Short title: **Supersaturation in airways and influenza activity in subtropical climate**

Full title: **Positive association of supersaturation effects in the human airways with influenza activity in subtropical climate: influenza seasons in Okinawa (2007-2012) – New method for analyzing and forecasting**

First preliminary results

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Summary

There are many theories of the seasonality of influenza for different climatic zones. But none of the known theories provides a clear explanation, especially for the tropical and subtropical climate.

Here we have originally analyzed the association/connection of activity of seasonal influenza in Okinawa (subtropical zone) with the probability of occurring of supersaturation in the human airways when inhaling environmental air under specific weather conditions.

We have shown for the first time that the effects of supersaturation in the human airways may be associated with main representative peaks of intensity/activity of influenza in Okinawa in the period of observation from Jan 2007 until Dec 2012 including 2009 pandemic.

Our observation is the first one which clearly shows in the practice that the effect of supersaturation in the airways can be used for understanding and forecast the influenza activity in subtropical and tropical zones. Because the effect of supersaturation may lead to an additional risk of acidification of epithelial lining fluid in the local areas of the respiratory tract and to additional risk of deposition of infectious agents from inhaled air in the upper airways.

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Introduction

Glossary

Supersaturation in the airways *— is the effect of a dramatic rise in local relative humidity (RH) inside the airways (RH >100%) due to inhaling cold/cool air. Under these conditions, the effect of the condensational growth of inhaled fine and ultrafine particles/droplets may occur.*

Condensational Growth *— effect of liquefaction of water vapor on fine/ultrafine particles/droplets under supersaturated conditions. Condensational Growth factor for airborne particles is determined by relative humidity in the airways RH>100% (oversaturated and supersaturated conditions). The growth of the fine and ultrafine particles/droplets by condensation is not particularly limited.*

Two distinct types of climatic conditions are associated with seasonal patterns of influenza were observed globally: “cold-dry” type (for temperate climate) and “humid-rainy” type (for tropical and subtropical countries). The main difference consists in the problem of understanding of influence/impact of the humidity and temperature on the seasonality of influenza in different climatic zones. One can read long series of studies and theories describing different kinds of hypotheses explaining the influenza seasonality in different climatic zones, but almost all authors of them agree on the one thing, that nobody has a good reliable theory of influenza seasonality in tropical and subtropical countries nor a single unified theory for all regions (see reviews in (Lipsitch and Viboud, 2009; Moura et al., 2009; Shaman et al., 2011; Tamerius et al., 2013; Ishmatov, 2016b; Kamigaki et al., 2016; Iha et al., 2016; Bjornstad and Viboud, 2016)).

As was mentioned by Marc Lipsitch and Cecile Viboud (2009) (Lipsitch and Viboud, 2009): *“Seasonal variation in the incidence of communicable diseases is among the oldest observations in population biology, dating back at least to ancient Greece, yet our understanding of the mechanisms underlying this phenomenon remains hazy at best.*“

Previously (Ishmatov, 2016a; 2016b; 2017b; 2017a) we have hypothetically shown that under specific climatic conditions which are peculiar to (associated with) the seasonal patterns of influenza (“humid-rainy” and “cold-dry”) the effect of supersaturation and condensational growth in the airways can act as additional common trigger for influenza, common colds and other respiratory infection in both mid-latitudes and in tropical and subtropical zones. We hypothetically shown the effects of supersaturation and condensational growth in the airways may lead to ***additional risk of acidification of epithelial lining fluid*** in the local areas of the respiratory tract, and, as a result, may lead to negative impacts on host cells and weakening of the defense mechanisms of the human

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airways (Ishmatov, 2017a), and moreover, we found this effect ***may lead to additional risk of deposition of infectious agents*** from inhaled air in the upper airways (Ishmatov, 2016b; 2016a; 2017b).

In this study, we aim to characterize the epidemic patterns of influenza and influenza-like illness in the subtropical zone and its association/connection with the effect of supersaturation and condensational growth in the human airways. It is the first preliminary attempt to use the effect of supersaturation in the human airways to analyze seasonal influenza and the 2009 flu pandemic.

We used Influenza surveillance in Okinawa because Okinawa is important for clarifying influenza patterns in both temperate and tropical regions (Iha et al., 2016).

Methods

Meteorological data

Okinawa (South Japan) (26.2˚N latitude, 127.7˚E longitude) is in the subtropical zone (subtropical climates) and consists of nearly 50 inhabited islands in the East China Sea. Most of the population (about 90%) lives on the main island of Okinawa (capital city is Naha).

Daily and hourly climate data including ambient temperature and relative humidity were retrieved from the Wunderground.com — Meteorological website (available from: https://www.wunderground.com/) (Wunderground, 2017) for Naha City from Jan 2007 until Dec 2012. In addition, we carried out the verification of the received data from Wunderground.com with the average daily and weekly climate datasets using datasets of (Kamigaki et al., 2016) and (Iha et al., 2016) and from the Japanese Meteorological Agency website (available from: http://www.jma.go.jp) (Japan, 2017).

Influenza surveillance data

We used influenza surveillance data collected by Iha et al. (Iha et al., 2016): the dataset of number and results of rapid antigen tests (RAT) performed in clinical laboratories of four representative hospitals in Okinawa, Naha City Hospital (470 beds), Okinawa Red Cross Hospital (314 beds), Okinawa Prefectural Nanbu Medical Center (434 beds), and Urasoe General Hospital (311 beds). Cases are pooled for each week from Jan 2007 until Dec 2012.

We also used a dataset of Kamigaki et al.(Kamigaki et al., 2016) on influenza cases in Okinawa in 2010-2012 for additional verification. These data were taken by Kamigaki et al from Okinawa Infectious Disease Information Center ((Okinawa Infectious Disease Information Center Homepage. Available from: http:// www.idsc-okinawa.jp/index.html.)) (Okinawa, 2017).

Evaluation of the intensity of supersaturation effect

The effect of supersaturation in the airways depends simultaneously (at the same time) on both temperature and relative humidity of inhaled air (see the reviews in (Ishmatov, 2016b) and (Jinxiang et al., 2015)). Thus, temperature and humidity are the parameters of one simple function (it is the effect of supersaturation and condensational growth) and here we used this function to analyze the correlation between climatic parameters and cases of influenza.

We used a simple physical and mathematical model from physics of heat-and-mass transfer (Shaviv, 2015) (see the descriptions in the Application1 in the end of the manuscript) to make a preliminary (rough) evaluation of the probability and intensity of the local supersaturation in the airways when mixed the inhaled ambient air and the warm moist air (whose parameters correspond to those inside the airways: RH=99%, T=37C). For these purposes, the psychrometric chart also can be used (Mollier's chart -- it is widely-used as the tool for determining of isobaric psychrometric processes of moist air (Barenbrug, 1974). Here the online resource (available from: http://www.sciencebits.com/ExhaleCondCalc?calc=yes) can be useful (Shaviv, 2015) for additional estimation and verification of our estimation data.

In the present study, we used this model (Shaviv, 2015) only to show a qualitative «picture» of the probability of occurring of the supersaturation effect and its intensity under specific environmental conditions. Of course these calculations cannot be used for to obtain accurate data for the real human respiratory tract (in this case the very complicated numerical calculations and models should be used – see some descriptions in the Application1 in the end of the manuscript). Some results of real measurements and calculations of parameters of supersaturation in the real human airways see in (table 1 in (Ishmatov, 2016b)).

Here the analysis and evaluation of the intensity/strength of the supersaturation effect were made for each single day. For this, we used the averaged parameters of temperature and relative humidity (RH) for each day from Jan 2007 until Dec 2012. Here we used the intensity of supersaturation effect as a probability parameter of an intensity of liquefaction of water vapor and indicate the concentration of liquid water in the mixed air (g/kg).

We additionally analyzed the short-term impacts of supersaturation effect for rainy days or days when it was abrupt changes in climatic parameters (Humidity and Temperature) during these days. Here we analyzed the hourly parameters of temperature and humidity in the periods from 6 am to 11 pm, because at this time the main activity of the population is observed and it is at this time when the influence of weather factors on the organism can be considered. Here we took into account only those days when the rain was strong enough and lasting (more than 1.5-2 hours in a row). In this case, the temperature was taken equal to the temperature at which was raining and the humidity was taken equal to RH=100%. This is a very strong simplification/assumption based on our hypothesis (see PANEL1).

PANEL 1

On the rainy days and supersaturation in the airways (Hypothesis)

Buie et al (Joung et al., 2017) have shown a new mechanism by which rain produces aerosols. The rain droplets, creating a spray at the moment of impact on the surface, this spray consists of much smaller water droplets, or aerosols (size of aerosol ranges from a few microns to a few hundred microns). In the present study, we hypothesized that these “secondary” fine droplets may play a role of condensational centers (lead to the local supersaturation and condensational growth) when these droplets inhaled by individuals.

For a deeper understanding of the effect of condensational growth of inhaled droplets the adaptable model for growth and/or shrinkage of droplets in the respiratory tract during inhalation of aqueous particles (Grasmeijer et al., 2016) is very useful.

As mentioned by Grasmeijer et al. (Grasmeijer et al., 2016) for inhaled cool/cold droplets: “When close to the wall (of the respiratory tract), the relatively cold droplet is quickly subjected to an environment of higher temperature and humidity which promotes condensational growth, resulting in larger droplets. However, droplets farther away from the wall will first evaporate before the heat and moisture from the airway wall will reach these droplets.” Grasmeijer et al. (Grasmeijer et al., 2016) have found the instead of reaching the lower airways, the droplets (about 5 microns) will be deposited higher in the respiratory tract than might be originally intended.

We use this important finding to make a hypothetical assumption that effect of supersaturation in the airways during rainy weather can occur and the main impact of this effect will be on the upper respiratory tract. We assume that such droplets may lead to additional acidification and cooling of epithelial cells of upper respiratory tract.

Here we are based on the fact that the temperature of these droplets below the air temperature inside the respiratory tract (droplets temperature <37C and equal the environmental temperature). Under these conditions (inside the airways), near of these droplets (in the boundary air layer), one should expect that the relative humidity will be

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above 100%. Thus, these droplets can be considered as an additional factor for the effect of condensation growth in the airways.

Thus, taking the humidity condition in rainy days equal to RH=100%, we are trying to show a picture of the possible intensity/strength of the supersaturation effect when condensation of water vapor takes place on the surface of inhaled fine “secondary” rain droplets which were produced by rain droplets. ***But it should be understood that this is a very rough simplification and here the evaluations of this type have a subjective nature. And in the future, a new methodology should be developed for this type of analyses***.

Results and Discussions

Association/connection of representative peaks of influenza with supersaturation

I analyzed the association/connection of the effect of supersaturation in the airways and influenza seasonality in Okinawa (subtropical zone) from Jan 2007 until Dec 2012 (the data are shown in Fig1). I used the numbered labels for indicating the main representative peaks of influenza and influenza-like illness (ILI) cases (Fig 1B and Fig 1C respectively). I connected/correlated and indicated these labels with representative peaks in the daily trends of intensity of the effect of supersaturation (Fig 1A). Practically all labels (main representative peaks of influenza and ILI cases) are associated with main peaks in the daily trends of intensity of the effect of supersaturation (Fig 1A), with the exception of few peaks in summer months (labels “9”, “12” and “18”).

It is important to note, I found practically no connection the intensity of peaks of influenza (number of cases of influenza and ILI) with the intensity of supersaturation in the airways (probability of liquefaction of water vapor; indicates the concentration of liquid water in the mixed air (g/kg)). I found that intensity of peaks of influenza is connected with the trend of critical days when supersaturation in the airways may occur (see the next sections).

Additional analysis with using the daily trends of critical short-term supersaturation intensity (when the supersaturation may occur during a few hours in a day) (Fig 1D) shows full compliance/correlation of the representative peaks of influenza cases with supersaturation effect. Here (Fig 1D) we found that labels always are correlated with some kind of “stair” on the blue trend line in the (Fig 1D). We do not know why the next peak of the flu outbreak should be preceded by a flat section on the graph (Fig 1D). Here we can only judge by the observed pattern. And it is important to understand that Fig 1D shows preliminary results of our evaluations based on our hypothesis (see above PANEL 1). And the “Stairs” on the line trend also shows the frequency of rainy days. Nevertheless, this aspect/issue (the aspect of supersaturation in the airways during rainy hours) should be investigated in the future.

***Thus**, days when the main peaks of influenza cases were registered in Okinawa it was those days when probability of occurring of effect supersaturation in the airways was high. During these days the effects of supersaturation in the airways could lead to an additional risk of acidification of epithelial lining fluid in the local areas of the respiratory tract (Ishmatov, 2017a) and to additional risk of deposition of infectious agents from inhaled air in the upper airways (Ishmatov, 2016b; 2016a; 2017b).*

The intensity of the incidence of influenza and ILI by year (2007-1012)

Form Fig1 I found that frequency of incidence of influenza (Fig1B) and ILI cases (Fig1C) is associated with a number of critical days in the year. A small number of critical days lead to a small number of influenza and ILI cases in this year – see the red trend line in Fig1A. Here the following years 2007, 2008, 2011 and 2012 are indicative, with the exception of the pandemic (2009) and post-pandemic year (2010). I believe/think the problem of the small number of influenza and ILI cases in 2010 may be due to the development of specific/adaptive immunity in the population after the 2009 pandemic (immunity builds up to a particular influenza strains). The problem of 2009 pandemic is discussed below.

The 2009 flu pandemic

In 2009, a new influenza virus (H1N1pdm) produced a significant pandemic in Okinawa (Sunagawa et al., 2016). This season appears quite different from typical influenza seasons in Okinawa. As suggested by Sunagawa et al. (Sunagawa et al., 2016) it was caused by appears of the new and more virulent virus.

Fig 1A shows that in 2009 the number of critical days when supersaturation in the airways could occur was small (in comparison with other years). And in accordance with above and with our theory (Ishmatov, 2016; 2017b; 2017c; 2017a), we should have expected that this year there should be a small number of cases of influenza and ILI (but it was not so in 2009 (Fig 1 B,C)). Nevertheless, all main peaks of influenza cases in 2009 correlated with fig 1A and Fig 1D.

Additionally, we found the pre-pandemic years (2007-2008) may be important and indicative here. Fig 1B,C shows that a small number of influenza and ILI cases in this period (from summer 2007 until winter 2008) was observed. Fig 1A shows that it correlates with a small number of critical days when the effect of supersaturation could occur (see the red trend line in Fig1A). Thus, in the pre-pandemic period (from the summer of 2007 until the end of 2008) the population of Okinawa rarely gets influenza and ILI. Respectively, it can be assumed that the population had weakened specific/adaptive immunity to the respiratory viruses in 2009 (immunological memory in the population was not developed).

In my opinion, the severity of the 2009 pandemic was caused both by the virulence of the new strain of influenza virus (H1N1pdm) and specific/adaptive immunity of the population of Okinawa after the pre-pandemic period when small number of critical days (when the effect of supersaturation could occur) were observed. In this issue, I do not have sufficient competence. This aspect is beyond the scope of this manuscript and in the future requires detailed study/investigation.

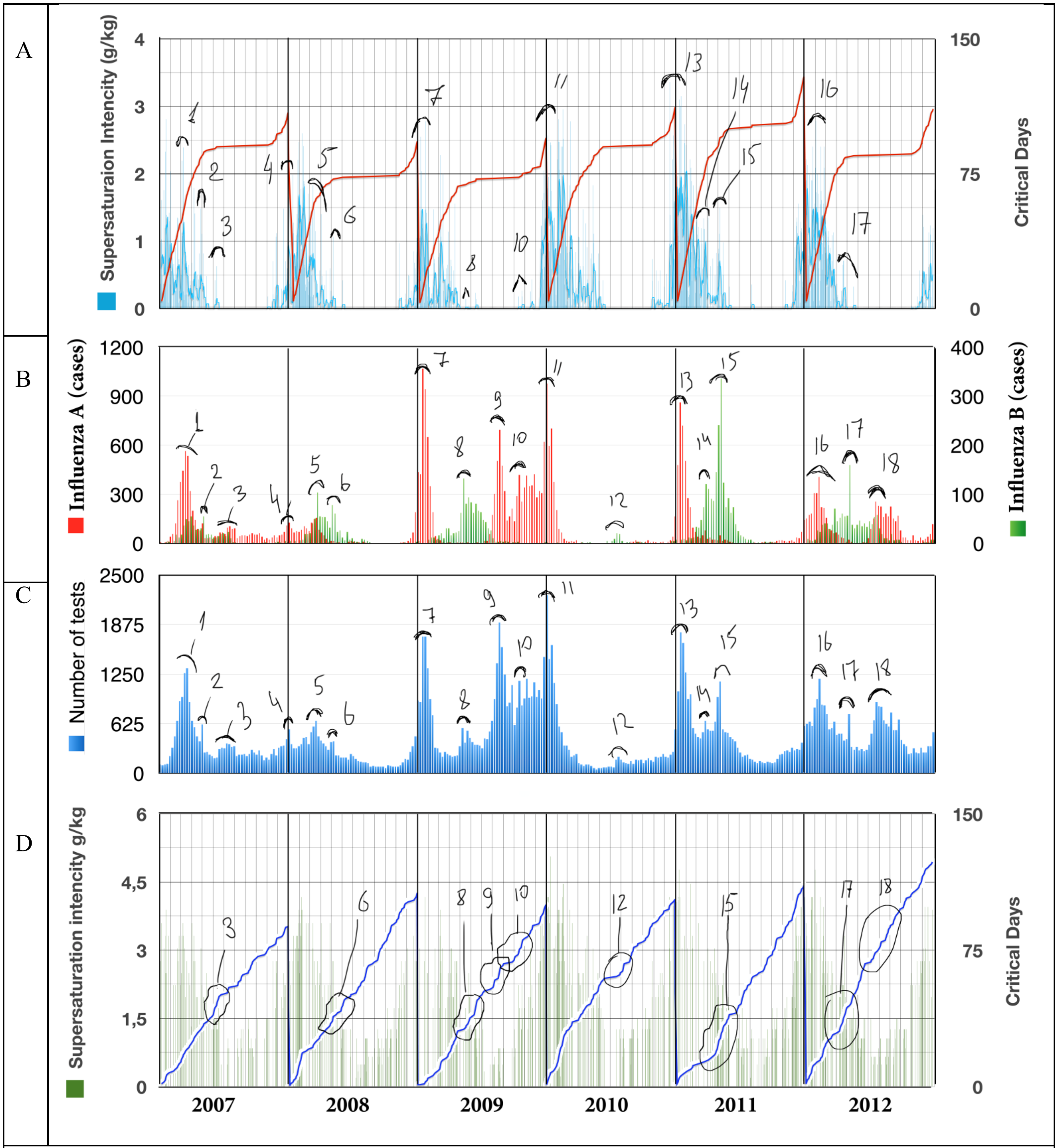

Fig 1 Trends of supersaturation intensity and influenza cases detected in Okinawa, from January 2007 until December 2012

Labels *"1"-"15"* – these labels indicate the representative peaks of cases of influenza

A) **Daily trends of supersaturation intensity.** The light blue bars indicate daily supersaturation intensity, the light blue line indicates the weekly trends of supersaturation intensity, and the red line indicates the summ of critical days when supersaturation occurred.

B) **A number of confirmed influenza A (red) and influenza B (green) cases by week detected in Okinawa, Japan**. Cases are pooled for each week from Jan 2007 until Dec 2012 using the rapid antigen tests (RAT) results from four representative hospitals in Okinawa (on the basis of dataset of Iha et al. (Iha et al., 2016)).

C) **A number of rapid antigen tests (RAT) performed in four representative hospitals in Okinawa, Japan.** Cases are pooled for each week from Jan 2007 until Dec 2012 (on the basis of dataset of Iha et al. (Iha et al., 2016)).

D) **Daily trends of critical short-term supersaturation intensity** (*Preliminary and subjective results; for preliminary analyzing only). The green bars indicate daily critical short-term supersaturation intensity (when the supersaturation may occur during a few hours in a day); the blue line indicates the summ of critical days when short-term supersaturation was occurring during the year.

**Supersaturation intensity is a parameter of probability of liquefaction of water vapor and indicates the concentration of liquid water in the mixed air (g/kg).*

<u>There were many limitations to our study.</u>

First, we used a very simple mathematical model for analyses of intensity of supersaturation effect. This model may be used only for rough and preliminary estimations. And for future research, the modern and complicated models of heat and mass transfer in the airways should be used.

Second, this study covers only a 6-year period and limited locations and climatic conditions. In the future, it should be necessary to analyze the more extended periods for many locations and climate zones.

Third, for additional analysis we took a very rough simplification to evaluate the supersaturation effect in rainy days. Here these results are preliminary and subjective nature. There are no accurate models now. It is necessary for the future studies to carry out the additionally estimations of the intensity of generating secondary aerosols by rain droplets in the surface/ground layer (1-3 meters). And also it is very important to conduct and use the accurate instantaneous measurements of the relative humidity (without using wet psychrometers, because this method gives a large lag and the error in the measurement under “rapidly” changing weather conditions).

Conclusion

The present study is the first research of this kind in which it is shown that during days of influenza seasons in the subtropical climate the effect of supersaturation has the high probability of occurring in the human airways.*

Results of the present study are simplified. But even in this form, these results had shown for the first time that there is an association/connection between the supersaturation effects in the airways with influenza activity in Okinawa from Jan 2007 until Dec 2012. It is the first observation of this type for subtropical climate. No one before looked at the problem of seasonal influenza and pandemics in the tropical/subtropical zones from this point of view.

The problem of prediction of influenza epidemics and pandemics is one of the greatest problems of our time. And as was mentioned by W. Zhang and R.G. Webster (Zhang and Webster, 2017) “*We…still fail to predict influenza pandemics, and this must change*.”

I believe the methods and results of our study, even in this preliminary form, can be used by researchers and institutions as a new additional method to analyze and predict/forecast influenza in different climatic conditions. At this stage of the study, it is essential to inform the public in a timely manner and create a forum on the new insight on the seasonality of respiratory infections and its connection with effects of supersaturation and condensational growth in the human upper airways when breathing cold/cool air.

* *The effect of supersaturation in the airways can be used for the analysis and predicting the outbreaks of influenza in subtropical climate, because this effect may lead to a risk of acidification of epithelial lining fluid in the local areas of the respiratory tract and to effective deposition of infectious agents from inhaled air in the human upper airways.*

Supporting information

Table S1-S6. Dataset of daily weather conditions in Okinawa and daily intensity of supersaturation effect under these conditions from 2007 until 2012.

Declaration of interests

I report no competing interests. The study was partially supported (in part of preparing of final version of manuscript) by a grant of Russian Scientific Foundation (project No. 17-44-07001)".

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APPLICATION 1

Estimation of supersaturation and condensation of water vapor in mixed air

(*Why so simple model as the model of Nir Shaviv*[1] *is sufficient for describing of probability of supersaturation in the human respiratory tract during INHALATION*)**.**

From the text above follows the next: "*In the study the author talking about the supersaturation in the human airways during INHALATION, but the author used the model that was created for estimation of mist formation during EXHALATION!*"

I deliberately used simple calculations in order to show only the essence of mixing process during inhaling cold/cool air. Of course, the using the complicated models seems more appropriate for our study. But the mechanics of the breathing have a complex mathematical implementation especially for nasal breathing and taking into account the anatomy and physiology of the nasal cavity of individuals (e.g. race, gender and age). I have no opportunity and resources to make these calculations in present time (I will be glad to any cooperation in this direction).

The effect of supersaturation in the human airways when breathing cold/cool air is the fact, the supersaturation is possible in the nasal turbinate region and upper airways (from [2-9]) (also see Table1 in [10]). And due to the above the simple model and calculation is used in present study only for to show the essence of probability of processes of supersaturation in the airways (it is not the main aim -- it is "background" for the present study). I believe, this type of estimation is sufficient to describe our hypothesis at this preliminary stage of research. But it is very important to understand as mentioned by professor Ferron in 1988 [9]: "*Supersaturation occurs only in small areas in airways cross sections in the trachea and upper bronchi. Not all of the particles will see this supersaturation.*"

Moreover, the simple model of Nir Shaviv will help to the readers to provide the first preliminary estimation by themselves "in online" -- it is a great opportunity for common readers and medical workers without specific knowledge in math to provide estimations of probability the mist formation in their observations of the seasonality of respiratory infections and diseases.

Thus the main aim of the study is to show to the readers the connection/correlation of the effects supersaturation and condensational growth in the upper respiratory tract with the seasonality of respiratory infections.

Model description

A model below describes the mixing of cold/cool air with warm and moist air. The model of Nir Shaviv[1] was created for estimation of the probability of mist formation during exhalation. This simple model describes, in an idealized manner, the mixing of cold air with exhaled warm moist air. The model has assumed an absence of effects of turbulent mixing and lack of airborne particles in the air. We used this model and online resource "breath condensation calculator" (ScienceBits.com) [1] with kind permission from Nir

Shaviv.

Here I considered the process of inhalation of cold/cool air as a simple process of air mixing in the upper respiratory tract under conditions of absence of the heat and mass exchange with walls (it is the rough assumption).

When inhaling cool/cold air mixes with the air located in the airways two processes occur: 1) warming of inhaled cool/cold air (for information: volume of inhaled air is 0.2-0.5 liters); 2) cooling of humid warm air inside the airways (for information: volume of warm air in upper airways before inhalation is 0.150-0.180 liter; the functional residual capacity of lungs is 3 liters). The process of local cooling of moist air is important for us because this air can become supersaturated. It may lead to processes of condensational growth in the airways. The air in the respiratory tract before inhalation (the functional residual capacity, approximately 3 liters) has the following parameters: T=37°C; RH=99.47% [2,3].

At this point of view, the using of the model of Nir Shaviv may be applicable for our task because it describes the idealized mixing of warm air and cold air. And this type of estimation is sufficient to describe our hypothesis at present stage of research.

Description of variables:

f – the mixing ratio of inhaled air and air in lungs

p – the pressure of the mixed gas, which remains constant;

Tc - the temperature in ˚C;

H – the enthalpy;

U – the internal energy;

V – the volume;

W – the work energy;

g - total water content of the mixed gas in lungs;

g_0, g_1 - water content in outside air and air in lung (gr/kg);

$g=(1-f)g_0+f\times g_1$ (from the fact that total amount of water remains constant).

To calculate g using the approximate relations:

$$g[gr/kg]=6\cdot 2\cdot 10^{-3}\,p_w[Pa].$$

To calculate water pressure:

$$p_w[Pa]=RH\times 610\cdot 8\exp\left(\frac{17\cdot 2694T_C}{T_C+238\cdot 3^{\circ}}\right).$$

The enthalpy of the system for mixing under constant pressure:

$H=U+PV$.

The first law of Thermodynamics (law of conservation of energy):

$$dH = dQ - dW + d(PV) = dQ - pdV + Vdp = 0.$$

For adiabatic process (the heat exchanged *dQ*=0) and under constant pressure (*dp*=0) we use an approximation for the enthalpy:

$$\frac{h}{[kJ/kg]} \approx (1\cdot007T_C - 0\cdot026) + \frac{g}{[gr/kg]} \times (2\cdot501 + 0\cdot00184T_C).$$

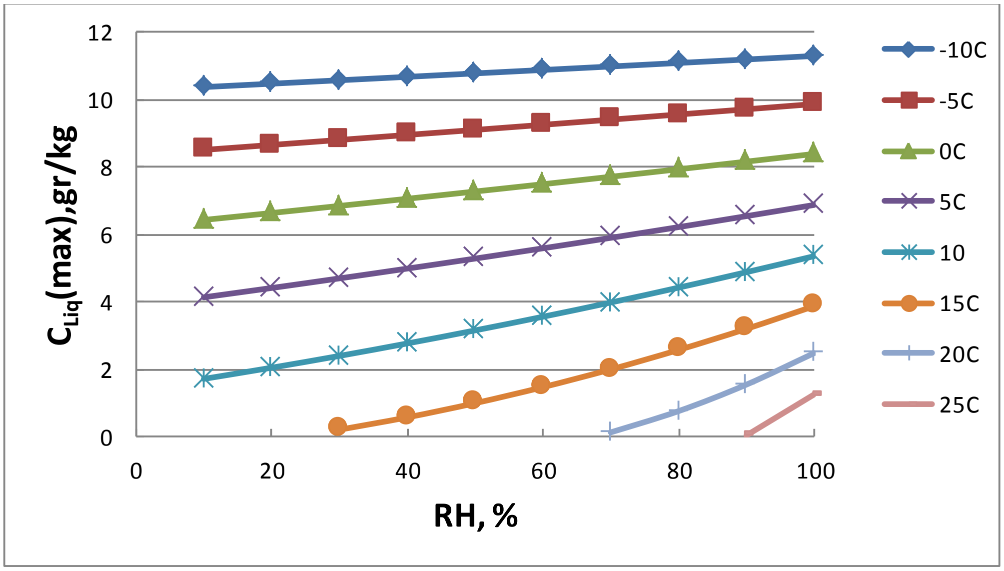
For RH>1 (condensation occurs) the enthalpy of the condensed water is lower by the heat of vaporization. Water content, for this reason, may be considered as content of vapor and condensed water, $g = g_v + g_c$, we have:

$$\frac{h}{[kJ/kg]} \approx (1\cdot007T_C - 0\cdot026) + \frac{g_v}{[gr/kg]} \times (2\cdot501 + 0\cdot00184T_C) + \frac{g_c}{[gr/kg]} \times 0\cdot00419T_C.$$

And for a mixed gas:

$$h = (1-f)h_0 + f \times h_1.$$

Results of preliminary estimation of supersaturation intensity in mixing gases are presented in Fig2.



(reprinted from (Ishmatov, 2016b))

Fig.2. The concentration of liquid water in the mixed air in the oversaturated state (mixture of the inhaled air at different humidity and temperatures with the air which parameters corresponding to the air inside of the airways (initial conditions: RH=99.47; T=37˚C)). C_{Liq}(max) – is maximal local concentration of liquid water in the mixed air (g of water / kg of air); RH – Relative humidity of the inhaled air, %.

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