Author's response to reviews

Title: Decline in temperature and humidity increases the occurrence of influenza in cold climate

Authors:

Kari Jaakkola (kari.jaakkola@mil.fi)
Annika Saukkoriipi (annika.saukkoriipi@thl.fi)
Jari Jokelainen (jari.jokelainen@oulu.fi)
Raija Juvonen (raija.juvonen@kainuu.fi)
Jaana Kauppila (jaana.kauppila@ppshp.fi)
Olli Vainio (olli.vainio@oulu.fi)
Thedi Ziegler (thedi.ziegler@thl.fi)
Esa Rönkkö (esa.ronkko@thl.fi)
Jouni JK Jaakkola (jouni.jaakkola@oulu.fi)
Tiina M Ikäheimo (tiina.ikaheimo@oulu.fi)

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Author's response to reviews:

Environmental Health
Editorial Office
COVER LETTER
Please find enclosed our revised manuscript entitled: “DECLINE IN TEMPERATURE AND HUMIDITY INCREASE THE OCCURRENCE OF INFLUENZA IN COLD CLIMATE” by Kari Jaakkola, Annika Saukkoriipi, Jari Jokelainen, Raija Juvonen, Jaana Kauppila, Olli Vainio,
Thedi Ziegler, Esa Rönkkö, Jouni JK Jaakkola, Tiina M Ikäheimo and the KIAS-Study Group
We appreciate the comments from the reviewers and have addressed the issues raised by them. Please find our point-to-point response below. In addition, we have modified the manuscript according to the guidelines provided by the Editorial office of Environmental Health and the journal's instructions for style. As a result of these revisions we feel that the manuscript has improved further. I would appreciate if you could consider this work to be published in Environmental Health. Looking forward to
hearing from you at your earliest convenience. On behalf of the co-authors, Oulu, Finland 25.02.2014

Tiina M Ikäheimo, Ph.D., Adjunct professor
University of Oulu; Finland, Center for Environmental and Respiratory Health Research
and the Institute of Health Sciences (corresponding author)
tiina.ikaheimo@oulu.fi

Responses to the issues raised by the editorial office and reviewers

Editorial Office:

As for the formatting, remove the running title and the word count. Only the first letter of the first word of the title should be capitalized except for proper nouns in the title. Remove the existing corresponding author information and replace with §Corresponding author: placing the § after his/her superscript number(s). Insert the heading Email addresses and underneath list as author's initials:email address e.g. JS:joe.shmoe@university.edu. Only the first letter of the headings should be capitalized. The first section should have the heading Background. Italicized and bolded text should be changed to normal except where indicated in the References. The abbreviation in the List of abbreviations section should be formatted as abbreviation: term separating the pairs with semi-colons in sentence format. The wording for the Competing interests section can be found in the Instructions for authors. In the References, list up to 30 authors before using et al, and use full page references e.g. #1, 938-943. Please also ensure that your revised manuscript conforms to the journal style (http://www.ehjournal.net/info/instructions/ ). It is important that your files are correctly formatted.

- We have followed the instructions and made the abovementioned corrections.

Reviewer 1

Fig2. The X-axis should be labeled "Clinic Day" to indicate what -10 to 7 correspond to.

- For figure 2 we have clarified the figure legend in the following way: “Mean daily temperature (°C) and absolute humidity (g/m³) during the hazard and reference periods starting 7 days before and after the beginning of the hazard period.
Reviewer 2

The timely communication attempts to address a currently popular question of how the onset of flu might relate to meteorological conditions. Authors rightfully noted the lack of a rigor analysis of factors associated with the flu onset in cold climates. They are exploring this question in unique settings of military training of healthy young recruits.

Authors employed a case-crossover design to test whether “the risk of influenza A and B virus infection increases with decreased daily temperature and humidity during the 3 days preceding the onset of disease.” They concluded that 1°C decrease in ambient temperature (AT) and 0.5 g decrease per m3 in absolute humidity (AH) increased the estimated risk by 11% and 58%, respectively.

With presented data, model and results I would derive one observation: for a presented study period in a specific population a single flu outbreak peaked when ambient temperature was about to drop to its minimum level. The modeling considerations ignore person-to-person transmission highly plausible for flu. I am not convinced that presented results are reproducible, as they are based on a single flu season. It is also not clear whether 66 cases distributed over ~20 days warrant statistical power to detect the effect of temperature variations to claim generalizability of results. However, a study of the proposed design with multiple flu seasons in multiple sites might provide an insight for the stated hypothesis.

We concur with the reviewer that having several cold periods would have strengthened our findings and have indicated this in the study limitations (“Yet, larger samples from equal climatic conditions and followup of multiple influenza seasons would be useful to strengthen our findings”. Given the gap in knowledge our results provided a good starting point and a priori hypothesis for further studies.” p. 14). Our analyses are based on 28 separate hazard periods and, we utilized meteorological information from 84 days. In terms of generalizability we have aimed to soften our conclusions taken into account the restricted sample.
It is correct that a larger sample would strengthen the evidence for the observed association between temperature, AH and influenza. However, our findings are supported by a larger sample of diagnosed upper and lower respiratory tract infections from the same population which demonstrated that RTI's in overall were clustered to ambient temperatures at or slightly below zero (Mäkinen et al. 2009). In addition, results from an experimental animal model are consistent with our findings (Lowen et al. 2007, indicated in the discussion).

According to our hypothesis we assumed that low temperature and humidity in combination with physical exercise would predispose subjects to an Influenza infection. This hypothesis is supported by questionnaire information where 69% of those receiving influenza (45/65) reported outdoor training, 76% (33/43) physical exercise and 72% (31/43) feeling cold during the previous three days (added this information to the methods, p. 6: “They were also asked to fill in a questionnaire assessing outdoor training and symptoms during the three preceding days” and to the results, p. 8: “69% (45/65) reported outdoor training, 76% (33/43) physical exercise and 72% (31/43) feeling cold during the previous three days”).

It is correct that our analyses do not take into account the person-to-person transmission which is likely to occur in a confined environment, such as during military training and would facilitate the spread of the disease. We have included this as a study limitation (p. 14). However, this type of crowding tends to occur during wintertime in many other institutions as well.

Major Compulsory Revisions
1. Based on the presented results, authors’ concluding statement “that a decrease rather than low temperature and humidity per se during the preceding three days increase the risk of influenza episodes in a cold climate” contradicts their own findings: “Our novel finding was that influenza risk is reduced at very low
temperatures as judged by the higher mean temperature for the hazard (-6.75°C) compared with the reference periods (-7.51 and -9.17°C).” It would be helpful to explain how heavily overlapping confidence intervals, say for AT [-17.736, 4.216] in hazard period and [-20.8516, 2.5116] in the post period can produce significant results at <0.001 and n=66. This requires re-thinking and re-phrasing.

· One of our main finding is that a decrease in temperature and AH (max change) involve an increased risk for influenza. However, at the same time the association between meteorological parameters and influenza is positive which indicates that the risk of influenza is lower at very cold temperatures or low humidity. Rather than referring to the information in Table 1, we now justify our observation by the fact that a majority (74%) of the flu infections occurred at a temperature range of +5 to -10°C (and 38% between +5°C to -5°C) which represents a rather typical, but not extremely cold condition. Furthermore, our previously published article from the same source population showed that upper and lower respiratory tract infections occurred commonly between 0 to -5°C (Mäkinen et al. 2009). We have added this information to the discussion (p. 10) and removed the text referring to Table 1.

· At the same time we calculated the CI’s for the hazards and reference period (see Table attached in the end of the document). Based on our examination the point estimate is overlapping, depending on the parameters, during one of the reference periods but not the other one. To our opinion this partial overlapping does not mean that no significant differences between the groups exist as 95% CI’s provide conservative results. We have also carefully examined our GLM Procedure and provided the correct significance levels for the different parameters. No differences in means were detected for mean AH or the level from where it declined. However, for the rest of the parameters we detected significant differences between the hazard and reference periods (Table 1). If the reviewer
considers this table more appropriate we would be happy to use this instead.

· After careful revision we detected two typos for the temperature and AH levels before they declined which we have now corrected to Table 1. However, the appropriate values were utilized in the analyses.

2. I found a few methodological aspects related to: a) study period, b) role of reference periods, and c) model outcomes confusing.

a. The Method section states the study period from June 2004 to June 2005. Figure 1 show 12 month time period starting from January (presumably 2005?). Both time frames are somewhat irrelevant for the analysis, where the study periods are restricted to one week before the first case and one week after the last case. Furthermore, for the 66 cases considered for the analysis, only a fixed number of days weighted based on the flu incidence are relevant for the main model. Based on Figure 1 there were about 20 days with at least one episode of flu; this implies that the conclusions are based on ~60 daily meteorological measurements. This issue is likely to confuse the readers.

· Thank you for this comment. The correct study period was from July 2004 to December 2005 which is now changed in the manuscript (p. 5). This included the follow-up of two intake groups completing their military service. For clarity reasons we have now redrawn the figure to include the correct follow-up period (see revised figure 1).

· For clarity reasons we have added the following sentence to the results section: “As the total number of days with episodes was 28, we utilized meteorological information from 84 days” (p. 8).

· In Figure 1 we wanted to demonstrate the remarkable variation in temperature and humidity during the study period, and on the other hand, the clustering of influenza cases to the coldest months.

b. Table 1 illustrates the following question: is the average temperature (humidity, and their derivatives) in 3 days preceding the doctor visits differ from the average temperature (humidity, and their derivatives) observed in two
other periods, 3d pre and 3d post. It is not clear how the reference periods were considered in the conditional logistic regression model.

- Table 1 describes the meteorological conditions during the hazard and reference periods which demonstrate slight differences in means between these conditions. We have described the calculation of the explanatory variables in the methods section both for the hazard and reference periods (exposure assessment, p. 7).

- The conditional regression model compares the hazard period with both of the reference periods. Although, some random variation occur in the weather a symmetrical selection of two reference periods closely before and after the hazard period controls for temporal and seasonal confounding (Bateson & Schwartz 1999 Epidemiol, Janes et al. 2005 Epidemiol).

- The procedure for forming an outcome for the logistic regression model has to be specified. It would be valuable to explain how considerations time progression, high probability for person-to-person transmission, and non-zero probability for re-infection are taken into account. It would be also helpful to show the incidence rate for flu A and B in relationship to AT, AH and their derivatives.

- In the case-crossover design the starting point is the identification of the cases of interest and the time of onset for the cases. The scientific inference is based on the comparison of the determinant phenomenon; here temperature levels between the so called hazard period and selected reference period(s). Any difference in temperature profile (level or change) between hazard and reference periods indicates causal effect (after consideration of confounding). The cases were systematically identified from the cohorts of conscript over the study period (time of their military service).

- In principle we could try to identify subcategories of cases for example on the basis of suspected reinfection, but given the number of cases the feasibility of using subcategories is quite low. Therefore we
need to consider the cases as a singular entity.

- In principle time progression could be analyzed by using calendar time as an effect modifier.

Unfortunately the amount of data is not sufficient.

- Non-zero probability of reinfection. It is probable that a majority of individuals were protected against reinfection during the influenza period of winter 2005. However, reinfection is sometimes possible even with the same strain pending that the individuals have a delayed or deficient humoral immune response (Camacho et al. Proc. R. Soc. B. 2011). Our data included two subjects that were infected twice during the follow-up. We cannot evaluate the role of reinfection in the study (included to the limitations), but consider that its influence on our results is not very significant.

- Unfortunately we did not have a possibility to analyze person-to-person transmission which is probably high in the military setting. This was included as a limitation (p. 14).

- According to the suggestion of the reviewer we have now calculated incidence rates for Influenza A (105/1000 person years) and B (16.5/1000 person years) separately which demonstrate high incidence of flu in our study population (information added to the results, p. 8). Modeling influenza A and B separately against temperature and AH is unfortunately not meaningful as this would reduce the number of observations substantially and it would not be possible to obtain accurate and reliable statistical estimates.

2. The modeling procedure is lacking details; therefore, in the absence of model's specification I am not convinced that the results presented in Table 2 and Figure 2 address the stated hypothesis.

- Conditional logistic regression is used to investigate the relationship between an outcome and a set of prognostic factors in matched case-crossover studies. In our case the used model consisted of comparing the environmental conditions between the hazard period and the two reference periods. Each hazard
period was matched for the two corresponding reference periods. The PHREG procedure was applied using the discrete logistic model and forming a stratum for each matched set. Thus the odds ratios represented the risk of the onset of influenza in relation to the level or change of temperature/relative humidity (included to the statistical analyses, p. 8).

4. It is necessary to clarify if separate models were executed for each explanatory variable or if some form of a parsimonious model with multiple explanatory variables was developed.

- We conducted separate models for each explanatory variable (mean temperature and AH, max change in temperature and AH)

5. The ambient temperature (AT) and absolute humidity (AH) are highly correlated and in many instances relative humidity was used for predicting flu. Thus, an explanation for selecting these two very similar meteorological characteristics would be helpful.

- Thank you for this comment. We recognize the existing dependence between temperature and humidity (e.g. the amount of water vapor in air is dependent on the pressure and temperature of the gas). Due to this we have avoided distinguishing these physical quantities and discussing their separate roles. We have also stated that they are interconnected in the text (discussion p.12 and 13).

- We have consulted our collaborators at the Finnish Meteorological Institute for the appropriate meteorological variables. Absolute humidity (AH) is a valid humidity measure to use in cold climates, as it reflects the actual water content of air both for outdoor and indoor conditions (as opposed to RH which may vary considerably being relatively high outdoors, despite of the low amount of water in air, but becoming significantly reduced indoors where the temperature is higher).

- AH describes the features of the air mass in a similar manner as the mass-based parameters (specific humidity, dew point temperature). Hence, this suggests that using an alternative humidity parameter would likely not change our main conclusions.

6. With respect to Figure 2, authors stated: “Of note, the temperature and AH levels before they declined were also higher during the hazard compared with the reference periods (p<0.001, Fig. 2).” I found this statement confusing. The figure indicates that within the hazard period the average temperature changes by 5 degrees from day -3 to day 0, which cause doubts for model’s sensitivity.

· We have removed the confusing statement. Here our purpose was to indicate that the slightly higher temperature observed during the hazard period could possibly improve virus transmission. Then, combined with a sudden decrease in temperature and AH (and possible further cooling of the subjects due to the combined effects of cold and physical exercise), this would lead to an actual infection and seeking of medical care.

· For maximum change in temperature and AH we adjusted for the initial level from where the decrease started in our model (included to statistical analyses, p. 8). This could have occurred at any stage within the three day period.
Minor Essential Revisions

7. In Table 2 superscripts 1 and 3 need to be clarified.
   · Superscript 1 denotes comparing means with ANOVA between the hazard and reference periods. The superscript has now been changed to *

8. Figure 1, the right axis, needs clarification whether the numbers reflect both AH and the number of cases. If yes, why a single case is less than 0.
   · The reviewer is correct in that the right axis reflects both AH and the number of flu episodes. Due to technical faults this appeared incorrectly which we have now corrected in our revised figure.

9. Figure 2 requires clarification; each interval contains 4 time points and it is not clear which three points within the intervals were considered for the analysis.
   · For the logistic regression analyses the mean temperature and AH was calculated as an average of the three preceding days (day -3 to 0) of the onset of an influenza infection and similarly for the reference periods 7 days before and after the start of the hazard period. A maximum decline in temperature and AH was calculated as the largest change (maximum versus minimum) occurring in these parameters within the three day period (e.g. day -3 versus day -2, day -3 versus day -1, day -2 versus day -1 etc.) and similarly for the reference periods. We have added this text to the figure legend (Figure 2).