## PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (see an example) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below. Some articles will have been accepted based in part or entirely on reviews undertaken for other BMJ Group journals. These will be reproduced where possible.

## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | **Effectiveness and safety of the A-H1N1 vaccine in children: a hospital-based case-control study** |
| **AUTHORS** | Menniti-Ippolito, Francesca; Da Cas, Roberto; Sagliocca, Luciano; Traversa, Giuseppe; Ferrazin, Fernanda; Santuccio, Carmela; Trataglia, Loriana; Trotta, Francesco; Di Pietro, Pasquale; Renna, Salvatore; Rossi, Rossella; Domenichini, Bianca; Gamba, Stefania; Trovato, Francesco; Tovo, Pier-Angelo; Bianciotto, Manuela; Calitri, Carmelina; Gabiano, Clara; Raffaldi, Irene; Urbino, Antonio |

## VERSION 1 - REVIEW

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| **REVIEWER** | ***Xavier Kurz***<br>Signal Detection Coordination and Project lead<br>European Medicines Agency (EMA)<br>United Kingdom<br>I have no conflict of interest. |
| **REVIEW RETURNED** | 24-May-2011 |

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| **THE STUDY** | One of the outcome measures for effectiveness of the pandemic vaccines was non-laboratory confirmed influenza-like illness, with an exposure time window any time before admission. This outcome does not allow to differentiate between ILI as a (known and expected) adverse reaction of pandemic vaccines and ILI as a possible A/H1N1 influenza disease. This definition of the outcome is not in line with the recommendations from the ECDC “Protocols for case-control studies to measure influenza vaccine effectiveness in the EU and EEA Member States (July 2009)” (http://ecdc.europa.eu/en/healthtopics/H1N1/vaccines/Pages/vaccine_effectiveness.aspx), where a pandemic influenza case is defined as an ILI case with a respiratory sample positive for A(H1N1)v and a patient is considered vaccinated when a first vaccine dose has been received more than 14 days prior to the event; a patient having received the vaccine in less that 15 days prior onset of symptoms is considered unvaccinated. Therefore, the data in Table 3, although useful from a descriptive point of view, are not relevant for the study of effectiveness. Only those shown in Table 4 are relevant (with the limitation of an inappropriate time window) but do not allow to measure effectiveness of A-H1N1 vaccine.<br>It does not seem appropriate in Table 4 to pool data for seasonal and pandemic A/H1N1 vaccines. |
| **RESULTS & CONCLUSIONS** | Due to low vaccination prevalence, it is felt the authors have not been able to answer the question, but this is not a problem of methodology, except the concern about the outcome definition explained above.<br>Comments regarding results:<br>- Table 1: if the the percentage of vaccinated children in Italy was about 4%, the results of exposure prevalence for the four conditions ranging from 13% to 28% could be seen as worrisome. Are there |

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| | any data on the percentage of vaccination in all hospitalised children of the same age? This (apparent?) discrepancy should be discussed. It does not provide evidence for a positive benefit-risk profile of the vaccine.<br>- Table 3: an OR>1 means that vaccine increase the risk of ILI and cannot be interpreted in terms of effectiveness. Adjusted OR of 1.3 to 2.1 rather indicate a risk of ILI being an adverse reaction of the vaccine (given the time window chosen for exposure). This is also suggested by the fact that only 11 of the 244 (4.5%) ILI cases seem to have tested positive to A/H1N1 virus.<br>- Table 4: An OR of 0.7 for seasonal vaccines means an effectiveness of 30%. This result could be discussed in light of other published results, considered this figure might be underestimated as vaccines administered shortly before the onset of symptoms may have not been protective yet.<br>It might be useful to also use the control group used for Table 3.<br><br>The authors appropriately discuss the limitations of their findings, but I suggest they should explain the difficulties for conducting an effectiveness study in the situation of this pandemic (short pandemic duration, vaccine available late, small vaccine exposure), which resulted in the inability to document the benefit-risk profile of the vaccines.<br>The comments above should be considered. The main result published by I-MOVE (reference 17) could be presented. |

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| **REVIEWER** | ***Professor Angus Nicoll CBE***<br>Senior Expert - Influenza Coordination<br>European Centre for Disease Prevention and Control (ECDC)<br>Tomtebodavagen 11A (this line not needed for posted material)<br>17183, Stockholm, SWEDEN<br>angus.nicoll@ecdc.europa.eu www.ecdc.europa.eu |
| **REVIEW RETURNED** | 02-Jun-2011 |

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| **THE STUDY** | This is a study which was well intended but was undermined simply by the low use of pandemic vaccination in the country concerned. As the authors recognise this was estimated to be low generally in the country (<5%) and especially low in children (<2%). See http://ecdc.europa.eu/en/ESCAIDE/ESCAIDE%20Presentations%20library/ESCAIDE2010_Late_Breakers_Mereckiene.pdf<br>Hence the study is simply underpowered to test its hypotheses.<br>One oddity in the statistical analysis in that it states that because there were no confirmed influenza cases that had been vaccinated they have had to focus on non-specific outcomes - influenza like illness. It would be possible to use what is known as the 'screening method' and estimate the likelihood of none of the eleven children with confirmed influenza having been immunised with the pandemic vaccine. It would not give a statistically significant result but it could be done |
| **RESULTS & CONCLUSIONS** | Through no fault of the authors this study ended up being simply underpowered<br><br>The statement that the result provide further evidence of a positive benefit-risk profile is unsupported by the evidence. |

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| **REVIEWER** | ***Bruno Christian Ciancio***<br>Head Epidemiological Methods Section, SRS |

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| | European Centre for Disease Prevention and Control, Sweden<br>I have no conflicts of interest to declare |
| **REVIEW RETURNED** | 03-Jun-2011 |

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| **RESULTS & CONCLUSIONS** | The study lacks the power to draw firm conclusions on the safety and effectiveness of pandemic vaccines. This was a problem that affected almost all vaccines evaluation studies during the 2009 pandemic because vaccination campaigns started when the virus was already circulating and because very low vaccination coverages were achiemed in many countries. The study represents a good integrated model for conducting evaluations of safety and effectiveness of vaccines in children. |
| **GENERAL COMMENTS** | General comment<br>An active surveillance system based on a network of large reference pediatric hospitals and running for several years, represents an excellent setting for performing evaluations of vaccines safety and effectiveness. The submitted article describes strenghts and limitations that such a system experienced while attempting to estimate effectiveness and safety of the pandemic vaccines used in Italy during the 2009 H1N1 influenza pandemic. Whereas there is value in presenting the system and the methodology employed during the pandemic as a model that can be used in future pandemics and similar events, the authors should avoid overinterpretation of their findings given the small sample size. As also mentioned in this manuscript, the evaluation of safety and effectiveness of 2009 pandemic vaccines was very challenging due to late start of vaccination campaigns and low vaccination coverages. As results only a few studies have been published on this and mostly based on very large multicentic international collaborations. The submitted article lacks the power to draw conclusion on the risk benefit evaluation of pandemic vaccines. I would suggest that a revised version of the manuscript is submitted with less enphasys on the results but focussing on the system and its characteristics. Results of the pandemic study should be presented as an example of the type of analyses that can be performed and the ways to deal with possibles biases and confounders in evaluating safety and effectiveness of influenza vaccines in children.<br><br>Specific comments<br>In the Introduction or at the beginning of the Results chapters, the authors should report a short overview of the epidemiology of H1N1 in the 2009/10 season. A graph describing the weekly incidence of ILI and indicating the date when the vaccination campaign started could help clarify the major limitation on the study i.e. the recruitment of vaccinated children.<br>When describing the objective of the study at the end of the Introduction, it should be specified that the outcome was ILI requiring hospitalisation.<br>In the description of the recruitment process it is not very clear whether the systematic approach to recruit patients during certain days of the week applies to the entire study population (all ILI patients) or only to those undergoing laboratory confirmation.<br>The authors should provide more details on the ascertainment of the vaccination status of the study population. Was this information collected by direct interviews? Were parents involved? Was it possible to validate this information through other sources (GP records?). Ascertaiment of vaccination status through interviews only could have led to misclassification of some cases especially in |

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| | the context of the 2009 pandemic when two influenza vaccines were used.<br>During the 2009 pandemic, the probability of being vaccinated increased with time and the risk of being infected decreased with time. Therefore time was a strong confounder in many vaccine effectiveness studies. Is it possible in the design proposed by the authors of this article to take time into account in the analysis? In addition it would be important to restrict the vaccine effectiveness analysis to the periods of influenza circulation. Extending the recruitment to periods when there was limited or no flu circulation means including a greater proportion of vaccinated ILI that are not flu, thus underestimating vaccine effectiveness. In the test negative design extending the recruitment to periods of no flu circulation means increasing artificially the number of vaccinated controls thus inflating vaccine effectiveness.<br>The fact that out of 244 ILI patients only 35 were swabed for laboratory confimation should prompt a revision of the recruitment process in future seasons to ensure an adequate number of subjects for the test-negative analysis. |

**VERSION 1 – AUTHOR RESPONSE**

Referee: Xavier Kurz

One of the outcome measures for effectiveness of the pandemic vaccines was non-laboratory confirmed influenza-like illness, with an exposure time window any time before admission. This outcome does not allow to differentiate between ILI as a (known and expected) adverse reaction of pandemic vaccines and ILI as a possible A/H1N1 influenza disease. This definition of the outcome is not in line with the recommendations from the ECDC “Protocols for case-control studies to measure influenza vaccine effectiveness in the EU and EEA Member States (July 2009)” (http://ecdc.europa.eu/en/healthtopics/H1N1/vaccines/Pages/vaccine_effectiveness.aspx), where a pandemic influenza case is defined as an ILI case with a respiratory sample positive for A(H1N1)v and a patient is considered vaccinated when a first vaccine dose has been received more than 14 days prior to the event; a patient having received the vaccine in less that 15 days prior onset of symptoms is considered unvaccinated. Therefore, the data in Table 3, although useful from a descriptive point of view, are not relevant for the study of effectiveness. Only those shown in Table 4 are relevant (with the limitation of an inappropriate time window) but do not allow to measure effectiveness of A-H1N1 vaccine.

It does not seem appropriate in Table 4 to pool data for seasonal and pandemic A/H1N1 vaccines.

Due to low vaccination prevalence, it is felt the authors have not been able to answer the question, but this is not a problem of methodology, except the concern about the outcome definition explained above.

Response 1

We decided to focus on both more specific and non-specific definition of ILI since both outcomes were considered of interest in evaluating the effectiveness of the influenza vaccines [Jefferson 2010].

In order to better explain our outcome definition we modified a paragraph in the Methods section: “Assessment via laboratory test for influenza A-H1N1 virus was not an inclusion requirement. Both clinically defined, and laboratory confirmed, hospitalisations for ILI were considered of interest in evaluating the effectiveness of the influenza vaccines [Jefferson 2010]. Given the non-interventional nature of the study design, we had to rely on the usual practice of the participating hospitals. Moreover, following a recommendation of the Italian Ministry of Health, laboratory confirmation of A-H1N1 virus was not routinely suggested after the declining phase of the epidemic (mid December

2009). In order to limit selection bias (i.e. selective enrolment of vaccinated children) participating centres were given the indication to enrol ILI cases on a given day of the week, up to 3-4 cases per week, blindly with regard to the vaccination status. This recruitment strategy applied to all ILI cases and was independent from the decision to perform laboratory confirmation."

With regard to the time interval between vaccination and the hospitalisations for ILI we specified, in the Results section: "All immunised children (both cases of ILI and controls) had received the influenza vaccines more than 14 days prior to the hospitalisation."

Comments regarding results:
- Table 1: if the percentage of vaccinated children in Italy was about 4%, the results of exposure prevalence for the four conditions ranging from 13% to 28% could be seen as worrisome. Are there any data on the percentage of vaccination in all hospitalised children of the same age? This (apparent?) discrepancy should be discussed. It does not provide evidence for a positive benefit-risk profile of the vaccine.

Response 2
A footnote was added on table 1 to clarify this point: "All vaccines administered to the study subjects, and not only influenza ones, are included".

- Table 3: an OR>1 means that vaccine increase the risk of ILI and cannot be interpreted in terms of effectiveness. Adjusted OR of 1.3 to 2.1 rather indicate a risk of ILI being an adverse reaction of the vaccine (given the time window chosen for exposure). This is also suggested by the fact that only 11 of the 244 (4.5%) ILI cases seem to have tested positive to A/H1N1 virus.
- Table 4: An OR of 0.7 for seasonal vaccines means an effectiveness of 30%. This result could be discussed in light of other published results, considered this figure might be underestimated as vaccines administered shortly before the onset of symptoms may have not been protective yet.
It might be useful to also use the control group used for Table 3.

Response 3
We clarified, in the above reported answers, the reasons for including both clinically and laboratory confirmed cases of ILI. Moreover, we pointed out in the Discussion section of the manuscript that the increase in the OR of ILI among immunised children should be attributed, in coherence with the literature, to a strong confounding effect (see fourth paragraph of the discussion section).

The authors appropriately discuss the limitations of their findings, but I suggest they should explain the difficulties for conducting an effectiveness study in the situation of this pandemic (short pandemic duration, vaccine available late, small vaccine exposure), which resulted in the inability to document the benefit-risk profile of the vaccines.
The comments above should be considered. The main result published by I-MOVE (reference 17) could be presented.

Response 4
The results of the I-Move study were commented in the Discussion section and the following sentence was added: "These difficulties were observed in other European Countries. A very low level of vaccination was found, for instance, in the multinational study "Influenza monitoring vaccine effectiveness in Europe (I-Move)", [14] which was a practitioner-based outpatient surveillance conducted in seven Countries. Five of the seven Countries were not able to contribute with more than 1 vaccinated patient with confirmed A-H1N1 influenza. Only the pooled analysis, derived from the

international collaboration, was able to estimate the effectiveness."

Reviewer: Angus Nicoll
This is a study which was well intended but was undermined simply by the low use of pandemic vaccination in the country concerned. As the authors recognise this was estimated to be low generally in the country (<5%) and especially low in children (<2%). See http://ecdc.europa.eu/en/ESCAIDE/ESCAIDE%20Presentations%20library/ESCAIDE2010_Late_Breakers_Mereckiene.pdf
Hence the study is simply underpowered to test its hypotheses.
One oddity in the statistical analysis in that it states that because there were no confirmed influenza cases that had been vaccinated they have had to focus on non-specific outcomes - influenza like illness. It would be possible to use what is known as the 'screening method' and estimate the likelihood of none of the eleven children with confirmed influenza having been immunised with the pandemic vaccine. It would not give a statistically significant result but it could be done

Response 5
Using Fisher exact probability test we calculated the probability of observing no immunised children among the 11 laboratory confirmed cases; we modified the last sentence of the Results section accordingly: "Among the 35 children who underwent the laboratory testing, 11 resulted positive and 24 negative for the A-H1N1 virus. Since none of the A-H1N1 positive children had a positive history for A-H1N1vaccination it was not possible to obtain an estimate of the vaccine effectiveness. Given the prevalence of exposure among controls, the likelihood of none of the 11 children with confirmed influenza having been immunised with the pandemic vaccine was 0.35. The OR of ILI associated with any influenza vaccination was slightly lower than one (OR=0.9; 95% CI 0.1-5.5)."

Though no fault of the authors this study ended up being simply underpowered
The statement that the result provide further evidence of a positive benefit-risk profile is unsupported by the evidence.

Response 6
We modified the sentence in the Discussion section according to the suggestion of the referee: "Despite the limitations, our study provided additional information on the benefit-risk profile of the pandemic vaccine."

Reviewer: Bruno Christian Ciancio
The study lacks the power to draw firm conclusions on the safety and effectiveness of pandemic vaccines. This was a problem that affected almost all vaccines evaluation studies during the 2009 pandemic because vaccination campaigns started when the virus was already circulating and because very low vaccination coverages were achieved in many countries. The study represents a good integrated model for conducting evaluations of safety and effectiveness of vaccines in children.

General comment
An active surveillance system based on a network of large reference pediatric hospitals and running for several years, represents an excellent setting for performing evaluations of vaccines safety and effectiveness. The submitted article describes strenghts and limitations that such a system experienced while attempting to estimate effectiveness and safety of the pandemic vaccines used in Italy during the 2009 H1N1 influenza pandemic. Whereas there is value in presenting the system and the methodology employed during the pandemic as a model that can be used in future pandemics and similar events, the authors should avoid overinterpretation of their findings given the small sample

size. As also mentioned in this manuscript, the evaluation of safety and effectiveness of 2009 pandemic vaccines was very challenging due to late start of vaccination campaigns and low vaccination coverages. As results only a few studies have been published on this and mostly based on very large multicentic international collaborations.

Response 7
The reference to the difficulties faced by the studies aimed at evaluating the benefit-risk profile of the pandemic vaccine is already reported as an answer to the first Referee (please, see the above reported sentence related to the I-Move study).

The submitted article lacks the power to draw conclusion on the risk benefit evaluation of pandemic vaccines. I would suggest that a revised version of the manuscript is submitted with less enphasys on the results but focussing on the system and its characteristics. Results of the pandemic study should be presented as an example of the type of analyses that can be performed and the ways to deal with possible biases and confounders in evaluating safety and effectiveness of influenza vaccines in children.

Response 8
To deal with the suggestion, a sentence was added in the Discussion section: “One of the main results of this experience was to test the usefulness of an integrated model for conducting evaluations of the benefit-risk profile of vaccines in children.”

Specific comments
In the Introduction or at the beginning of the Results chapters, the authors should report a short overview of the epidemiology of H1N1 in the 2009/10 season. A graph describing the weekly incidence of ILI and indicating the date when the vaccination campaign started could help clarify the major limitation on the study i.e. the recruitment of vaccinated children.
When describing the objective of the study at the end of the Introduction, it should be specified that the outcome was ILI requiring hospitalisation.

Response 9
We added, in the revised version, a Figure describing the epidemic curve of the pandemic influenza in Italy, together with the starting point of the vaccination campaign.

We specified, in the last paragraph of the Background section, that the study focused on children with ILI requiring hospitalisation: “The purpose of this study was to assess effectiveness of the influenza A-H1N1 vaccination in the paediatric population in preventing the occurrence of Influenza Like Illnesses (ILI) requiring hospitalisation. Moreover, we assessed the safety of the vaccine, in particular by evaluating all the Adverse Events of Special Interest (AESI) reported in the exposed population.”

In the description of the recruitment process it is not very clear whether the systematic approach to recruit patients during certain days of the week applies to the entire study population (all ILI patients) or only to those undergoing laboratory confirmation.

Response 10
To better explain this point, we modified the second paragraph of the Methods section (this paragraph also includes one of the answers to Referee 1): “We decided to focus on both more specific and non-specific definition of ILI since both outcomes were considered of interest in evaluating the effectiveness of the influenza vaccines [Jefferson 2010]. Given the non-interventional nature of the

study design, we had to rely on the usual practice of the participating hospitals. Moreover, following a recommendation of the Italian Ministry of Health, laboratory confirmation of A-H1N1 virus was not routinely suggested after the declining phase of the epidemic (mid December 2009). In order to limit selection bias (i.e. selective enrolment of vaccinated children) participating centres were given the indication to enrol ILI cases on a given day of the week, up to 3-4 cases per week, blindly with regard to the vaccination status. This recruitment strategy applied to all ILI cases and was independent from the decision to perform laboratory confirmation."

The authors should provide more details on the ascertainment of the vaccination status of the study population. Was this information collected by direct interviews? Were parents involved? Was it possible to validate this information through other sources (GP records?). Ascertainment of vaccination status through interviews only could have led to misclassification of some cases especially in the context of the 2009 pandemic when two influenza vaccines were used.

Response 11
We specified this point by adding a sentence in the first paragraph of the "Data source" in Methods section: "The interview was aimed at acquiring the anamnesis and at ascertaining drug use and vaccination status. As reported in the study protocol, no validation of the information on drug and vaccine exposure was conducted."

During the 2009 pandemic, the probability of being vaccinated increased with time and the risk of being infected decreased with time. Therefore time was a strong confounder in many vaccine effectiveness studies. Is it possible in the design proposed by the authors of this article to take time into account in the analysis? In addition it would be important to restrict the vaccine effectiveness analysis to the periods of influenza circulation.
Extending the recruitment to periods when there was limited or no flu circulation means including a greater proportion of vaccinated ILI that are not flu, thus underestimating vaccine effectiveness. In the test negative design extending the recruitment to periods of no flu circulation means increasing artificially the number of vaccinated controls thus inflating vaccine effectiveness.
The fact that out of 244 ILI patients only 35 were swabed for laboratory confirmation should prompt a revision of the recruitment process in future seasons to ensure an adequate number of subjects for the test-negative analysis.

Response 12
A sentence was added in the Result section: "No difference in the OR estimates was observed when the analysis was restricted to the pandemic period (i.e., October 2009 – January 2010)."

**VERSION 2 - REVIEW**

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| **REVIEWER** | ***Xavier Kurz*** |
| **REVIEW RETURNED** | 17-Jun-2011 |

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| **GENERAL COMMENTS** | I thank the authors for their responses and I agree with the main conclusions of the article, which has been much improved.<br>I still have some minor comments:<br>- Page 6: A paragraph on the recruitment procedure has been added. Lines 20 to 24 state that, in order to limit selection criteria (i.e. selective enrolment of vaccinated children), participating centres were given the indication to enrol ILI cases on a given day of the week, up to 3-4 cases per week, blindly with regard to the vaccination status. Could the authors clarify what means "up to 3-4 |

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| | cases per week" ? Selection bias would be avoided only if all ILI cases were included. How were these 3 to 4 cases per week chosen? Were they consecutive cases?<br><br>- Table 1 includes the percentages of children with different conditions exposed to any vaccine administred. It would be useful to add a column with the % of children exposed to the influenza A/H1N1 vaccine.<br><br>- I agree that the study provides additional information on the benefit-risk profile of the H1N1 vaccine. However, the suggestion (page 9, line 27) that the OR of 0.9 may be intereted as a sign of a beneficial effect of the vaccination is not supported. Besides the large confidence interval, an OR of 0.9 means an effectiveness of 10% only.<br><br>- Editorial comments:<br>page 6,line 44: vaccine effectiveness<br>page 9,line 9: a word is missing; "was derived" ? |

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| **REVIEWER** | ***Bruno Christian Ciancio*** |
| **REVIEW RETURNED** | 06-Jul-2011 |

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| **GENERAL COMMENTS** | Reviewer completed checklist only. No further comments were made |

**VERSION 2 – AUTHOR RESPONSE**

Dear Mr. Sands,

First of all I would like to thank you for the decision to accept our manuscript for publication. Please find attached the revised version which includes the revisions suggested by the reviewer (we used the track changes mode to highlight the modified sentences). In the following we specify how we dealt with the reviewer's comments.

With regard to the first comment made by Dr. Xavier Kurz, we would like to clarify that the decision to limit the enrolment to one day per week, up to 3-4 consecutive ILI cases, was taken under the assumption of an expected large number of ILI hospitalizations during the pandemia. This decision was stated in the study protocol and agreed with all participating centres. We consequently specified in the methods section that the enrolment procedure was based on "consecutive" cases.

With regard to the second comment, the following sentence was added in the footnote (as an alternative to adding further columns) of Table 1: "... 25 children exposed to the pandemic vaccine were distributed as follows: 6 cases (2.5%) were hospitalized for neurological disorders; 4 (2.8%) for muco-cutaneous diseases and vasculitis; 2 (4.3%) for gastroduodenal lesions; and 13 (5.3%) for ILI."

With regard to the third comment, we agreed with the suggestion and we substituted the mentioned sentence with the following one: "To support the assumption of a beneficial effect of the pandemic vaccination, our findings need to be corroborated by those of similar studies."

Also on behalf of my co-authors I wish to thank the reviewers for the comments that were made both on the first and second version of the manuscript.

Please do not hesitate to contact me for any possible clarification.

Sincerely,
Francesca Menniti-Ippolito