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Environmental pesticide exposure and the risk of irritable bowel syndrome: A case-control study

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ABSTRACT

The agricultural model in southern Spain is highly productive, mainly due to the intensive cultivation under plastic. Despite strict pesticide regulation, human exposure to pesticides in the environment has been connected to an increase in diseases such as celiac disease. Certain pesticides have also been associated to the disruption of the intestinal microbiota, which has been tied to the development of irritable bowel syndrome (IBS). A case-control study was conducted in Andalusia, south Spain, to assess the prevalence and risk of IBS related to pesticide exposure. This research found a high prevalence of IBS in Andalusia between 2000 and 2021 in areas with high pesticide exposure using agronomic criteria. Furthermore, the odds ratio for IBS was significantly higher in the population with high pesticide exposure. This study suggests that pesticides may be involved in IBS, whereas more research is needed to determine the role of pesticides in IBS symptomatology.

1. Introduction

Pesticides are used in crop maintenance and have a wide range of active substances available, including herbicides, fungicides, and insecticides against weeds, fungi, and insects, respectively. In this context, pesticides have shown a high occupational and environmental exposure due to their widespread use in agricultural fields (Tudi et al., 2021). Daily exposure to pesticides could have serious consequences on the intestine, an organ that is highly exposed when contaminated food is ingested. In this regard, irritable bowel syndrome (IBS) is one of the most common functional digestive disorders, characterized by recurrent abdominal pain and constipation or diarrhea, which do have a negative impact on quality of life (Reygner et al., 2016). While the etiology of IBS is unclear, and there is no specific diagnosis or treatment, it is known that stress, infections, and alcohol can all affect the intestinal mucosa. All of these increase the permeability of the mucosa, allowing substances or molecules to pass through that can disrupt the functioning of the digestive system (Kim et al., 2010; Reding et al., 2013; Vanuytsel et al., 2014). In this vein, affecting the intestinal environment has also been shown to reach the intestinal microbiota in IBS patients (Ghoshal et al., 2016; Jeffery et al., 2012).

The intestinal microbiota is known to have metabolic, trophic, and defensive functions in the mucosa. Some bacterial populations ferment dietary fiber into short-chain fatty acids (SCFA), which serve as an energy source, improve transit, and support the immune system (Wichmann et al., 2013), while others are involved in vitamin B and K synthesis (Cooke et al., 2009; Park et al., 2007; Uebanso et al., 2020). Certain lifestyle and dietary factors can contribute to a qualitative and quantitative imbalance of the intestinal microbiota. These factors can alter mucosal permeability and cause inflammation, resulting in pain, transit disorders, and abdominal distension, all of which are symptoms of IBS (Conlon and Bird, 2015; Kang et al., 2011; Villarreal et al., 2012). In terms of gut bacterial richness, diarrhea-predominant IBS patients have a lower abundance of Firmicutes, Fusobacteria and Actinobacteria phyla, and an increased abundance of Proteobacteria phylum compared to healthy subjects (Mei et al., 2021). This imbalance in gut microbiota (GM) composition has also been observed following exposure to certain fungicides and insecticides, which may cause inflammation associated

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with the development of IBS (Jin et al., 2016; Joly et al., 2013; Reygner et al., 2016). All things considered, the current study aims to assess whether continued environmental exposure to pesticides in people living near areas of intensive agriculture is associated with an increased prevalence and risk of developing IBS over a 20-year period.

2. Methods

2.1. Design

A population-based case-control study IBS cases was conducted. The study population was drawn from the autonomous community of Andalusia (southern Spain) to ascertain whether pesticide exposure is associated to the disease.

2.2. Criteria for selecting study areas and categorizing pesticide exposure

The geographical locations corresponded to Andalusian health districts and were classified into two groups, high and low pesticide use, based on 777. The high pesticide use areas included four health districts: West of Almeria, Almeria Center, South of Granada, and Huelva Litoral. The health districts of Axarquia (Malaga), the coast of Jerez (Cadiz), East Almeria, North-East Jaen, North Cordoba, and North Seville were low pesticide use areas.

For the crop season 2016–2017, the consumption of pesticides in areas of high use of pesticides amounted to 14.002 tons (91.4%) of pesticides, and occupied 93.2% of the total surface of plastic-covered greenhouses in Andalusia where vegetables and fruits are grown. Conversely, areas of low use of pesticides consumed 1306 tons (8.6%) of pesticides, and occupied only 6.8% of the total surface of greenhouses in Andalusia (Consejería de Agricultura Pesca y Desarrollo Rural, 2017).

In these areas, the most commonly used insecticides are organophosphates (primarily chlorpyrifos), N-methylcarbamates, macrocyclic lactones, neonicotinoids, and pyrethroids. Fungicides frequently used in plastic greenhouses include (di) thiocarbamates, conazoles, dicarboximide), anilino-pyrimidines, and copper salts. Bipyridyl (paraquat, diquat), organophosphonates (glyphosate), chlorotriazine, and phenylurea are the herbicides most popularly used in the study areas (García-García et al., 2016).

2.3. Study population and IBS

In both areas, the study population totaled 1,450,720 citizens. Between 2000 and 2021, 18,807 were diagnosed with IBS. The control population was taken from the general population living in the same study areas as the cases, and was age and sex matched.

Cases and controls between 2000 and 2021 were collected from the computerized registry of the Andalusian Public Health Service, known as the Basic Minimum Data Set (BMDS), from 2000 to 2021. The BMDS collects information on discharges from public hospitals, including coded clinical data of hospitalized patients. The main cause for admission (major diagnosis) and other secondary medical diagnoses are recorded routinely in the BMDS, together with age, sex, and place of residence. The validity of the data collected in the BMDS is determined by the quality of the discharge report with respect to the collection of the major and secondary diagnoses and procedures, and the comprehensiveness in the coding of hospital discharges.

Individuals in the control group were selected by stratified random sampling, considering health districts (geographic areas) as strata. The controls were a random sample of Andalusian patients, belonging to each stratum, who did not have digestive pathology and were matched by sex and age. The number of controls in each stratum was selected based on the number of cases diagnosed in that stratum during the study period.

Regarding exclusion criteria, patients under 18 years of age were excluded in both groups. As for the control group, people who did not reside in the selected geographical areas and diagnosed with digestive pathologies were also excluded.

The diagnosis of IBS was defined using the World Health Organization's ninth and tenth revisions of the International Classification of Diseases (ICD-9 and ICD-10) in effect during the study period. The ICD-9 code for irritable bowel was 564.1. The ICD-10 codes used were irritable bowel syndrome with diarrhea (ICD-9 code K58.0), irritable bowel syndrome with constipation (ICD-9 code K58.1), mixed irritable bowel syndrome (ICD-9 code K58.2), other irritable bowel syndrome (ICD-9 code K58.8) and irritable bowel syndrome without diarrhea (ICD-9 code K58.9).

2.4. Statistical analysis

Frequencies and percentages were measured for categorical variables, and means and standard deviations were estimated for quantitative variables. Furthermore, the Chi-square test was used to calculate the prevalence proportion and risk of IBS in areas with high and low pesticide use. Odds ratios (OR) and 95% confidence interval (CI) were also calculated. To compare population age differences between the two study areas, the Kolmogorov-Smirnov normality test was used, followed by the Mann Whitney test. Multiple binary logistic regression was carried out to assess the risk of having IBS adjusted for age, gender, and areas of high versus low pesticide use as a surrogate for exposure, as these factors were considered to have the capacity to influence the statistical models. The statistical significance level was set at $p < 0.05$. SPSS 26 statistical software was used to analyze the data.

2.5. Ethical considerations

In addition to adhering to the ethical principles established for human research in the Helsinki Declaration and its subsequent revisions, ethical approval was granted from the Provincial Research Ethics Committee of Almeria (UALMICROBIOTA21, 28 July 2021). Throughout the data collection process, anonymity, privacy, and confidentiality of the data will be ensured. All records were kept in accordance with the principles established in current data protection legislation, including Organic Law 3/2018 on the Protection of Personal Data and the Guarantee of Digital Rights, R.D. 994/99 and the provisions of Art. 5 of the General Data Protection Regulation and 14 November Law 41/2002, which regulates Patient Autonomy and the Rights and Obligations Regarding Clinical Information and Documentation.

3. Results

The study population included 1,450,720 people who lived in the study areas. Between January 2000 and December 2021, 18,807 people were diagnosed with IBS. Ten thousand three hundred and two people with IBS lived in pesticide-heavy areas, while 8506 lived in pesticide-light areas. In high pesticide use areas, the average age of IBS patients was 47.93 (19.12) years, while in low pesticide use areas, the average age was 48.12 (10.87) years. There were no statistically significant differences ($p > 0.05$). The average age of the control population was 47.30 (18.09) years in high pesticide use areas and 47.93 (16.31) years in low pesticide use areas. There were no significant differences between the groups ($p > 0.05$) (Table 1).

IBS prevalence proportion per 100 inhabitants were significantly higher in geographical areas with higher pesticide use than in areas with lower pesticide use ($p < 0.05$). After stratifying the data by sex, both female and male prevalence proportion were significantly higher in pesticide-heavy areas ($p < 0.05$) (Table 2). Table 2 also shows the odds ratio (OR) for developing IBS in pesticide-heavy areas versus pesticide-light areas. When compared to lower pesticide use areas, higher pesticide use areas had a significant ($p < 0.05$) increase in the odds of IBS (OR: 1.16). In terms of sexes, women had the highest odds, with an OR of

Table 1
Comparation of the mean age by the geographical areas studied (high and low use pesticide) and sex.

Irritable Bowel Syndrome	Exposure	Age (Mean (SD))	p value*
Females	High pesticide use	48.28 (19.26)	0.79*
	Low pesticide use	48.43 (17.27)	
Males	High pesticide use	46.95 (18.71)	0.41*
	Low pesticide use	47.37 (18.45)	
Total	High pesticide use	47.93 (19.12)	0.70*
	Low pesticide use	48.12 (10.87)	

* p-value obtained by Mann Whitney U test

Table 2
Prevalence (proportion per 100 inhabitants), odds ratio (OR), and 95% confidence interval (95% CI) for Irritable Bowel Syndrome in the population living in areas of high pesticide use relative to areas of low pesticide use.

Irritable Bowel Syndrome	High pesticides use	Low pesticides use	OR (95% CI)	p value*
Females	15.77	12.40	2.62 (2.54–2.70)	< 0.001
Males	6.79	4.64	1.33 (1.26–1.41)	< 0.001
Total	11.31	9.01	1.16 (1.13–1.20)	< 0.001

* p- value obtained by Pearson's Chi-Squared test

2.62.

A multiple logistic regression analysis of IBS was performed, which was adjusted for age, sex, and environmental pesticide exposure (Table 3). People who lived in pesticide-heavy areas were more likely to be affected by the pathology (OR: 1.26) with women having significantly higher odds (OR: 2.26). In all cases, the results were statistically significant ($p < 0.05$).

4. Discussion

The aim of this study was to assess whether continued environmental exposure to pesticides in people living near areas of intensive agriculture is associated with an increased prevalence proportion and risk of developing IBS. Pesticide use in Spain is diverse, but agriculture ranks first, with agricultural workers being the most affected by occupational exposure (González et al., 2021). According to the Labour Force Survey, Andalusia is the Spanish region with the largest number of employees in the agricultural sector. In 2019, there were around 255,500 farmers which represents an 8.2% of active workers in Andalusia, double that in the country as a whole (4.0%) (International Labour Organization, 2019). However, food and environmental contamination, as well as proximity to pesticide-heavy areas, can have an impact on the general population (Linhart et al., 2019; Mesnage et al., 2022; Rubio et al., 2014). To our knowledge, this is the first time that the relationship between pesticide exposure and the prevalence of IBS has been studied.

Table 3
Stepwise multiple logistic regression analysis of Irritable Bowel Syndrome, adjusted for geographical areas studied (high and low pesticide exposure), gender, and age.

	Risk factor	OR*	95% CI	p value
Irritable Bowel Syndrome	Areas of high pesticide use	1.26	1.12–1.19	< 0.001
	Female	2.26	2.19–2.33	< 0.001
	Age	1.03	1.01–1.02	< 0.001

* Models were adjusted for the following variables: age, sex (1: female; 0: male), environmental pesticide exposure (1: areas of high pesticide use; 0: areas of low pesticide use).

Overall, the findings of this study indicate a correlation between pesticides and IBS, with higher prevalence and risk of developing IBS in areas with high pesticide exposure.

While the cause of IBS is still unknown, it is suggested to be a multifactorial disease. Different scenarios have been proposed as disease triggers, including the consumption of high-carbohydrate and sugar-containing foods, air pollution, antibiotic overuse, and pesticide exposure (Kaplan et al., 2012; Marynowski et al., 2015; Nilholm et al., 2019; Villarreal et al., 2012). At the environmental level, both atmospheric pollutants and pesticides have been shown to induce systemic inflammation (Sun et al., 2005), an oxidative environment in the intestinal mucosa (Dybdahl et al., 2003), or changes in GM composition (Choi et al., 2013; Kish et al., 2013), all of which may aggravate IBS symptoms. In this sense, an increased prevalence of non-specific abdominal pain, such as pain in IBS, has been reported in individuals exposed to high concentrations of particle manner, carbon monoxide, sulphur dioxide, and nitrogen dioxide in the atmosphere (Kaplan et al., 2012). As for pesticide exposure, the available data emerge from experimental animal and in vitro tests, which showed how many synthetic chemicals used in agriculture alter GM homeostasis (Jin et al., 2018; Reygner et al., 2016).

There is a large body of clinical evidence demonstrating a strong connection between the GM disturbance known as gut dysbiosis and symptoms in IBS patients (Ghoshal et al., 2016; Jeffery et al., 2012). In this manner, elevated levels of endotoxins, such as lipopolysaccharides (LPS) derived from the outer membrane of gram-negative bacteria in the bloodstream, constitutes a very common condition that can cause an immune response characterized by increased release of interleukin-1β (IL-1β) and interleukin-6 (IL-6), leading to systemic inflammation (Liebregts et al., 2007). Moreover, changes in GM composition are related to a decrease in SCFA production and an increase in intestinal mucosal permeability, as well as a constant activation of the enteric nervous system (ENS), which may cause visceral pain and increased intestinal motility (Downs et al., 2017).

A wide range of environmental pollutants, including heavy metals, insecticides, herbicides, and pesticides, can cause gut dysbiosis (Jin et al., 2015; Lin et al., 2020; Reygner et al., 2016). As suggested in our findings, there is also an increased risk of IBS precisely in pesticide-heavy areas and particularly in women. Previous research have found sexually dimorphic metabolic effect after pesticides exposures (Lukowicz et al., 2018). In this regard, Roundup®, a glyphosate-based herbicide (GBH) widely used in Andalusian crops, can increase the Bacteroidetes group while decreasing the Lactobacillaceae family in fecal samples of female rats (Lozano et al., 2018). Similarly, chronic glyphosate exposure reduces *Corynebacterium*, Firmicutes, Bacteroidetes, and *Lactobacillus* in mice following GBH administration (Aitbali et al., 2018). Exposure to fungicides such as imazalil can also alter the composition of the GM after acute and chronic administration. Specifically, in mice, imazalil increases the relative abundance of Firmicutes, Proteobacteria and Actinobacteria while decreasing the abundance of Bacteroidetes (Jin et al., 2016). Insecticides such as chlorpyrifos, diazinon and aldicarb also disrupt the GM of rats and mice (Aitbali et al., 2018; Gao et al., 2019, 2017; Joly et al., 2013). This modification has been associated with increased intestinal permeability and bacterial translocation in the case of chlorpyrifos (Condette et al., 2014). Diazinon inhibits several SCFA-producing genera in the Lachnospiraceae family, which operate as an energy substrate for colonic epithelial cells, altering energy harvesting in the intestinal epithelium (Gao et al., 2017).

Pesticide-induced imbalances in the GM may trigger an immune response that would negatively affect IBS patients by promoting intestinal inflammation, increased permeability and thus exacerbating the pathology. Glyphosate can increase mRNA expression of IL-1β, IL-6 and tumor necrosis factor-alpha (TNF-α) in different segments of the gut of glyphosate-treated rats, which is accompanied by a change in the abundance of certain phyla and genera of the GM (Tang et al., 2020). Similar results were found after 28 days of imazalil fungicide ingestion in the colon of mice (Jin et al., 2016). The role of tight junctions in IBS is noteworthy. These proteins are multiprotein complexes that hold two adjacent cells together and control the transport of solutes and water, although they are dysregulated in IBS patients (Cheng et al., 2015). In this respect, it has been demonstrated that exposure to the insecticides imidacloprid and chlorpyrifos reduces claudin-1, occludin, and ZO-1 proteins in the ileum and colon of rodents (Liang et al., 2019; Zhao et al., 2021).

After controlling for age and sex, our study revealed a higher risk of IBS in areas with higher pesticide use. In areas of high exposure, it is reasonable to consider sex and age as potential predictors of IBS. As previously stated, female rodents are considered to be more vulnerable to pesticides than males (Lozano et al., 2018; Lukowicz et al., 2018). Male and female immune systems, on the other hand, have been proposed to differ. Females are more prone to autoimmune disorders, whereas males are more susceptible to infections (Fish, 2008; vom Steeg and Klein, 2016; Whitacre, 2001). In terms of IBS and GM and its metabolites, male patients with diarrhea-predominant IBS had higher levels of *Prevotella* genus and fecal propionate than healthy individuals, while female patients and healthy subjects showed no differences (Sun et al., 2021). These findings may help to understand the relationship between pesticide exposure and IBS; however, more epidemiological and experimental research is needed to understand and confirm the role of these exposures in the development of IBS.

4.1. Limitations and strengths

The ecological nature of the exposure data is the major limitation of this study. As specific data on frequency and duration of exposure to pesticides were not available at individual level, we used aggregated measures of exposure to calculate the risk of having IBS. While exposure at the aggregated level may not reflect that at the individual level, the categorization made for health districts (i.e. high versus low pesticide use) intended to make exposure rather homogeneous, close to the average, for all individuals living in those two study areas.

Potential bias from socioeconomic factors was partially controlled by the lack of significant differences in the distribution of the employed population across primary, secondary and tertiary sectors of the economic activity between areas with high and low pesticide use (Parrón et al., 2011).

Conversely, the approach to a real-life scenario of pesticide exposure in a large area of intensive agriculture over a 20-year period is foremost strength of this study, contributing to a better understanding of the real-life threat posed by exposure to multiple pesticides from different sources.

5. Conclusions

Our findings suggest that environmental pesticide exposure may contribute to the development of IBS. The widespread use of pesticides has raised concerns about environmental pollutants, and policies to address these concerns should be implemented. However, more research is needed to confirm our findings since epidemiological evidence is scarce even though data obtained in experimental animals supports the deleterious effects of pesticides on intestinal health.

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CRediT authorship contribution statement

Lola Rueda-Ruzafa: Validation, Investigation, Data curation, Writing – original draft. **Pablo Roman:** Conceptualization, Investigation, Data curation, Writing – review & editing, Supervision. **Diana Cardona:** Investigation, Data curation, Writing – original draft, Writing – review & editing. **Mar Requena:** Conceptualization, Methodology, Formal analysis, Visualization. **Carmen Ropero-Padilla:** Writing – original draft, Visualization. **Alarcon Raquel Alarcón:** Conceptualization, Methodology, Validation, Formal analysis, Writing – review & editing, Supervision. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The authors do not have permission to share data.

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