An exploration of selective trends and seasonality in viral respiratory tract infections

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Note added March 2015: I have now written a shorter version of this paper, which I hope is argued better. It is available at http://vixra.org/abs/1406.0140. In the process of writing it my ideas have of course moved on. Rather than rewriting this paper yet again, I have added comments in blue text.

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Abstract

Current explanations of the seasonality of colds and influenza are incompatible with observations of the incidence of these diseases in the tropics. Many or most wild respiratory viruses possess temperature sensitivity (with less activity at higher temperatures) and it has been suggested that this prevents them from moving down the respiratory tract and infecting the lungs and internal organs of birds and mammals. This temperature sensitivity seems to be finely balanced, and to be continuously adjusted by natural selection, but it may be lost very rapidly in laboratory cultures. Nevertheless, many biochemical studies show decreased viral activity at elevated temperatures. Overdue weight seems to have been given to early volunteer investigations into viral respiratory tract infections (VRTIs) that often used recycled viral strains. [These "pedigree" strains were established by collecting nasal secretions from volunteers with colds, and inoculating subsequent batches of volunteers with the secretions.] Clear-cut evidence that outbreaks of VRTIs are closely (and inversely) correlated with ambient temperature, and that individuals are more likely to develop VRTIs after chilling may therefore have been overlooked. In the laboratory, the following unexpected observations need to be explained: (1) persistent viral infections of cell cultures often yield spontaneously-generated temperature-sensitive (*ts*) viral strains, and, (2) on at least two occasions, temperature sensitivity was *lost* when *ts* influenza A strains were incubated *at a low temperature* (33°C) in conditions that allowed rapid replication. In this review I note that diverse viral species cause very similar VRTIs, that the incubation periods of VRTIs have frequently been underestimated, that influenza A and B may be shed by asymptomatic patients who have not seroconverted, and that colds and influenza often infect only a subset of the susceptible individuals who are exposed to them. Mechanisms where temperature *fluctuations* can increase viral replication and transmission are considered, and explanations of VRTI seasonality in both temperate and tropical regions are discussed.

Key index phrases

Respiratory tract infections, viral infections, temperature changes, influenza seasonality, viral epidemiology.

Introduction - epidemiological anomalies that need to be explained

Before the discovery of influenza A virus in 1933, many physicians doubted that influenza was contagious [1]. For example, in 1775 Thomas Glass of Exeter wrote of influenza, 'Nor does this distemper seem to arise ... from contagion. For in this city, in the year 1729, it was conjectured that two thousand persons at least were seized with it in one night' [1]. During the 19th century, the question of whether influenza was contagious was the subject of intense medical debate, and a major focal point of medical research [133]. Many agreed with August Hirsch, who claimed in 1883 that influenza was not communicable because it spread "quite independently of intercourse" [1]. Today, in spite of intense study and public interest, it has often been noted that influenza and other VRTIs spread in patterns that are difficult to predict or explain [2]. Hope-Simpson listed several unexplained features of the epidemiology of influenza A, including its abrupt cessation when numerous susceptible individuals remain in the population (discussed below), and its reappearance after long absences [3]. It often appears explosively over wide areas at the same time. For example, Magrassi was impressed by cases of influenza in 1948 among shepherds living in complete social isolation in open country in Sardinia, who developed influenza contemporaneously with the inhabitants of towns on the same island [113]. Hope-Simpson also noted the low attack rate of influenza A within households, often contrasting with a high attack rate within institutions [3]. For example, he found that during the "Hong Kong" (H3N2) epidemics of 1968/69 and 1969/70, 70% of infected households in Cirencester (UK) had only one case of influenza (a total of 134 households were infected) [3], and 81% of cases occurred on the first day that influenza arrived in each household. (The low attack rate within households could not be explained by the susceptibility or ages of patients; the H3N2 subtype arrived in Britain for the first time in 1968, and 70% of patients in the first two epidemics in Cirencester were adults [98].) Moreover, no serial interval, dividing introducing cases from those infected by them,

could be found. Hope-Simpson concluded that the disease was not spreading within households. In a similar study of household transmission of 2009 pandemic influenza A (H1N1) virus, the proportion of household contacts in whom acute respiratory illness developed decreased with the size of the household, from 28% in two-member households to 9% in six-member households [64]. (In this context, the authors comment that that "the sociologic, environmental, and biologic mechanisms available to explain the relationship between secondary attack rates and household size are still limited", but conclude that "the transmissibility of the 2009 H1N1 influenza virus in households is lower than that seen in past pandemics".) Van Loghem looked for similar patterns of infection with common colds in a very large survey in Holland in the 1920s [4]. He noted that the attack rate for members of families was the same as the general population, and that the chance that family members would get colds was almost the same for small families as for large families, which would not be expected if the disease were contagious within families. The low attack rate of both colds and influenza contrasts with e.g. measles, where up to 90% of susceptible persons who are exposed to the measles virus develop the disease [12]. Moreover, measles and similar diseases mainly infect young children, who have not been exposed to the disease and therefore have no immunity. Although children often have a higher infection rate than adults, influenza usually infects all ages. For example, 76% of patients infected by influenza A (including both H2N2 and H3N2 strains) in Cirencester between 1960 and 1976 were aged 16 or over [98] (in spite of a higher *proportion* of children being infected). [Therefore the ages of patients did not explain the low attack rate within families.]

A more recent study in Chicago noted great variation in the transmission of other respiratory viruses in a school that was unexplained [5]. Of the 14 viruses identified, seven spread to one or more children, and three of these spread quite extensively, infecting from 42% to 77% of the children. However the remaining seven viruses infected one child only. The difference was not explained by the characteristics of the associated illness, patterns of viral shedding, or levels of immunity [5].

Another puzzle is the seasonal appearance in temperate regions of colds and influenza each winter, so familiar that we take it for granted. A satisfactory model of VRTI seasonality needs to explain (1) the high levels of VRTIs in winters in temperate regions, (2) the very low levels of VRTIs in temperate summers, (3) the moderate levels of VRTIs seen in the tropics throughout the year, and (4) the seasonal fluctuations in VRTIs seen in some tropical locations. Points (2) and (3) seem paradoxical, since any climate or behavioral factor that reduces VRTIs in the temperate summer is likely to have more extreme values in the tropics. Since over 200 serologically distinct viruses are responsible for human colds [29], and since we experience far fewer colds in summer than in winter, it is clear that the great majority of cold viruses are more active in temperate winters than summers. [The seasonality of a range of VRTIs was later directly confirmed by PCR tests in hospitals. See Viegas *et al.* (2004). Respiratory viruses' seasonality in children under five years of age in Buenos Aires, Argentina; A five-year analysis. *Journal of Infection*, *49*(3), 222-228; and Du Prel *et al.* Are meteorological parameters associated with acute respiratory tract infections? *Clinical infectious diseases*, *49*(6), 861-868.] It also seems reasonable to assume that the seasonality of colds and influenza are caused by the same general factors [120]. Two recent reviews concluded that the seasonality of influenza cannot be explained by existing proposals [94, 95]. Tamerius *et al.* noted that differences in "crowding" between summer and winter are minimal since the amount of time people spend *indoors* in e.g. the USA varies by less than 10% between summer and winter, and also that school term-times are not well-correlated with influenza prevalence. They also note that differences in viral survival outside the body e.g. due to changes in absolute humidity cannot explain the prevalence and reduced seasonality of influenza in the tropics [94]. Lofgren *et al.* also concluded that theoretical and empirical studies do not adequately explain influenza A seasonality, noting that no published studies directly show that variations in crowding cause influenza seasonality, and also that a linkage between viral evolution and the wide assortment of other proposed factors in influenza seasonality is lacking [95]. (They dismissed decreased ambient temperature as a cause of influenza seasonality since "no direct biological justification for this effect has emerged".) The inability of such proposals to explain simultaneously both the seasonality VRTIs and their occurrence in the tropics will be discussed below, and explanations that focus on temperature *fluctuations* will be considered.

There is a widespread belief among scientists and doctors that chilling does not increase the likelihood of acquiring a VRTI. As discussed below, this belief seems to have arisen from bad experimental design or misinterpretation of early

volunteer experiments. (One study, however, is noted below that does seem to support this belief.)

Review

Overview of respiratory viruses

It is a remarkable fact that over 200 serologically distinct DNA and RNA viruses are responsible for human upper respiratory tract infections [29]. Some of the viruses that cause VRTIs and are spread by coughing, sneezing, and runny noses are listed in Table 1. It is striking that completely unrelated strains have very similar lifecycles [I mean in terms of their transmission and tropism, not their genetics and biochemistry] and produce indistinguishable symptoms, especially in the early stages of infection. Respiratory viruses clearly occupy a very popular and well-defined ecological niche. The number of different routes for human viral infection is limited, and most other routes (such as fecal-oral transfer, transfer in saliva, transfer in blood - for example in fights, sexual transmission, and the transfer of viruses causing warts and cold sores by contact) can be eliminated by modern preventative measures. It is difficult, however, to eliminate the transfer of VRTIs by contact mechanisms, aerosols or large droplets [30].

Table 1. *Characteristics of virus families and strains that cause human VRTIs.*

Family	*Viral strains that cause human VRTIs*	*Primary genetic material*	*Replication site in the cell*	*Presence of lipid envelope*	*Virion shape*
Adenoviridae	Adenovirus	Double-stranded DNA	Nucleus	lacking envelope	icosahedral
Coronaviridae	Coronavirus, severe acute respiratory syndrome virus	Positive-sense single-stranded RNA	Cytoplasm	Enveloped	spherical with projections
Orthomyxoviridae	Influenza virus	Negative-sense single-stranded RNA	nucleus	Enveloped	spherical or filamentous
Paramyxoviridae	Measles, mumps, parainfluenza and respiratory syncytial viruses, human metapneumovirus	Negative-sense single-stranded RNA	Cytoplasm	Enveloped	spherical or variable
Picornaviridae	Hand foot and mouth virus, rhinovirus	Positive-sense single-stranded RNA	Cytoplasm	lacking envelope	icosahedral
Togaviridae	Rubella virus	Positive-sense single-stranded RNA	Cytoplasm	Enveloped	icosahedral

The economic impact and human cost of VRTIs

The direct medical costs of influenza in the USA have been estimated to be over $10 billion annually [33], based on the 2003 US population. Projected lost earnings due to illness and loss of life from the disease in the USA amounted to over $16 billion. Worldwide, influenza epidemics result in about three to five million cases of severe illness each year, and about 250,000 to 500,000 deaths [34]. A 21st century estimate of the global mortality from the 1918-1920 "Spanish" influenza pandemic puts the total at 50 to 100 million [35]. Other VRTIs also have high costs. The economic cost of lost productivity due to the common cold is close to $25 billion [36].

Evidence that exposure to cold can promote VRTIs.

It is interesting to look at the data of early and mid-twentieth century studies of VRTIs, several of which show the incidence of colds and influenza alongside outside air temperature. (I see no reason to doubt the accuracy or

integrity of these reports, since the pressures and temptations to "massage" data are if anything greater now than they were several decades ago.) These reports show the seasonality and other clear patterns of VRTI outbreaks that need to be explained, and they have the advantage of being recorded in an era when people travelled less than they do today, which simplifies interpretation.

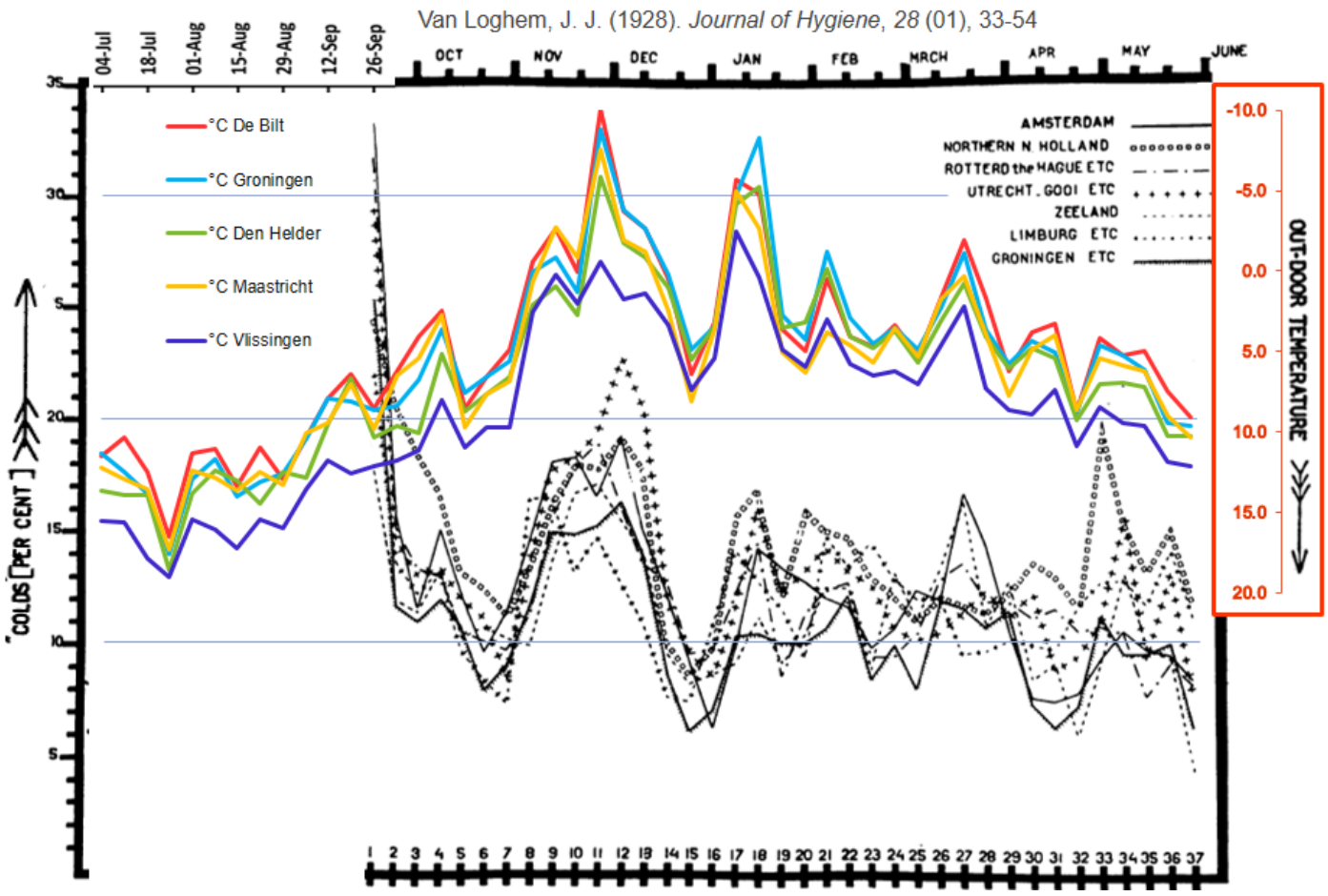

Figure 1. *Graph II from van Loghem's report [4] on the epidemiology of VRTIs in Holland in the winter of 1925/26, with ambient temperature superimposed*. The graph shows the percentages of persons with colds in seven regions of Holland for 37 weeks. The data was compiled from the reports of 6933 correspondents that were submitted by post each week. Amsterdam had the largest number of informants (1159) and Noord-Holland the fewest (581). I have added the daily minimum outdoor air temperature (averaged over 7 days at weekly intervals) from five Dutch weather stations, with the temperature scale inverted (lowest temperatures at the top). Note that by far the highest rate of VRTIs was at the beginning of the study (September 1925), and that VRTIs in different regions are closely correlated with each other and with inverted temperature. These correlations are strongest in the first half of the cold season.

Van Loghem carried out a very extensive survey of the common cold during the winter of 1925/26, collecting data from almost 7,000 participants who were distributed throughout Holland [4] (see Figure 1). The data showed that (1) the development of cold outbreaks in widely-separated parts of Holland was synchronized, (2) the number of colds was closely and inversely correlated with outdoor air temperature (lower air temperature gave more colds), (3) the highest levels of colds that he recorded were in September (see section S17 in the Supplementary Information), (4) a better correlation was observed from September to January than in the period from February onwards, (5) the various forms of "colds" (coryza, bronchitis, angina, influenza, laryngitis) were highly correlated with each other and inversely correlated with air temperature, and (6) the chance that family members would get colds was almost the same for small families as for large families. Note that the development of epidemics is very rapid with outbreaks starting one week after air temperature drops or simultaneously, and that outbreaks are extraordinarily well-synchronized across geographical regions. For example, van Loghem recorded a cold snap

lasting from late October to late December in 1925 (Figure 1). Outbreaks of VRTIs in all seven regions of Holland were recorded that paralleled the progress of the cold snap. Initially all regions were synchronized to within a few days. The typical incubation periods of VRTIs [58] are reported to be 2 to 10 days (see the following section, however). It is therefore highly improbable that VRTIs could have spread between regions and then developed into epidemics during those few days, especially since the population of Holland in 1926 was less mobile than today. Van Loghem himself concluded that the colds were not spread by contagion during the period of observation, but that microbes that were previously present as commensals were able to cause VRTIs because of disturbances to thermoregulation in their hosts [4].

Hope-Simpson carried out a similar survey in Cirencester (UK) in 1954/55 [107]. Again a close negative correlation of colds with ambient temperature could be seen. Figure 1 of his report is particularly striking. It is noticeable in these data that cold outbreaks are closely related to temperature *drops* (as opposed to constant low temperatures), with rapid drops followed one to three weeks later by increases in colds; constant low temperatures tend to be followed by a reduction in colds. Again, a closer correlation is apparent before the end of January.

A third report by Milam & Smillie that dates from 1931 described similar events on the isolated tropical island of St. John, which is one of the Virgin Islands [126]. The population of St. John was then about 700 people, who lived in huts scattered around the island. Most were very poor, and there was very little movement of the population. Throughout the year the temperature on the island drops sharply by about 7°C between mid-afternoon and midnight, and this difference is approximately the same as the difference between the mean temperatures of summer and winter. In spite of this small seasonal temperature variation in comparison to the daily variation, there was a marked seasonality in colds, with almost no colds during the slightly warmer period from June to the second half of October. During the year that was investigated four outbreaks of colds were recorded [Figure S5, which is Chart 1 of ref. 126]. Three of the four began a week after unusually large drops in night temperature (in that climate "unusually large" is only 1 – 2°C). The outbreak that was most spread out in time ran from late April to the end of June, which was a period of generally rising temperatures, and it was interrupted by a sudden temperature rise at the beginning of May, when the incidence of colds temporarily fell to low levels.

Recently, Jaakkola *et al.* studied the incidence of influenza A and B among military conscripts in northern Finland [127]. They found that a sudden decline in both air temperature and absolute humidity (in the three days that preceded reporting the sickness) increased the incidence of influenza A and B. Paradoxically, the incidence of influenza was lower at very low temperatures, and it was the sudden decline of temperature rather than low temperature and humidity *per se* that increased the risk of influenza.

Mourtzoukou & Falagas reviewed the evidence that chilling increases the risk of developing VRTIs and dying from them, and concluded that the general public and public health authorities should reduce exposure to cold to prevent increases in morbidity and mortality due to VRTIs during the winter months [45]. Hajat *et al.* found [46] that both upper and lower respiratory tract infections were associated with cold weather, with general practitioner consultations for lower respiratory tract infections in one UK City (Norwich) increasing by 19% for every degree that average temperature dropped below 5°C, observed 0 - 20 days before the consultation.

Costilla-Esquivel et al. found a relationship between weather and acute respiratory illnesses in Monterrey, which they were able to model very accurately using only three weather parameters: weekly accumulated rainfall, minimum temperature in the week, and weekly median relative humidity [139]. [Temperature was negatively correlated, while rainfall and humidity were positively correlated.] Of the three parameters rainfall had the highest impact, humidity the lowest. High relative humidity is expected as a consequence of rainfall and low temperature.

Table 2. *Data from the Eurowinter Group* [47]. Regression coefficients (R) and their significance (p), for cause-specific indices of respiratory disease-related mortality on personal cold-exposure factors standardized at 7°C mean daily temperature.

Cold exposure factor	R	p-value
Mean duration of going out	0	0.922
Living room temperature	-1.8	0.001
Frequency of going out	-1.9	0.116
Whether going out	-2	0.623
Clothing area (fraction of body surface)	2.2	0.183
Bedroom heating	-2.8	0.053
Long underpants	-3.8	0.022
Gloves	-3.9	0.065
Long-sleeved vest	-4.5	0.072
Hat	-4.7	0.004
Long trousers	-6.6	0.005
Anorak	-6.7	0.001
Overcoat	7.3	0.002
Skirt	8.3	0.005
Sweater	9.5	0.001
Stationery >2 mins	13.2	0.04
Sweating outside	-17.5	0.02
Shiver	23.8	0.001

Table 2 shows data generated by the Eurowinter Group [47]. The table shows the regression coefficients (R) and their significance (p-value), for indices of respiratory disease-related mortality on personal cold-exposure factors standardized at 7°C mean daily temperature with allowance for age and sex. These data are plotted in Figure 2. It can be seen that shivering while outside was by far the most dangerous activity that was investigated ($p<0{\cdot}001$), while the indices for respiratory disease were also positively related to the fraction who kept still for at least two minutes ($p<0{\cdot}04$) and to wearing a sweater, overcoat or skirt, probably because these items are not usually worn with the more protective anorak and trousers. The indices were negatively related to heat stress sufficient to cause outdoor sweating ($p<0{\cdot}02$) and to the wearing of anoraks, trousers, and hats outdoors ($p<0{\cdot}004$ for all). Warm living rooms were also protective ($p<0{\cdot}001$). The protective effect of outdoor exercise is discussed below.

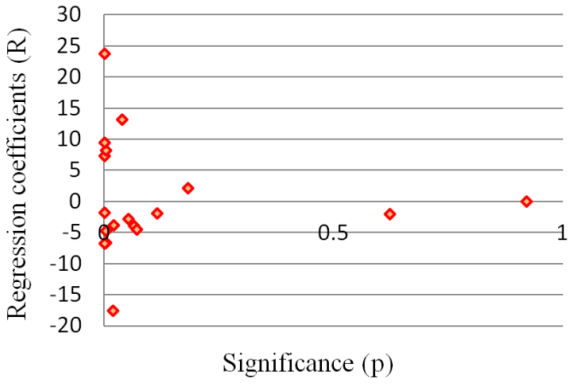

Figure 2. *Data from the Eurowinter Group* [47]. The regression coefficients (R) of Table 2 are plotted against their significance (p).

Several studies found that the induction of hypothermia in patients suffering from brain or other injuries increased the likelihood of also contracting pneumonia. An interesting study by Yanagawa *et al.* examined a group of patients who had suffered cardiopulmonary arrest but had a spontaneous return of circulation [48]. Eleven of 13 patients who were treated with mild hypothermia developed pneumonia, as compared to 6 of 15 controls who were maintained at normal body temperature ($p<0.02$).

Two reports from Antarctica appear to show the "activation" of dormant respiratory viruses by chilling. A geologist at the Mawson research station in 1966 picked up a virus from a visiting field party after 12 months of complete isolation [61]. 17 days later he was exposed to cold and damp conditions. Thirty-six hours after that he developed the symptoms of a VRTI including muscle aches and a sore throat that lasted for a week. The second report recorded VRTIs at the British Antarctic Survey Base at Adelaide Island in 1969 [121]. After 17 weeks of complete isolation several men developed colds four days after the air temperature fell in one day from 0°C to -24°C. [Another study at Adelaide Island in 1969 found that after 17 weeks of complete isolation several men developed colds four days after the air temperature fell in one day from 0°C to -24°C. Allen *et al.* (1973). An outbreak of common colds at an Antarctic base after seventeen weeks of complete isolation. *Journal of Hygiene*, *71*(04), 657-667.] These studies are discussed in section S8 of the Supplementary Information.

The abrupt cessation of influenza epidemics

Hope-Simpson noted that all of the major influenza epidemics that he recorded in Cirencester, UK, (1951, 1957,

1959, 1969 and 1973) ceased within 5 - 9 weeks [116] [after a single peak.]. In at least one case this was clearly not due to a lack of susceptible persons: the H2N2 subtype arrived explosively for the first time in Cirencester in September 1957, with over 100 individuals suffering from acute febrile respiratory diseases by the third week of October. This epidemic abruptly ceased after only six weeks. It is known for certain that many susceptible individuals remained in the population because there was a second major H2N2 epidemic the following winter [116]. The abrupt cessation of the first epidemic is therefore unexplained.

Difficulties with current explanations of VRTI seasonality

1. Evolutionary considerations

Colds and influenza are seasonal, with much higher disease rates in the winter than in the summer in temperate regions [4, 94, 95, 107]. If all other considerations were equal, respiratory viruses that could replicate during the summer months would have a clear selective advantage over those that did not. This suggests that, for a wide variety of respiratory viruses, selective pressures associated with the mechanisms of replication or transmission during the colder months outweigh the selective disadvantages of inactivity during the summer. (It also suggests that surviving the summer months is not a major difficulty.) We therefore need to look for a mechanism of replication or transmission, shared by a wide variety of respiratory viruses, that would have the side-effect of summer inactivity. All VRTI-causing viruses that are less prevalent in the summer can be considered, including influenza.

2. Problems with explanations based on changes in transmission rates or host susceptibility

The data of van Loghem, Hope-Simpson, Milam, Hajat, Jaakkola etc. show that VRTI outbreaks often follow sudden drops in outside air temperature [4, 107, 126, 46, 127]. Laboratory studies have found that transmission of influenza increases as the temperature drops [123], and we might assume that other viruses behave similarly. Therefore, a seemingly-plausible explanation of these observations is that low-level epidemics were present before each temperature drop, and that at the lower temperature the virus survived outside the body for longer, which increased transmission and initiated each new outbreak. However, this reasonable-sounding suggestion has several major problems. Firstly, as discussed below, it cannot explain the distribution of tropical VRTIs, including influenza. Secondly, the scales are wrong, because the sensitivity of the virus to temperature is too great. Milam & Smillie noted VRTI outbreaks on a tropical island following temperature declines of only 1.0 – 1.9°C (Figure S5), which is presumably a small part of the temperature range over which viral transmission varies. It is difficult to imagine mechanisms where such small temperature changes consistently increase transmission sufficiently to initiate epidemics. Moreover, the three outbreaks on the island noted above started after temperature drops that began and ended at different temperatures, and they occurred at different times of the year. In another example, Jaakkola *et al.* reported that "sudden declines" of around 5°C preceded the onset of influenza [127]. These events were observed at temperatures above 15°C and also below -15°C, which implies that transmission needs to vary over about 30°C. Temperature drops of only one-sixth of this range would not be expected to have an effect that was easy to observe. Visual inspection of Hope-Simpson's colds data confirms the same pattern [107]. Another very interesting and clear-cut example comes from Singapore, where Hii *et al.* studied the strong association of hand, foot and mouth disease with the weather [137]. The risk of the disease increased by 41% for every 1°C that the weekly temperature *difference* increased above 7°C. The authors concede that the "exact reasons for the relationship between weather and HFMD are not known" [137]. Again, it is difficult to imagine that weather anomalies of the order of 1°C can change either the general susceptibility of human hosts to VRTIs or the survival of respiratory viruses outside the body.

A third difficulty arises from the need to explain why major VRTI epidemics are not more common in midwinter and high latitudes. Van Loghem recorded a doubling of cases of colds in three weeks in the autumn of 1925 when the temperature dipped below 12°C. If this and similar autumn outbreaks were caused by an increase in viral transmission, why do we not experience multiple overlapping epidemics every winter when the temperature hovers around 0°C? Instead, the number of VRTIs in mid-winter is (only) roughly double the number in autumn and spring [4, 94, 107]. (We also need to explain why, in midwinter when transmission should be greatest, major influenza epidemics rarely last for more than 6 weeks in any particular location [116]).

An interesting and ingenious recent review looked directly at the seasonality of immune responses in humans by investigating antibody responses of individuals following

vaccination [140]. Although the authors found that seasonal variation in immunity appears to occur in humans, the direction was not appropriate: seven of the studies of vaccines reported a stronger immune response in the winter than in the summer, with only 1 showing the opposite seasonality. There was no clear trend with regard to the dry and rainy seasons in tropical regions. (Seven studies showed no clear seasonality.) This study therefore strongly suggests that variations in host susceptibility cannot explain the seasonality of VRTIs.

3. VRTIs in the tropics and elsewhere

Tamerius *et al.* reviewed the many mechanisms that have been put forward to explain human influenza seasonality [94], including factors that may change contact rates (school closures, ambient temperature and precipitation), factors that may influence virus survival outside the body (relative humidity, absolute humidity, solar radiation and temperature), and factors that may change the immunity of hosts (humidity, photoperiodicity, temperature, viral interference, and selenium, vitamin C, vitamin D [49] and vitamin E deficiency) [for references see 94]. It is, however, very difficult for these mechanisms to explain influenza seasonality while being at the same time compatible the moderate levels of influenza that are encountered in the tropics year-round [94]. For example, influenza is present throughout the year in Singapore [94] (with peaks during the two monsoons, discussed below). Compared to summer conditions in temperate zones, all of the factors mentioned above have values that are either similar or more extreme in the tropics. If influenza is virtually eliminated in the summer in temperate zones by these factors it should not be present in the tropics at all. In reality there are as many cases of influenza in the tropics as in some temperate regions (for example, compare Singapore to Sidney, Australia, in Figure 1 of the publication by Tamerius, [94]), although cases in the tropics are spread more evenly throughout the year. Similar variations in the seasonal activity of influenza and RSV were reported more recently by Tamerius *et al.* and Bloom-Feshbach *et al.* [118, 119] in their detailed global surveys. Conventional explanations therefore cannot explain the patterns observed [94, 95, 125]. Two very popular explanations [94, 95] for influenza seasonality are (1) that increased contact rates during school term-times drive seasonality, and (2) that viruses can survive outside the body for longer in conditions of low absolute humidity and temperature (winter) than high (summer). However, Tamerius *et al.* note the *U-shaped* relationship between the likelihood of an influenza peak and average monthly absolute humidity in their global survey, and comment that the mechanism that causes the *increase* in influenza peaks (occurring in the tropics) when absolute humidity is high (above 15 g/kg) "is not readily explained" [118]. For example, the marked seasonality of influenza in Fortaleza, Brazil, [94] is very closely correlated with precipitation occurring during the rainy season. (A similar pattern was reported recently for RSV. RSV incidence in Southeast Florida was strongly correlated with rainfall two months earlier, whereas RSV incidence in North Florida was strongly correlated with temperature [122].) Absolute humidity levels are far higher in Singapore throughout the year than in northern European countries and northern US states. There are small variations in ambient temperature and humidity in Singapore, but they are not in fact correlated with influenza rates. Moreover, there are two clear peaks of influenza in Singapore, and one of them coincides with the holidays in June, which opposes the first explanation, above. Other factors, however, show clearer correlations: the two influenza peaks in Singapore that begin in June and December coincide with the two monsoons, the Southwest and the Northeast monsoons respectively. Monsoons are associated with strong winds and fewer thunderstorms. The December monsoon is associated with increased rainfall, and one explanation is that the increased rainfall causes greater crowding as individuals spend more time indoors to escape the wet weather [94]. Rainfall throughout the rest of the year, however, remains roughly constant, including the June monsoon. Perhaps we should focus instead on the strong winds that occur during both monsoons, which may make it difficult for individuals to keep dry outside, especially since the lack of thunderstorms in June suggests persistent light rainfall. Both monsoons may increase the level of personal chilling as strong monsoon winds chill individuals who may be wearing damp clothing (and it's difficult to make good use of an umbrella in strong winds!).

4. Animal experiments with human respiratory viruses

Experiments with guinea pigs show that the transmission of influenza A is more efficient at lower temperatures and lower relative humidity. For example, Lowen *at al.* found a 3.5-fold increase in the transmission of human influenza A between guinea pigs at 5°C compared to at 20°C [123] (in fact this was true only at 50% relative humidity; at higher and lower humidity, transmission rates were either similar at both temperatures or higher at 20°C). These differences in agreement with measurements of the stability of

influenza A virions (generated in cell cultures) in air of different temperatures and humidities [124]. Variations in transmission due to weather changes may therefore contribute to the seasonality of influenza (and other VRTIs) in temperate regions. A more recent study by same authors found that the transmission of influenza between guinea pigs by medium-range aerosol was eliminated at 30°C, although transfer between animals in the same enclosure by short-range aerosol or direct contact was as efficient at 30°C as at 20°C [125]. The authors postulate that the normal mode of influenza transmission varies depending on climate: in temperate regions aerosol transmission may predominate, while in the tropics short-range and contact transmission may be more important. Epidemics of H3N2 influenza in the temperate regions, however, are seeded each year from a network of temporarily overlapping epidemics in East and Southeast Asia [112]. There is therefore no reason why influenza cannot be transmitted by the contact route in the summer months into and within the temperate regions. Lowen *et al.* recognize this difficulty, and they postulate the existence of "additional factors, other than warm temperature and high relative humidity, which suppress influenza transmission by all routes during the summer months" in temperate regions. Although the authors may have identified the routes of influenza transmission at different latitudes, they have therefore not provided a complete explanation for its seasonality.

5. Difficulties with other proposed explanations

Few of the remaining mechanisms proposed above can adequately explain VRTI seasonality. For example, school closures in Bismark, ND, USA are not well-correlated with VRTI epidemics since influenza and colds decrease long before the summer holidays begin. Moreover, the main increase in influenza (but probably not colds) comes long after the autumn term begins [94]. Similarly, the number of school-days in the UK during the coldest six months (October to April) is only 10% higher than in the warmest six months, but, like all temperate countries, the UK has marked VRTI seasonality. Vitamin D deficiency, photoperiodicity and other factors that may change the immunity of hosts cannot explain why VRTI epidemics begin in the early autumn before vitamin D deficiencies etc. can arise from lack of sunshine. For example, simultaneous outbreaks of "Spanish" influenza occurred in Alaska, Spitsbergen (Svalbard) and Kentucky in the same months, September and October 1918 [26].

Viral incubation periods and dormancy

The incubation periods reported [58] for many respiratory viruses may have been underestimated because of reporting biases that arise because (1) the route of infection is likely to be clearer when incubation periods are shorter, and (2) very long incubation periods may not be picked up if by studies that are too brief. Observations at three Antarctic research stations show that the incubation periods of parainfluenza [62] and two unidentified common cold viruses [61, 121] have been underestimated in the literature, since the observed incubation periods were much greater than the normally-accepted values [58]. (The incubation periods recorded in these reports ranged from 18 days to 17 weeks, while a recent review of the literature found that the longest reported incubation period for a VRTI was 14 days, for measles [58].) These and other anomalous observations are discussed in detail in section S8 of the Supplementary Information.

Studies indicate that VRTIs can spread through human populations too rapidly to be explained by a chain of person-to-person viral transfers that take place during the epidemic [4, 107, 113]. This suggests that VRTI viruses may be dormant before being activated by stimuli that include environmental temperature changes. Viruses such as adenovirus [114], respiratory syncytial virus (RSV) [115], and foot-and-mouth virus [20] - all of which can spread via the respiratory tract - are known to become dormant within their hosts. Other respiratory viruses may show similar behavior. Influenza viruses have been detected several times in the absence of symptoms or an immune response from the host, which indicates that dormant influenza virus is present. In the course of a double-blind trial in the USA of alpha$_2$ interferon for the prevention of the common cold, Foy *et al.* identified 37 individuals who were shedding influenza B virus, of whom 12 were asymptomatic, and 10 did not respond with antibody by any of the five test methods employed (complement fixation, hemagglutination inhibition, enzyme linked immunosorbent assay (ELISA), neutralization, and Western blot) [128]. During the 2009 influenza A (H1N1) pandemic, Tandale *et al.* found that, of 65 asymptomatic individuals with PCR-confirmed H1N1, 12 had not seroconverted [129]. During the same pandemic, Papenburg *et al.* found five individuals in Quebec City with PCR-confirmed infections who were asymptomatic, and two of these had not seroconverted [130]. Lastly, Thai *et al.* found in Vietnam that of 11 individuals shown by PCR to have been infected

with pandemic H1N1 by other members of their household, 5 remained asymptomatic [131]. One of these had not seroconverted. The authors commented that this "may indicate that viral RNA remained in the respiratory tract without being internalized and eliciting an immune response" [131].

An explanation of several anomalous epidemiological features of VRTIs including VRTI seasonality

Mudd and Grant suggested in 1919 that chilling of the skin of individuals could cause respiratory tract infections [92]. Van Loghem (and others) noted that falls in outside air temperature were often followed by VRTI outbreaks, and he suggested that disturbance of the regulation of the heat of the body could cause respiratory pathogens that were already present in the body as commensals to infect the host [4]. These suggestions can be extended by the following hypothesis:

(1) The activity (virulence) of viruses including respiratory viruses is continuously adjusted by natural selection. One consideration is that transmission is normally maximized by a moderate level of virulence (too much virulence and hosts may die or be immobilized, too little and the shedding of viruses is likely to be reduced).
(2) The temperature sensitivity of respiratory viruses is also adjusted to ensure that they infect only the upper respiratory tract and do not invade the lungs and internal organs.
(3) Natural selection generally allows respiratory viruses to adapt to their geographical location and climate by acquiring appropriate temperature sensitivity.
(4) A temperature gradient exists in the respiratory tract from nose (air temperature) to the lungs (body temperature). This gradient can allow respiratory viruses with naturally-occurring temperature sensitivity to become immobilized and to accumulate in parts of the respiratory tract that happen to be at the appropriate temperature, where they can become dormant.
(5) If the host is chilled or breaths cold air, the temperature of the membranes of the respiratory tract will fall and a batch of viruses can become activated simultaneously, causing the host to develop a VRTI.

An important distinction here is that the hypothesis suggests that temperature changes may trigger VRTIs, whereas the mechanisms reviewed by Tamerius and Lofgren [94, 95] are related to the absolute values of temperature, relative humidity and other parameters. Preliminary models based on these ideas are presented in sections S2.1 S2.2 and S3 of the Supplementary Information. The proposed accumulation might take place over a few hours, or over several months. In other cases (such as measles and pandemic influenza), very active viruses might infect individuals immediately without any accumulation being necessary.

Recent observations of infections of humans with avian H5N1 influenza, discussed below in the section about sites of infection, are relevant to point (2) above.

This hypothesis can explain several anomalous features of VRTIs, as follows.

1. The rapid development of VRTIs.

Since many individuals in a community may harbor similar viruses, a temperature fluctuation can activate an epidemic without the need for a chain of person-to-person viral transfers that takes place during the epidemic. This can explain the very rapid development of VRTI outbreaks seen by van Loghem, Hope-Simpson, Hajat and others [4, 107, 46].

2. The simultaneous appearance of VRTI outbreaks over wide geographical areas.

Similarly, viruses could spread throughout a geographical region during warm weather, and then become activated throughout the region during sudden temperature dips. The explosive arrival of H2N2 influenza throughout southern England was noted by Hope-Simpson [3], while the data of van Loghem showed that VRTI outbreaks in the winter of 1925 were very closely synchronized in all seven regions of Holland (Figure 1).

3. The seasonality of VRTIs in temperate regions.

When outdoor temperature is generally rising, as in spring and early summer in temperate regions, the respiratory tract of mammals is less likely to experience major downward temperature fluctuations. Therefore any viruses that are immobilized in the respiratory tract are, according to this hypothesis, unlikely to become active. Conversely, in the autumn and winter,

temperature fluctuations can expose the respiratory tract to temperatures that are lower than those experienced for several previous weeks or months. This is could activate immobilized viruses, producing seasonal VRTI outbreaks.

4. The prevalence of VRTIs in the tropics, and their association with increased rainfall

Influenza and other VRTIs are more common in the tropics than in temperate zones during summers, but less common than in temperate winters. Viruses have high rates of mutation, and it is proposed that changes to their temperature sensitivity can be selected, allowing them to spread quickly to regions with differing climates. Moreover, in the tropics they may become immobilized in positions that are closer to the nose, in temperate regions closer to the lungs. Hence viruses that are common in the tropics can adapt to temperate regions and *vice versa*. The chilling of individuals in the tropics, for example by rainfall or strong winds, is nevertheless likely to lower the temperature of the respiratory tract, thereby (it is proposed) activating viruses that are immobilized there. This can explain the association [94] of influenza with rainy seasons and monsoons in tropical regions. Similarly, the hypothesis can explain the finding by Hii *et al.* that temperature fluctuations are associated with dramatic increases in cases of hand, foot and mouth disease [137]. During warmer weather the virus may move up the respiratory tract, and towards the extremities of the hands and feet. Sudden cooling can then activate or mobilize the virus.

5. The sensitivity of respiratory viruses to relatively small temperature changes, starting at various absolute temperature levels and in different seasons.

As noted above, it is difficult to imagine mechanisms that cause VRTI outbreaks after changes of only a few degrees. For example, Milam & Smillie recorded VRTI outbreaks when the temperature fell by 1.0 – 1.9°C [126], and Jaakkola *et al.* noted that cases of influenza after temperature falls of about 5°C [127]. In both studies the triggering temperature fall occurred at a variety of absolute temperatures and at different times of the year. The hypothesis suggests that viruses with appropriate natural temperature sensitivity can accumulate in the respiratory tract at various positions (temperatures), and be activated when the temperature falls.

6. The abrupt cessation of VRTI epidemics

The hypothesis suggests that VRTI epidemics frequently arise when batches of viruses are simultaneously activated in many hosts by changes in the ambient temperature in a region. During the epidemic the viruses involved may be passed to other hosts, but they may not initially be activated, becoming dormant instead. The epidemic therefore ceases. Subsequent epidemics may arise when dormant viruses are activated by later temperature falls.

7. Polar observations of VRTIs

The model predicts that respiratory viruses can be dormant in the respiratory tract for periods ranging from days to months, and that VRTIs can be precipitated by chilling. Polar observations bear out these predictions.

8. The low attack rate of influenza within households

Different household members are likely to have different histories of chilling. If, for example, some members of a household are exposed to cold at regular intervals, temperature-sensitive (*ts)* viruses that may be present in their respiratory tracts may be activated regularly so that the body is able to remove each small batch without apparent ill-health. Other members may not be exposed to chilling until e.g. the weather turns cold in the autumn or winter. Therefore much larger batches of viruses may become active simultaneously, generating the symptoms of a VRTI. These trends may explain the low attack rate within households.

9. The protective effects against VRTIs of warm clothing and outdoor exercise.

As discussed above, the Eurowinter group found that warm outdoor clothing and outdoor exercise sufficient to cause sweating in the winter months (which is associated with *cooling* of the respiratory tract [32]) are protective against VRTIs [47]. Warm clothing may reduce the magnitude of temperature drops in the respiratory tract [92] and also prevent reduction of blood flow to the respiratory tract [54, 92], reducing viral activation. Regular outdoor exercise may allow the body to eliminate viruses in small batches.

The rapid development of VRTI outbreaks suggests that dormant viruses may already be physically associated with the cells that they subsequently infect. The exact cellular location of dormant viruses in humans is unknown and may be variable; viruses might become dormant on or near the surfaces of their target cells, or within them. Viral development might be stopped before entry into cells, transcription, genome replication, transport of viral materials to cell membranes, exit from cells, or the release of virions from the surface of cells.

Studies with volunteers who were chilled

In an experimental study, Johnson & Eccles investigated whether acute cooling of the feet causes the onset of common cold symptoms [51]. When the total symptom scores for the first 4 or 5 days after chilling were analyzed as dichotomous data, 26/90 (28.8%) of the chilled subjects and 8/90 (8.8%) of the control subjects were deemed to be suffering from a cold, and this difference was significant (p=0.001).

Several earlier studies of viral inoculation and chilling were reviewed by Eccles [54], including three influential early experimental studies [55, 56, 57]. These studies failed to demonstrate any effect of chilling on susceptibility to common cold viruses. In the study by Douglas *et al.* 44% of the volunteers who were chilled developed illness, whereas 29% became ill in the group that remained warm [57]. However, because the numbers were small (16 volunteers in all) and the results were not statistically significant, the authors reported a failure to demonstrate a "significant influence" on the incidence of infection. Eccles noted that the study by Dowling *et al.* [56] was complicated by the fact that 11% of the volunteers who had not been exposed to viral challenge developed colds, which casts doubt on the validity of the results. However, the real reason why these and other studies did not show a significant increase in colds after chilling may be that the investigators worked with viral strains that were not *ts*, even though the wild-type strains from which they were derived were. Serial passage experiments with animal parasites are known to produce rapid changes in the parasite (often increasing virulence) [102]. The investigators recycled secretions from volunteers with colds to inoculate new volunteers in subsequent experiments [56], and it would be natural to recycle strains that had shorter incubation times. While it is likely that they actively selected milder strains that prevented unnecessary distress to volunteers, they also wanted results to appear conveniently quickly. This may have resulted in the loss of temperature sensitivity in the early stages of infection (since *ts* strains might not have produced VRTIs during the time allocated). This suggestion is the converse of the effect that was noted by Preble & Youngner with persistent infections of cell cultures [15], discussed below. They reported that selection for less active strains often produces strains that possess *ts* mutations. Andrewes, Dowling and Douglas may have demonstrated that selection for short incubation periods eliminates temperature sensitivity. These authors worked with recycled strains and saw no clear temperature effect, whereas Johnson & Eccles used "natural" strains that the participants were already carrying by chance, and saw an effect of chilling [51]. (Both trends are shown schematically in Figure 3, below.)

It should, however, be noted that one study gave results that are not in agreement with those of Johnson and Eccles, or the observations of van Loghem, Hope-Simpson, Milam, Jaakkola and others [4, 107, 126, 127]. Jackson *et al.* performed many experiments with recycled viruses, but they also reported one set of experiments with "wild" viruses that the volunteers happened to be carrying at the time of the investigation [132]. In some experiments, volunteers in scant dress were exposed to 15.5°C air for four hours. In others, warmly-dressed volunteers breathed air at -12°C for two hours. Of those who were chilled, only 10% developed colds in the next 7 days, whereas 12% who were not chilled developed colds. Several comments can be made: (1) the results are, if anything, the opposite of those predicted by the proposals made in this review. (2) The authors do not tells us what proportion of volunteers were chilled by breathing cold air and what proportion by wearing scant clothing; in some cases breathing cold air can be protective [47], presumably because viruses are activated but they can be removed by the immune system. (3) These simple experiments need to be repeated, perhaps using different methods of chilling subjects including chilling the feet.

Eccles suggested that chilling the host may cause reflex vasoconstriction of the blood vessels of the upper airways, thereby reducing host defenses against infection [51, 54, 120]. This explanation may be partly right because the converse trend is observed - outdoor exercise is protective against VRTIs [47], presumably because of the increased blood flow during exercise. Moreover it can explain the appearance of VRTIs simultaneously across a wide geographical region, such as the influenza epidemics among

shepherds and others in Sardinia that were noted by Magrassi [113]. There are many problems with the proposal however. It cannot explain the failures of Andrewes, Dowling and Douglas to find an effect of chilling on their volunteers; or the abrupt cessation of influenza epidemics when many susceptible individuals remain in the population, in the middle of winter [116], when individuals' immune systems would be predicted to be at their weakest as a result of vasoconstriction. Another problem is the low attack rate within families, contrasting with the high attack rate in institutions [3, 4, 64]. In addition, the temperature fluctuations that we need to consider sometimes appear to be too small to have a significant effect on the immune system, but they nevertheless trigger VRTI outbreaks. For example, Milam & Smillie found that sudden drops in the minimum daily temperature of only 1.0 – 1.9°C triggered VRTI outbreaks on a tropical island (Figure S5) [126]. (The daily temperature fluctuations encountered on that island are about 7°C, which is about the same as the difference between summer and winter [126].) In addition the biochemical evidence reviewed in this article includes many examples of temperature sensitivity in respiratory viruses, but there is little evidence that the immune response of the host is inhibited by chilling, and some evidence points in the opposite direction [54]. A final problem for Eccles' theory is the observation that popular decongestants such as phenylephrine and pseudoephedrine alleviate the symptoms of colds and influenza by causing potent vasoconstriction of nasal blood vessels, which reduces mucosal edema. If nasal vasoconstriction alone strongly exacerbated VRTIs these decongestants would not be effective cold remedies.

Sites of infection and viremia

Obviously the cells that line the upper respiratory tract are typically below body temperature, and temperature-dependent binding to or entry into cells may allow viruses to target these tissues. Normal "seasonal" influenza mainly infects the upper respiratory tract, with limited infection of the lungs. However avian influenza A (H5N1) that has crossed from poultry to humans infects the lungs and other internal organs and is often fatal [135]. This may be because its normal site of replication is at 41°C, so it would not be expected to possess temperature sensitivity that could prevent replication in the internal organs. Moreover, H5N1 does not usually infect the human upper respiratory tract, which may explain why human-to-human transfer by sneezing and coughing has not been observed [135]. The presence of a lysine at position 627 of the viral polymerase protein PB2 may play a role in viral temperature sensitivity, and this residue has been found in human H5N1 isolates (avian H5N1 isolates typically carry a glutamic acid in this position) [136].

This raises the interesting question of whether human respiratory tract viruses can enter cells in other organs that happen to be at low temperatures. Measles and rubella spread to the skin, presumably via the blood. Moreover, mumps, measles, and rubella virus can frequently be found in the urine without any associated symptoms of the urogenital system [68]. Urine samples from patients with mumps collected in the first five days of facial swelling revealed mumps virus in 72% of cases [69]. Measles virus was found in the urine of 6 of 8 patients just before or after the rash appeared [70]. Hand, foot and mouth disease causes a skin rash in parts of the body that are likely to be colder in young children – the feet (especially the soles), the hands and around the lips [71]. As noted below, foot-and-mouth disease in livestock may be another VRTI where the virus becomes localized in the coldest parts of the body [20].

Human VRTIs that do not usually cause skin rashes or blisters may nevertheless involve viremia. There are several reports of viremia from rhinovirus [72] and human influenza [73 - 76], including three children who were infected by H1N1 influenza and presented in 2009 with petechial rashes [103]. Influenza A caused hemorrhagic cystitis in 33 patients who were infected by the H3N2 strain [77]. Khalpour *et al.* [78] found influenza virus by chance in the blood of an asymptomatic patient who subsequently developed influenza, suggesting that viremia may exist only in the very early stages of the disease when it might not normally be noticed. Note that even very low and transient levels of virus in the blood might allow the virus to reach tissues other than the respiratory tract. Deposits of viruses in other parts of the body may provide reservoirs that can be activated later on (in both animals and humans).

Persistent viral infections in cell cultures and animals: unusual selective pressures

In an interesting review of 1975 [15], Preble & Youngner suggested that viruses are subject to unusual selective pressures when they establish persistent infections in cultured cells, and when they become dormant in animals. These pressures often give a surprising result: *ts* strains (with less activity at higher temperatures) may appear

spontaneously. The authors noted the spontaneous generation of *ts* mutants in cell cultures with persistent infections of many unrelated viral species (see section S4 of the Supplementary Information for more details.) Similarly, three more recent reports described the recovery of spontaneously-generated *ts* strains of influenza A from persistent infections of cell cultures [16, 17, 110]. Preble & Youngner point out that *ts* strains tend to be less virulent and suggest that they may allow persistent infections to become established. They do not, however, explain why *ts* mutations in particular should be selected in persistent infections, as opposed to other non-*ts* attenuating mutations. The selection of *ts* mutations in conditions that favor lower viral activity, together with the converse observation (see below), is shown schematically in Figure 3.

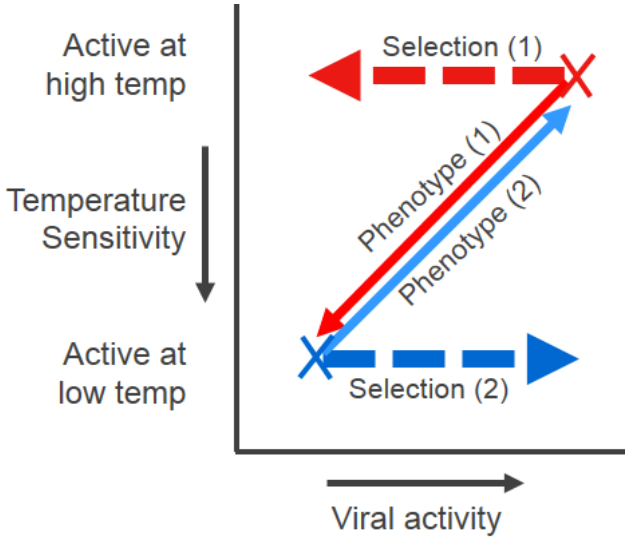

Figure 3. *The observed effect on temperature sensitivity of selection for increased and decreased viral activity.* Selective pressures are indicated by dotted arrows, while the resulting changes to viral phenotype are indicated by solid arrows. The establishment of persistent viral infections of cell cultures generally depends on reduced viral activity [15]. The corresponding selective pressure is indicated by the dotted red arrow. Unexpectedly, reduced activity is often accompanied by the spontaneous appearance of temperature (heat) sensitivity. This is indicated by the solid red arrow. See the main text for examples [15, 16, 17, 110]. The converse trend is equally surprising: when *ts* viruses are propagated in conditions that allow rapid growth (thereby selecting the most active mutants, dotted blue arrow), heat sensitivity is often lost (solid blue arrow) even when selection takes place at low temperatures (see main text [23, 24]).

It is likely that all present-day viruses have undergone many cycles of increased and decreased virulence (activity) in their histories (partly because viruses occasionally jump species barriers [9, 11, 26, 27] as discussed in section S1 of the Supplementary Information.) We can therefore speculate that biochemical routes exist that allow the level of virulence to respond quickly to selective pressures by the reversal of previous mutations. The implication of Preble & Youngner's observations is that changes in virulence are often brought about by the reversal of a previous loss or acquisition of temperature sensitivity.

Preble & Youngner also noted in their review [15] that foot-and-mouth virus that was recovered from carrier cattle after several months was more *ts* than virus recovered from acute infections. Foot-and-mouth disease is an interesting example of a (partly respiratory) disease that often gives rise to persistent infections. It is often spread by aerosols and the early replication sites of the virus are typically the lungs, pharynx, and soft palate [18]. After causing a high fever for two or three days, the virus causes blisters on the lips, in the mouth and on the feet. Note that these are the parts of the body that tend to be at lower temperatures, especially if the animal cannot breathe through its nose. Profuse nasal discharge is a common symptom of the disease [19]. Infected animals frequently become disease carriers, and quantitative RT-PCR has indicated that the main sites of viral persistence are the tongue and feet [20.] As well as frequently being ts [21], viruses recovered from carrier animals show evidence of high rates of mutation with frequent amino acid substitutions and rapid antigenic variation [22]. These findings suggest that temperature may play an important part in the life-cycle of foot-and-mouth virus and other viruses. This is discussed further in section S2 of the Supplementary Information.

The loss of naturally occurring ts *phenotypes in conditions that select rapidly growing variants*

Since *ts* mutations generally slow up the replication of viruses, it seems possible that selective pressures when viruses replicate rapidly in previously uninfected cells might lead to the loss of *ts* mutations, even at low temperatures. (This would be the reverse of the selective process put forward by Preble & Youngner to explain the generation of *ts* strains in persistent infections. Both trends are shown schematically in Figure 3.) Remarkably, this outcome has been observed by chance on at least two occasions. Chu *et al.* [23] found a naturally occurring *ts* influenza A strain that

was a subclone of the H3N2 strain Ningxia/11/72. When they passaged the strain three times through chicken embryos at 33°C, a non-ts strain was unexpectedly produced. Similarly, Oxford *et al.* [24] found that a naturally occurring *ts* virus, A/Eng/116/78 (H1N1), progressively lost its *ts* character during five passages at low temperature (33°C). Both groups concluded that even at the permissive temperature (33°C) the *ts* phenotype may confer a selective disadvantage in eggs. These studies are described in more detail in section S5 of the Supplementary Information.

The prevalence of naturally occurring ts *viral strains and the temperatures that are used for virus isolation and propagation*

Numerous studies have found that it is easier to propagate respiratory viruses that are freshly collected from patients by incubation at temperatures below 37°C. Rhinoviruses were first isolated at 35°C but a greater variety of rhinoviruses was discovered at 33°C [41], and this is the temperature that is recommended today for their isolation by the Clinical and Laboratory Standards Institute [42]. Coronaviruses were first isolated at 33°C [38] although laboratory strains are now frequently propagated at 37°C. Naturally occurring influenza strains are also frequently *ts*. For example, in 1962 Stern & Tippett [43] collected five viral specimens from patients with H2N2 "Asian" influenza. For specimens could be propagated in cell cultures and all of them were *ts*. They all gave cytopathic effects in monkey cells and agglutination in eggs at 33°C but not at 37°C. Subcultures were able to adapt to culture at 37°C but grew more slowly than at 33°C. In 1977, Kung *et al.* found that nine of ten isolates of the newly emerged "Russian" H1N1 influenza were *ts* [44]. Oxford *et al.* found that 17 of 26 recent H1N1 isolates, and 2 of 11 recent H3N2 isolates were *ts*, producing cultures that gave at least 10 times more viral plaques at 34°C than at 38.5°C [24]. Chu tested seven H1N1 strains with varying degrees of temperature sensitivity in volunteers and found a correlation between temperature sensitivity and the severity of VRTI symptoms [23]. More details of these studies are given in section S6 of the Supplementary Information.

Today, influenza A is frequently isolated from patients by propagation at 37°C [42]. This may be because the viral interactions in human cells that give temperature sensitivity are not present in infections of cell-cultures derived from other species.

Biochemical studies of VRTIs

For several decades virologists have found that maximum RNA transcription in influenza viruses occurs below normal body temperature. In 1977, Plotch & Krug [79] reported optimum activity of the RNA polymerase of WSN virus at 30 – 32°C. This is similar to the optimum of the polymerase of influenza C, which is 33°C [80, 81]. Ulmanen *at al.* [83] found that the rate of transcription by detergent-treated wild-type WSN viruses was about 10 times greater at 33°C than at 39.5°C, and that the binding of a cleaved primer cap, which they called the A13 fragment, to the viral cores was "unexpectedly" much weaker at 39.5°C than at 33°C. Once the heterologous RNAs were cleaved, the subsequent steps of transcription were temperature insensitive. This suggests the presence of one or more *ts* switches that initiate transcription.

Scholtissek & Rott [82] showed that the optimum for the polymerase of the Rostock strain of fowl plague virus was 36°C, five degrees below chickens' normal body temperature (41°C). However, other avian viruses are cold-sensitive; investigation showed that the replication of two avian influenza A strains was delayed at 33°C compared to 37°C, with sensitivity to cold determined mainly by residue 627 of PB2 [111, 136].

At least two reports show that temperature affects the balance between transcription and viral replication. Kashiwagi *et al.* looked at the effect of temperature on RNA production for five varied influenza A strains [84]. For all strains, vRNA unexpectedly decreased when the temperature was increased from 37°C to 42°C (cRNA production also decreased for two of the five strains.) The PA subunit of the viral polymerase caused this thermal sensitivity. In another interesting study, Dalton *et al.* showed that the production of mRNA by the PR8 influenza strain is favored at a higher temperature (41°C), with very little vRNA being produced at that temperature [85]. A plasmid-based recombinant system showed that as the incubation temperature increased from 31°C to 39°C the amount of replicative RNA products (c- and vRNA) decreased and a greater accumulation of mRNA was observed [Figure 2 of ref. 85]. The cRNA that is used as a template to make the vRNA formed a complex with the polymerase that was particularly heat-labile, showing rapid dissociation even at 37°C. The authors suggested that the "switch" that regulates the transition from transcription to replication is dependent on temperature, but made no comments about how shifts in the host's body or

respiratory tract temperature may influence this transition. They did suggest that this mechanism may have implications for the exchange of influenza between birds and man, given the different body temperatures of birds and mammals.

In all the cases mentioned above, the temperature sensitivity identified may be a remnant of a switch that is useful in nature but is a disadvantage in the laboratory setting.

Much recent attention has focused on the role of RNA secondary structure in influenza A. Little secondary structure is predicted in vRNA outside the untranslated terminal ends of the vRNA strands that form the "panhandle" structure [89]. However the positive-sense RNA is predicted to have extensive secondary structure, which is conserved, in segments 1, 2, 5, 7 and 8. Since the ordered RNA is conserved it must have some biological function, and ordered RNA is intrinsically thermally sensitive. It is therefore almost inevitable that ordered RNA provides biochemical thermal switching to a greater or lesser extent, and that the switching temperatures are varied by natural selection.

It is interesting that the most stable (+)RNA sequences tend to be avian, followed by swine, then human [90]. This sequence corresponds to the temperature of the site of replication in these strains: the avian gut is at 42°C, while the swine and human respiratory tracts are at roughly 37°C and 33°C respectively. It seems likely that RNA structures must be stable enough, but must not be too stable, to perform their functions at each temperature.

More details of these and other biochemical studies are given in the Supplementary Information, sections S9 to S11.

Membrane fusion and ts entry into cells

Takashita *et al.* found that, in influenza C (C/Ann Arbor/1/50), roughly half the amount the hemagglutinin-esterase-fusion protein (HEF) was found on the cell surface at 37°C compared to 33°C [66]. (HEF in influenza C carries out the functions of both hemagglutinin (HA) and neuraminidase (NA) in influenza A or B.) Moreover, membrane fusion mediated by HEF was observed at 33°C but not at 37°C. This was found to be due to instability of the trimeric form of HEF at 37°C.

In an interesting study, Russell measured the uptake of two influenza viruses and Newcastle disease virus into canine cells [65]. The uptake of Newcastle disease was sensitive to cold, remaining at low levels from 0 to 30°C, then increasing rapidly as temperature rose beyond 30°C. However the triple reassortant influenza virus A/Jap/Bel gave a different result that Russell described as "unexpected". Uptake of the virus increased steadily from 0°C, with 100% of the virus entering the cells at 30°C. However, at 34°C and 38°C less A/Jap/Bel was taken up than at 30 °C [Figure2 of ref. 65]. This was repeated on two separate occasions using a chicken anti-H2 serum when 100% of virus escaped neutralization at 30°C, compared to 50% at 38°C, suggesting that viral entry into cells was *ts*.

Vaccines

Live vaccines are often more effective than inactivated (killed) vaccines. Live influenza vaccines may be administered to individuals who are at greater risk from infection, such as the very young, the elderly and the immune-repressed. One reason for studying temperature sensitivity of viruses is to make live vaccines for influenza, measles, mumps, rubella etc. However, the observed spontaneous generation of non-*ts* mutants in conditions that encourage rapid replication [23, 24] suggests that this approach may be unsafe, since non-*ts* revertants may arise spontaneously with relatively high frequency. This point was made by Preble & Youngner [15], who noted that rats that were inoculated with *ts* reovirus mutants developed a slowly progressing hydrocephalus, and they cited Fields who postulated that *ts* virus mutants may be involved in similar chronic diseases of humans [117]. A safer approach the manufacture of live vaccines may be to make them from attenuated strains with non-*ts* mutations (especially to proteins other than HA and NA).

[Note added March 2015. I now disagree with my previous ideas. I now think that temperature sensitivity is essential to prevent viruses from multiplying in the internal organs.]

Conclusions

Explanations of the effect of temperature on VRTIs

No satisfactory explanations have been available for the close inverse correlation of VRTIs with ambient temperature [5, 107] or the seasonal appearance of VRTIs [94, 95]. Moreover, the low attack rate of influenza in families [3, 4, 64] and the tendency for personal chilling to

increase mortality from VRTIs [45, 47, 48] have been unexplained.

However these observations can be at least partly explained by the following suggestions:

1. Viruses continually adjust their temperature sensitivity to maximize transmission and replication.
2. Mutations that confer decreased viral activity tend (unexpectedly) to increase temperature sensitivity and *vice versa.*
3. Laboratory virus strains may rapidly lose their natural temperature sensitivity in conditions that allow rapid replication.
4. Respiratory viruses often target the respiratory tracts of their hosts by refraining from developing at normal body temperature, being instead active only in tissues at lower temperatures.
5. Respiratory viruses may become dormant for hours to months (in some unknown cellular or extracellular location) in or near the cells that line the respiratory tract or in other tissues, and temperature fluctuations may subsequently provide a signal that activates or mobilizes many viruses simultaneously, thereby overcoming the hosts' immune defenses.
6. Temperature-sensitive mechanisms may inhibit activity at one or several stages in the viral life-cycle, including viral attachment, entry into cells, transcription, genome replication, exit from cells, and release from the surface of cells. It seems likely that a few strategically placed *ts* viral processes can allow respiratory viruses to be activated in bursts, to infect a subset of susceptible individuals, and to establish persistent infections.

Recommendations for research into VRTIs

The following recommendations may increase the understanding of VRTIs:

1. Study the biochemistry, genetics and infectiousness of respiratory viruses using recently isolated strains. Avoid selective conditions that may alter the temperature sensitivity of viral processes.
2. Identify sequence differences in naturally-occurring *ts* strains in comparison to related non-*ts* strains including those that give amino acid sequence changes in HA, NA and viral RNA polymerase (which are known to provide spontaneous *ts* mutants) and those that may change RNA secondary structure.
3. Investigate the response of viral processes to temperature shifts and temperature cycling in animals, tissue cultures and *in vitro.*
4. Use temperature shift-up and shift-down experiments to investigate the effect of temperature on the synthesis of viral proteins.
5. Use temperature-shift experiments to investigate the effect of temperature on the synthesis of viral mRNA, cRNA and vRNA.
6. Study the adhesion, release and entry of viruses into cells in temperature-shift experiments.
7. Using a wide variety of virus strains, look for immobilized and dormant viruses in cold parts of the bodies of humans and animals, including the upper respiratory tract, lips, ears, feet and digits.

These and other suggestions for the experimental investigation of the temperature sensitivity of respiratory viruses are shown in Figure S3.

A shorter, more recent, version of this paper is available at http://vixra.org/abs/1406.0140

List of abbreviations used

HA: hemagglutinin
HEF: hemagglutinin-esterase-fusion protein
NA: neuraminidase
RSV: respiratory syncytial virus
Ts or ts: temperature-sensitive
VRTI or VRTIs: Viral respiratory tract infection or infections

In this article, unless otherwise stated, temperature-sensitive or ts refers to viruses that are more active at lower temperatures, i.e. they are heat-sensitive.

Competing interests

I declare that I have no competing interests.

Author's information

I am one of the two founders and Directors of Douglas Instruments Ltd, a small UK company that manufactures automatic systems for protein crystallization. I worked with Professor David Blow in the 1990s, and have published 15 papers about protein crystallization that have together

been cited over 720 times. A few years ago I began to think about respiratory viruses when a friend bet me that I couldn't find biochemical evidence that chilling could trigger VRTIs. I started to write a short note, but everything fell into place so neatly that it grew until it became the current document. In an effort to keep the length down, much of the original paper, including the more speculative parts, have been moved to the Supplementary Information.

I'm very interested to hear of any evidence either for or against the ideas in this paper. Please get in touch with me at pshawstewart@gmail.com.

Supplementary Information

S1. Patterns of viral evolution

Hope-Simpson and others have been puzzled by the patterns of VRTIs, but it can be seen that they may give the viruses involved long-term selective advantages. Viruses can be divided into four rough categories (A to D) based on their level of activity and virulence: (A) some viral diseases infect and kill a high proportion of the host population. The viruses involved may have crossed recently to the current host from another species, and may spread rapidly, often using insect vectors. Examples are myxomatosis (crossed from New World rabbits to European rabbits), ebola (crossed from bats and other species to humans) and HIV (crossed from various primates to humans). Both theoretical work on virulence evolution during disease emergence [6] and empirical studies [7] suggest that at high host densities and in conditions that favor transmission, selective pressures may increase virulence. Such viruses are in danger of killing all of their hosts that do not become immune, and they risk becoming extinct unless the host population (such as the population of European rabbits in Australia) is large. If the virus reduces the density of the host population, or a proportion of the host population becomes immune, more virulent virus strains may spread less efficiently than less virulent strains because infected individuals die before they can infect many other potential hosts [8]. Less virulent variants may therefore arise, a trend that is consistent with the well-known "trade-off" model [6, 7]. During the first year after its introduction, myxomatosis in Australia is estimated to have killed 99.5% of the rabbits that it infected [9]. However, even this high rate allowed around 0.5% to recover and breed. By the third year the mortality rate was down to 65%, probably due to a combination of increased host resistance and decreased viral virulence [9]. (B) Once equilibrium is approached, viral species that are highly infectious but rarely fatal may arise [10]. For example, measles is highly infective, predominantly infecting children because most adults are immune. Measles is, however, a fairly recent human VRTI, having diverged from the formerly widespread rinderpest virus, which infects cattle, roughly 1000 years ago [11]. Deaths from measles are comparatively rare (about 1 death in 1000 cases). Both categories A and B need relatively large host populations to survive, so that enough susceptible progeny can be born to allow the virus to replicate (it is estimated that measles requires aggregations of over 500,000 people to become endemic [13]). (C) Influenza and colds (generally) use more subtle patterns of infection, where a low rate of attack [3, 5] allows the virus to linger in a community for longer, and to return to the same community in subsequent seasons. To use a wildfire analogy, myxomatosis is like a forest blaze that burns very fiercely and rapidly consumes its fuel, while measles burns more gently, allowing most trees in the forest to survive and replicate. Influenza is like a fire that has the unusual property of burning only some trees, leaving others intact. (D) Many viruses, including some influenza strains, can also "smolder" by generating asymptomatic infections [14] that can be reactivated and become infective. Which of these strategies is followed by a particular viral strain depends on its history and the selective pressures that it experiences.

S2.1. Viral responses to temperature shifts – general comments

In principle, respiratory viruses might use temperature shifts to synchronize their development at critical stages in their life cycles. One or more temperature cycles (cold - warm - cold - warm etc.) might be required to complete the life-cycle of the virus. [This is possible but I suspect that most respiratory viruses simply slow down/stop at higher temperatures.] The temperature shifts might in principle be upwards or downwards, but the epidemiological evidence reviewed above suggests that activation of respiratory viruses most commonly follows drops in temperature. Moreover, viral activation following upward temperature shifts would be in danger of causing infections that would spread to the lungs. The biochemical evidence reviewed in

the main article and below suggests that entry into the cell, the initiation of transcription, and the initiation of genome replication are important steps that can be regulated by temperature. Such temperature sensitivity might give respiratory viruses several important advantages: firstly, the virus can in effect increase its virulence (activity) temporarily, allowing a contagious infection to develop, but with a low chance of killing or immobilizing its host (immobilization often reduces viral transmission [8]). Virus numbers can also build up before a temperature shift, such that when the shift eventually occurs viruses move into or out of cells in rapid bursts that can overcome their hosts' immune defenses. [I no longer think that this is the main point. I suspect that most viruses can easily overcome the immune defenses of hosts that lack specific immunity. I think temperature sensitivity is mainly about *moderating* the activity of viruses In order to maximize transmission.] This argument is similar to the explanation given for the synchronized hatching of mayflies when they emerge from lakes in order to breed: by synchronizing their appearance they increase their chances of evading predators. Similarly, viruses may evade the host's immune system by appearing in bursts. Secondly, since human family and community members have different roles and activities, they generally experience different patterns of temperature exposure. Therefore a subset of the group may become infected by a *ts* virus at a given time during an epidemic, which can allow the virus to linger for longer and leave some individuals as potential hosts in future epidemics, a trend that was noted in influenza epidemics, including the H3N2 epidemics of 1968-1970, by Hope-Simpson [3]. A third advantage is that *ts* mutations may help viruses to establish persistent infections. We can speculate that the viruses that can become dormant such as foot-and-mouth disease virus need to reduce their activity to evade detection by the host's immune system - less active viral clones may remain undetected and therefore be selected, while more active ones are destroyed. Any mutation that damps down activity could be used, but *ts* mutations have the advantage of encouraging the concentration of viruses in the coldest parts of the body, which often have lower metabolic rates. Foot-and-mouth virus recovered from carrier animals is frequently *ts* [21]. Similar selection may take place among viral clones in the respiratory tract. Since the evolutionary history of most respiratory viruses has probably included multiple episodes of selection for *ts* and non-*ts* forms, it is likely that both can be readily generated by limited nucleotide substitutions. These trends are shown schematically in Figure 3 in the main article. To use another analogy, the establishment of persistent viral infections may be like the strategy followed by a plant that produces a batch of seeds that become dormant and germinate over several years. This can decrease the probability that the whole batch will be destroyed by a catastrophe after germination such as drought or late frost [31]. Similar reasoning suggests that periods of inactivity may help viruses to achieve long-term transmission. This point is also related to the first point, above: if viruses use *ts* mutations to colonize the coldest parts of the body, they may automatically possess a mechanism that allows them to be reactivated or released when the temperature changes (possibly rising or falling) and they can emerge in a burst, which may increase their chances of survival.

Note that as host densities increase and conditions conducive to greater transmission arise, the selective advantages of *ts* strains may decrease. Taking an extreme example, during a deadly avian influenza epidemic in a chicken barn we would expect that strains that produce transient infections, or those that infect only a minority of the host population, would be at a selective disadvantage. This may be a general trend, with selection for greater virulence frequently being associated with selection for decreased temperature sensitivity.

S2.2. Viral responses to temperature shifts – a preliminary model

Temperature can obviously be used by respiratory viruses to identify the respiratory tract during infection, since the upper respiratory tract is one of the coldest parts of the body. [This is the main point!] For example, if a group of (heat-sensitive) *ts* virus particles are inhaled into a mammal or bird's lung they may remain inactive since the temperature of the lung is close to body temperature. Alternatively, they may be carried to the cooler [32] upper respiratory tract by the mucociliary escalator. There they may become immobilized (at some unknown cellular or extracellular location) possibly forming a cluster. Bear in mind that there is a temperature gradient within the respiratory tract, with temperatures in humans varying from around 24°C at the glottis to around 35.5°C at the subsegmental bronchi [32] (these measurements were made with 19°C air that was inhaled at a rate of 30 ventilations per minute). Mudd & Grant showed in 1919 that cooling the skin of human volunteers caused rapid cooling and constriction of blood vessels in the pharynx and tonsils [92]. Therefore chilling may activate one or more

clusters of *ts* viruses. This mechanism can work well if immobilization and activation of viruses are both *ts* processes, but the immobilization temperature is the higher of the two. A model based on this mechanism is shown in Figure S1. After a VRTI infection is established it may spread towards the nose (since the large number of virions released may overwhelm the host immune system, and since, moreover, mutants with greater virulence are expected to arise) or towards the lungs (since mutants with decreased temperature sensitivity are also expected). Note also that this mechanism can apply in the tropics since viruses may adapt to warmer temperatures by (1) colonizing parts of the respiratory tract nearer the nose or (2) by reducing their temperature (heat) sensitivity by mutation, i.e. strains with higher *ts* transition temperatures may evolve. Temperature dips below normal tropical temperatures can then allow viral activation in spite of generally warmer ambient temperatures.

This model is compatible with the observation (Table 2 and Figure 2) by the Eurowinter Group that outdoor exercise is protective: breathing cold air rapidly for a limited period may activate a proportion of the viruses that were previously immobilized and inactive within the respiratory tract, since breathing cold air reduces the temperature of the lining of the respiratory tract [32].

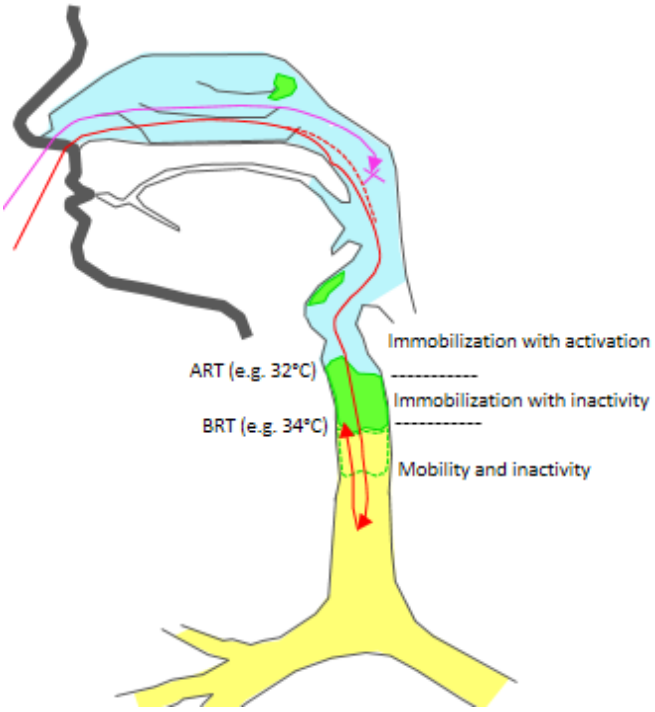

Figure S1. *A model of the typical infective route of VRTIs.* It is known that there is a temperature gradient in the respiratory tract of mammals, with lower temperatures at the nose and higher temperatures in the lungs [32]. This raises the possibility that many viruses possess *ts* properties that allow them to be activated only below a "activation restrictive temperature" (ART). In addition, viruses may possess other *ts* properties that cause them to become attached to cells (or to enter them) only below a "binding restrictive temperature" (BRT) that is above the ART. For example, the ART of a respiratory virus might be 30°C and the BRT a few degrees higher, say 34°C. This defines a transitional band of temperatures, which corresponds to one or more areas - shown in green in the figure - where the viruses will be immobilized within or near cells but not activated. One larger and two smaller areas are shown schematically in Figure S1. If a virus particle that is inhaled lands in a part of the tract (shown as pale blue) that is at a temperature below the ART, it will be immobilized and activated. This is indicated by the purple arrow. If the strain is not highly virulent or if the viral dose received is not great, the virus is likely to be destroyed by the immune system. If a virus particle is inhaled and lands in an area of the tract (shown as yellow) that is above the BRT, it will not be immobilized, and so may be carried by the mucociliary escalator to the transitional band (green), where it may bind. This route is indicated by the red arrows. (If the particle is delivered in e.g. a larger droplet it may land in a cooler area, but it may subsequently be inhaled again [solid red line]. Alternatively it may be inhaled in a fine aerosol and so travel straight to the warmer parts of the tract [dotted red line]). If the host is chilled the tract will rapidly cool [108] and the position of both the ART and the BRT will move further down the tract (dotted green lines). The result is that any viruses that were previously immobilized in the green area (or areas) will be activated in a burst. Respiratory viruses in the tropics may colonize regions of the respiratory tract that are closer to the nose. This model is hypothetical and needs to be confirmed by experiment. Many other possible mechanisms could exist. For example the BRT is not necessary – similar mechanisms could exist where viruses bind at all temperatures.

At the biochemical level, temperature sensitivity might result from inactivation at any critical step in viral development. Inactivation could occur e.g. when previously-bound viruses enter cells, at the initiation of transcription, at the initiation of viral genome replication, or when virus particles are released from cells. Biochemical evidence for several *ts* steps is discussed in the main article and in sections S9 to S11 below. Factors that may have hidden respiratory virus's temperature sensitivity from scientists are described in section S7 below.

S3. Modelling the seasonality of VRTIs

The model discussed above (Figure S1) suggests that temperature dips may trigger VRTIs. This can provide an

explanation for the seasonality of VRTIs. Figure S2 shows an extension of the model including seasonal variation. Note that this figure suggests that batches of similar virions may be released in the absence of symptoms (this accords with the ideas of Hope-Simpson, who asserted that the peculiar epidemiology of influenza required the release of virions from symptomless individuals [3]). Figure S3 shows schematically the strange global distribution of influenza epidemics, in particular the presence of the illness year-round in the tropics while it is almost absent from temperate regions during the summer months. Figure S3 also shows the seasonal movements of respiratory viruses that are predicted by the model since infection is more likely to be triggered when humans (or other mammals and birds) travel to cooler climates (for roughly the same reasons that VRTIs are most prevalent in the colder months in temperate regions).

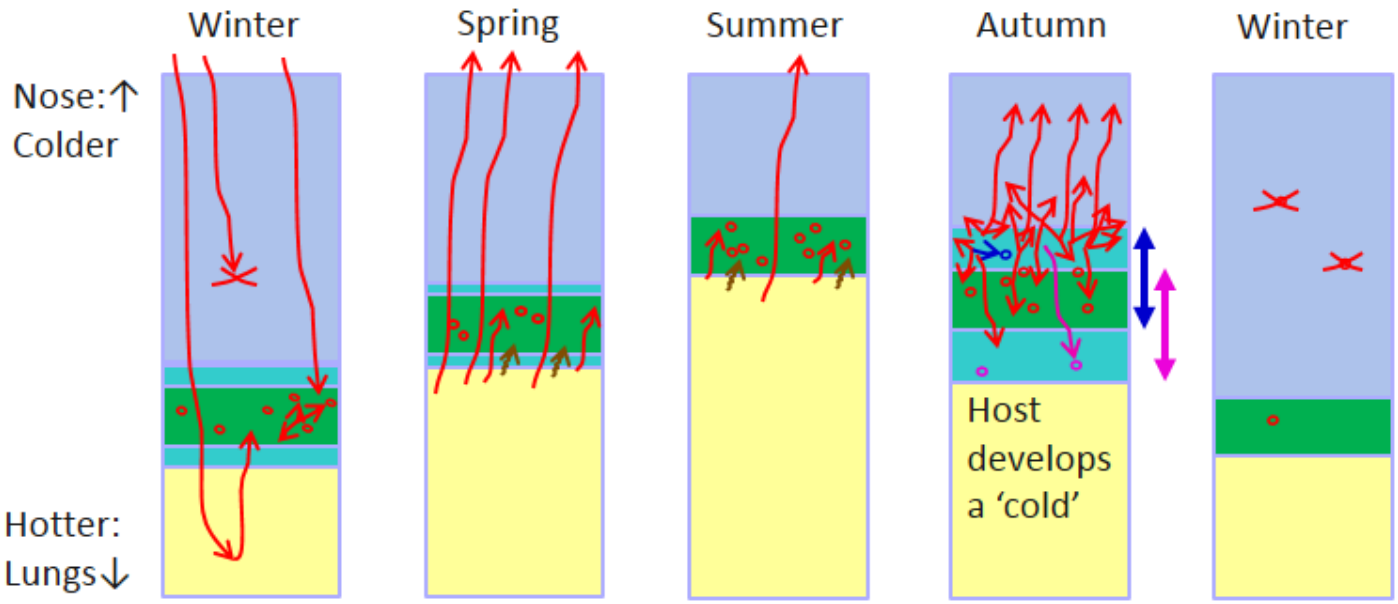

Figure S2. *An extension of the model of Figure S1, showing the predicted effects of seasonal temperature changes.* The figure shows one possible pathway that might be followed by a cluster of viruses that enter the respiratory tract (RT) in the winter months. The first panel (winter) shows the routes of entry that are suggested in Figure S1, above. In warmer weather (spring), the BRT will move up the RT and virions may be released and may bind higher up the RT, or they may leave the body by being breathed, coughed or sneezed out as indicated. This may be an important transmission route in the absence of symptoms. Note that the viruses released at any time are predicted to have similar temperature sensitivity since they derive from a particular physical region of the RT. Several mechanisms where viruses move up the respiratory tract can be considered. They may be released into the mucocillary escalator, and rebind higher up if they happen to come into contact with the cells that line the RT (short red arrows). Alternatively they may remain associated with the cell membrane and move up the RT in many small steps (brown arrows). See main text for further comments. A similar argument suggests that viruses may be released in the summer as the temperature continues to rise (middle panel). At the end of the summer, a large number of inactive viruses may be present in relatively confined areas of the RT. When the temperature dips (autumn) many viruses may be activated simultaneously, causing the symptoms of a VRTI. Some viruses will be shed, while mutants with less *ts* binding properties may travel down the RT and bind at higher temperatures (thin purple arrow). The increased physical binding range of such viruses, which have a higher BRT, is indicated by the thick purple double-headed arrow. Similarly, mutants with activation that is more heat-sensitive may become stably attached to cells higher up the RT (giving viral clones with a decreased HAT, having an increased physical binding range that is indicated by the thick white double-headed arrow). This model can explain many of the strange features of influenza and cold epidemics that puzzled Hope-Simpson [1, 3, 98, 107, 110] and others [2, 4, 5, 23, 24, 45 – 47, 51, 54, 55, 61, 94, 95]. It should be emphasized that many other pathways are possible and that this is a preliminary hypothetical model that needs to be verified by experiment. [This is all very hypothetical, but may be correct for some respiratory viruses.]

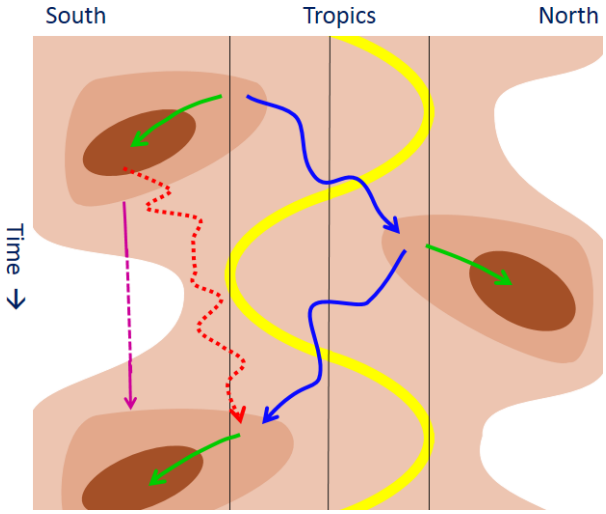

Figure S3. *The global spread of respiratory viruses predicted by the model*. Levels of VRTIs are indicated by the brown shading, with dark brown indicating the highest rates of infection, while the yellow curve shows the path of vertical solar radiation. The strange distribution of VRTIs is shown, with more VRTIs in the tropics throughout the year than in temperate regions during the summer [94, 95]. Figures 2, S1 and S2 suggest that personal chilling will increase the prevalence of VRTIs, and, since travel away from the tropical regions is generally associated with a decrease in temperature, the model suggests that VRTIs may spread more quickly from the tropics to the temperate regions (green arrows) than in the opposite direction (dotted red arrow). VRTIs are, however, expected to circulate in the tropics (blue arrows) since individuals experience climate-driven and daily temperature fluctuations in all climates. This matches the pattern of dispersion of influenza A (H3N2) reported by Russell et al. [112] who showed that seed strains circulate continuously in a network in East and Southeast Asia (blue arrows) and spread to temperate regions from this network (green arrows). The degree to which viruses remain dormant during the summer in temperate regions (dotted purple arrow) is unknown. Again the figure shows schematically a preliminary hypothetical model that needs to be verified experimentally.

S4. Studies of where ts strains were recovered from persistent infections – further details

As described above, Preble & Youngner noted many examples where cell cultures with persistent viral infections yielded virus that was *ts* [15]. These included cell cultures infected by Newcastle disease virus that was both less virulent and possessed less HA activity than the parent strain. The virus recovered had *ts* variants of HA, NA and RNA polymerase. *Ts* mutants were also recovered from cell cultures with Western equine encephalitis virus, Sendai virus, measles virus (in both human and hamster cells), stomatitis virus, Sindbis virus, and from other systems including a bacteriophage that had a polymerase that was defective at higher temperatures. Conversely, an artificially generated *ts* strain of reovirus that was injected into rats caused a slowly progressing encephalitis, while the wild-type strain caused acute encephalitis [for references, see 15].

Spontaneous *ts* persistent infections of cell cultures by influenza A have also been noted several times. Frielle *et al.* recovered *ts* influenza A strains from persistent infections of the WSN (H1N1) strain in hamster cells [16]. The *ts* virus recovered from the infection could not be amplified in eggs or several cell-lines, and expressed larger amounts of NP and dramatically reduced amounts of matrix protein in comparison to the parental strain. Similarly, Liu *et al.* established a persistent infection of influenza A (E61-24-P15) in canine cells, and recovered *ts* virus with an M1 protein that had two amino acid substitutions and was defective at 38°C [17]. In another study that used a persistent infection of canine cells with the influenza A/Victoria/35/72 virus (H3N2), after 158 days the population consisted entirely of *ts*, inhibitor-resistant small plaque clones [110]. Less than 0.05% of the cells contained the influenza virus antigen. Multiple *ts* mutations were found in the genes coding for P2, NP, NA, M and NS proteins.

Preble & Youngner [15] also found many examples where stable infections of cell-cultures by viruses involved increased production of defective interfering particles (DIPs). These include infections with vesicular stomatitis virus, measles in various cell-types, Western equine encephalitis virus, lymphocytic choriomeningitis, Newcastle disease virus, Parana virus, and Sindbis virus. As they pointed out, increased DIPs and *ts* mutants can contribute simultaneously to reduction of viral activity in a system. A more recent report suggested that the loss of DIPs caused a dramatic increase in the virulence of influenza A in chickens [25].

S5. Laboratory studies where temperature sensitivity was lost – further details

Chu *et al.* [23] identified a naturally occurring *ts* influenza A strain, Xia-*ts,* which was a subclone of the H3N2 strain Ningxia/11/72. In an attempt to generate a cold-adapted strain, they treated Xia-*ts* with dimethyl sulfate and passaged it through chicken embryos three times at 25°C, and once at 33°C. To their surprise, a non-*ts* mutant, Xia-*ts*+, was selected by this process. They later found that the

infective titres of Xia-*ts* grown at 33°C in both eggs and hamster cells were one to two logs lower than Xia-*ts*+, and this lower replication rate of the *ts* strain may explain the unexpected selection and appearance of Xia-*ts*+. They also tested the replication of both strains in the lungs of live hamsters, and found that Xia-*ts* was much less active, replicating more slowly and with a maximum titre that was two logs lower than that of Xia-ts+.

Oxford *et al.* made similar observations [24] when they also investigated naturally occurring *ts* influenza A strains that had been isolated from patients shortly before the study. They noticed that a *ts* virus, A/Eng/116/78 (H1N1), progressively lost its *ts* character during five passages at 33°C. The authors then looked carefully at a similar virus, A/Eng/772/78 (H1N1), that had minimal *ts* properties, and found that it contained a mixture of *ts* and non-*ts* viruses. The authors reached a similar conclusion to Chu: even at the permissive temperature (33°C) the *ts* character may confer a selective disadvantage in eggs.

S6. Studies of naturally occurring ts viral strains – further details

In 1963 Stern & Tippett [43] collected five viral specimens from patients with H2N2 "Asian" flu. One could not be propagated, but the remaining four were *ts*, showing cytopathic effects in monkey cells at 33°C but not at 37°C. Inoculation of eggs showed the same pattern, with agglutination at 33°C but not 37°C. Limited growth was observed in some of the cell cultures at 37°C, and subcultures of isolates obtained from them were able to grow at 37°C, albeit more slowly that at 33°C, confirming that the viruses could adapt to culture at 37°C. The authors also looked at older egg-adapted strains including FM1 (H1N1, 1947) and PR8 (H0N1, 1934). Both strains (in 1962) grew much more slowly on both cynomolgus and rhesus kidney cells at 37°C than at 33°C.

In 1977, Kung *et al.* found by accident that nine of the ten isolates of the newly emerged "Russian" H1N1 influenza that they looked at were *ts* [44]. These isolates had been passaged one to five times in eggs. The "cut-off" temperature (at which at least 90% of virus replication at inhibited) was determined for six of the nine *ts* isolates, and it was found to be roughly 38°C for three isolates, and 39°C for the remainder. (These relatively high temperatures may be due to the adaptation of isolates to growth in eggs. Note also that viral temperature sensitivity in the human respiratory tract may be finely tuned, and that viral interactions with chicken cells may give different results to those with human cells, possibly having higher cut-off temperatures.)

Around the same time, Oxford *et al.* [24] tested 26 recently-isolated H1N1 influenza strains, and found that 17 were *ts*, producing at least 10 times more virus at 34°C than at 38.5°C. Two of eleven H3N2 isolates from that period were also *ts* (H3N2 was then in circulation concurrently with H1N1). These isolates were obtained from the World Health Organization and had been passaged two to five times in eggs or cell cultures. These results were compared to those from standard laboratory strains. Of 17 older laboratory H1N1 strains originally isolated between 1947 and 1963, only three were *ts.*

Chu tested seven H1N1 strains with varying degrees of temperature sensitivity in volunteers and found a correlation between temperature sensitivity and the severity of VRTI symptoms [23]. The four with cut-off temperatures of 38°C or below gave milder fevers when administered to volunteers, and were variable in eliciting an antibody response. Those with cut-off temperatures of 39°C or above gave fevers above 38.6°C in eight volunteers and generally gave strong antibody responses.

S7. Factors that may have hidden respiratory viruses' temperature sensitivity from researchers

There are many reasons why scientists may have worked with viral strains that were not obviously *ts,* even when the wild strains from which they were derived were. Viruses have high mutation rates, which ensures that they adapt quickly to new growth conditions. Twenty-two codons in the HA1 segment of HA (hemagglutinin) have been identified that frequently mutate in embryonated chicken eggs [39]. Another study found that after the human influenza virus A/Fujian/411/2002 (H3N2) had passaged six times in eggs both the HA and NA protein sequences were altered, with four amino acid substitutions in HA and one in NA [40]. It is clear that the virus rapidly adapts to new hosts by the mutation of residues in critical positions in viral proteins. The key point, however, may be the unexpected correlation between viral activity and temperature sensitivity, discussed above in the main article (Figure 3). This may have resulted in the loss of temperature sensitivity almost as soon as viral strains were isolated, which is suggested by the findings of Chu and Oxford described above [23,24]. Normally newly-isolated strains

are passaged several times before they are fully characterized. For example, when the discovery of human coronaviruses was first published, the strain had already been passaged seven times in various human cells [38]. Other laboratory viruses that are used routinely in research were isolated from wild strains more than 60 years ago, and have been passaged hundreds of times. Chu *et al.* noted in 1982 that the WSN virus strain in their lab had "undergone many passages in ferrets, mouse lung, mouse brain and chicken embryos" [23].

Changes to non-coding RNA and synonymous mutations that do not change amino acid sequences may nevertheless change the secondary structure of RNA. The ordered RNA sequences that are the targets for such mutations are relatively large, which increases the probability of mutation. As discussed below, it is likely that changes to RNA secondary structure can influence the temperature sensitivity of viruses.

Another reason why the importance of viral temperature sensitivity has not been recognized may be that many researchers deliberately work with artificial *ts* mutants, either for biochemical studies or to make vaccines. This may have obscured the frequent temperature sensitivity of wild-type viruses. (This also implies that some laboratory *ts* mutants may be closer to actual wild-type viruses than the corresponding lab strain that is regarded as "wild-type".) Researchers have also focused on the factors that allow or prevent viruses from transferring between species, particularly the temperatures of the sites where viruses replicate in different animals [37], rather than the effect of temperature *fluctuations.* (The temperature of the replication site may not be an important barrier in practice because the respiratory tract of mammals and birds possesses sites that encompass almost the full range of temperatures from body to ambient air temperature, as discussed above.)

S8. Viral Incubation periods – detailed discussion

Lessler *et al.* reviewed published articles on nine VRTIs [58], namely adenovirus, human coronavirus, severe acute respiratory syndrome coronavirus, influenza A, influenza B, measles, parainfluenza, RSV, and rhinovirus, and they found median incubations periods that ranged from 0.6 days (influenza B) to 12.5 days (measles). The authors included 15 experimental studies and 24 observational studies. However, it can be argued both approaches are likely to underestimate incubation periods.

With experimental studies, everything depends on the choice of viral strain that is used to inoculate volunteers. As discussed above, experimenters may consciously or unconsciously choose strains that rapidly and consistently cause infection. The typical "wild" respiratory virus would probably give fewer colds in the time available for the study.

Observational studies are likely to underestimate incubation periods for a different reason. Consider the following well-known incident from 1977 [59], which was included in Lessler's analysis: a Boeing 737 airliner was delayed on the ground for three hours because of engine failure, with the air-conditioning turned off. One passenger was in the early stages of influenza A, and within 72 hours 72% of passengers developed the symptoms of influenza. If, however, the disease had taken, say, a week to develop, it is likely that the incident would never have been reported, partly because it would not have been clear that the sufferers had been infected on the plane. A similar case was reported in 1918, when two doctors travelled from London to York sharing a railway compartment an airman who had severe symptoms of "Spanish" influenza [60]. The doctors became ill in 41 hours and their families caught the disease from them in a similar period. The clear-cut origin and timing of the disease encouraged them to report the observed incubation period to the British Medical Journal [60].

In normal human societies, individuals are typically exposed to many different viruses every day, and it is difficult to trace the source of any particular infection. One of the few settings where it is possible to follow the progress of individual viral epidemics is in isolated Antarctic communities (in recent decades this isolation has been confined to the winter months). Such captive groups have limited and known contacts with outsiders and provide an excellent opportunity for epidemiological studies [61]. In their review of incubation periods, Lessler *et al.* found literature estimates of the incubation period of parainfluenza of 2 - 6 days [58]. However, Muchmore *et al.* reported persistent shedding of parainfluenza by healthy young adults throughout the 8½-month winter isolation period at Amundsen–Scott South Pole Station during 1978 [62]. Two episodes of respiratory illness caused by parainfluenza were observed that year after 10 and 29

weeks of complete social isolation. Parainfluenza virus in this environment is unable to survive for more than 17 days either inside or outside of the polar station [109]. It is clear that (1) the virus was able to become dormant in asymptomatic carriers who nevertheless shed virus, (2) the incubation period (or the period of harboring the virus before passing it to other individuals) was much greater than 2.6 days, and (3) the illness was able either to flare up in a dormant carrier, or to be passed from a dormant carrier to a new host who developed a VRTI. In another Antarctic study [61], Cameron & Moore reported the case of a geologist ("J.E.H.") who, after twelve months of isolation, picked up a virus from a visiting Russian field party. J.E.H reported minor abdominal discomfort a few days later, then, 17 days after leaving the field party, participated in an out-door activity that caused his hands and some outer clothing to become cold and damp. Thirty-six hours after that, J. E. H. noted the onset of sore throat, mild rhinorrhea and muscle aches, as did two of the three men who worked out-doors with J.E.H. In spite of attempts, the virus involved was not isolated or identified. This case shows again that a respiratory virus could remain largely dormant for 18 days, during which period it was passed to other individuals, and also that the incubation period was far greater than Lessler reported for any respiratory virus. It is also a case of apparent "viral activation" by exposure to cold. A third Antarctic report from Adelaide Island in 1969 showed that the incubation period of an unknown respiratory virus was at least 17 weeks [121].

Studies in temperate regions have also identified asymptomatic carriers who failed to seroconvert as described in the main article.

A reasonable interpretation of the evidence above is that incubation periods are often close to those reported by Lessler [58], but that longer incubation periods and extended viral dormancy also exist and can have profound effects on the long-term survival and evolution of respiratory viruses and the re-emergence of VTRI epidemics.

S9. Studies of transcription in influenza – further details

Scholtissek and Rott conducted a study [82] with the Rostock strain of fowl plague virus, which infects chickens. Figure 3 of their report shows a maximum activity of viral RNA polymerase at 36°C, five degrees below chickens' normal body temperature (41°C). Polymerase activity fell off rapidly above 36°C. At 4l°C the virus RNA polymerase was unstable in vitro as well as in vivo. The synthesis of virus HA and NA was, however, unimpaired at 4l°C.

The genome of influenza A consists of eight single-stranded RNA segments, referred to as viral RNA (vRNA). These segments are negative-sense, meaning that they must be transcribed by the viral polymerase to produce viral messenger RNA (mRNA). This mRNA is translated to produce the 11 viral proteins. However the mRNA is not a complete copy of the vRNA and in the early stages of viral infection a second batch of positive-sense RNA is also produced called cRNA. This is an exact copy of the vRNA, and serves as a template to generate the vRNA. Ulmanen *et al.* looked at the first step of influenza viral mRNA synthesis [83]. This is the endonucleolytic cleavage of heterologous RNAs containing cap 1 (m7GpppNm) structures to generate capped primers that are 10 to 13 nucleotides long, which are then elongated to form the viral mRNA chains. When they investigated these steps using detergent-treated wild-type viruses they found that the rate of transcription was about 10 times greater at 33°C compared to 39.5°C [Figure 1A of ref. 83]. Since they wanted to use artificial *ts* mutants at 39.5°C, they did not use this detergent-treated material. Instead they used purified "viral cores" that were more stable at 39.5°C. (Even using the purified material, activity was reduced by about 20% at 39.5°C in comparison to 33°C [Figure 1B of 83].) The unpurified material apparently contained "an undefined nuclease", which was more active at 39.5°C than at 33°C and which degraded the primer fragments generated by the viral cap-dependent endonuclease. (Whether this nuclease was contributed by the virus or the host is not important. The virus-host system was *ts*.) This temperature dependence was not investigated further, and purified material lacking the nuclease was used in the studies. The authors later investigated the binding of a cleaved primer cap, referred to as the A13 fragment, to the viral cores. "Unexpectedly", the wild-type viral cores bound much more weakly to A13 at 39.5°C than at 33°C [Figure 5 of ref. 83]. (This difference was eliminated by adding ATP, GTP and CTP to the mixture so that further work could be carried out with *ts* mutants.) Once the heterologous RNAs were cleaved, the subsequent steps of transcription were temperature insensitive [Figure 4 of ref. 83 focusing on the first five minutes]. These studies suggest the presence of one or more *ts* switches that initiate transcription.

Kashiwagi *et al.* looked at the effect of temperature on RNA production for five influenza A strains [84]. These strains came from a wide variety of sources, and included WSN (an H1N1 strain that was isolated in 1933), 1968 H3N2 ("Hong Kong flu"), 2009 H1N1 ("swine flu"), and two strains of H5N1 "bird flu". For all strains, mRNA production increased with increasing temperature. While vRNA production increased when the temperature was increased from 33°C to 37°C, it unexpectedly decreased for all five strains when the temperature was further increased from 37°C to 42°C [Figure 1B and Table 1 of ref. 84] (cRNA production also decreased for two of the five strains). By creating artificial hybrids containing PA subunits from different influenza strains, the authors showed that the PA subunit of the viral polymerase caused this thermal sensitivity. Since only three temperatures (33°C, 37°C and 42°C) were examined, the study did not show at what temperature the maximum vRNA production would have occurred. The maximum may have been below 37°C. Moreover, the viral strains used are typically propagated at 37°C and can be presumed to have adapted to this temperature. All of the strains except for A/Kurume/K0910/2009 had been maintained in a laboratory environment for at least five years prior to the experiments (in the case of WSN around 76 years).

Working with the PR8 influenza A strain (isolated in Puerto Rico in 1934) [85], Dalton *et al.* showed [Figure 1 of ref. 85] that the production of mRNA is favored at high temperatures (41°C), with very little vRNA being produced. A plasmid-based recombinant system that recreates functional influenza virus RNPs in cells was also used in the study. Using this, it was clear that as the incubation temperature increased from 31°C to 39°C the amount of replicative RNA products (c- and vRNA) decreased and a greater accumulation of mRNA was observed [Figure 2 of ref. 84]. mRNA is produced by the viral polymerase using vRNA as a template. The complex of the polymerase with vRNA degrades very slowly at 37°C [Figure 7 of ref. 85], allowing efficient production of mRNA. However, the cRNA that is used as a template to make the vRNA forms a complex that is particularly heat-labile, showing rapid dissociation even at 37°C. The authors suggest that a *ts* "switch" regulates the transition from transcription to replication.

Note that Stern & Tippett found in 1962 that PR8 grew more slowly in monkey cells at 37°C than at 33°C [43].

Some steps of viral development were not affected by higher temperatures. Scholtissek found that the synthesis of virus HA and NA was unimpaired at 4l°C [82]. Kashiwagi and Dalton both found that mRNA production increased above 37°C [84, 85]. Ulmanen found this to be the case from the point where transcription was initiated by the cleavage of the heterologous RNAs [83].

S10. RNA secondary structure – further details

Brinson *et al.* studied infectious salmon anemia virus (ISAV), which is a member of the family *Orthomyxoviridae*, and a distant cousin of the influenza viruses (like influenza A and B, but not C, the virus has eight genomic segments of single-stranded RNA with negative polarity) [86]. They predicted a "temperature-dependent switch in RNA secondary structures between 15°C, the temperature of the North Atlantic and the optimal temperature for ISAV replication, and 37°C", noting that the virus does not replicate above 25°C. The authors also tested the temperature dependency of RNA structures using high-resolution NMR spectroscopy and thermal melts.

The secondary structure of the RNA of influenza A is conserved in both negative-sense (vRNA) and positive-sense RNA (mRNA and cRNA). The untranslated 3' and 5' ends of vRNA strands contain complimentary sequences that can hybridize to circularize the strand forming a panhandle structure both *in vitro* and *in vivo* [87]. The structure formed creates the promoter that is needed for vRNA to be copied to make positive-sense RNA. On binding the viral polymerase, both termini form hairpins, a rearrangement referred to as the "corkscrew" structural model. A 2010 study suggests that virus-encoded short RNA transcripts that are complimentary to the 3' termini of cRNA may allow viral RNA synthesis to switch from transcription (production of mRNA) to replication (production of vRNA) [88]. All these interactions between complimentary RNA sequences provide opportunities for changes in temperature to switch viral biochemistry on or off.

The secondary structure of influenza A segment 7 mRNA, which encodes the M1 matrix protein and the M2 ion channel, has been studied in detail [89]. This region can fold as either a hairpin or a pseudoknot, and (if either interacted with a protein that would increase stability) a *ts* switch could presumably be created.

S11. Viral functionality that is affected by naturally occurring temperature sensitivity

It may be helpful to compare the viral functionality that was affected by *ts* mutations in the studies of influenza described above. The uptake into cells of the triple assortment virus A/Jap/Bel (H2N2/H1N1/H1N1) used by Russell was *ts* [65]. Ulmanen *at al.* found that the very first steps of influenza A mRNA synthesis were *ts*, but that subsequent steps of transcription were not sensitive to temperature [83].

Liu *et al.* recovered *ts* virus with defective M1 protein from a persistently-infected cell culture [17]. Frielle *et al.* recovered *ts* virus from a persistently infected cell culture that expressed larger amounts of NP and dramatically reduced amounts of matrix protein in comparison to the parental strain [16]. Chu *et al.* looked at naturally occurring *ts* strains and found lesions in the NP gene of two H3N2 strains and in a matrix protein gene of two H1N1 strains [23]. Oxford *et al.* found that the *ts* lesions in naturally occurring *ts* H1N1 strains were not located in the NA or HA proteins [24]. Priore *et al.* showed that the positive-sense RNA in influenza A is predicted to have extensive conserved secondary structure in segments 1, 2, 5, 7 and 8 and that the predicted thermal stability of (+)RNA is correlated with the temperature of the site of viral replication in different species [90]. Kashiwagi *et al.* found that, for five diverse influenza A strains, mRNA production was insensitive to temperature, but that vRNA production was *ts* [84]. They found that the PA subunit of the viral polymerase caused this thermal sensitivity. Dalton *et al.* showed that the production of mRNA is favored at high temperatures (41°C), but that very little cRNA and vRNA are produced at high temperatures [85]. They also showed that the cRNA that is used as a template to make the vRNA forms a complex that degrades rapidly at 37°C. Finally, Takashita *et al.* found that less hemagglutinin-esterase-fusion protein in influenza C (C/Ann Arbor/1/50) was found on the cell surface at 37°C compared to 33°C, and less membrane fusion was observed [66]. The authors attributed the reduction of HEF at the higher temperature at the cell surface to the thermal instability of the HEF trimer.

S12. Possible sites for persistent viral infections resulting from local chilling

Viruses such as foot-and-mouth virus seem to locate the tissues that they will infect using temperature, including the lips, tongue, soft palate, pharynx and feet [18]. VRTIs sometimes affect tissues outside the respiratory tract that are colder than the individual's general body temperature, such as the feet and head in birds. Possible benefits to the virus include (1) the provision of a reservoir of virus that can become activated when the temperature of those tissues subsequently rises or falls, and (2) the transmission of viruses to other hosts from, for example, blisters on feet. Human respiratory viruses may also affect sites outside the respiratory tract, although it seems unlikely that they frequently spread by viral shedding from these sites (with the exception of VRTIs such as chickenpox and measles that cause skin eruptions). It is possible, however, that human tissues may become infected "accidentally". Chilblains are a poorly understood tissue injury that can occur when a predisposed individual is exposed to cold [100]. They most often affect the toes, but many parts of the body that tend to be cold can be affected, including the fingers, ears and nose. Chilblains can occur in one geographical area but not in another that has the same general characteristics of temperature and humidity. Occasionally the illness persistently affects one side of the body [100]. These observations are compatible with a viral involvement in the disease, suggesting that chilblains may be analogous to the blisters generated by foot-and-mouth disease in cloven-hoofed mammals (although, unlike those blisters, chilblains are probably "accidental" in that they may not contribute to transmission of the virus involved).

Chapped lips (cheilitis) can be caused by cold weather, often when the individual has a cold, but they are also associated with high fevers, including fevers caused by VRTIs [101 – better ref?]. The conventional explanation is that breathing through the mouth when the nose is blocked causes the lips to become "dried out". However, it is not clear how the extra humidity of breath can cause "drying". An alternative explanation is that the virus itself is immobilized in the lips and causes chapping, since the lips tend to be cold, especially when breathing through the mouth.

An interesting parallel may be drawn between the study by Johnson & Eccles where volunteers' feet were chilled and a study by Baerheim & Laerum, who found that chilling of the feet was associated with the subsequent development of bacterial symptomatic lower urinary tract infection in a sample of cystitis-prone women [52]. This is consistent with the widespread belief in the Norwegian lay population that cystitis may be induced by having cold feet. Respiratory viruses including adenoviruses, mumps and

influenza A virus can play a role in cystitis or kidney infections [53, 68, 77].

S14. VTRIs in institutions

Hope Simpson pointed out that the attack rates of influenza in families are generally low, whereas attack rates in institutions such as boarding schools and army camps are often high [1]. Consider a respiratory virus in a family setting that evolves increased virulence. The likely result is that a higher proportion of family members will become sick and remain at home. Since these patients will not be at work or school there may be *fewer* opportunities for transmission to other families than would be the case for a milder strain that allows its hosts to carry on working. (Animals may show similar behavior because they often keep away from other members of their species when they are sick. For example, cattle with foot-and-mouth disease often stand alone in a field and may be reluctant to move [19].) Contrast this with the situation in an institution. There, patients may be forced to remain close to their colleagues whether they are sick or well, and extra virulence may increase viral shedding and allow greater transmission. This may explain the sudden appearance of "Spanish" Influenza in the autumn of 1918 [99]. During World War I, unprecedented numbers of soldiers were stationed in military camps and trenches, many concentrated on the Western Front (for example, 1.1 to 1.2 million British and Dominion soldiers were there at any one time during the war [50]). Moreover, unlike most civilians, they engaged in *synchronized* activities and were therefore subjected to simultaneous temperature fluctuations, including fluctuations from weather in tents and trenches. The high density of susceptible hosts, combined with high transmission rates resulting in part from temperature fluctuations, may have given rise to non-equilibrium selection that increased virulence [6]. The 1918 pandemic appears to have begun in a sparsely-populated rural in Kansas, where it caused several deaths [134]. However the strain decreased its incubation period [60] and increased its virulence after it spread into the armed forces. Once this high level of virulence was attained in the military, similar selection may have maintained virulence and hastened the virus's transmission through civilian populations.

Wild viruses may constantly adjust their virulence by rapidly evolving strains with increased or decreased virulence in response to changing opportunities for transmission. In practice the evolution of animal viruses may not be characterized by an "arms race" between animals and viruses, since viruses may in general moderate their virulence. The arms race may be between different members of the host species, as each "aims" to be less susceptible than the average. This point is illustrated by a joke about two scientists who are running away from a bear. One says to the other, "it's no use, you can never outrun a bear". The other replies, "I don't have to outrun the bear, I just have to outrun you."

S15. The fever response

Many vertebrates raise their body temperature in response to microbial infection, either by seeking a warmer environment or by expending energy [91]. However the usefulness of this ancient response is controversial, and studies have failed to show any benefit in helping recovery from infection in humans, or any undesirable effects of treating fever with antipyretics [91]. One possible benefit is that higher body temperature does not aid recovery but may prevent new acute viral infections by keeping the body permanently above the range of temperatures where other existing persistent infections can be activated. This may partly explain one of the features of influenza that perplexed Hope-Simpson [3]: the tendency for each new epidemic of influenza to replace all previous strains with very little overlap. (One can also argue that fever may reduce the risk of predation etc. by speeding up recovery. A higher body temperature will in principle speed up the metabolism of both the host and the pathogen – if anything the pathogen can more easily adapt to higher temperatures since it is much less complex and can mutate much more rapidly. However the outcome is likely to be arrived at faster if the body temperature of a sick animal rises, reducing the likelihood of discovery by a predator or competitor.)

S16. The usefulness of absolute and relative humidity for research into VRTIs

Relative humidity is the appropriate variable to consider when investigating the drying of virions in aerosols and on surfaces, since it is a measure of the drying capacity of air. In the biolab setting, absolute humidity has little physico-chemical meaning (since biological entities are produced at moderate temperatures and at atmospheric pressure). Absolute humidity is, however, the appropriate meteorological parameter to record if you are interested in drying effects indoors since indoor temperatures vary little in developed countries throughout the year and humidity is not precisely controlled in most homes and workplaces.

Outdoor absolute humidity can therefore provide an estimate of indoor relative humidity.

S17. Google Flu Trends

Google Flu Trends (GFT) provides a wealth of information in a form that is far easier to access and evaluate than the data that is provided by the national research institutes that Google uses to calibrate its models. There are some interesting variations in the data presented by GFT. For most countries, GFT attempts to model and predict influenza-like illness (IFI). IFI is defined by the Centers for Disease Control and Prevention as a fever above 37.8°C accompanied by a cough or sore throat in the absence of a known cause other than influenza. (Note, however, that only 20 – 70% of IFIs are caused by influenza [138] and that other viruses including Rhinoviruses, RSV, adenoviruses and parainfluenza viruses commonly cause IFIs.) However, GFT for Germany, South Africa and Ukraine models acute respiratory infection (ARI) rather than IFI. The main difference is that ARI includes illnesses that do not cause fevers. The GFT plots at the time of writing for southwestern Germany (Baden-Württemberg) and the closest region of France (Alace) are shown in Figure S4. Striking differences are apparent. IFI epidemics in Alsace typically develop in late December, which may imply that influenza strains (and similar viruses) initially cause colds, and evolve into strains that cause fevers later in the cold season. However ARIs in Germany arrive explosively at the beginning of September and peak in October. If one ignores anomalous peaks in particular years (which may be caused by IFIs) it can be seen that ARIs then decline slightly, forming a nearly constant plateau until a second peak in February. The sudden arrival of ARIs in the autumn, which agrees with the observations of e.g. van Loghem [4] and Milam [126], suggests that respiratory viruses that were present in individuals' respiratory tracts in the summer were activated when the temperature dipped in the autumn.

S18. Viral selection in laboratory strains

Many of the arguments in this paper hinge on the differences that may exist between laboratory viral strains and the corresponding wild-type strains. This in turn depends on how laboratory strains are propagated. Serial passages of viruses in cell cultures tend to select the fastest-growing variants, and, as discussed above, this selection would be expected to reduce temperature sensitivity if it is present. Loss of temperature sensitivity has been observed in the first few passages of newly isolated viruses [23, 24]. The entities being selected here are virus particles, and the selection may be similar to that experienced by novel infectious diseases in nature [6] with "colonizers" having the greatest replicative success [97]. Such selection would be expected to predominate, for example, in an embryonated egg inoculated with a laboratory influenza strain. In a typical experiment, the virus would grow rapidly before being harvested (in part to provide a viral stock for future experiments). Here the host cells cannot reproduce quickly enough to influence selection. By contrast, medium-term selection in cell-cultures that contain viruses tends to reduce viral activity and virulence and increase temperature sensitivity [15]. Here selection may predominantly be of cells (containing viruses), and cellular repair mechanisms and cell division must keep up with viral activity. The viruses selected may be "competitors" that are less virulent but can outcompete colonizers in co-infected cells or prevent subsequent infection. This type of selection is likely to dominate when persistently infected cell lines are established [15].

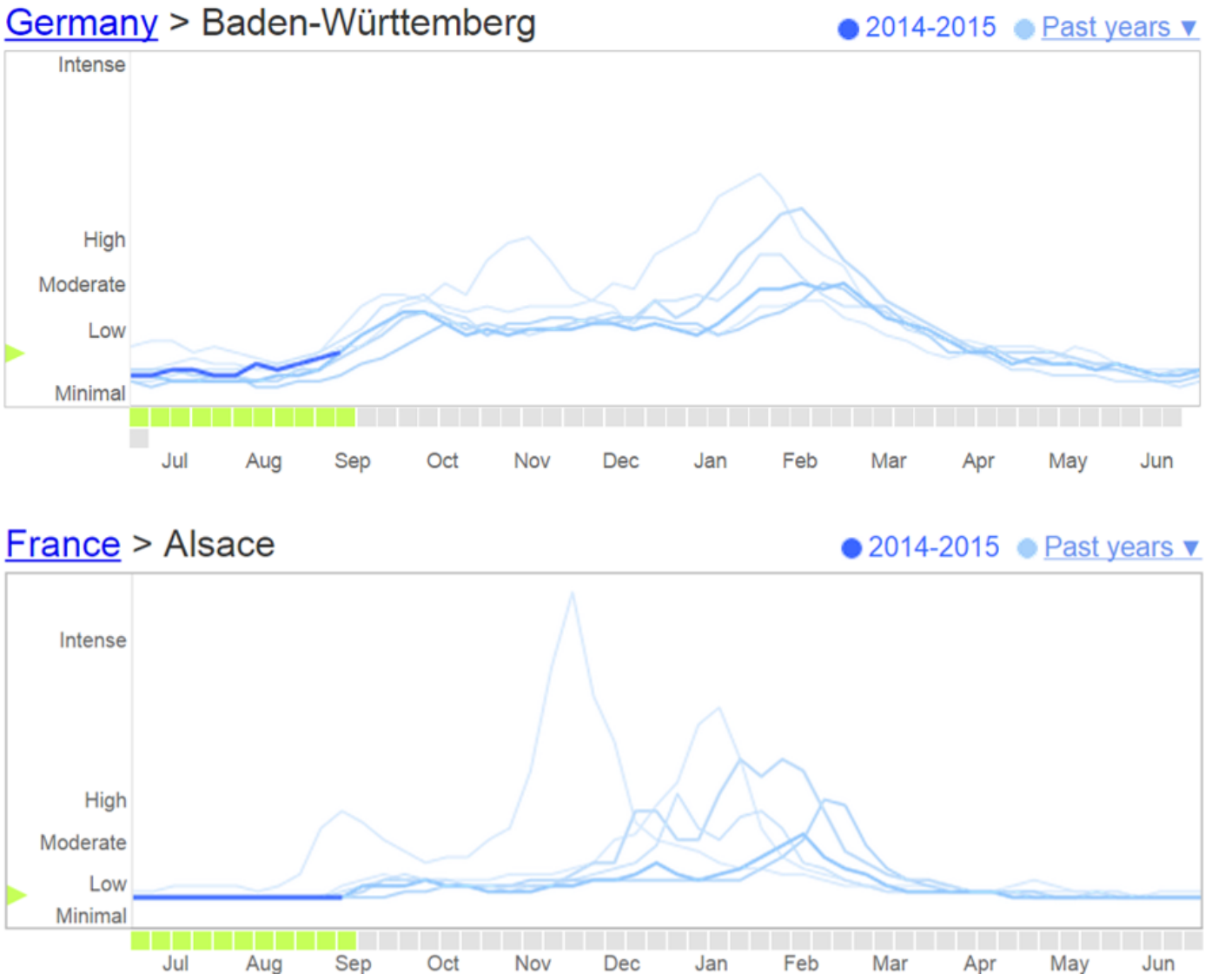

Figure S4. *Google Flue Trends (GFT) in neighboring areas of Germany and France.* GFT in Germany attempts to model acute respiratory infection (ARI), which includes illnesses that do not cause fevers. In France it models influenza-like illness (ILI), which is defined as a fever in the presence of either a cough or a sore throat. Note the sudden onset of ARI in Germany, as soon as the temperature dips at the end of summer. In contrast, ILI in France typically develops at the end of December. See section S17 for further discussion.

S19. Some natural history

The scientist and writer Rupert Sheldrake once remarked to me, "All science starts with natural history" – that is, with disinterested observation of natural phenomena. I would like to include some anecdotes about VTRIs, not to draw definite conclusions, but to suggest opportunities for experimentation.

- In February 2011, I travelled to Mumbai, and woke up on the first morning with few bedclothes covering me, but with the air conditioning on, and I found that I had a distinct sore throat. Steam inhalation had previously prevented a VRTI from developing when I used it as soon as the infection became detectable, so I used inhalation for ten minutes in my hotel room each morning for five days, which made my throat feel much better for about 30 minutes on each occasion. However the cold progressed to a fever which lasted for ten days and seemed unusually severe.
- In September of that year, I noticed a scratchy throat. I used steam inhalation which removed all symptoms of a VRTI. Four weeks later I again had a scratchy throat, which was this time not cured by steam inhalation, and was followed by feverish cold.
- In September 2012 I developed a cold and sore throat. This time I did not use steam inhalation and instead I avoided hot drinks. I completely

recovered within two days, which was faster than I had expected.

My interpretation of these and other events is: (1) in the early stages of a VRTI, heat can interfere in some way with viral replication. (2) Once a substantial VRTI has developed heat can spread the infection or hinder the immune system in eliminating the infection. (3) Heat may sometimes suppress a VRTI but not eliminate it.

S20. Conversations with virologists

I have heard the statements below from virologists or medics. All need to be examined carefully.

"Viruses cause colds and flu, not exposure to cold." This assertion, which appears in medical textbooks [104], is based on a false dichotomy. Very few educated people believe today that colds and flu are not viral diseases that can be caught from infected individuals. However many people (whether ignorant or educated) believe that chilling can cause the symptoms of VRTIs to appear by activating viruses that are already present. This distinction was made very clearly by van Loghem in 1928 [4].

"Influenza viruses can't become dormant." It is difficult to generalize about the behavior of viruses because they have a very high rate of mutation and occasionally jump species barriers. However, it is known that many viruses, including foot-and-mouth virus, chickenpox virus, HIV, parainfluenza and Epstein-Barr virus, can become dormant. Avian influenza, including the highly pathogenic H5N1 strain, can be transmitted by ducks that are themselves asymptomatic [14]. Several studies reported that asymptomatic individuals harbored or shed influenza A virus, although they did not have antibody in their blood [128 - 131].

"Viruses can't survive in the bloodstream." Many viruses cause viremia, including those causing mumps, rubella and measles, all of which can also be found in the urine of patients [68-70]. Avian influenza infects most organs of the chicken [67], and human influenza can infect mice, again invading multiple organs [106]. Several reports describe viremia in human influenza A [73 - 77], including the study of Khakpour *et al.* who found viremia during the incubation period of the virus in one patient [78]. The presence of viruses in the blood was picked up by chance and would not normally have been noticed, so it is not known how common this is. Influenza A can cause skin rashes [103] and hemorrhagic cystitis [77].

"Bugs, including viruses, can be very sensitive to temperature". This is true, but one needs to ask why. A virus could very quickly develop more heat-stable proteins by mutation. It seems that heat-sensitivity is important in some aspect of viral replication or transmission.

"The idea that being cold can give you a cold is an old wives' tale". I have tried to show that the scientific evidence demonstrates that the idea is much more than an old wives' tale. However, traditional beliefs throughout the world are remarkably consistent in suggesting that personal chilling often causes respiratory disease. A famous English folk song holds that visiting Ilkley Moor bart 'at (without a hat) could be a fatal. Although many UK citizens accept the idea, my Russian friends assure me that Russians are much more conscious than the British of the dangers of chilling for children and others. In Brazil, India, Eastern China and Malaysia, cold weather including wet weather is held to increase the risk of respiratory illness. An Indian friend pointed out that *increases* in temperature can cause the symptoms of a cold, but that a cold bath would usually eliminate the problem. He told me that both rain and wind are held to bring on colds in India. In the South of France a cold draught is thought to cause colds, while in Japan cold, clear weather is held responsible. An Indonesian friend emphasized that it was not a question of temperature, but that rain and wind could precipitate a cold. It is interesting that in many cultures the feet are worthy of special attention in this context. In Germany and the Netherlands a traditional cold cure is to go to sleep wearing socks that have been soaked in water or vinegar. The feet play a very important part in traditional Chinese medicine, while in northern Brazil there is a widespread belief that walking or running on hot asphalt in the cool of the evening will bring on a cold. Note that Johnson & Eccles found that chilling the feet does indeed cause colds [51].

To any readers who doubt the ideas put forward in this review, I would ask the following question: would you go swimming in cold water when you had a cold?

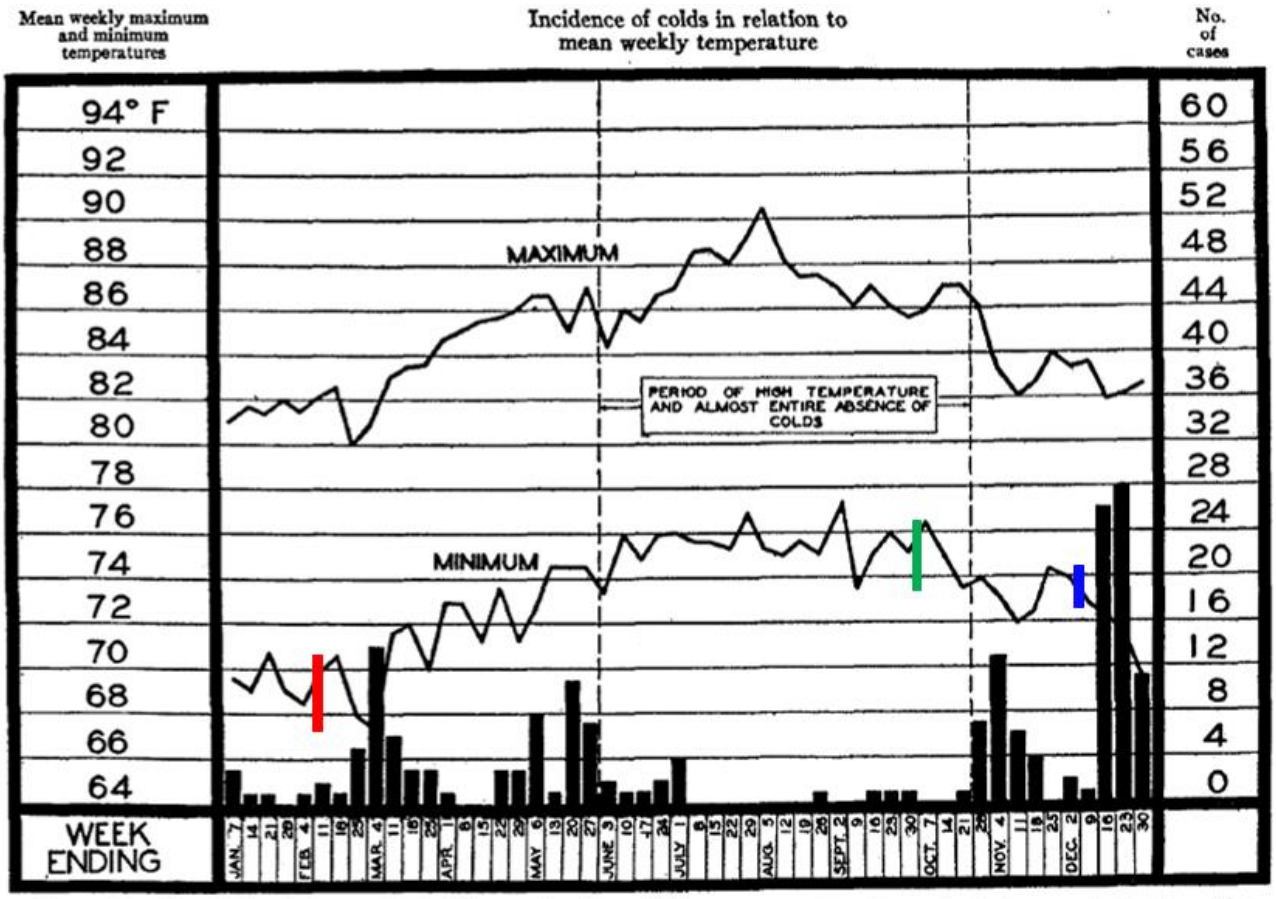

CHART 1. Correlation between the incidence of colds and the mean weekly atmospheric temperature, Cruz Bay, St. John, Virgin Islands, 1929.

Figure S5. *Chart 1 from Milam and Smillie's 1929 study of colds on an isolated tropical island.* The authors noted that outbreaks of colds often followed temperature drops, and were almost entirely absent in the summer months. The red, green and blue bars indicate temperature fluctuations of 1.9, 1.7 and 1.0°C respectively. See the main text for more details.

Proposed experiments

	Binding	*Entry into cells*	*Transcription*	*Replication of genetic material*	*Release*	*Complete viral replication*
Human	Investigate chilblains and chapped lips	Compare the number of colds in volunteers with and without chilling making use of respiratory viruses that they happen to be carrying.		"Deep-sequencing" of fresh viral isolates		Subject volunteers to cyclical chilling, using viruses that are naturally present. Compare *ts* properties of early and late isolates.
Animals	Image naturally-occurring and inoculated virions that may be immobilized in patches in the respiratory tract. Attempt to release viruses by changing temperature					Cyclical chilling of e.g. ferrets with newly-isolated strains
Cell culture	Pull virions out of solution by washing cells at low temperature, then release at high temperature (rather like phage display, the genetic material remains attached).				Look for release of (newly-generated) virions after temperature shifts	Simple temperature-cycling experiments with newly-isolated strains
Cell culture - focussing on RNA		Follow transcription, cRNA production and vRNA replication (using what? Real-time PCR, biochips, gels, radionucleotide labeling?)				
Cell culture - focussing on protein		Follow synthesis of viral proteins (using what? MS, SPR, gels, labeling, immunoassay, NMR?)				
In vitro	ITC / thermal shift of hemagglutinin/ receptor complex?	Measure entry into cells using something like the Peter Russell method	Measure thermal stability of secondary structures of RNA and RNA/protein complexes		Follow thermal kinetics of NA	
Bioinformatics and theoretical	Analysis of HA structures and sequences		Prediction of stability of secondary structures of RNA and RNA/protein complexes; analysis of polymerase structures and sequences		Analysis of NA structures and sequences	Epidemiological simulations etc.

Figure S6. *Proposals for biochemical studies of VRTIs.* Many other possibilities exist! Suggestions are welcome.

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