

Preventive Medicine

Vaccination against seasonal influenza and socio-economic and environmental factors as determinants of the geographic variation of COVID-19 incidence and mortality in the Italian elderly. --Manuscript Draft--

Manuscript Number:	YPMED-20-1203R1
Article Type:	Short communication
Keywords:	COVID-19; Influenza; Vaccine; epidemiology; Public Health
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Abstract:	<p>Background . A possible protective effect of seasonal influenza vaccination against the spread of the COVID-19 epidemic has been suggested. Methods . We used publicly available data bases to explore the hypothesis as well as the effect of multiple social and environmental factors in the 20 Italian regions. Results . Our results suggest that vaccination against seasonal influenza might beneficially impact on incidence and severity of the novel corona virus epidemic. Population density and vehicular traffic were also moderately associated with cumulative incidence of COVID-19. None of the other variables we considered showed an effect on cumulative incidence, case fatality rate or mortality from COVID-19. Conclusions . Extending influenza vaccination coverage particularly among the elderly, vulnerable individuals with specific chronic medical conditions, health care workers, and workers in other essential services, early in the upcoming 2020 influenza season, might help reduce the health impact of a second epidemic wave of COVID-19.</p>



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Dear *Editor*,

We would like to thank you for the opportunity to re-submit an amended version of our article now entitled "Vaccination against seasonal influenza and socio-economic and environmental factors as determinants of the geographic variation of COVID-19 incidence and mortality in the Italian elderly" for publication in Preventive Medicine.

We would like also to thank the Reviewers for their very helpful comments and suggestions that we have addressed in the enclosed point-by-point response.

We hope that our revised article is now suitable for publication in your journal.

Best regards,

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Preventive Medicine

YPMED-20-1203 *"Vaccination against seasonal flu and socio-economic and environmental factors as determinants of the geographic variation of COVID-19 incidence and mortality in Italy"*.

Response to the editorial instructions

1) *Include a separate document uploaded as "Response to Review" that fully addresses the issues raised in the reviewers' comments and the applicable editorial instructions below, point by point.*

We have addressed at our best reviewers' comments point by point in the enclosed 'Response to review'.

2) *In the response document, copy the entire text from the reviewer's comment and insert your answer below each of the points made in the review. You must also include a suitable justification to each specific request for changes that you decided not to accept.*

Below are our answers to each point raised by the four reviewers, numbered consecutively.

3) *It is essential that you highlight or mark all changes to the manuscript that were prompted by the reviewers' or editors' suggestions or by your own revisions.*

All changes to the original text have been marked and saved in a marked copy of the revised version. An unmarked copy is also being submitted.

4) *Include word counts for abstract and text (exclusive of in-line references) from beginning of introduction to end of conclusions.*

The first page of the original version already included word counts for abstract and text.

5) *As in #2 above, include also your response to the mandatory editorial instructions.*

Done.

6) *Before resubmitting, consult our instructions to authors in the journal's website and a recent issue of Preventive Medicine for guidance on how to comply with our formatting and stylistic requirements.*

We have consulted the instructions to authors and formatted accordingly the revised version.

Response to the reviewers' comments.

Reviewer # 1

1. *"...general editing for grammar and punctuation."*

In the revised version, we made the corrections to the grammar and punctuation errors we could detect.

2. *"...changing "flu" to "influenza" in the title (and possibly throughout, at the discretion of the journal). Add in population studied (i.e., in "older adults" or "elderly" or "among those aged >=65").*

In the revised version, we modified the title and made the requested correction throughout the abstract and text.

3. *"...reference a more specific time point in paragraph one, specifically at line 4, re: "Italy has been [...]"*

In the revised version (first paragraph of the introduction), we indicated clearly the time frame which our statement refers to.

4) *"...Add reference at line 12 or soften language to "may have acted..."*

We modified the whole sentence and changed the reference, as, at a more careful glance, the one we cited presented faulty results due to some miscalculations. Also, we included two additional citations about viral interference, showing that the interaction between influenza vaccination and other viral infections might be more complicated.

5) *"At the start of line 13 use "As of this writing [...]", or a specific time point"*.

We amended the paragraph, and we added the specific time point as suggested.

6) *"Line 21 - do the authors mean the hypothesis stated above? If yes, please clarify language for readers."*
We modified the sentence on line 21 of the previous version, so to explicit what hypothesis we refer to.

7) *"It would be useful to include recent influenza vaccination rates in this population, as this has implications on your analysis."*

By the time we submitted our manuscript, the National Institutes of Health published on its web site also the influenza vaccinations rates for the season 2019-2020. Therefore, in the revised version, we clarified that the influenza vaccination rates refer to the 2019-2020 season, and we repeated the analysis with the new data.

8) *"Ensure that limitations of public data are included in discussion."*

In the revised version, we mentioned ecological fallacy as a known problem in interpreting the results of ecological studies, such as the one we conducted. We added also a reference in this regard.

9) *"The methods are not clear if this is limited to outcomes in the ≥ 65 year old population, as the variables and wording are not consistent throughout - needs clarification in this section and should be discussed earlier, ideally in the transition from intro to methods. (3) Line 50, again, if this is restricted to outcomes in the ≥ 65 population, add that here (as done at line 61)".*

We indicated in the title that our analysis refer to the Italian elderly, and clarified at the end of the first paragraph of the Methods section that, although the case fatality rate referred to the total population, comparing it to the vaccination rates in subjects aged ≥ 65 years is still valid as, 94% of deaths occurred among this age group. Also, regional socio-economic and environmental indicators refer to the whole population of that region and not to the specific age group object of this study. This is a further limitation that we addressed in the discussion. Wherever necessary, we now indicate that the outcomes we considered refer to the ≥ 65 year old segment of the population.

10) *"Figure 1 axes need to be labeled."*

We labeled the axes in figure 1

11) *"Is Deprivation Index missing from Table 1?"*

In the previous version, we did not include deprivation index as it was not associated with the COVID-19 outcomes, and it was strongly correlated with average income and the crowding index. After re-analyzing data with the most recent vaccination rates, seeing the fully adjusted model, and looking at the correlation matrix (now table 1), deprivation index was the socioeconomic covariate that best correlated with the COVID-19 outcomes. Therefore, based on the p-value of the regression coefficient (now shown in the new Table 2 of the revised version) we retained deprivation index, and excluded average income and crowding index.

12) *"Need to add basic limitations to this section -- issues with observational/missing data, generalizability, possible confounding due to other factors associated with influenza vaccination not explored here."*

Issues of generalizability are now discussed in the new paragraph we added to the discussion to discuss ecological fallacy. We did not have to deal with missing data and retrieved from official sources the information on socioeconomic and environmental variables. In the revised version, we added a new sentence on possible confounding due to other factors associated with influenza vaccination we did not consider.

Reviewer #2

We thank you reviewer # 2 for the favorable comments.

1) *"... put legends in the Figure to make it easy to understand."*

Please see our response to point # 10 of reviewer # 1.

Reviewer # 3.

- 1) *"...It appears that vaccination data were limited to this age group [NdA: ≥ 65 years], but there is no mention of a particular age group in the title or abstract." From the methods, it is not clear why the analyses were limited to vaccination of ≥ 65 year olds ...". "Individuals ≥ 65 years have a lower response to influenza vaccine than other groups. Why would their vaccination against influenza affect the entire population's susceptibility to COVID-19?"*

Please see our response to point #9 of reviewer # 1.

- 2) *"In the results, it is not clear on what basis the outlier areas were determined to be so and thus removed from further analyses. Please clarify the criteria. Should these have been determined a priori?"*

In the previous version, we considered as outliers the observed values beyond the upper or lower 95% confidence interval of the predicted value along the regression line. In the revised version, we dropped the point on outliers, as they were much less far from the predicted regression line when using the 2019-20 vaccination rates instead of those in 2018-19. Besides, we reconsidered adding details on the outliers as redundant and out of scope in relation to the paper.

- 3) *"the authors report a relationship between circulating vehicles and population density as predictors of cumulative incidence, but they are not significant if the standard 0.05 P value is used. They should not be reported as significant. The same is true for vaccination rate and cumulative deaths."*

We replaced the 2018-19 vaccination rates with the 2019-2020 rates that became available in the meanwhile. We conducted a new analysis and present the results in the revised version. We now present the results with due consideration of the comments raised by reviewer # 3. As described in the results section of the revised version, our findings were confirmed as significant for COVID-19 mortality and case-fatality rate, and of borderline significance for cumulative incidence.

- 4) *"Why is deprivation index not included in the results?"*

Please see our response to point # 11 of reviewer # 1.

- 5) *"The conclusions, especially in the abstract, talk about reducing spread of COVID-19 but no data were presented to support that statement."*

The reviewer is right: we have no data to support our final statements. In fact, it is just a hypothesis suggested by a few publications, mechanistic theories and the findings of our analyses in the present work. Our auspices, and the scope of our paper, are for further observational epidemiological studies to provide more data in this regard as we stated in the last paragraph of the Discussion.

- 6) *"Further no data were presented regarding health care workers or other vulnerable subjects"*

As mentioned in the text, we used publicly available data to conduct our analysis. We did not have access to information on the prevalence of specific occupations or health conditions within each region. In the previous version, we mentioned that we did not find data on long-term health care facilities for the elderly; in the revised version, we added a mention on occupational groups as well.

- 7) *"The focus on public health response and impact are important points for discussion, i.e., preventing more flu will help focus efforts on identifying COVID cases."*

Our discussion already stresses the public health impact of preventing the seasonal influenza epidemic superimposing its effects on the COVID-19 pandemic, in worsening the prognosis of individual cases and in overloading the health care system with sudden, massive emergencies. In particular, we stated at the end of the Discussion that '...beneficial effects might result from excluding influenza in the differential diagnosis of COVID-19-like symptomatic cases, which would restrict the number of cases requiring a deeper diagnostic work up, so lightening the burden on the health system'.

Reviewer # 4

- 1) *"Have there been cross strain protections by coronaviruses in the past? If so, documenting that would strengthen the validity of the conclusions. One that seems somewhat relevant is Am J Epidemiol. 2018 Dec 1;187(12):2530-2540, although that is H1N1, and for prior disease, not vaccination."*

In the revised version, we mentioned the reference reviewer # 4 suggested and two additional reports, suggesting a long lasting, age-related cross—protection from pre-existing antibodies against the same H1N1 virus, or different H1N1 strain. We added a short sentence at the beginning of the second paragraph of the discussion section, and we mentioned as a limitation of our study the fact that vaccination coverage was available for subjects aged ≥ 65 only, and not by age group.

- 2) *"...show the intercorrelations of the predictor variables considered. For example, is the rate of vaccination correlated with population density? If so, it may be that vaccination rate is serving as an index of population density."*

We thank the reviewer for the suggestion. In the revised version we have added as new Table 1 the results of the correlation matrix, where it is shown that there is no correlation between population density and influenza vaccination rate in the elderly.

- 3) *"discussion of the potential effect of general health care as a potential confounder is needed: do people who get vaccinations take better care of their health in general?"*

In the revised version, we mentioned this important point raised by reviewer # 4 as a limitation in interpreting the results of our ecological analysis, as an example of the confounders mentioned in point 12 of reviewer # 1.

- Influenza vaccination is inversely associated to COVID-19 incidence and mortality
- Potential protective effect of influenza vaccination is stronger among elderly
- Extension of influenza vaccination coverage could prevent COVID-19

Vaccination against seasonal ~~flu~~influenza and socio-economic and environmental factors as determinants of the geographic variation of COVID-19 incidence and mortality in the ~~Italy~~Italian elderly

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Word count: abstract: 147; text: ~~1338~~1983

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Abstract

Background. A possible protective effect of seasonal [flu-influenza](#) vaccination against the spread of the COVID-19 epidemic has been suggested. *Methods.* We used publicly available data bases to explore the hypothesis as well as the effect of multiple social and environmental factors in the 20 Italian regions. *Results.* Our results suggest that vaccination against seasonal [flu-influenza](#) might beneficially impact on incidence and severity of the novel corona virus epidemic. Population density and vehicular traffic were also moderately associated with cumulative incidence of COVID-19. None of the other variables we considered showed an effect on cumulative incidence, case fatality rate or mortality from COVID-19. *Conclusions.* Extending [flu-influenza](#) vaccination coverage particularly among the elderly, vulnerable individuals with specific chronic medical conditions, health care workers, and workers in other essential services, early in the upcoming 2020 [flu-influenza](#) season, might help ~~reduc~~^{ing} the health impact of a second epidemic wave of COVID-19.

Keywords: COVID-19; influenza; vaccine; epidemiology.

Introduction

The 2019-2020 novel corona virus SARS-CoV-2 pandemic hit at different times, with variable force worldwide. Reasons for such geographical variation in virulence are not clearly understood; various factors have been suggested, including environmental pollution (1), temperature, humidity, -UV index (2,3), and smoking (4). [In the first quarter of year 2020](#), Italy has been the first and one of the worst hit western countries, with COVID-19 showing a peculiar, southward decreasing trend in cumulative incidence and mortality (5). Also, consistently with what reported in other countries, almost 60% deaths occurred among the elderly living in retirement homes (5), where social contact ~~was~~ [is](#) considered a benefit; therefore, vulnerable subjects were not appropriately isolated from the rising epidemic, and the staff taking care of them often acted as carriers.

One hundred fifteen cases of co-occurrence of influenza A and COVID-19 have been described in Wuhan, China (6), and several clinical reports have described the same co-occurrence worldwide (7). [A survey conducted in Italy between April and June 2020](#) ~~a intriguing online report described~~ [reported a significant reduction in risk of SARS-CoV-2 nasopharyngeal swab test positivity associated with previous influenza and pneumococcal vaccination among younger subjects](#). The statistical power was not sufficient to exclude chance as the determinant of ~~the same size reduction in risk observed among subjects aged ≥65 years~~ (8). [On the other hand](#), ~~a~~ [an](#) ~~inversed~~ [inverse](#) direct correlation between influenza (H1N1) vaccination status and ~~mortality from~~ the attack rate of COVID-19 as a measure of outbreak severity [was observed](#) in 34 world countries, ~~limited to the 15 days between 27 February and 12 March 2020~~ (8,9). ~~which the author interpreted as suggestive of influenza vaccine possibly conferring partial protection against~~ [favouring](#) COVID-19 respiratory tract infections, due to epitope sharing and evolutionary closeness between SARS-CoV-2 and H1N1 vaccine interference. [However, another cross-sectional survey, conducted among the U.S.A. military in 2019, observed that influenza vaccination conferred a reduction in risk for several other influenza and parainfluenza infections, while vaccine interference showed its effects in increasing risk of coronavirus and human metapneumovirus infection](#) (10).

We explored the [hypothesis-relationship between vaccination against seasonal influenza in subjects aged ≥65 years](#) and the concurrent effect of multiple [social-socio-economic](#) and environmental factors; ~~including influenza vaccination status, and COVID-19 cumulative incidence, cumulative death rate, and case fatality rate in the 20 Italian regions, using publicly available data bases.~~

Methods

We used publicly available online resources to extract data on the following socio-economic and environmental variables for each of the 20 Italian regions: average number of ~~household residents/home~~ [\(100 x residents/m²\)](#), average *per capita* income ([€/1000](#)), deprivation index ([modified from http://istat.it/-](#)), circulating vehicles per 1000 residents ([www.comuni-italiani.it/statistiche/-](#); [www.comuni-italiani.it/statistiche/](#)), average March temperature in [March in the main urban area of the region in the Celsius scale](#) ([https://www.ilmeteo.it/portale/archivio-meteo/-](#); [https://www.ilmeteo.it/portale/archivio-meteo/](#)), and population density ([https://www.tuttitalia.it/regioni/densita/](#)). The Italian National Institute of Statistics (ISTAT) deprivation index combines characteristic, such as educational level, percent of unemployed, housing and familiar conditions, to express the level of social disadvantage of a given population (11). As the ISTAT ~~(Italian National Institute of Statistics)~~ deprivation index varies around zero, for the purposes of our analysis, we modified it by eliminating the negative values through adding an equal quantity to each regional value, corresponding to 1+ the lowest negative value, so to have the lowest representing the wealthiest region. [We extracted Data on the 2019-2020 seasonal flu-influenza vaccination rates among the resident population aged ≥ 65 by region from the Italian National Institute of Health \(ISS\) web site](#) ([https://www.epicentro.iss.it/influenza/copertura-vaccinali](#)). ISS was also the source for the number of total incident cases [\(all ages, both genders\)](#) of, and the number of deaths from COVID-19, and number of deaths in March 2020 by region, age, and gender, and for the case fatality rate (deaths/100 diagnoses, all ages, both genders) by region ~~were available online from the Italian National Institute of Health~~ ([https://www.epicentro.iss.it/en/coronavirus/sars-cov-2-integrated-surveillance-data](#)). The resident population of each region aged 65 years or older was extracted from the ~~Italian National Institute of Statistics~~ ISTAT web site ([https://www.istat.it/](#)). Seasonal ~~flu-influenza~~ vaccination rates among the Italian population [in the winter 2019-2020](#) were available by region for the total population aged ≥65, but not gender stratified. Also, ~~Incident cases of COVID-19 incidence and case fatality rate (deaths/100 diagnoses) was were~~ available for the total population [\(all ages, both genders\)](#) at the regional level; however, as [91% of incident cases of, and 94% of deaths from COVID-19 occurred among subjects aged ≥65](#), we ~~considered used~~ the overall [cumulative incidence and case fatality rate](#) ~~as proxies for~~

close enough to represent the mortality experience among the elderly. Also, However, based on the nationwide proportion of COVID-19 cases aged ≥ 65 years or older, we estimated the number of incident cases among elderly aged 65 or older by region, and the cumulative incidence rate in the corresponding age group. Of note, we could not find data on the number of residents in retirement homes for the elderly and disabled, nor on the prevalence of occupational groups at higher risk, such as health-care workers, broken by region. The deprivation index combines characteristic, such as educational level, percent of unemployed, housing and familiar conditions, to express the level of social disadvantage of a given population (9).

We first conducted univariate linear regression analyses to assess the relationship between seasonal flu-influenza vaccination rate, and COVID-19 cumulative incidence, case-fatality rate, and cumulative deaths, and case-fatality rate in from the date of registration of the first case through 31 March 2020. The month of March 2020 This period was selected because it included the phase of logarithmic increase-growth of the epidemic curve in Italy, and because death statistics were more extensively available. We used the Pearson's correlation statistics to test the chance probability associated with the observed regression line.

Based on the available data, we calculated the cumulative COVID-19 incidence ($1,000 \times$ number of cases diagnosed with COVID-19 in 1-31 March 2020 among subjects aged ≥ 65 years / total residents aged ≥ 65 years as of 1 January 2020), and the cumulative COVID-19 death rate among the population aged ≥ 65 years ($10,000 \times$ number of deaths in 1-31 March 2020 among subjects aged ≥ 65 years / total residents aged ≥ 65 years as of 1 January 2020) by Italian region among the population aged ≥ 65 . We used multiple regression analysis to predict COVID-19 cumulative incidence of COVID-19, cumulative death rate, and case fatality rate, and COVID-19 mortality in March 2020 as a function of average number of household residents/home, average per capita income, deprivation index, circulating vehicles per 1000 residents, average March temperature in March the region capital, population density, and seasonal flu-influenza vaccination rate among subjects aged ≥ 65 . We followed a stepwise backward procedure, and retained the variables included in the best fitting model, as indicated by the adjusted R^2 value.

We used SPSS® 20.0 to conduct the analysis.

Results

Figure 1 shows significant inverse correlations trends negative association between between COVID-19 cumulative incidence (a), cumulative death rate case-fatality rate (b), and case fatality rate cumulative deaths (c) in the Italian regions and the respective seasonal flu-influenza vaccination rates among the population aged ≥ 65 years in the 20 Italian regions. The inverse trend by vaccination rate did not change after replacing the estimated cumulative incidence in the elderly with that over the total resident population ($r = 0.546$, $p = 0.006$). The distance of the observed point from the predicted from the regression equation would suggest that other factors, whether environmental or genetic, might have contributed. Two outliers stand out: the island of Sardinia, and Umbria, the first with an observed/expected ratio (calculated from the regression equation for the corresponding vaccination rate) of 0.13 (p -value 0.019 derived from the probability function associated with the distribution of the observed/expected ratio), and the second with an observed/expected ratio of 11.13 (p -value 1.23×10^{-13} , as derived from the same probability function). After excluding these two outliers, the Pearson's correlation coefficient was 0.795 (p -value $= 1.14 \times 10^{-4}$).

We explored a few potentially contributing factors from environmental and socio-economic origin with multiple regression analysis. Table 1 shows the correlation matrix of the selected predictive variables we considered. As expected, average income, crowding index, and deprivation index showed a strong reciprocal correlation. Average income and crowding index were not significant predictors of COVID-19 cumulative incidence in the multiple regression analysis, and therefore these variables are not included, and only deprivation index is retained in the best fitting regression models, which results are shown in Table 2, the results. After the reciprocal adjustments, Vaccination rate against seasonal flu-influenza was still showed an inversely associated association of borderline significance, which was weakened, but was still visible, when using the crude cumulative incidence calculated over the whole resident population. The association was stronger with with incidence, cumulative death rate, and with severity of the disease, as represented by the case fatality rate. An inverse association was also visible, although weaker, with the cumulative death rate. Population density and vehicular traffic also moderately increased cumulative incidence of COVID-19 among the elderly, perhaps by reducing social distance and increasing the susceptibility of the respiratory system

already affected by the particulate emissions in the atmosphere. Deprivation index was instead inversely associated with cumulative incidence of COVID-19, which might be suggestive of an increased risk among the wealthier, possibly because of a more intense social activity. Contrasting previously reported effect of a higher average temperature in reducing the impact of the COVID-19 epidemic (2,3), average temperature did not affect any of the outcomes, and circulating vehicles did not affect cumulative mortality and case fatality rate. Being restricted to the 20 Italian regions, our study was not powerful enough to detect the previously reported effect of a higher average temperature in reducing the impact of the COVID-19 epidemic (2,3), although the suggestive inverse regression coefficient we observed seems to provide support to the hypothesis. None of the other variables we considered showed an effect on cumulative incidence, case fatality rate, or cumulative deaths from COVID-19 (Table 1).

Discussion

Our results suggest that vaccination against seasonal flu-influenza might beneficially impact on incidence and severity of the novel corona virus epidemic in subjects aged 65 years or older. Underlying genetic features of the resident population (10), and/or environmental and socio-economic conditions in two outlier regions might have outstripped their cumulative incidence downwards and upwards, respectively, from the expected value.

Our findings are in agreement with a recent ecological study that evaluated this hypothesis in Italy, but it did not have access to the most recent 2019–2020 regional influenza vaccination coverage rate (12); therefore, our work confirms and extends these results.

Long lasting cross-protection has been described in the elderly from pre-existing antibodies against different H1N1 viral strains or previous contacts with the same strain (132–154). SARS-CoV-2 genomic sequence has been recently characterized as closely related to two SARS-like coronaviruses, with a similar receptor-binding-domain structure, and some amino acid variation at key residues (141,65), allowing its identification as a new human-infecting *b* coronavirus. However, the pathogenesis of influenza and SARS-CoV-2 viruses requires similar hemagglutinin-esterase proteins (121,76); both share spike protein features with class 1 viral membrane fusion proteins (187,3,141,98); and, alike SARS-CoV-2, influenza A viruses also link to the ACE-2 receptors in the lung (152,019).

However, although suggestive, such the circumstantial evidence and the results of our ecological analysis do not prove that seasonal flu-influenza vaccination would prevent COVID-19 to occur through inducing antibodies against common specific antigens. First, we conducted an exploratory analysis of public data on influenza vaccine coverage, which were available for subjects aged ≥ 65 years only; therefore, we could not explore the suggested age-related varying response to viral infections. In fact, Second, the so-called ecological fallacy is a main problem limiting the interpretation of the ecological studies, as the geographic area and not the individual is the unit of measurement, and all subjects within the same area are considered equally exposed to the same factors, while we could not assess whether the within region variance in exposure to each factor would be smaller, similar, or even greater than the between regions variance. Therefore, it is never justified interpreting such results in terms of the individuals composing the population of the area (20). Third, the outcomes we explored refer to the ≥ 65 year old population segment, and not to the whole population, while the socio-economic and environmental indicators we used refer to the total population of each region. This is a further reason of concern in interpreting our findings. Finally, we cannot exclude that possible confounding, due to factors associated with influenza vaccination other than those that we selected, might have affected our results. For instance, as influenza vaccination is a personal choice, it is quite plausible that the resulting benefits against other infections would result from a generic healthier attitude of the persons who choose to be vaccinated in respect to those who did not, and not from the vaccine itself. Still, as multiple reports suggested (6,7), and the U.S. Centers for Disease Control recommends vaccination against seasonal influenza for poultry workers to prevent avian flu (162,24), and a few reports suggested (6,7), that it might prevent seasonal flu-influenza with vaccination would worsening the prognosis through also avoid superimposing its effects on COVID-19, thus worsening the prognosis. On the other hand, mathematical models suggest that efforts in extending public health measures against seasonal flu-influenza would prevent the burden on overburdening the health care system from co-occurring respiratory outbreaks, and the shortage of laboratory equipment and treatment devices (172,32). For instance, beneficial effects might result from excluding influenza in the differential diagnosis of COVID-19-like symptomatic cases, which would restrict the number of cases requiring a deeper diagnostic work up, so lightening the burden on the health system.

Further formal [epidemiological-observational epidemiological](#) analyses are warranted to test this hypothesis. Nonetheless, 1) a specific vaccine against SARS-CoV-2 would not be expected to become available to the general population worldwide in a short time; and 2) benefits and no harm would result from a more extensive coverage of the general population worldwide with the seasonal [flu-influenza](#) vaccine. Besides social distancing and use of face masks, enforcing the WHO recommendation on the use of seasonal [flu-influenza](#) vaccination ([48243](#)), and extending the coverage particularly among the elderly, vulnerable individuals with specific chronic medical conditions, health care workers, and workers in other essential services, early in the upcoming 2020 [flu-influenza](#) season, might help reducing the health impact of a second epidemic wave of COVID-19.

Contributors

PC and SDM conceived the study; PC conducted the analysis and wrote the manuscript; FM, AC, and DS contributed to the analysis and the revision of the manuscript; MC and SDM revised the manuscript. All authors participated in the interpretation of the results, provided critical feedback, and approved the final version.

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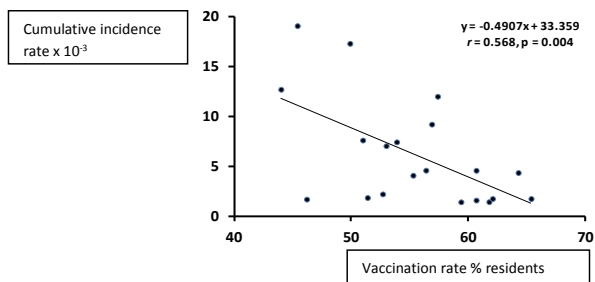
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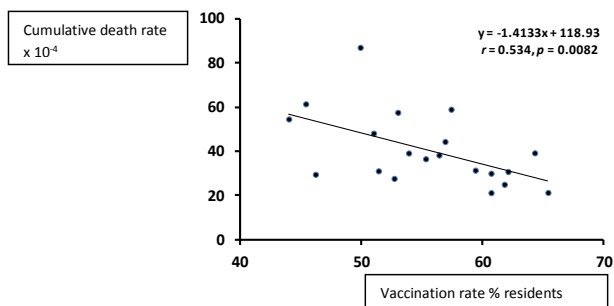
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Figure 1. Effects of seasonal flu vaccination rate on COVID-19 cumulative incidence (A), [cumulative death rate ease-fatality rate](#) (B), and [case-fatality rate cumulative deaths](#) (C) in March 2020 among the Italian population aged ≥ 65 resident in the 20 Italian regions. The equation of the regression line and the related statistics are included in the graphs.

A.



B.



C.

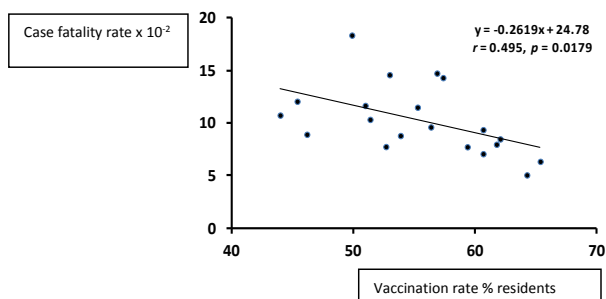


Table 1. [COVID-19 cumulative incidence up to 31 March 2020, influenza vaccination rate in the elderly, and environmental and socio-economic variables in the 20 regions of Italy](#). Correlation matrix.

	COVID-19 cumulative incidence rate x 10 ⁻³	Crowding (residents/100 m ²)	Deprivation index (modified)	income (Euro/1000)	circulating vehicles/ 100 inhabitants	average temperature in March	Population density	influenza vaccination rate (age >65)
COVID-19 cumulative incidence rate x 10 ⁻³	1.000	-0.239	0.468	0.654	0.600	-0.430	0.060	-0.568
Crowding (residents/100 m ²)	-0.239	1.000	0.786	-0.453	-0.180	0.286	0.419	0.141
Deprivation index (modified)	0.468	0.786	1.000	-0.784	-0.203	0.606	0.270	0.102
income (Euro/1000)	0.654	-0.453	-0.784	1.000	0.183	-0.418	0.144	-0.361
circulating vehicles/100 inhabitants*	0.600	-0.180	-0.203	0.183	1.000	-0.374	-0.501	-0.426
average March temperature	-0.430	0.286	0.606	-0.418	-0.374	1.000	0.324	-0.078
Population density	0.060	0.419	0.270	0.144	-0.501	0.324	1.000	0.011
influenza vaccination rate (age >65)	-0.568	0.141	0.102	-0.361	-0.426	-0.078	0.011	1.000

Table 42. Parameters (β = regression coefficient; se = standard error, t = t test; p = p -value) of the multiple regression analyses predicting COVID-19 cumulative incidence, case fatality rate and cumulative deaths in March 2020 among the [Italian](#) population aged ≥ 65 by region ~~in Italy~~.

Variables	Outcomes											
	Cumulative incidence				Cumulative death rate				Case fatality rate			
	β	se	t	p	β	se	t	p	β	se	t	p
Constant	10.94	13.67	0.80	0.437	114.0	26.36	4.33	0.0005	23.44	5.317	4.41	0.0004
Deprivation index	-0.919	0.434	-2.12	0.052	-3.151	1.535	-2.05	0.057	-0.601	0.310	-1.94	0.070
Circulating vehicles (N/1000 residents)	0.227	0.068	3.35	0.005	-				-			
Average March temperature	-0.641	0.561	-1.14	0.272	-				-			
Population density	0.024	0.007	3.45	0.004	0.057	0.026	2.16	0.046	0.013	0.006	1.89	0.075
Vaccination rate	-0.265	0.128	-2.06	0.058	-1.322	0.469	-2.82	0.012	-0.245	0.094	-2.59	0.020
Adjusted R²	0.723				0.410				0.401			

Vaccination against seasonal influenza and socio-economic and environmental factors as determinants of the geographic variation of COVID-19 incidence and mortality in the Italian elderly

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Abstract

Background. A possible protective effect of seasonal influenza vaccination against the spread of the COVID-19 epidemic has been suggested. *Methods.* We used publicly available data bases to explore the hypothesis as well as the effect of multiple social and environmental factors in the 20 Italian regions. *Results.* Our results suggest that vaccination against seasonal influenza might beneficially impact on incidence and severity of the novel corona virus epidemic. Population density and vehicular traffic were also moderately associated with cumulative incidence of COVID-19. None of the other variables we considered showed an effect on cumulative incidence, case fatality rate or mortality from COVID-19. *Conclusions.* Extending influenza vaccination coverage particularly among the elderly, vulnerable individuals with specific chronic medical conditions, health care workers, and workers in other essential services, early in the upcoming 2020 influenza season, might help reduce the health impact of a second epidemic wave of COVID-19.

Keywords: COVID-19; influenza; vaccine; epidemiology.

Introduction

The 2019-2020 novel corona virus SARS-CoV-2 pandemic hit at different times, with variable force worldwide. Reasons for such geographical variation in virulence are not clearly understood; various factors have been suggested, including environmental pollution (1), temperature, humidity, UV index (2,3), and smoking (4). In the first quarter of year 2020, Italy has been the first and one of the worst hit western countries, with COVID-19 showing a peculiar, southward decreasing trend in cumulative incidence and mortality (5). Also, consistently with what reported in other countries, almost 60% deaths occurred among the elderly living in retirement homes (5), where social contact is considered a benefit; therefore, vulnerable subjects were not appropriately isolated from the rising epidemic, and the staff taking care of them often acted as carrier.

One hundred fifteen cases of co-occurrence of influenza A and COVID-19 have been described in Wuhan, China (6), and several clinical reports have described the same co-occurrence worldwide (7). A survey conducted in Italy between April and June 2020 reported a significant reduction in risk of SARS-CoV-2 naso-pharyngeal swab test positivity associated with previous influenza and pneumococcal vaccination among younger subjects. The statistical power was not sufficient to exclude chance as the determinant of the same size reduction in risk observed among subjects aged ≥ 65 years (8). On the other hand, a direct correlation between influenza (H1N1) vaccination status and the attack rate of COVID-19 as a measure of outbreak severity was observed in 34 world countries, limited to the 15 days between 27 February and 12 March 2020 (9) which the author interpreted as suggestive of influenza vaccine possibly favouring COVID-19 respiratory tract infections, due to vaccine interference. However, another cross-sectional survey, conducted among the U.S.A. military in 2019, observed that influenza vaccination conferred a reduction in risk for several other influenza and parainfluenza infections, while vaccine interference showed its effects in increasing risk of coronavirus and human metapneumovirus infection (10).

We explored the relationship between vaccination against seasonal influenza in subjects aged ≥ 65 years and the concurrent effect of multiple socio-economic and environmental factors and COVID-19 cumulative incidence, cumulative death rate, and case fatality rate in the 20 Italian regions.

Methods

We used publicly available online resources to extract data on the following socio-economic and environmental variables for each of the 20 Italian regions: average number of household residents ($100 \times \text{residents}/\text{m}^2$), average *per capita* income (€/1000), deprivation index (modified from <http://istat.it>), circulating vehicles per 100 residents (www.comuni-italiani.it/statistiche/), average temperature in March in the main urban area of the region in the Celsius scale (<https://www.ilmeteo.it/portale/archivio-meteo/>), and population density (<https://www.tuttitalia.it/regioni/densita/>). The Italian National Institute of Statistics (ISTAT) deprivation index combines several characteristics, such as educational level, percent of unemployed, housing and familiar conditions, to express the level of social disadvantage of a given population (11). As the ISTAT deprivation index varies around zero, for the purposes of our analysis, we modified it by eliminating the negative values through adding an equal quantity to each regional value, corresponding to 1+ the lowest negative value, so to have the lowest representing the wealthiest region. We extracted the 2019-2020 seasonal influenza vaccination rates among the resident population aged ≥ 65 by region from the Italian National Institute of Health (ISS) web site (<https://www.epicentro.iss.it/influenza/copertura-vaccinali>). ISS was also the source for the number of total COVID-19 incident cases (all ages, both genders), number of COVID-19 deaths in March 2020 by region, age, and gender, and for the COVID-19 case fatality rate (deaths/100 diagnoses, all ages, both genders) by region (<https://www.epicentro.iss.it/en/coronavirus/sars-cov-2-integrated-surveillance-data>). The resident population of each region aged 65 years or older was extracted from the ISTAT web site (<https://www.istat.it/>). Seasonal influenza vaccination rates among the Italian population in the winter 2019-2020 were available by region for the total population aged ≥ 65 , but not gender stratified. Also, COVID-19 incidence and case fatality rate were available for the total population at the regional level; however, as 91% of incident cases, and 94% of deaths from COVID-19 occurred among subjects aged ≥ 65 , we used the overall cumulative incidence and case fatality rate as *proxies* for mortality among the elderly. Also, based on the nationwide proportion of COVID-19 cases aged ≥ 65 years, we estimated the number of incident cases among elderly by region, and the cumulative incidence rate in the corresponding age group. Of note, we could not find data on the number of residents in retirement homes for the elderly and disabled, nor on the prevalence of occupational groups at higher risk, such as healthcare workers by region.

We first conducted univariate linear regression analyses to assess the relationship between seasonal influenza vaccination rate, and COVID-19 cumulative incidence, cumulative deaths, and case-fatality rate from the date of registration of the first case through 31 March 2020. This period was selected because it included the phase of logarithmic growth of the epidemic curve in Italy. We used the Pearson's correlation statistic to test the chance probability associated with the observed regression line.

Based on the available data, we calculated the cumulative COVID-19 incidence (1,000 x number of cases diagnosed with COVID-19 among subjects aged ≥ 65 years/ total residents aged ≥ 65 years as of 1 January 2020), and the cumulative COVID-19 death rate among the population aged ≥ 65 years (10,000 x number of deaths among subjects aged ≥ 65 years /total residents aged ≥ 65 years as of 1 January 2020) by Italian region. We used multiple regression analysis to predict COVID-19 cumulative incidence, cumulative death rate, and case fatality rate, as a function of average number of household residents, average *per capita* income, deprivation index, circulating vehicles, average temperature in March, population density, and seasonal influenza vaccination rate among subjects aged ≥ 65 . We followed a stepwise backward procedure, and retained the variables included in the best fitting model, as indicated by the adjusted R^2 value.

We used SPSS® 20.0 to conduct the analysis.

Results

Figure 1 shows significant negative association between COVID-19 cumulative incidence (a), cumulative death rate (b), and case fatality rate (c) and the respective seasonal influenza vaccination rates among the population aged ≥ 65 years in the 20 Italian regions. The inverse trend by vaccination rate did not change after replacing the estimated cumulative incidence in the elderly with that over the total resident population ($r = 0.546$, $p = 0.006$).

We explored a few potentially contributing factors from environmental and socio-economic origin with multiple regression analysis. Table 1 shows the correlation matrix of the selected predictive variables. As expected, average income, crowding index, and deprivation index showed a strong reciprocal correlation. Average income and crowding index were not significant predictors of COVID-19 cumulative incidence in the multiple regression analysis, and therefore these variables are not included, and only deprivation index is retained in the best fitting regression models, which results are shown in Table 2. After the reciprocal adjustments, vaccination rate against seasonal influenza still showed an inverse association of borderline significance, which was weakened, but still visible, when using the crude cumulative incidence calculated over the whole resident population. The association was stronger with cumulative death rate, and severity of the disease, as represented by the case fatality rate. Population density and vehicular traffic increased cumulative incidence of COVID-19 among the elderly, perhaps by reducing social distance and increasing the susceptibility of the respiratory system already affected by the particulate emissions in the atmosphere. Deprivation index was instead inversely associated with cumulative incidence of COVID-19, which might be suggestive of an increased risk among the wealthier, possibly because of a more intense social activity. Contrasting previously reported effect of a higher average temperature in reducing the impact of the COVID-19 epidemic (2,3), average temperature did not affect any of the outcomes, and circulating vehicles did not affect either cumulative mortality or case fatality rate.

Discussion

Our results suggest that vaccination against seasonal influenza might beneficially impact on incidence and severity of the novel corona virus epidemic in subjects aged 65 years or older.

Our findings are in agreement with a recent ecological study that evaluated this hypothesis in Italy, but it did not have access to the most recent 2019–2020 regional influenza vaccination coverage rate (12); therefore, our work confirms and extends these results.

Long lasting cross-protection has been described in the elderly from pre-existing antibodies against different H1N1 viral strains or previous contacts with the same strain (13-15). SARS-CoV-2 genomic sequence has been recently

characterized as closely related to two SARS-like coronaviruses, with a similar receptor-binding domain structure, and some amino acid variation at key residues (16), allowing its identification as a new human-infecting *β* coronavirus. However, the pathogenesis of influenza and SARS-CoV-2 viruses requires similar hemagglutinin-esterase proteins (17); both share spike protein features with class 1 viral membrane fusion proteins (18,19); and, alike SARS-CoV-2, influenza A viruses also link to the ACE-2 receptors in the lung (20).

Although suggestive, the circumstantial evidence and the results of our ecological analysis do not prove that seasonal influenza vaccination would prevent COVID-19 occurrence by inducing antibodies against common specific antigens. First, we conducted an exploratory analysis of public data on influenza vaccination coverage, which were available for subjects aged ≥ 65 years only; therefore, we could not explore the suggested age-related varying response to viral infections. Second, the so-called ecological fallacy is a main problem limiting the interpretation of the ecological studies, as the geographic area and not the individual is the unit of measurement, and all subjects within the same area are considered equally exposed to the same factors, while we could not assess whether the within region variance in exposure to each factor would be smaller, similar, or even greater than the between regions variance. Therefore, it is never justified interpreting such results in terms of the individuals composing the population of the area (21). Third, the outcomes we explored refer to the ≥ 65 year old population segment, and not to the whole population, while the socio-economic and environmental indicators we used refer to the total population of each region. This is a further reason for suggesting caution in interpreting our findings. Finally, we cannot exclude that possible confounding, due to factors associated with influenza vaccination other than those selected, might have affected our results. For instance, as influenza vaccination is a personal choice, it is quite plausible that the resulting benefits against other infections would result from a generic healthier lifestyle of the persons who choose to be vaccinated in respect to those who did not, and not from the vaccine itself. Still, the U.S. Centers for Disease Control recommends vaccination against seasonal influenza for poultry workers to prevent avian flu (22), and a few reports suggested (6,7), that preventing seasonal influenza via vaccination would also avoid superimposing its effects on COVID-19, thus worsening the prognosis. On the other hand, mathematical models suggest that efforts in extending public health measures against seasonal influenza would prevent overburdening the healthcare system from co-occurrence of respiratory outbreaks, and shortage of laboratory equipment and treatment devices (23). For instance, beneficial effects might result from excluding influenza in the differential diagnosis of COVID-19-like symptomatic cases, which would restrict the number of cases requiring a deeper diagnostic work up, so lightening the burden on the healthcare system capacity.

Further formal observational epidemiological analyses are warranted to test this hypothesis. Nonetheless, 1) a specific vaccine against SARS-CoV-2 would not be expected to become available to the general population worldwide in a short time; and 2) benefits and no harm would result from a more extensive influenza vaccination coverage in the general population worldwide. Besides social distancing and use of face masks, enforcing the WHO recommendation on the use of seasonal influenza vaccination (24), and extending the coverage particularly among the elderly, vulnerable individuals with specific chronic medical conditions, health care workers, and workers in other essential services, early in the upcoming 2020 influenza season, might help reduce the health impact of a second epidemic wave of COVID-19.

Contributors

PC and SDM conceived the study; PC conducted the analysis and wrote the manuscript; FM, AC, and DS contributed to the analysis and the revision of the manuscript; MC and SDM revised the manuscript. All authors participated in the interpretation of the results, provided critical feedback, and approved the final version.

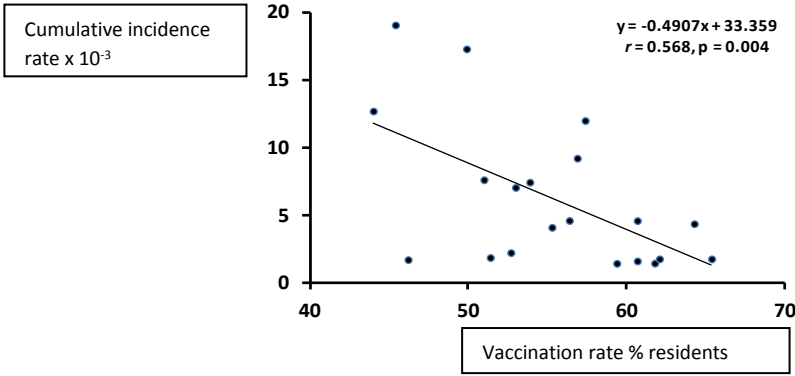
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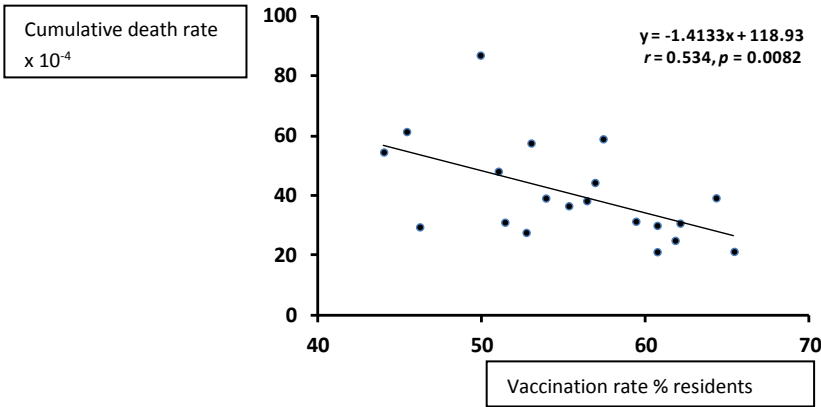
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Figure 1. Effects of seasonal flu vaccination rate on COVID-19 cumulative incidence (A), cumulative death rate (B), and case-fatality rate (C) in March 2020 among the Italian population aged ≥ 65 resident in the 20 Italian regions. The equation of the regression line and the related statistics are included in the graphs.

A.



B.



C.

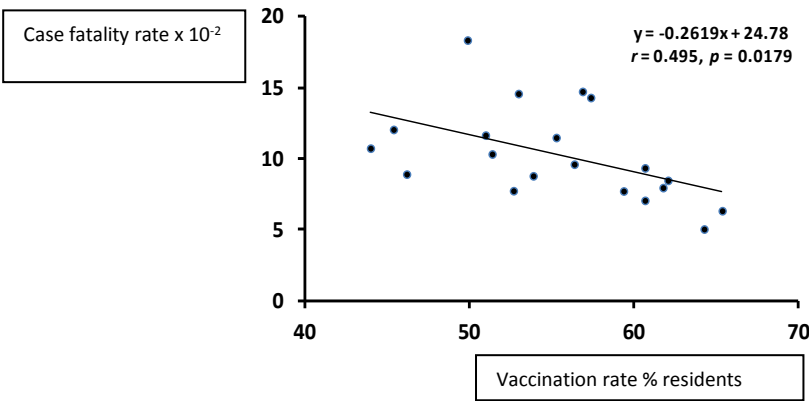


Table 1. COVID-19 cumulative incidence up to 31 March 2020, influenza vaccination rate in the elderly, and environmental and socio-economic variables in the 20 regions of Italy. Correlation matrix.

	COVID-19 cumulative incidence rate x 10 ⁻³	Crowding (residents/100 m ²)	Deprivation index (modified)	income (Euro/1000)	circulating vehicles/ 100 inhabitants	average temperature in March	Population density	influenza vaccination rate (age >65)
COVID-19 cumulative incidence rate x 10 ⁻³	1.000	-0.239	0.468	0.654	0.600	-0.430	0.060	-0.568
Crowding (residents/100 m ²)	-0.239	1.000	0.786	-0.453	-0.180	0.286	0.419	0.141
Deprivation index (modified)	0.468	0.786	1.000	-0.784	-0.203	0.606	0.270	0.102
income (Euro/1000)	0.654	-0.453	-0.784	1.000	0.183	-0.418	0.144	-0.361
circulating vehicles/100 inhabitants*	0.600	-0.180	-0.203	0.183	1.000	-0.374	-0.501	-0.426
average March temperature	-0.430	0.286	0.606	-0.418	-0.374	1.000	0.324	-0.078
Population density	0.060	0.419	0.270	0.144	-0.501	0.324	1.000	0.011
influenza vaccination rate (age >65)	-0.568	0.141	0.102	-0.361	-0.426	-0.078	0.011	1.000

Table 2. Parameters (β = regression coefficient; se = standard error, t = t test; p = p -value) of the multiple regression analyses predicting COVID-19 cumulative incidence, case fatality rate and cumulative deaths in March 2020 among the Italian population aged ≥ 65 by region.

<i>Variables</i>	<i>Outcomes</i>											
	<i>Cumulative incidence</i>				<i>Cumulative death rate</i>				<i>Case fatality rate</i>			
	β	se	t	p	β	se	t	p	β	se	t	p
Constant	10.94	13.67	0.80	0.437	114.0	26.36	4.33	0.0005	23.44	5.317	4.41	0.0004
Deprivation index	-0.919	0.434	-2.12	0.052	-3.151	1.535	-2.05	0.057	-0.601	0.310	-1.94	0.070
Circulating vehicles (N/1000 residents)	0.227	0.068	3.35	0.005	-				-			
Average March temperature	-0.641	0.561	-1.14	0.272	-				-			
Population density	0.024	0.007	3.45	0.004	0.057	0.026	2.16	0.046	0.013	0.006	1.89	0.075
Vaccination rate	-0.265	0.128	-2.06	0.058	-1.322	0.469	-2.82	0.012	-0.245	0.094	-2.59	0.020
Adjusted R ²	0.723				0.410				0.401			

PC and SDM conceived the study; PC conducted the analysis and wrote the manuscript; FM, AC, and DS contributed to the analysis and the revision of the manuscript; MC and SDM revised the manuscript. All authors participated in the interpretation of the results, provided critical feedback, and approved the final version.