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Revisiting Cancer 15 Years Later: Exploring Mortality Among Agricultural and Non-Agricultural Workers in the Serrana Region of Rio de Janeiro

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Abstract

Background—Agricultural production has expanded dramatically throughout Brazil. Previous research in the Serrana Region found that from 1979 to 1998, agricultural workers experienced high mortality rates from certain cancers compared to non-agricultural workers [Meyer et al. (2003): *Environ Res* 93:264–271].

Methods—New data were obtained for 1999–2013 and Mortality Odds Ratios (MORs) were utilized to compare cancer and other mortality between male agricultural workers in the Serrana Region and non-agricultural workers in the Serrana Region, Rio de Janeiro, and Porto Alegre, and to compare mortality odds to previous decades.

Results—Respectively, compared to aforementioned reference-groups, agricultural workers experienced highest MORs for stomach (1.55 [95%CI: 1.13–2.12], 2.30 [95% CI: 1.72–3.08], 2.28 [95%CI: 1.69–3.08]) and esophageal cancers (95%CI: 1.93 [1.38–2.7], 1.93 [95%CI: 1.38–2.71], 3.12 [95%CI: 2.30–4.24]), greater than reported in previous decades. Agricultural workers

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AUTHORS' CONTRIBUTIONS

NK contributed to analysis and interpretation of the data and drafted the manuscript. ASES contributed to the acquisition, analysis, and interpretation of the data. JL contributed to the conception of the work and reviewing the manuscript. AM contributed to the conception and design of the work, and to reviewing and revising the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

ETHICAL APPROVAL AND INFORMED CONSENT

In accordance with the National Council of Health resolution that establishes rules and regulations related to the ethics of research with human subjects in Brazil, IRB review was not deemed necessary due to the lack of identifying information on subjects from which mortality data were extracted [Conselho Nacional de Saude, 2012].

DISCLOSURE (AUTHORS)

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experienced higher mortality for external-causes, respiratory, and cardiovascular problems compared to urban reference-groups.

Conclusion—Agricultural workers may be at increasing risk for cancer and other mortality. Efforts are needed to investigate distinct risk-factors among this group.

Keywords

cancer; agricultural health; occupational exposures; mortality; ecological study

INTRODUCTION

In recent decades, agricultural production in Brazil has drastically risen. According to the World Trade Organization, in 2014, Brazil was the third largest exporter of agricultural products in the world: In 2013 alone, total agricultural product exports for Brazil equaled 90,664 million US dollars, over a fivefold increase as compared to the 15,464 million US dollars in exports Brazil had in the year 2000 [WTO, 2015]. Agricultural workers are constantly exposed to occupational toxins via contact with the skin, inhalation, and ingestion, which along with rigorous farm work and precarious work and psychosocial conditions can be detrimental to the health of workers [Cordes and Foster, 1988; Pratt, 1990; WHO, 1990; El Batawi, 2003; Zukiewicz-Sobczak et al., 2013]. The rise in agricultural work in Brazil and other developing countries has sparked the interest of health professionals about long-term effects of exposure to chemicals used in agriculture. Cancer, in particular, has been a topic of discussion among international researchers in regards to the risks associated with agricultural work and whether stressors associated with the nature of the work, particular dietary, or substance use habits, or potentially harmful occupational toxins may play a role in cancer morbidity and mortality. Certain cancers have been repeatedly found to be more common in agricultural workers than in the general population, especially non-Hodgkin's lymphoma, leukemia, lip, stomach, brain, and prostate cancers [Blair et al., 1992; Blair and Zham, 1995; Viel et al., 1998; Pearce and McLean, 2005; Mills and Yang, 2007; Mills et al., 2009; Ragin et al., 2013].

Although farm workers may experience several distinct risk and lifestyle factors for cancer that may contribute to these higher rates, one potential contributor is their frequent contact with toxic substances and pollutants. Agricultural workers often utilize fertilizers, fuels, oils, engine exhausts, zoonotic microbes, and other hazards that may impact the carcinogenic process and other long-term health effects [Blair and Zahm, 1995; El Batawi, 2003; Zukiewicz-Sobczak et al., 2013]. These exposures can be especially risky in developing countries where there is limited education regarding safe handling of neurotoxic and carcinogenic substances and little regulation to ensure safe working conditions [Rathinam et al., 2005; Kesavachandran et al., 2009; London, 2011]. Special attention has been given to the potential harms associated with the rise in pesticide use in Brazil in recent years. Reports by FAOSTAT suggest that in 2013 Brazil was the top world consumer of insecticides, herbicides, fungicides, and bactericides [FAOSTAT, 2015]. Just in the past decade alone, pesticide consumption in Brazil has increased significantly: While in 2002, Brazil used 599.5 millions of liters of pesticides, by 2011 this number rose by almost 50% to 852.8 million liters. Pesticide use has also risen in proportion to the amount of agricultural

production is it used for: From 2002 to 2011, Brazil went from using 10.5 to using 12 L of pesticides per planted hectare [ABRASCO, 2010]. Certain pesticides have been found to be carcinogenic through their activation of oncogenes and through their disruption of the actions of tumor suppressor genes that in turn can lead to uncontrolled cell proliferation and tumor formation [Vakonaki et al., 2013]. Studies linking pesticide exposure and cancer have mostly focused on the actions of organochlorines, but other classes of pesticides, including organophosphates, carbamates, and pyrethroids have also been linked to carcinogenic mechanisms and to higher risk for cancers in population-based studies [George and Shukla, 2011].

Still, data regarding cancer incidence varies greatly between studies and the relationship between the particular lifestyles of agricultural workers, exposure to occupational toxins, and the risk of cancer remains poorly understood. Despite the country's heavy agricultural production, there is limited research in Brazil exploring the relationship between agricultural occupations and cancer. Preliminary studies have shown that Brazilian agricultural workers may be at higher risk for certain types of cancers: Chrisman et al. [2009] found that areas in Brazil with more pesticide sales showed a significant correlation with mortality rates for leukemia, and cancers of the prostate, soft tissue, lip, esophagus, and pancreas. Stoppelli and Crestana [2005] found an almost two times higher probability of developing cancer among rural workers as compared to non rural workers in São Paulo, with cancers of the skin and digestive system being the most prevalent. A study in Southern Brazil found that agricultural workers were at significantly higher risk of dying from esophageal cancer than non-agricultural workers [Meyer et al., 2011]. Miranda-Filho et al. [2012] observed that in Rio de Janeiro state, agricultural workers were at higher risk of brain cancer mortality than non-agricultural workers.

Rio de Janeiro, in particular, has a large and concentrated agricultural community in the Serrana Region, which is a lead producer of fruits, vegetables, and flowers, crops that demand continuous use of several types of pesticides and other chemicals [Camara et al., 2008; Miranda Filho et al., 2014]. It has been estimated that workers in this region may be exposed to up to 56 kg of pesticide/year [Peres et al., 2001]. Organophosphates, carbamates, pyrethroids, and other pesticides that have associated with certain cancers are commonly used for agriculture in this region [Meyer et al., 1999; Rangel et al., 2011], and it has been suggested that the soil may still be contaminated by certain carcinogenic organochlorines even though they were officially banned from use in Brazil in 1985 [Paumgartten et al., 1998]. And while laws exist to limit workers' exposure to dangerous toxins, in reality, little enforcement exists on the ground to regulate the marketing and handling of these products.

A study on cancer mortality in the Serrana Region by Meyer et al. [2003] found that between 1979 and 1998, there were higher mortality rates from stomach and esophageal cancer among agricultural workers from this region when compared to non-agricultural reference populations in both local municipalities and other cities in Brazil. As agricultural production in Brazil has been rising since this study was conducted [WTO, 2015], we aimed to re-explore mortality rates in the Serrana Region and investigate how death by cancer and other health complications has changed over time in comparison to local and other urban populations. In this study, we add new mortality data from the Serrana Region for the 1999–

2013 period and investigate whether agricultural workers continue to be at higher risk for certain cancers and other negative health outcomes.

MATERIALS AND METHODS

This study adapted the methods described by Meyer et al. [2003] in order to compare mortality data on cancer and other causes of death among agricultural workers and three non-agricultural worker reference populations in Brazil. As in the aforementioned paper, the primary study population included male agricultural workers aged 30–69 from six municipalities in the Serrana Region of Rio de Janeiro, including Teresopolis, Nova Friburgo, Bom Jardim, Cantagalo, Duas Barras, and Sumidouro. Together, these municipalities span an area of 3,609 km² and have a population of 416,821 people, of which about 17% live in rural areas [IBGE, 2010]. This region contains the largest concentration of agricultural production in Rio de Janeiro State with an estimated 6,274 establishments that produce crops. About 5.3% of workers in this region are involved in agricultural activities [IBGE, 2006], and the illiteracy rate in the region is around 13% [IBGE, 2010]. Unlike many other agricultural regions where migrant workers perform much of the work, the Serrana Region is characterized by mostly long-term and family run farming with little geographic mobility between workers. Thus, this region makes for an appropriate area to study the long-term effects of agricultural exposures.

Mortality data for the study population were compared against three reference populations: The general non-agricultural worker male population of the local municipalities in the Serrana Region of study, the general non-agricultural worker male population of the capital city of Rio de Janeiro, and the general non-agricultural worker male population of Porto Alegre, the capital city of the Southern state of Rio Grande do Sul. The local municipalities reference group was chosen to compare agricultural workers from the Serrana Region with non-agricultural workers with similar geographic and socioeconomic conditions. The Rio de Janeiro reference group was chosen due to the city's geographic proximity to the Serrana Region and yet very different socio-structural makeup as a large metropolitan and capital city. The Porto Alegre reference group was chosen due to the city's especially high incidence of cancers that can be used to recognize elevated cancer risk among the population of study.

Data on mortality from certain types of cancer, cardiovascular, and respiratory problems and external causes, along with age and sex were obtained for the study and reference populations for the years 1999–2013 using the Brazilian Health Ministry's National Mortality System, which classifies causes of death according to the International Classification of Diseases (ICD-10). Occupation codes 60–63, which include the categories, “administrators and overseers of farming and forestry,” “agricultural producers,” “multipurpose agricultural workers and similar occupations,” and “specialized agricultural workers” were used to distinguish deaths of agricultural workers from those of other occupations [DATASUS, 2008]. In accordance with the National Council of Health resolution that establishes rules and regulations related to the ethics of research with human subjects in Brazil, IRB review was not deemed necessary due to the lack of identifying

information on subjects from which mortality data were extracted [Conselho Nacional de Saúde, 2012].

Mortality between groups was compared by dividing the number of deaths for each cause of interest by the number of deaths from all other causes in that population, generating a death-by-cause ratio. This ratio was divided by the death-by-cause ratio of the reference population, generating a mortality odds ratio (MOR). Respective 95% confidence intervals (CI) were recorded for each MOR. The methods for calculating MOR has been previously described by Miettinen and Wang [1981].

RESULTS

A total of 146,487 deaths for outcomes of interest were reported for males aged 30–69 between the years 1999 and 2013 and were included in our analysis. Table I presents number of deaths by specific cause and group as well as mortality odds ratios (MORs) comparing death-by-cause ratios of the study population with those of the three reference populations. Overall, total cancer mortality among the study population was similar to that of the local reference population and that of the Rio de Janeiro reference population, and lower than that of the Porto Alegre reference population.

The most common forms of cancers among agricultural workers in the Serrana Region were stomach cancer (27% of cancer deaths) and esophageal cancer (25% of cancer deaths), with MORs indicating that agricultural workers in the Serrana Region are at significantly higher risk of death from these cancers as compared to all three-reference populations. Although lung cancer accounted for 10% of cancer deaths, agricultural workers appeared to be at significantly lower risk of lung cancer mortality than their non-agricultural worker counterparts in all three reference groups. Prostate cancer was another common cause of cancer mortality among agricultural workers (10% of cancer deaths), but there was no significant difference in risk as compared to the three reference groups. For liver, pancreatic, and colon cancers, agricultural workers appeared to have significantly lower risk of death as compared to the Porto Alegre reference group, but showed no difference in mortality risk as compared to the other reference groups. Agricultural workers had a similar risk of death from cardiovascular problems, respiratory problems, and external causes as the local reference group, but significantly higher risk of death from these causes than the Rio de Janeiro and Porto Alegre reference groups.

Table II presents number of deaths by specific cause as well as MORs for cancer and other mortality causes of interest among younger males aged 30–49. Risk of any cancer mortality was significantly higher in the study population than in the local and Rio de Janeiro reference groups and similar to that of the Porto Alegre reference population. The most common death by cancer among the study population was stomach cancer (36% of cancer deaths), and mortality odds was almost threefold that of the local reference population and as much as fourfold that of the Rio de Janeiro and Porto Alegre reference populations. All other cancer mortalities did not show a significant difference between study and reference populations. Within this age group, agricultural workers were at lower risk of death by

external causes as compared to the Rio de Janeiro reference group, while at a higher risk of death from cardiovascular problems than the Porto Alegre reference group.

Table III presents number of deaths by specific cause as well as MORs for cancer and other mortality causes of interest among older males aged 50–69. This age group had cancer mortality similar to that of local and Rio de Janeiro reference groups and slightly lower than that of the Porto Alegre reference group. Esophageal cancer was the most common form of cancer mortality among this group (accounting for 30% of cancer mortalities), and mortality was significantly higher among the study population than all three-reference populations. Stomach cancer was the next most common form of cancer mortality (24% of cancer mortalities), significantly higher than the Rio de Janeiro and Porto Alegre reference groups. Prostate cancer mortality, responsible for 13% of the cancer deaths in this group, was similar as compared to the three reference groups. Lung cancer accounted for 12% of all cancer deaths in this study population. However, risk of lung cancer mortality was significantly lower in the study population than in the Rio de Janeiro and Porto Alegre reference groups and borderline lower than in the local reference group. Liver and Colon cancer in the study population had a very low mortality risk as compared to the Porto Alegre reference population, and pancreatic cancer had a lower mortality risk as compared to both the Rio de Janeiro and Porto Alegre reference populations, but these results were based on a small number of deaths from these cancers in the study population. As compared to the local reference group, agricultural workers of this age group were at higher risk of death from respiratory problems, and as compared to the Rio de Janeiro and Porto Alegre reference groups, agricultural workers were at higher risk of death from cardiovascular problems, respiratory problems, and external causes.

The graphs in Figure 1 display MOR and respective confidence intervals for esophageal and stomach cancer mortality among the study population as compared to the local (Part A), Rio de Janeiro (Part B), and Porto Alegre (Part C) reference populations in the time periods of 1979–1998 and 1999–2013 (MOR data for 1979–1998 are based on results presented by Meyer et al. [2003]). These graphs show that over the last decades, mortality risks from esophageal and stomach cancer have been consistently higher among the study population than non-agricultural workers in all reference groups, and that these disparities appear to be on the rise.

In Part A, the graph shows that the study population has had a consistently higher MOR for esophageal cancer as compared to the local Serrana Region reference population since the 1979–1998 period. The MOR for stomach cancer appears to have risen since the 1978–1999 period, with the odds of mortality becoming statistically significantly higher than that of the reference group in the most recent study period. In Part B, the graph shows that since the 1978–1999 period, both esophageal and stomach cancer mortality have been consistently higher among the study population as compared to the Rio de Janeiro general population. Although confidence intervals for MOR overlap between the two time periods, the MOR appears to have generally risen in recent years. In Part C, the graph shows again that both esophageal and stomach cancer risk have been consistently higher among the study population relative to the Porto Alegre general population, and that the MOR for both

esophageal and stomach cancer has increased in the most recent time period, although the confidence intervals still overlap with those of the MOR from the earlier period.

DISCUSSION

In the current study, we aimed to update the literature on mortality in the Serrana Region of Rio de Janeiro and discuss its possible association with occupational risks of working in agriculture. In concurrence with previous decades' findings in the Serrana Region, results display that in the years 1999–2013, agricultural workers ages 30–69 were at highest risk for stomach and esophageal cancer mortality, with mortality odds among this group being higher than in all three reference groups. Mortality for both these cancers has been consistently high over the last few decades, suggesting ongoing disparities in cancer mortality between agricultural workers and non-agricultural workers, and that these disparities may be growing. Among the younger study population of agricultural workers aged 30–49, our findings for stomach cancer mortality were most alarming because this is a relatively young age group for cancer mortality. Stomach cancer accounted for over a third of cancer deaths among this group, with agricultural workers presenting much higher mortality as compared to all three reference populations. As mortality was more elevated in agricultural workers than even the local Serrana Region non-agricultural workers, this suggests workers' risk for stomach cancer may be directly related to their status and lifestyle as an agricultural worker and not necessarily regional habits or exposures that affect the entire population.

Among the older study population of agricultural workers aged 50–69, findings suggest esophageal cancer is not only the most common cause of death from cancer among this group, but that cancer mortality is significantly higher among the study population than non-agricultural workers in this and other regions. The high mortality by esophageal cancer as compared to the third-reference group of non-agricultural workers in Porto Alegre is especially notable as Rio Grande do Sul, the state of which Porto Alegre is the capital, experiences some of the highest rates of esophageal cancer in the country [Pinto et al., 2003]. Again, the high odds of esophageal cancer among agricultural workers as compared to non-agricultural workers in the local Serrana Region suggests workers may experience certain risk factors related to their status as an agricultural worker that may increase mortality risk for this cancer.

Stomach and esophageal cancers are, respectively, the current third and fifth most common causes of cancer mortality in Brazil (GloboCan, 2015), and such cancers of the digestive system have been repeatedly associated with farm work in Brazil: In a case control study, Meyer et al. [2011] found that agricultural workers in Brazil were at significantly higher risk of esophageal cancer mortality as compared to non-agricultural workers. In a study on pesticide exposure and cancer among rural workers from São Paulo, cancers of the digestive system represented 36% of the cancer cases found among workers, of which six cases were specifically of gastric cancer, implying these cancers are common among agricultural workers in other parts of Brazil as well [Stoppelli and Crestana, 2005]. International studies have also found associations between agricultural exposures and higher risk for these cancers. Lee et al. [2008] found that regions in South Korea with higher farming indexes had

higher rates of esophageal and gastric cancers. Colt et al. [2001] found excess deaths by esophageal, stomach, and others cancers among U.S. farmworkers. In Taiwan, Huang et al. [2012] found that farmers, gardeners, and workers with exposure to dust were at high risk for esophageal cancer. In Sweden, studies have found associations between pesticide exposure, in particular, and increased risk for esophageal and stomach cancer [Ekstrom et al., 1999; Jansson et al., 2006].

Certain pesticides such as trifluralin, chlorthalonil, and dichlorvos have been associated with gastric tumors [Cocco et al., 1996]. In a study of Hispanic workers in California, Mills and Yang [2007] found gastric cancer to be elevated in farm workers that had been exposed to herbicides 2,4-D and trifluran, insecticides chlordane, and malathion, fungicides mancozeb, and maneb, fumigants methyl bromide, or acaricide propargite, chemicals of which many are classified as possible and probable carcinogens by the United States Environmental Protection Agency. While there is limited information on the exact types of pesticides used in the Serrana Region, certain fungicides listed above, such as mancozeb have been specifically cited as being used by workers in the surrounding regions [Meyer et al., 1999; Rangel et al., 2011]. Other agricultural exposures that have been associated with elevated risk for gastric cancer include organic and inorganic dusts, fertilizers, and diesel fuels [Cocco et al., 1996], but limited information exists about the exposure to such chemicals specifically in the Serrana Region of Brazil.

It is possible that other lifestyle factors may be implicated in the higher observed odds of cancer mortality. Dietary habits, for example, including the high consumption of dietary nitrate, has been associated with esophageal and gastric cancers [Lijinsky, 1999; Pinto et al., 2003; Berretta et al., 2012; Palladino-Davis et al., 2015], and alcohol and tobacco use have been repeatedly associated with the pathologies of such cancers both in Brazil and elsewhere [Gammon et al., 1997; Kollarova et al., 2007]. Agricultural workers may also have higher rates of exposure to *Helicobacter pylori*, which is a known risk factor for stomach cancer and that has been observed to be more prevalent among some rural populations [Elitsur et al., 1998; Ito et al., 2001]. Growing disparities in cancer mortalities may also reflect socioeconomic disadvantages and stressors that can impact the health of rural workers [Peres, 2009]. This may include difficulty in accessing health services and infrequent monitoring of health [Silva et al., 2005], which may be especially relevant to esophageal and stomach cancer, which progress rapidly, have low recovery rates [Holmes and Vaughan, 2007; Kollarova et al., 2007; Brenner et al., 2009] and may be difficult to diagnosis at early stages [Palladino-Davis et al., 2015]. However, due to the limits of information available about the subjects in this study, we could not investigate these factors among our study population.

Consistent with findings from previous decades, lung cancer was one of the most common causes of cancer death among the Serrana Region study population, and yet agricultural workers appeared to be at a significantly lower risk for lung cancer mortality than all three reference groups. The especially lower odds of mortality from lung cancer as compared to the Porto Alegre reference group is consistent with the generally lower prevalence of smoking rates in the state of Rio de Janeiro as compared to the state of Porto Alegre that have been reported in national surveys [Ministério da Saude, 2004]. This also concurs with

findings from other studies that have found a lower incidence of lung cancer among farm workers [Blair et al., 1992; Fincham et al., 1992; Levêque-Morlais et al., 2015]. Lower rates of lung cancer as well as generally lower rates of cardiovascular and respiratory problems among agricultural workers have been hypothesized to demonstrate a “healthy worker effect” that results from lower rates of smoking among this group and workers’ overall healthier diet and engagement in physical activity [Blair et al., 1992, 2005; Levêque-Morlais et al., 2015]. Moreover, since early scientific findings [Henderson and Enterline, 1973], workers have been found to have lower lung cancer rates in manufacturing settings when exposed to endotoxins, an integral component of the outer membrane of the cell walls of gram-negative bacteria, whose inflammatory-induced pathways have protective effects against lung cancer [Lenters et al., 2010].

Interestingly, while farm workers in the Serrana region had lower lung cancer mortality relative to other groups, they were at higher risk for mortality due to respiratory and cardiovascular problems than the two urban reference populations. This is in contrast to previous findings by Meyer et al. [2003] in which agricultural workers had similar or even lower odds of death from cardiovascular and respiratory problems than non-agricultural worker reference groups, and suggests that farmers may not be experiencing the same benefits that were once assumed to come along with rural lifestyles. Epidemiological studies have found associations of occupational pesticide exposure with asthma and chronic bronchitis [Mamane et al., 2015] and several other respiratory problems including wheezing, airway irritation, dry, or sore throat, cough, breathlessness, and chest tightness [Ye et al., 2013]. A review found that pesticides may act as sensitizers or irritants that directly damage the bronchial mucosa and increase bronchial hyper-responsiveness [Hernández et al., 2011]. Such exposures, combined with increasing smoking rates among Brazilians and changes in Brazilian diet [Koifman and Koifman, 2003], may help account for the rising cardiovascular and respiratory problems observed among this group. The higher risk of death from external causes among the study population, which include injuries and poisonings, is also consistent with results from previous decades. The high odds of these deaths among agricultural workers as compared to the urban populations is especially alarming among older populations. The cause for such high odds of death by external-causes among this group should be further investigated.

The current study contains several limitations. First off, the databases from which mortality data were gathered do not contain information on socioeconomic factors, alcohol, dietary, or smoking habits of subjects, so we could not control for these confounding factors that may play an important role in cancer morbidity and mortality. The nature of this ecological study also did not allow us to determine direct associations between agricultural exposures and cancer mortality: While most Brazilian farms in the Serrana Region utilize pesticides and other chemicals, we cannot determine to what extent the agricultural workers included in this study came in contact with specific toxins during their working life. Moreover, information is not available regarding the specific types of chemicals workers are exposed to in the region so we could not determine whether particular toxins may be involved in the abnormal mortality rates observed. Despite these limitations, the consistency of these results with previous years’ data and with mortality risk patterns generally found among agricultural workers support the robustness of our findings.

CONCLUSION

The current study adds valuable data on mortality in a region with intensive agricultural production in the state of Rio de Janeiro, Brazil. Our results largely corroborate the findings from the previous 2003 study in the Serrana Region by Meyer et al. [2003] and suggest that agricultural workers are experiencing high rates of mortality from esophageal and stomach cancers as compared to non agricultural workers and that these disparities may be rising. While agricultural workers have previously shown lower rates of mortality from injuries and cardiovascular and respiratory problems, mortality from these causes is becoming more common and appears to be even surpassing that of local and urban non-agricultural workers, emphasizing the need to investigate the source of these changes. More controlled studies that take into account individual, lifestyle and occupational risk factors are needed to understand the causes of such higher mortality rates in this region and help plan appropriate interventions to protect the health of workers.

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REFERENCES

- Berretta M, Cappellani A, Lleshi A, Di Vita M, Lo Menzo E, Bearz A, Galvano F, Spina M, Malaguarnera M, Tirelli U, et al. 2012 The role of diet in gastric cancer: Still an open question. *Front Biosci* 17:1640–1647.
- Blair A, Zahm SH. 1995 Agricultural exposures and cancer. *Environ Health Perspect* 103:205. [PubMed: 8741784]
- Blair A, Zahm SH, Pearce NE, Heineman EF, Fraumeni JF Jr., 1992 Clues to cancer etiology from studies of farmers. *Scand J Work Environ Health* 18:209–215. [PubMed: 1411362]
- Blair A, Sandler DP, Tarone R, Lubin J, Thomas K, Hoppin JA, Samanic C, Coble J, Kamel F, Knott C, et al. 2005 Mortality among participants in the agricultural health study. *Ann Epidemiol* 15:279–285. [PubMed: 15780775]
- Brasileian Association for Collective Health (ABRASCO). 2010 Agrotóxicos, segurança alimentar e nutricional e saúde. [agrottoxins, food and nutritional security, and health]. http://www.abrasco.org.br/site/wp-content/uploads/2015/03/Dossie_Abrasco_01.pdf
- Brenner H, Dietrich R, Volker A. 2009 Epidemiology of stomach cancer. *Methods Mol Biol* 472:467–477. [PubMed: 19107449]
- Camara MCC, Cost L, Marinho CL, Guilam MCR. 2008 A produção científica sobre intoxicações por agrotóxicos na região serrana do Rio de Janeiro [in Portuguese]. *O Mundo da Saúde* 32:268–274.
- Chrisman J, Koifman S, de Novaes Sarcinelli P, Moreira JC, Koifman RJ, Meyer A. 2009 Pesticide sales and adult male cancer mortality in Brazil. *Int J Hyg Environ Health* 212:310–321. [PubMed: 18838335]
- Cocco P, Ward MH, Buiatti E. 1996 Occupational risk factors for gastric cancer: An overview. *Epidemiol Rev* 18:218–234. [PubMed: 9021314]
- Colt JS, Stallones L, Cameron LL, Dosemeci M, Zahm SH. 2001 Proportionate mortality among US migrant and seasonal farmworkers in twenty-four states. *Am J Ind Med* 40:604–611. [PubMed: 11675631]
- Conselho Nacional de Saúde. Resolução 466/12. Trata de pesquisas em seres humanos e atualiza a resolução 196. 2012. [Diário Oficial da União]. <http://conselho.saude.gov.br/resolucoes/2012/Reso466.pdf>

- Cordes DH, Foster D. 1988 Health hazards of farming. *Am Fam Physician* 38:233–244. [PubMed: 3051979]
- DATASUS (Unified Health System Informatics Department). 2008 Mortality Information System. Available from: <http://www2.datasus.gov.br/DATASUS/index.php?area=01>
- Ekström AM, Eriksson M, Hansson LE, Lindgren A, Signorello LB, Nyrén O, Hardell L. 1999 Occupational exposures and risk of gastric cancer in a population-based case-control study. *Cancer Res* 59: 5932–5937. [PubMed: 10606238]
- El Batawi MA. 2003 Health of workers in agriculture. Cairo: World Health Organization Regional Office for the Eastern Mediterranean. Available from: <http://applications.emro.who.int/dsaf/dsa226.pdf>
- Elitsur Y, Short JP, Neace C. 1998 Prevalence of *Helicobacter pylori* infection in children from urban and rural West Virginia. *Dig Dis Sci* 43:773–778. [PubMed: 9558033]
- Fincham SM, Hanson J, Berkel J. 1992 Patterns and risks of cancer in farmers in Alberta. *Cancer* 69:1276–1285. [PubMed: 1739927]
- Food and Agriculture Organization of the United Nations: Statistics Division (FAOSTAT). 2015 Download Data: Production; Inputs. Available from: <http://faostat3.fao.org/download/Q/QC/E>
- Gammon MD, Schoenberg JB, Ahsan H, Risch HA, Vaughan TL, Chow WH, Rotterdam H, West AB, Dubrow R, Stanfoed JL, et al. 1997 Tobacco, alcohol, and socioeconomic status and adenocarcinomas of the esophagus and gastric cardia. *J Natl Cancer Inst* 89:1277–1284. [PubMed: 9293918]
- George J, Shukla Y. 2011 Pesticides and cancer: Insights into toxicoproteomic-based findings. *J Proteomics* 74:2713–2722. [PubMed: 21989265]
- Henderson V, Enterline PE. 1973 An unusual mortality experience in cotton textile workers. *J Occup Med* 15:717–719. [PubMed: 4738625]
- Hernández AF, Parrón T, Alarcón R. 2011 Pesticides and asthma. *Curr Opin Allergy Clin Immunol* 11:90–96. [PubMed: 21368619]
- Holmes RS, Vaughan TL. 2007 Epidemiology and pathogenesis of esophageal cancer. *Semin Radiat Oncol* 17:2–9. [PubMed: 17185192]
- Huang SH, Wu IC, Wu DC, Wu CC, Yang JF, Chen YK, Chai CY, Chiu YW, Huang CT, Lee TC, et al. 2012 Occupational risks of esophageal cancer in Taiwanese men. *Kaohsiung J Med Sci* 12:654–659.
- International Agency for Research on Cancer (IARC). 2015 GLOBACAN: Estimated cancer incidence, mortality, and prevalence worldwide in 2012. Available from: <http://globocan.iarc.fr/Pages/Map.aspx>
- IBGE (Brazilian Institute of Geography and Statistics). 2006 SIDRA: Agricultural Census 2006. Available from: <http://www.sidra.ibge.gov.br/bda/agric/default.asp?z=t&o=11&i=P>
- IBGE. 2010 Cidades: Population Census 2010. Available from: <http://www.cidades.ibge.gov.br/xtras/home.php>. Accessed 05.01.2015.
- Ito LS, Oba SM, Hamajima N, Marie SK, Uno M, Shinjo SK, Kino A, Lavilla F, Inoue M, Tajima K, Tominaga S. 2001 *Helicobacter pylori* seropositivity among 963 Japanese Brazilians according to sex, age, generation, and lifestyle factors. *Jpn J Cancer Res* 92:1150–6. [PubMed: 11714438]
- Jansson C, Plato N, Johansson ALV, Nyrén O, Lagergren J. 2006 Airborne occupational exposures and risk of oesophageal and cardia adenocarcinoma. *Occup Environ Med* 63:107–112. [PubMed: 16421388]
- Kesavachandran CN, Fareed M, Pathak MK, Bihari V, Mathur N, Srivastava AK. 2009 Adverse health effects of pesticides in agrarian populations of developing countries. *Rev Environ Contam Toxicol* 200:33–52. [PubMed: 19680610]
- Koifman S, Koifman RJ. 2003 Environment and cancer in Brazil: An overview from a public health perspective. *Mutat Res* 544:305–311. [PubMed: 14644332]
- Kollarova H, Machova L, Horakova D, Janoutova G, Janout V. 2007 Epidemiology of esophageal cancer-an overview article. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 151:17. [PubMed: 17690734]

- Lee WJ, Son M, Chun BC, Park ES, Lee HK, Coble J, Dosemeci M. 2008 Cancer mortality and farming in South Korea: An ecologic study. *Cancer Causes Control* 19:505–13. [PubMed: 18197459]
- Lenters V, Basinas I, Beane-Freeman L, Boffetta P, Checkoway H, Coggon D, Portengen L, Sim M, Wouters IM, Heederik D, et al. 2010 Endotoxin exposure and lung cancer risk: A systematic review and meta-analysis of the published literature on agriculture and cotton textile workers. *Cancer Causes Control* 21:523–555. [PubMed: 20012774]
- Levêque-Morlais N, Tual S, Clin B, Adjemian A, Baldi I, Lebailly P. 2015 The AGRiculture and CANcer (AGRICAN) cohort study: Enrollment and causes of death for the 2005–2009 period. *Int Arch Occup Environ Health* 88:61–73. [PubMed: 24599726]
- Lijinsky W N-Nitroso compounds in the diet. 1999 *Mutat Res* 443: 129–38. [PubMed: 10415436]
- London L. 2011 Human rights and health: Opportunities to advance rural occupational health. *Int J Occup Environ Health* 17:80–92. [PubMed: 21344823]
- Mamane A, Baldi I, Tessier JF, Raherison C, Bouvier G. 2015 Occupational exposure to pesticides and respiratory health. *Eur Respir Rev* 24:306–19. [PubMed: 26028642]
- Meyer A, Sarcinelli PN, Moreira JC. 1999 Are some Brazilian population groups subject to endocrine disrupters? *Cad Saúde Pública* 15:845–50. [PubMed: 10633206]
- Meyer A, Chrisman J, Moreira JC, Koifman S. 2003 Cancer mortality among agricultural workers from Serrana Region, state of Rio de Janeiro, Brazil. *Environ Res* 93:264–271. [PubMed: 14615236]
- Meyer A, Alexandre PCB, Chrisman J, Markowitz SB, Koifman R, Koifman S. 2011 Esophageal cancer among Brazilian agricultural workers: Case-control study based on death certificates. *Int J Hyg Environ Health* 214:151–155. [PubMed: 21159552]
- Miettinen OS, Wang JD. 1981 An alternative to the proportionate mortality ratio. *Am J Epidemiol* 114:144–148. [PubMed: 7246522]
- Mills PK, Yang RC. 2007 Agricultural exposures and gastric cancer risk in Hispanic farm workers in California. *Environ Res* 104:282–289. [PubMed: 17196584]
- Mills PK, Dodge J, Yang R. 2009 Cancer in migrant and seasonal hired farm workers. *J Agromed* 14:185–191.
- Ministério da Saúde, Instituto Nacional de Câncer. 2004 Rio de Janeiro: Coordenação de Prevenção e Vigilância. Inquerito domiciliar sobre comportamentos de risco e morbidade referida de doenças e agravos não transmissíveis: Brasil, 15 capitais e Distrito Federal, 2002–2003. p.55 Available from: <http://www.inca.gov.br/inquerito/docs/completa.pdf>
- Miranda-Filho AL, Monteiro GTR, Meyer A. 2012 Brain cancer mortality among farm workers of the State of Rio de Janeiro, Brazil: A population-based case-control study, 1996–2005. *Int J Hyg Environ Health* 215:496–501 [PubMed: 22118878]
- Miranda Filho AL, Koifman RJ, Koifman S, Monteiro GT. 2014 Brain cancer mortality in an agricultural and a metropolitan region of Rio de Janeiro, Brazil: A population-based, age-period-cohort study, 1996–2010. *BMC Cancer* 14:320 [PubMed: 24884498]
- Palladino-Davis AG, Mendez BM, Fisichella PM, Davis CS. 2015 Dietary habits and esophageal cancer. *Dis Esophagus* 28:59–67. [PubMed: 23795778]
- Paumgartten FJ, Delgado IF, Oliveira ES, Alleluia IB, Barretto HH, Kussumi TA. 1998 Levels of organochlorine pesticides in the blood serum of agricultural workers from Rio de Janeiro State, Brazil. *Cad Saude Publica* 14:S33–9.
- Pearce N, McLean D. 2005 Agricultural exposures and non-Hodgkin's lymphoma. *Scand J Work Environ Health* 31:18–25. [PubMed: 16190145]
- Peres F. 2009 Saúde, trabalho e ambiente no meio rural brasileiro. *Cien Saude Colet* 14:1995–2004. [PubMed: 20069168]
- Peres F, Rozemberg B, Alves SR, Moreira JC, Oliveira-Silva JJ. 2001 Comunicação relacionada ao uso de agrotóxicos em região agrícola do Estado do Rio de Janeiro. *Rev Saude Pública* 35:564–70. [PubMed: 11799470]
- Pinto LF, Rossini AM, Albano RM, Felzenszwalb I, de Moura Gallo CV, Nunes RA, Andreollo NA. 2003 Mechanisms of esophageal cancer development in Brazilians. *Mutat Res* 544:365–73. [PubMed: 14644339]

- Pratt DS. 1990 Occupational health and the rural worker: Agriculture, mining and logging. *J Rural Health* 6:399–418. [PubMed: 10107682]
- Ragin C, Davis-Reyes B, Tadesse H, Daniels D, Bunker CH, Jackson M., Taioli E 2013 Farming, reported pesticide use, and prostate cancer. *Am J Men Health* 7:102–109.
- Rangel CD, Rosa AC, Sarcinelli PD. 2011 Uso de agrotóxicos e suas implicações na exposição ocupacional e contaminação ambiental. *Cad. Saúde Colet.*, (RioJ.) 19.
- Rathinam X, Kota R, Thiyagar N. 2005 Farmers and formulations-rural health perspective. *Med J Malaysia* 60:118. [PubMed: 16250298]
- Silva JM, Novato-Silva E, Faria HP, Pinheiro TM. 2005 Agrotóxico e trabalho: Uma combinação perigosa para a saúde do trabalhador rural. *Ciênc Saúde Coletiva* 10:891–903.
- Stoppelli IMDBS, Crestana S 2005 Pesticide exposure and cancer among rural workers from Bariri, Sao Paulo State, Brazil. *Environ Int* 31:731–738. [PubMed: 15910970]
- Vakonaki E, Androutsopoulos VP, Liesivuori J, Tsatsakis AM, Spandidos DA. 2013 Pesticides and oncogenic modulation. *Toxicology* 307:42–5. [PubMed: 23354457]
- Viel JF, Challier B, Pitard A, Pobel D. 1998 Brain cancer mortality among French farmers: The vineyard pesticide hypothesis. *Arch Environ Health* 53:65–70. [PubMed: 9570310]
- World Health Organization (WHO). 1990 Geneva: Public health impact of pesticides used in agriculture. Available from: <http://apps.who.int/iris/bitstream/10665/39772/1/9241561394.pdf>
- World Trade Organization Secretariat (WTO). 2015 International Trade Statistics 2015: World and Regional Export Profiles 2014. Available from: https://www.wto.org/english/res_e/statistics_e/world_commodity_profiles14_e.pdf and WTO Statistics Database Available from <http://stat.wto.org/Home/WSDBHome.aspx>
- Ye M, Beach J, Martin JW, Senthilselvan A. 2013 Occupational pesticide exposures and respiratory health. *Int J Environ Res Public Health* 10:6442–6471. [PubMed: 24287863]
- Zukiewicz-Sobczak WA, Chmielewska-Badora J, Wroblewska P, Zwolinski J. 2013 Farmers' occupational diseases of allergenic and zoonotic origin. *Postepy Dermatol Alergol* 30:311. [PubMed: 24353492]

FIGURE 1.

Mortality odds ratios and 95% confidence intervals comparing Serrana Region agricultural workers to three reference populations over two study periods; 1979–1998 and 1999–2013. Part **A** compares study population to Serrana Region non-agricultural workers, Part **B** compares study population to Rio de Janeiro non-agricultural workers, and Part **C** compares study population Porto Alegre non-agricultural workers.

Mortality Odds Ratio for Cancer Among Male Agricultural Workers Ages 30–69 Years, Serrana Region, Rio de Janeiro, 1999–2013 (N=146,487)

TABLE I.

Cause and number of deaths among serrana region agricultural workers	Serrana region non-agricultural workers (first reference group)			Rio de Janeiro non-agricultural workers (second reference group)			Porto Alegre non-agricultural workers (third reference group)		
	(n)	MOR	95%CI	(n)	MOR	95%CI	(n)	MOR	95%CI
All cancers (166)	1,156	1.10	0.95–1.28	20,771	0.99	0.86–1.14	6,465	0.64	0.56–0.74
Stomach cancer (45)	223	1.55	1.13–2.12	2,472	2.30	1.72–3.08	532	2.28	1.69–3.08
Esophageal cancer (41)	163	1.93	1.38–2.71	1,663	3.12	2.30–4.24	642	1.72	1.26–2.34
Lung cancer (17)	217	0.59	0.36–0.96	6,309	0.33	0.20–0.53	2,086	0.21	0.13–0.33
Prostate cancer (16)	81	1.50	0.88–2.56	1,883	1.06	0.65–1.72	447	0.95	0.58–1.56
Larynx cancer (10)	89	0.85	0.44–1.63	1,296	0.96	0.52–1.78	361	0.73	0.39–1.37
Brain cancer (9)	84	0.81	0.41–1.61	1,317	0.85	0.44–1.63	350	0.68	0.35–1.32
Colon cancer (7)	69	0.77	0.35–1.67	1,813	0.48	0.23–1.00	588	0.31	0.15–0.66
Liver cancer (5)	68	0.56	0.22–1.38	1,267	0.49	0.20–1.17	635	0.21	0.09–0.50
Leukemia (5)	47	0.81	0.32–2.02	816	0.76	0.32–1.83	234	0.57	0.23–1.37
Pancreatic cancer (4)	63	0.48	0.18–1.32	1,325	0.37	0.14–1.00	422	0.25	0.09–0.67
Penile cancer (2)	11	1.38	0.31–6.22	78	3.19	0.78–12.97	13	4.09	0.92–18.10
Oral cavity cancer (2)	20	0.76	0.18–3.24	172	1.45	0.36–5.82	36	1.48	0.36–6.12
Liposarcoma(2)	19	0.80	0.19–3.42	305	0.82	0.20–3.27	78	0.68	0.17–2.77
Testicular cancer (1)	2	3.80	0.34–41.83	55	2.26	0.31–16.32	41	0.65	0.09–4.70
Cardiovascular problems (445)	3,533	0.94	0.87–1.01	52,233	1.08	1.01–1.16	9,901	1.28	1.19–1.37
Respiratory problems (133)	913	1.12	0.94–1.33	14,135	1.19	1.01–1.39	2,809	1.28	1.09–1.51
External causes (254)	1,838	1.06	0.94–1.19	26,404	1.24	1.11–1.38	5,331	1.32	1.18–1.48
Total deaths (998)	7,440	—	—	113,543	—	—	24,506	—	—

Bold values are significant at $P < 0.05$.

Mortality Odds Ratio for Cancer Among Male Agricultural Workers Ages 30–49 Years, Serrana Region, Rio de Janeiro, 1999–2013 (N=44,418)

TABLE II.

Cause and number of deaths among serrana region agricultural workers	Serrana region non-agricultural workers (first reference group)			Rio de Janeiro non-agricultural workers (second reference group)			Porto Alegre non-agricultural workers (third reference group)		
	(n)	MOR	95%CI	(n)	MOR	95%CI	(n)	MOR	95%CI
All cancers (47)	224	1.49	1.10–2.01	3,159	1.54	1.17–2.03	964	1.16	0.87–1.53
Stomach cancer (17)	44	2.72	1.56–4.72	407	4.30	2.67–6.94	102	4.00	2.41–6.63
Esophageal cancer (5)	29	1.19	0.46–3.07	266	1.90	0.79–4.58	115	1.02	0.42–2.49
Brain cancer (5)	27	1.28	0.50–3.31	405	1.25	0.52–3.00	100	1.17	0.48–2.87
Lung cancer (3)	29	0.71	0.22–2.33	651	0.46	0.15–1.43	207	0.34	0.11–1.05
Larynx cancer (3)	18	1.15	0.34–3.90	192	1.58	0.51–4.91	61	1.15	0.36–3.67
Pancreatic cancer (3)	15	1.38	0.40–4.76	207	1.46	0.47–4.55	55	1.28	0.40–4.08
Leukemia (3)	16	1.30	0.38–4.43	289	1.05	0.34–3.25	69	1.02	0.32–3.23
Colon cancer (2)	17	0.81	0.19–3.50	300	0.67	0.17–2.68	81	0.58	0.14–2.34
Liver cancer (2)	10	1.38	0.30–6.29	205	0.98	0.24–3.94	93	0.50	0.12–2.03
Testicular cancer (1)	1	6.91	0.43–110.39	40	2.52	0.35–18.29	35	0.67	0.09–4.88
Penile cancer (1)	2	3.46	0.31–38.06	21	4.80	0.65–35.62	4	5.87	0.66–52.43
Oral cavity cancer (1)	6	1.15	0.14–9.54	22	4.58	0.62–33.93	3	7.86	0.82–75.12
Liposarcoma(l)	9	0.77	0.10–6.04	108	0.93	0.13–6.66	26	0.90	0.12–6.63
Prostate cancer (0)	1	—	—	46	—	—	13	—	—
Cardiovascular problems (107)	839	0.85	0.72–1.02	9,802	1.12	0.95–1.33	1,815	1.47	1.24–1.75
Respiratory problems (31)	235	0.91	0.63–1.30	3,336	0.93	0.66–1.31	617	1.19	0.84–1.69
External causes (164)	1,222	0.90	0.79–1.02	18,069	0.88	0.78–0.99	3,787	1.02	0.91–1.15
Total deaths (349)	2,520	—	—	34,366	—	—	7,183	—	—

Bold values are significant at $P < 0.05$.

Mortality Odds Ratio for Cancer Among Male Agricultural Workers Ages 50–69 Years, Serrana Region, Rio de Janeiro, 1999–2013 (N=102,069)

TABLE III.

Cause and number of deaths among serrana region agricultural workers	Serrana region non-agricultural workers (first reference group)			Rio de Janeiro non-agricultural workers (second reference group)			Porto Alegre non-agricultural workers (third reference group)		
	(n)	MOR	95%CI	(n)	MOR	95%CI	(n)	MOR	95%CI
All cancers (119)	932	1.03	0.86–1.22	17,612	0.93	0.79–1.10	5,501	0.56	0.47–0.66
Esophageal cancer (36)	134	2.20	1.53–3.15	1,397	3.68	2.66–5.09	527	1.98	1.42–2.76
Stomach cancer (28)	179	1.26	0.85–1.87	2,065	1.91	1.32–2.76	430	1.88	1.29–2.74
Prostate cancer (16)	80	1.61	0.95–2.75	1,837	1.21	0.74–1.98	434	1.05	0.64–1.72
Lung cancer (14)	188	0.59	0.34–1.01	5,658	0.33	0.20–0.56	1,879	0.20	0.12–0.34
Larynx cancer (7)	71	0.79	0.36–1.71	1,104	0.88	0.42–1.84	300	0.66	0.31–1.40
Colon cancer (5)	52	0.77	0.31–1.92	1,513	0.46	0.19–1.09	507	0.28	0.11–0.67
Brain cancer (4)	57	0.56	0.20–1.54	912	0.61	0.23–1.62	250	0.45	0.17–1.21
Liver cancer (3)	58	0.41	0.13–1.31	1,062	0.39	0.13–1.21	542	0.16	0.05–0.48
Leukemia (2)	31	0.52	0.12–2.15	527	0.53	0.13–2.10	165	0.34	0.09–1.38
Penile cancer (1)	9	0.89	0.11–7.02	57	2.44	0.34–17.58	9	3.17	0.40–24.95
Oral cavity cancer (1)	14	0.57	0.08–4.34	150	0.93	0.13–6.61	33	0.86	0.12–6.30
Pancreatic cancer (1)	48	0.17	0.02–1.20	1,118	0.12	0.02–0.87	367	0.08	0.01–0.54
Liposarcoma(1)	10	0.80	0.10–6.25	197	0.70	0.10–5.02	52	0.55	0.08–3.95
Testicular cancer (0)	1	—	—	15	—	—	6	—	—
Cardiovascular problems (338)	2,694	1.01	0.94–1.09	42,431	1.17	1.09–1.25	8,086	1.30	1.21–1.40
Respiratory problems (102)	678	1.23	1.01–1.50	10,799	1.35	1.12–1.62	2,192	1.37	1.13–1.64
External causes (90)	616	1.19	0.96–1.47	8,335	1.55	1.28–1.89	1,544	1.73	1.42–2.12
Total deaths (649)	4,920	—	—	79,177	—	—	17,323	—	—

Bold values are significant at $P < 0.05$.