUER, 2005; RAUDENBUSH, 2009). The aggregation of multiple effect sizes and outcomes was done according to the method of Borenstein *et al*. (2009). In order to evaluate the heterogeneity between studies, the Cochran's Q test, p value, heterogeneity index (I^2), and variability index (H^2) were performed. For bias analysis, the asymmetry test funnel plot and sensitivity analysis was employed (EGGER *et al*., 1997).

3. RESULTS

3.1 INCLUDED STUDIES

The assessment of eligibility of identified studies was conducted as recommended by the PRISMA 2020 updated guideline for reporting systematic reviews (PAGE *et al*., 2021). At first, the search generated a total of 136 results. Out of these, 73 were excluded due to duplication. It is important to mention that records marked as ineligible by automation tools as well as records removed for other reasons were not necessary. After screening based on titles, abstracts by the attendance of eligibility criteria, a total of 50 articles were excluded thus remaining a total of 9 studies. A total of 1868 individuals participated in the 9 countries, and 41 different parameters were analyzed. Figure 1 illustrates the process of literature screening. The included papers were tabulated with data on (I) First author/publication year, (II) Region (III) Study design, (IV) Pesticides characteristics, (V) Exposed Group, (VI) Control Group, (VII) Population aspects. (VIII) Summary, (IX) Immunological Parameters, (X) Results, (XI) p value, (XII) CI (Exposed vs. Control) and SD (Exposed vs. Control). The tabulated Immunological Parameters (IX), a total of 259, utilized to determine the presence of autoimmunity and autoimmune diseases included Ethylenethiourea-ETU urine, serum immunoglobulins (IgA, IgE, IgG, IgM), complement fractions (C3 and C4), lymphocyte subpopulations (CD3, CD4, CD19, CD25) and autoantibodies such as anti-nuclear, anti-mitochondrial, and anti-smooth muscle (ANA, AMA, ASMA). The tabulated information extracted can be found in Table 1 (Complete version in Table S1, Supplementary material).

Table 1 - Characteristics of included studies

First author/publication year	Country/Region	Study design	Pesticides characteristics	Exposed Group (n)	Control Group (n)	Population aspects	Summary	Evaluated Immunological Parameters
Jacobsen-Pereira (2020)	South Brazil	control vs. case	Mixture of pesticides (fungicides. insecticides and herbicides)	43	30	31 men and 12 women	Investigation of the relationship between occupational exposure to pesticides and the immunological profile in farmers exposed to mixtures of pesticides for 15 years.	TNF (pg/ mL) IL2 (pg/ mL) IL4 (pg/ mL) IL6 (pg/ mL) IL10 (pg/ mL) IFN-y (pg/ mL) IL17A (pg/ mL) Total leukocytes (cells/mm³) Total T cells (cells/mm³) Total monocytes (cells/mm³) Total lymphocytes (cells/mm³) Neutrophils (cells/mm³) Eosinophils (cells/mm³) Basophils (cells/mm³) Total B cells (cells/mm³) Total NK (cells/mm³) Total DCs (cells/mm³)
Corsini (2005)	Northern Italy	cross-sectional	Mancozeb®	13	13	13 vineyard workers (12 males and 1 female; mean age: 41. range: 25– 54) and 13 matched healthy (12 males and 1 female; mean age: 40. range: 25 – 50)	Investigated the immunological profile of farmers exposed to Mancozeb®, an EBDC fungicide, by determining several serum cellular and functional immune parameters	IgA (ng/dL) IgG (ng/dL) IgE (ng/dL) IgM (ng/dL) CD3 (a.n) CD19 (a.n) CD25 (a.n) CD4 (a.n) Eosinophils (%) Basophils (%) Neutrophils (%) Lympocytes (%) Monocytes (%) TNF-alfa (pg/cell)
El Rahman (2017)	Dakahleya region, Egypt	cross-sectional	Mixture of pesticides (fungicides. insecticides and herbicides)	50	25	50 workers (Gender distribution: Male 98%. Female 2%; Mean age: 40.92) and 25 healthy (Gender distribution: Male 68%. Female 32%; Mean age: 29.24)	Screened sera of subjects chronically exposed to mixtures of pesticides, mainly organophosphorus compounds and others, for autoantibodies against cytoskeletal neural proteins in the presence of neurological symptoms.	S100-B (ng/lane) GFAP (ng/lane) Tau (ng/lane) MAP-2 (ng/lane) NFP (ng/lane) TUBULIN (ng/lane) MBP (ng/lane) CaMKII (ng/lane) MAG (ng/lane) alfaSyN (ng/lane)
Santos (2022)	Southeast, Brazil	cross-sectional	Mixture of pesticides (fungicides. insecticides and herbicides) - not specified	52	68	52 Agricultural workers vs nonagricultural workers (median age value = Exposure 43 vs. Control 38.5; p < 0.001). (Gender distribution: Male 53.8% vs Male control: 26.5%0.002)	Assessed the link between pesticide use and anti-cyclic citrullinated peptide (anti-CCP) antibodies and antinuclear antibodies (ANA) levels.	ANA (U/mL) Anti-CCP (U/mL)
Steerenberg (2008)	Bulgaria Finland Italy Netherlands	cross-sectional	Mixture of pesticides (fungicides. insecticides and herbicides) - not specified	248	231	Netherlands: 40 workers employed in flower bulb growing industry and 39 non-(mostly office employees). Italy: 47 vineyard workers and 45 non-controls. Finland: 52 potato farmers and 48 non-biological farmers. Bulgaria: 49 employees from a factory that produced mainly zineb (an EBDC pesticide) and 53 controls. and 61 greenhouse workers producing cucumbers and tomatoes and 56 controls. (Gender distribution: Male Exposure: 66.5% vs Male Control: 62.4%-) (median age value = Exposure 42.4 vs. Control 42.2-)	Evaluated the effect of prolonged exposure to low doses of pesticide mixtures in occupationally people and measured its effects on hematological parameters and immune defense components.	IgG1 (mg/mL) IgG4 (mg/mL) IgM (mg/mL) IgA (mg/mL) Complement C3 (g/L) Complement C4 (g/L) Glycoproteins (g/L) ESR (g/L) Leucocyte count (10³/mm³) Lymphocytes (%) Monocytes (%) Neutrophils (%) Eosinophils (%) CD3 (a.n) CD4 (a.n) CD8 (a.n) CD19 (a.n) ANA (odds ratio) AMA (odds ratio) SMA (odds ratio) Creatinine (µg/g)

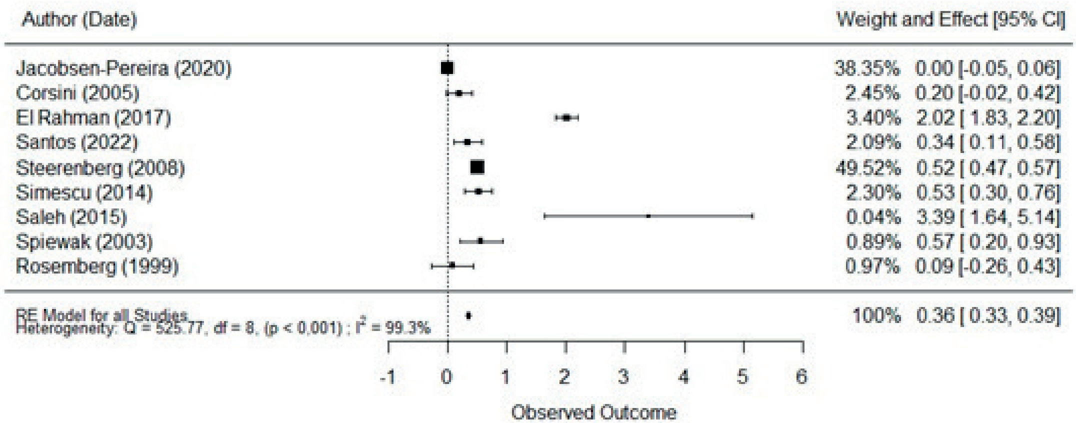
Simescu (2014)	Bucharest, Romania.	Coorte	Chlorpyrifos. trichloropyridinol (TCP). carbofurane. cypermethrin. dimethoate	108	27	18–78 years old; 19 males and 89 females	Evaluated the potential correlation between pesticide exposure and thyroid function, autoimmunity, and morphology in greenhouse workers.	TSH (µIU/mL)
				108	28			Free T4 (µIU/mL)
				18	15			Cholinesterase april 2010 (U/L)
				18	25			Cholinesterase august 2010 (U/L)
				18	12			Cholinesterase april 2011 (U/L)
				18	14			Cholinesterase may 2011 (U/L)
				18	12			Cholinesterase august 2011 (U/L)
Saleh (2015)	Egypt	Cross-sectional	-	10	200	10 patients with pemphigus vulgaris (4 of them exposed to pesticides). 20 healthy first-degree relatives of patients with pemphigus vulgaris (parents. siblings. or children) (4 exposed to pesticides). and 200 healthy human (6 of them exposed to pesticides).	Investigated anti-Dsg3 antibodies in healthy Egyptians to determine reasons for increase in pemphigus patients in Egypt.	Dsg3 positivity in patients of rural areas Dsg3 positivity in patients living near farms Dsg3 positivity in patients to pesticides
Spiewak (2003)	Lublin Region, Eastern Poland	Cross-sectional	-	90	50	Group: 25 men and 65 women. aged 18–82 (median 44) years Control Group: 50 men aged 21–55 (median 35) years	Proceeded with a comparison of antinuclear antibody frequency between rural inhabitants exposed to pesticides and urban blood donors in Eastern Poland.	ANA positivity (Number of cases) anti-dsDNA
Rosenberg (1999)	Saskatchewan, Canada	Cross-sectional	Mixture of pesticides (fungicides. insecticides and herbicides) - especified	209	97	54.3% males and the remaining female (mean age 49.3 ± 14.7 yr; range 16–87 yr).	Determined the prevalence of ANA in a rural population and evaluated its association with environmental and occupational exposures.	ANA positivity Pesticides (Number of cases) ANA positivity Herbicide (Number of cases) ANA positivity Insecticide (Number of cases) ANA positivity Fungicide (Number of cases) ANA positivity Carbamatos (Number of cases) ANA positivity Organoclorados (Number of cases)

Legend: *mean; **median; - non specified; Values of each parameter in supplementary material.

3.2 RELATIONSHIP BETWEEN OCCUPATIONAL EXPOSURE TO PESTICIDES AND IMMUNE ALTERATIONS

Our study aimed to observe in a broad and general way the research involving immunotoxicity of farmers exposed occupationally to pesticides; thus, 8 out of the 9 studies included in this meta--analysis showed immune alterations in the exposed group, demonstrating the topic's relevance. The random effect for all analyses was RE 0.36 (0.33- 0.39; p<.0001), indicating a significant impact on immune changes and damage for the pesticide-exposed group (Figure 2). All included studies had their effects greater than zero, except the study by Jacobsen-Pereira *et al*. (2020) which showed no difference between the evaluated groups (RE 0.00 [-0.05, 0.06]).

Figure 2 - Forest plot of the meta-analysis evaluating the immunotoxic effects in farmers occupationally exposed to pesticides. Random effects (RE) model for all analyses showed a pooled effect of 0.36 (95% CI: 0.33-0.39; $p < 0.0001$), indicating a significant impact on immunological changes and damage in the exposed group. Heterogeneity was high ($Q = 525.77$, $p < 0.001$; $I^2 = 99.3\%$), highlighting the variability among the studies analyzed.



Considering the weight of the effect, it is possible to see that it was not similar in all studies, these discrepancies can be explained by the number of studied individuals as well as by the number of immune parameters evaluated in each study. The average number of individuals analyzed in the 9 studies was 60 in the control group and 73 in the exposed group. Variation of studied individuals influenced the weight, as can be seen by the weight of the study from Jacobsen-Pereira *et al*. (2020) which included a number of 73 individuals and presented an effect of 38.35%, and by the study from Steerenberg *et al*. (2008) evaluating 479 individuals which presented a higher effect (49.52%). Furthermore, the number of evaluated immune parameters was not similar among the studies, with an average of 16 studied parameters, but with discrepancies, as the study from Jacobsen-Pereira *et al*. (2020) involving the evaluation of the biggest number of immunological parameters (n=73), and the studies performed by Saleh and El-Bahy (2015) and Rosenberg *et al*. (1999), the ones presenting the lowest number of evaluated parameters (n=1).

Moreover, the meta-analysis showed a significant heterogeneity among the studies. Cochran's Q statistic presented the notable result of 525.7692, as well as the I^2 with the value of 99.30% and the H^2 with the result of 143.31. All together, these results are a consequence of the variation in the immune parameters evaluated by each selected study and in the different groups of individuals evaluated among the included studies.

3.3 BIAS ANALYSIS

Trying to improve the understanding of the meta-analysis heterogeneity, both funnel plot and an asymmetry test were performed (Figure 3). Seven out of the nine selected studies showed lower grade of variance between them, as can be seen by the seven dots close to the overall effect line of the funnel plot, and the remaining two are dispersed to the right side revealing the chance of causing biases.

Figure 3 - Funnel plot for bias analysis. Most studies (7 of 9) showed low variance among themselves, demonstrated by the proximity of the points to the overall effect line in the graph. However, two studies are scattered on the right side of the graph, indicating possible publication bias or heterogeneity in the results. The observed asymmetry suggests the possibility of bias, which may have influenced the pooled estimate of the effect.

The bias analysis was complemented by the sensibility analysis, which showed that withdrawing one study, the overall effect remains positive, and the heterogeneity does not change significantly. Study from El Rahman *et al*. (2018) presented a relevant effect, however, if removed, the estimation of the overall effect continues relating to an association between pesticides and immune changes.

Likewise, study by Saleh and El-Bahy (2015) shows a greater effect than the others, with the possibility of bias, which was corrected by the meta-analysis, due to its low weight. The sensibility analysis reveals that the study does not present a notable modification in the overall result and in the association between pesticides and immune alterations.

3.4 IMMUNE CELLS QUANTIFICATION

Exposure to different pesticides resulted in varied leukocyte counts in populations. According to the results of Corsini *et al*. (2005), exposure to the fungicide Mancozeb resulted in a significant decrease in the percentage of eosinophils (p= 0.0001) and a significant increase in CD3+, CD4+, CD19+, and NK cells (p = 0.016; p = 0.006; p = 0.004; p = 0.034; p = 0.049, respectively). This scenario was reported by Steerenberg *et al*. (2008) during an examination of the effects of prolonged low-dose exposure to mixtures of pesticides on farmers from various countries. The study found an increase in total leukocyte count (p=0.0005) and the number of CD8 cells (p = 0.01), and also changes in the kidney function of the exposed groups, due to an increase in serum creatinine levels An increase in T-cells subpopulations was also observed in the case-control study conducted by Jacobsen-Pereira *et al*. (2020) The study revealed a significant increase in classical monocytes ($p<0.05$), dendritic cells ($p < 0.05$), and total T-cells ($p = 0.04$) in the exposed group, as compared to the control group. Furthermore, the B cells levels, especially the regulatory ones, were significantly lower in the exposed group ($p = 0.01$).

3.5 CYTOKINE LEVELS

When investigating the impact of exposure on immune functions, Corsini *et al*. (2005) results did not exhibit significant differences between IL-4 and g-IFN among the groups or alterations in NK cytolytic activity. Yet, it revealed a significant reduction in TNF-a levels in post-exposure samples ($p=0.0001$). However, when examining Jacobsen-Pereira *et al*. (2020), compared to controls, farmers showed significantly elevated levels of pro-inflammatory IL-6 in their plasma ($p = 0.04$).

3.6 IMMUNOGLOBULINS AND COMPLEMENT SYSTEM FACTORS LEVELS

Similarly, exposure to different pesticides was linked to changes in immunoglobulin levels. Corsini *et al*. (2005) demonstrated a significant decrease in serum IgE levels ($p = 0.016$). On the other hand, there were no changes observed in other immunoglobulins. Additionally, alterations in the levels of these constituents were also documented by Steerenberg *et al*. (2008) reporting higher levels of Complement Factors ($p = 0.01$) and IgG4 ($p = <0.0001$), while lower levels of IgA ($p = <0.0001$) were observed.

3.7 AUTOANTIBODIES LEVELS

The levels of autoantibodies in the blood showed significant variations, which were associated with changes in the clinical symptoms of autoimmune disorders. El Rahman *et al*. (2018) found that farmers submitted to chronic pesticide exposure and presenting neurological symptoms had a statistically significant increase in autoantibodies to CNS proteins, such as mielin basic protein-MBP (MBP, 7.67, MAG 5.89, CaMKII 5.50, GFAP 5.1, TAU 4.96, MAP2 4.83, SNCA 4.55, NFP 4.55, S-100B 2.43), as compared to the control group. Significant autoantibody levels were also observed by Saleh and El-Bahy (2015). The positivity for the Anti-desmoglein antibody found in patients with pemphigus was significantly related to the following population and behavioral characteristics of the studied population: rural areas residents ($p < 0.0001$), farmers ($p = 0.0025$), exposure to pesticides ($p = 0.0025$). Similar findings were seen by Spiewak and Stojek (2003). In a group of rural community residents exposed to pesticides, the frequency of Antinuclear Antibodies, mainly anti-double-stranded DNA (anti-dsDNA) autoantibodies, was 2.5 times higher than the unexposed group ($p = 0.0175$). Rosenberg *et al*. (1999) were also able to measure which class of agrochemicals used most influenced ANA positivity in rural Canadian populations exposed to, mainly, insecticides ($p = 0.006$) and compounds containing carbamate ($p = 0.023$) and organochlorines ($p = 0.016$). Santos *et al*. (2022) also demonstrated an increased incidence of positive serum antibody levels in rural workers, but with results that were not statistically relevant ($p > 0.05$).

4 DISCUSSION

In this systematic review and meta-analysis, we comprehensively evaluated available data on the effects of occupational pesticide exposure on the immune system. All evaluated studies showed a positive link between long-term pesticide exposure in farmers and rural populations and an increased risk of autoimmune events and diseases. The meta-analysis results also revealed significant associations between long-term exposure to pesticides and alteration in autoimmunity parameters.

The estimates indicate a greater likelihood of exposure to pesticides bearing insecticides and fungicides functions and, considering agrochemical classes, compounds particularly containing carbamates or organochlorines. Actually, it is difficult to assess the health effects of individual pesticides, because these substances are rarely used isolated. For example, out of the nine studies that were selected for meta-analysis, only one evaluated the immunomodulatory effect of an isolated pesticide (dithiocarbamate, a fungicide) (CORSINI *et al*., 2005). Meanwhile, all the other studies evaluated the exposure to multiple pesticides or complex mixtures of pesticides. In this sense, further analysis of individual pesticides exposure effect could better show us the type of immune alteration mediated by the specific pesticide evaluated. On the other hand, it has been evidenced that synergistic effects of multiple pesticides can be missed when studying the effects of a single pesticide (WANG *et al*., 2022).

The meta-analyses heterogeneity found was expected, since the studies analyzed a plethora of different parameters and also by the fact that the included studies were performed with different people in different settings, and these aspects such as ethnic characteristics and popular country lifestyle directly influence the results. All the highlighted studies involved a population approximately in the fourth decade of life, which can be explained by the fact that this is the median age of economically active rural workers (USDA, 2023). Furthermore, a high prevalence of male sex was observed in the evaluated populations of the selected studies and there were no particular analyses in the studies regarding sex influence towards immune alterations. The fact that the sample previously chosen in the studies was massively made up of male workers corroborates the demographic characteristics of rural workers (USDA, 2023).

It's worth noting that there are regional and socioeconomic differences in the analysis of autoantibody parameters. Interestingly, the less developed countries, such as Egypt, had more significant results (SALEH; EL-BAHY, 2015). This can be partly explained by the predominant social and economic structure of these countries, which have a primary economy focused mainly on agribusiness. Consequently, there is a greater investment in agricultural inputs, pesticides, and rural workers, leading to more significant exposure to agrochemicals among workers in these regions (LIGNANI; BRANDÃO, 2022).

When jointly analyzing the parameters of all the studies included, it can be observed that the majority of relevant immunological alterations involve the detection and also the quantification of high titers of antinuclear antibodies, ANA (ROSENBERG *et al*., 1999; SPIEWAK; SOJEK, 2003; STEERENBERG *et al*., 2008; SALEH; EL-BAHY, 2015). This immune status alteration might be explained by the induction of autoantibodies due to an endogenous antigen that became more immunogenic by the exposition to the toxic agent, which may result from contact with the active principle of the pesticide itself or the adjuvant compounds present in these products, such as solvents and additives (RODGERS *et al.*, 1986), especially due to the low molecular weight of these molecules, which allows greater permeability through mucosa and epithelia, and the combination with self-proteins (MOKARIZADEH *et al.*, 2015). Furthermore, it is important to mention that the simple detection of ANA autoantibodies does not imply in a clinical manifestation or active disease related to autoimmunity (SANTOS *et al.*, 2022), and therefore, simply indicate a subclinical condition, a subclinical marker that can, with continuous exposure to pesticides, be able to evolve to the autoimmune disease itself (ASSMANN *et al.*, 2014; SCHINASI; LEON, 2014).

Exposure to pesticides also appears to be related to immunological alterations related to metabolic/endocrine parameters. Simescu *et al.* (2014) revealed an increase in serum levels of hormones related to thyroid function (TSH, free T4), results which corroborate other epidemiological studies that evaluated the same parameters in rural populations exposed to pesticides and who presented hypothyroidism or subclinical hyperthyroidism (GOLDNER *et al.*, 2010).

In general, the presence of autoantibodies is also accompanied by other signs of immunological changes, such as immune cell functional or quantitative alterations (CORSINI *et al.*, 2005; STEERENBERG *et al.*, 2008; JACOBSEN-PEREIRA *et al.*, 2020). In this regard, all the selected articles which evaluated white blood cells demonstrated some degree of quantitative alteration of leukocytes. Most of the changes were concentrated in cells of the adaptive immune system: TCD4, TCD8 and B cells, which can be explained by the immunoproliferative mechanism, especially related to T cells (CORSINI *et al.*, 2005). This characteristic may mimic the autoimmune characteristics of chronic immune dysregulatory diseases, in which, due to the chronicity of exposure, these cell types have a greater predilection due to the greater cytotoxic and humoral effect, by modificating the Th1/Th2 cytokine profiles (KOUREAS *et al.*, 2017). Most of these changes are related to granular exocytosis and incongruity in the activation of apoptosis inducers related to immunoregulatory mechanisms such as the Fas ligand (FasL) (JACOBSEN-PEREIRA *et al.*, 2020). This change enhances T-cell cytotoxicity, decreases auto apoptosis and alters B-cell function, especially its humoral effect. Other cell lineages, especially from the innate immune system, have also been studied. Jacobsen-Pereira *et al.* (2020) showed that the amount of monocytes and dendritic cells are significantly increased in farmers' blood, demonstrating intense cell recruitment by the immune system. These cell lines play a crucial role in the immune system's initial defense mechanism against external substances. This is particularly important for mucous membranes and lining tissues that have more direct contact with environmental exposure, like the lungs and skin, which is relevant to airborne exposure to pesticides (COOPER *et al.*, 2004).

However, although all evaluated studies demonstrated some degree of alteration in leukocyte count (i.e. lymphocytes, neutrophils and basophils), the immune function showed considerable heterogeneity. Corsini *et al.* (2005) revealed a relevant seric TNF-alpha decrease after occupational exposure to the fungicide dithiocarbamate (Mancozeb, registered mark). On the other hand, Jacobsen-Pereira *et al.* (2020) demonstrated opposite results, showing no decreased TNF-a levels. Although not statistically significant, the authors evidenced some increase in TNF-alpha levels accompanied by a significant increase of IL-6 levels, and the authors attributed this to a possible compensatory attempt of the immune system due to the depletion of B cells also found in their study. Still, the authors believe that a secondary lymphoid organ's direct immunotoxicity may be happening since there is no depletion of other immune cells lineages nor of cytokines. In addition, changes in immunoglobulin levels were also observed (CORSINI *et al.*, 2005, STEERENBERG *et al.*, 2008). The main change occurred in IgA concentrations. One of the likely explanations is due to the function of this antibody IgA class itself, which is primarily responsible for protecting mucosal surfaces, mainly exposed to pesticides (COOPER *et al.*, 2004). However, both studies are inconclusive. While Corsini *et al.* (2005) reveals a slight increase in IgA levels, Steerenberg *et al.* (2008), reveals an intense and significant suppression. In this sense, further studies, focused on this immunological parameter, can be considered to determine the real effect of exposure.

Furthermore, increases in other factors related to autoimmunity, such as tubular proteins, were observed, which result in autoimmune neurological diseases such as Parkinson's and Alzheimer's disease (EL RAHMAN *et al.*, 2018).

Conjunction overall data, despite the several gaps of knowledge in this field of immune alterations related to pesticide exposure, some mechanisms by which pesticides exposure leads to the development of immune alterations have been speculated (RODGERS *et al.*, 1986; CORSINI *et al.*, 2005; SIMESCU *et al.*, 2014; EL RAHMAN *et al.*, 2018; JACOBSEN-PEREIRA *et al.*, 2020), as summarized in Figure 4.

Figure 4 - Proposed mechanisms of immunological alterations associated with pesticide exposure. Despite the knowledge gaps in this area, some studies suggest that exposure to pesticides can lead to the development of immunological alterations through different mechanisms.

Considering pesticide-exposure outcomes, literature has brought evidences of its relation with some chronic health problems as follows: I) Neurological diseases (EL RAHMAN *et al.*, 2018); II) Thyroid diseases and hormonal/metabolic disorders (SIMESCU *et al.*, 2014); III) Renal diseases (STE-

ERENBERG *et al*., 2008); IV) Autoimmune disease (ROSENBERG *et al*., 1999; SPIEWAK; STOJEK, 2003; STEERENBERG *et al*., 2008; SALEH; EL-BAHY, 2015). Other outcomes can also be observed: Allergic Rhinitis (KOUREAS *et al*., 2017), hepatocellular carcinoma (ABOU EL AZM *et al*., 2014).

In the course of the systematic review's research and screening, some issues were observed. Beyond the restricted number of articles found in the research, some limitations were perceived, such as individual self-reports of autoimmune disease diagnosis, without any clinical or laboratory proof, bringing the chance of a fake report. Other limitation of some evaluated studies was the low population sample which reduces the statistical power and inflates the effect size estimation of the study. Still, even the studies which specified the pesticides used together, there is no guarantee that the exposure was isolated and that there was no interference from another class of agrochemical, as this exposure was simply self referred by population. Besides, there were no studies regarding age stratification.

Moreover, in the meta-analyses' included articles, the inconsistency and the incongruence among the chosen immune parameters was a limitation, i.e. just one study (JACOBSEN-PEREIRA *et al*., 2020) performed IL-4 analysis. One of the main problems found in the literature included is due to the heterogeneity of the quantity of exposed and control individuals, which often results in inaccurate results.

5 CONCLUSION

Despite the limitations pointed above, our findings indicate that exposure to pesticides might increase the risk of autoimmunity in farmers and rural workers.

REFERENCES

ABOU EL AZM, A.R. *et al*. New insights on non-B non-C hepatocellular carcinoma in mid Delta Region, Egypt. **J Gastrointest Cancer**, v. 45, n. 3, p. 276–283, 2014.

ASSMANN, G. *et al*. Prevalence of anti-citrullinated protein antibodies (ACPA) in patients with diffuse large b-cell lymphoma (DLBCL): A Case-Control Study. **PLoS One**, v. 9, n. 2, p. e88177, 2014.

BORENSTEIN, M. *et al*. **Introduction to meta-analysis**. 1a Ed. John Wiley & Sons, Ltd.: United Kingdom. 2009.

COOPER, G.S. *et al*. Associations between plasma DDE levels and immunologic measures in African-American farmers in North Carolina. **Environ Health Perspect**, v. 112, n. 10, p. 1080–1084, 2004.

CORSINI, E. *et al*. Immunomodulatory effects of the fungicide Mancozeb in agricultural workers. **Toxicol Appl Pharmacol**, v. 15, n. 2, p. 178–185, 2005.

DALVIE, M.A.; LONDON, L. The impact of aerial application of organophosphates on the cholinesterase levels of rural residents in the Vaalharts district, Northern Cape Province, South Africa. **Environ Res**, v. 102, n. 3, p. 326–332, 2006.

EGGER, M. *et al*. Bias in meta-analysis detected by a simple, graphical test. **BMJ**, v. 13, p. 624–624, 1997.

EL RAHMAN, H.A.A. *et al*. A Panel of Autoantibodies Against Neural Proteins as Peripheral Biomarker for Pesticide-Induced Neurotoxicity. **Neurotox Res**, v. 33, p. 316–336, 2018.

FREIRE, C. *et al*. Long-term exposure to organochlorine pesticides and thyroid status in adults in a heavily contaminated area in Brazil. **Environ Res**, v. 127, p. 7–15, 2013.

GOLDNER, W.S. *et al*. Pesticide use and thyroid disease among women in the Agricultural Health Study. **Am J Epidemiol**, v. 171, n. 4, p. 455–464, 2010.

JACOBSEN-PEREIRA, C.H. *et al*. Immune response of Brazilian farmers exposed to multiple pesticides. **Ecotoxicol Environ Saf**, v. 202, p. 1-8, 2020.

KOUREAS, M. *et al*. Increased Frequency of Rheumatoid Arthritis and Allergic Rhinitis among Pesticide Sprayers and Associations with Pesticide Use. **Int J Environ Res Publ Health**, v. 14, n. 8, p. 1-8, 2017.

LEE, Y.H. An overview of meta-analysis for clinicians. **Korean J Intern Med**, v. 33, n. 2, p. 227–283, 2018.

LIGNANI, L.B.; BRANDÃO, J.L.G. A ditadura dos agrotóxicos: o Programa Nacional de Defensivos Agrícolas e as mudanças na produção e no consumo de pesticidas no Brasil, 1975–1985. **Hist Cienc Saude Manguinhos**, v. 29, n. 2, p. 337–359, 2022.

LLEO, A. *et al*. Definition of human autoimmunity — autoantibodies versus autoimmune disease. **Autoimmun Rev**, v. 9, n. 5, p. A259–A266, 2010.

MAKSYMIV, I. Pesticides: Benefits and Hazards. **J Vasyl Stefanyk Precarpath Nat Univ**, v. 2, n. 1, p. 70–76, 2015.

MILLER, F.W. The increasing prevalence of autoimmunity and autoimmune diseases: an urgent call to action for improved understanding, diagnosis, treatment, and prevention. **Curr Opin Immunol**, v. 80, p. 1-9, 2023.

MOKARIZADEH, A. *et al*. A comprehensive review of pesticides and the immune dysregulation: mechanisms, evidence and consequences. **Toxicol Mech Methods**, v. 25, n. 4, p. 258–278, 2015.

MORGAN, R.L. *et al* Identifying the PECO: A framework for formulating good questions to explore the association of environmental and other exposures with health outcomes. **Environ Int** v. 121, n. 1, p. 1027–1031, 2018

PAGE, M.J. *et al*. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. **BMJ**, v. 372, n. 160, p. 1-36, 2021.

PISETSKY, D.S. Pathogenesis of autoimmune disease. **Nat Rev Nephrol**, v. 19, p. 509–524, 2023.

RAUDENBUSH, S.W. Analyzing effect sizes: Random effects models. In: Cooper, H.; Hedges, L.V.; Valentine, J.C. (Ed.) **The handbook of research synthesis and meta-analysis**, 2a Ed. Russell Sage Foundation: New York. 2009.

RODGERS, K.E. *et al*. Organophosphorus pesticide immunotoxicity: effects of O,O,S-trimethyl phosphorothioate on cellular and humoral immune response systems. **Immunopharmacology**, v. 12, n. 3, p. 193–202, 1986.

ROSENBERG, A.M. *et al*. Prevalence of antinuclear antibodies in a rural population. **J Toxicol Environ Health A**, v. 57, n. 4, p. 225–236, 1999.

SALEH, M.A.; ELBAHY, M.M. Do normal Egyptians possess antidesmoglein 3 antibodies?. **Int J Dermatol**, v. 54, p. 1145–1149, 2015.

SANTOS, A.S.E. *et al*. Levels of anti-cyclic citrullinated peptide and antinuclear antibodies in Brazilian agricultural workers exposed to pesticides and fertilizers. **Sci Total Environ**, v. 838, n. 4, p. 1-6, 2022.

SCHINASI, L.; LEON, M.E. Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: A systematic review and meta-analysis. **Int J Environ Res Public Health**, v. 11, p. 4449–4527, 2014.

SIMESCU, M. *et al*. Multiple pesticides exposure of greenhouse workers and thyroid parameters. **Int J Sustain Dev Plan**, v. 9, p. 15–28, 2014.

SPIEWAK, R.; STOJEK, N. Antinuclear antibodies among eastern-Polish rural inhabitants. **Ann Agric Environ Med**, v. 10, n. 2, p. 207–209, 2003.

STEERENBERG, P. *et al*. Toxicological evaluation of the immune function of pesticide workers, a European wide assessment. **Hum Exp Toxicol**, v. 27, n. 9, p. 701–707, 2008.

USDA. United States Department of Agriculture. **Farm Labor**. Disponível em: https://www.ers.usda.gov/topics/farm-economy/farm-labor/#size. Acesso em: 21 nov. 2023.

VIECHTBAUER, W. Bias and efficiency of meta-analytic variance estimators in the random-effects model. **J Educ Behav Stat**, v. 30, n. 3, p. 261–293, 2005.

WANG, H. *et al*. Ambient air pollutants increase the risk of immunoglobulin E–mediated allergic diseases: a systematic review and meta-analysis. **Environ Sci Pollut Res Int**, v. 29, p. 49534–49552, 2022.

Recebido em: 13 de Setembro de 2024
Avaliado em: 24 de Fevereiro de 2025
Aceito em: 6 de Maio de 2025

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