Seasonality and selective trends in viral acute respiratory tract infections ☆

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ABSTRACT

Influenza A and B, and many unrelated viruses including rhinovirus, RSV, adenovirus, metapneumovirus and coronavirus share the same seasonality, since these viral acute respiratory tract infections (vARIs) are much more common in winter than summer. Unfortunately, early investigations that used recycled "pedigree" virus strains seem to have led microbiologists to dismiss the common folk belief that vARIs often follow chilling. Today, incontrovertible evidence shows that ambient temperature dips and host chilling increase the incidence and severity of vARIs. This review considers four possible mechanisms, M1 - 4, that can explain this link: (M1) increased crowding in winter may enhance viral transmission; (M2) lower temperatures may increase the stability of virions outside the body; (M3) chilling may increase host susceptibility; (M4) lower temperatures or host chilling may activate dormant virions. There is little evidence for M1 or M2, which are incompatible with tropical observations. Epidemiological anomalies such as the repeated simultaneous arrival of vARIs over wide geographical areas, the rapid cessation of influenza epidemics, and the low attack rate of influenza within families are compatible with M4, but not M3 (in its simple form). M4 seems to be the main driver of seasonality, but M3 may also play an important role.

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The seasonality of colds and 'flu'

When you have eliminated all which is impossible, then whatever remains, however improbable, must be the truth.
[A. Conan-Doyle, the Case-Book of Sherlock Holmes, 1927.]

The lack of a sound explanation for the seasonality of viral acute respiratory tract infections (vARIs) is a major problem for microbiology [1–4]. Other anomalous features of vARIs need to be explained too. For example, vARI epidemics often erupt very rapidly after ambient temperature drops. The increase may, however, be too rapid and too short-lived to be the result of increased transmission [5], as discussed below. Surveys also show that epidemics often occur simultaneously throughout wide geographical areas [5–7] (see Figs. 1 and 2). Moreover, influenza epidemics often cease very abruptly, even when many susceptible individuals remain in the population [7].

The viruses that cause vARIs include many unrelated families such as double-stranded DNA viruses (e.g. adenovirus), positive-sense single-stranded RNA viruses (e.g. coronavirus), negative-sense single-stranded RNA viruses (e.g. respiratory syncytial virus (RSV), influenza, measles, mumps and parainfluenza virus), and positive-sense single-stranded RNA viruses (e.g. hand foot and mouth virus, rhinovirus, rubella virus). They differ in their physical forms, with some having a lipid envelope (e.g. coronavirus, influenza, and parainfluenza viruses), which many others (e.g. adenovirus and rhinovirus) lack. Some are icosahedral (e.g. adenovirus. rhinovirus and Rubella virus) whereas many are spherical, filamentous or variable (e.g. RSV, influenza, and measles viruses). It is notable that the great majority of these diverse and often distantly-related strains share the same seasonality in temperate regions. For example, Hope-Simpson found in both 1954 and 1955 that the number of people suffering from "colds" in a sample of 380 volunteers was roughly 50 times greater in February than at the beginning of September [3] (Fig. 3). The common cold is caused by over 200 serologically-distinct strains [8]. It is clear that the great majority of these strains share the same seasonality in temperate regions, since colds in general show such strong seasonality. However, variations in the precise timing of the various respiratory viruses within the cold season have been reported, and it has been
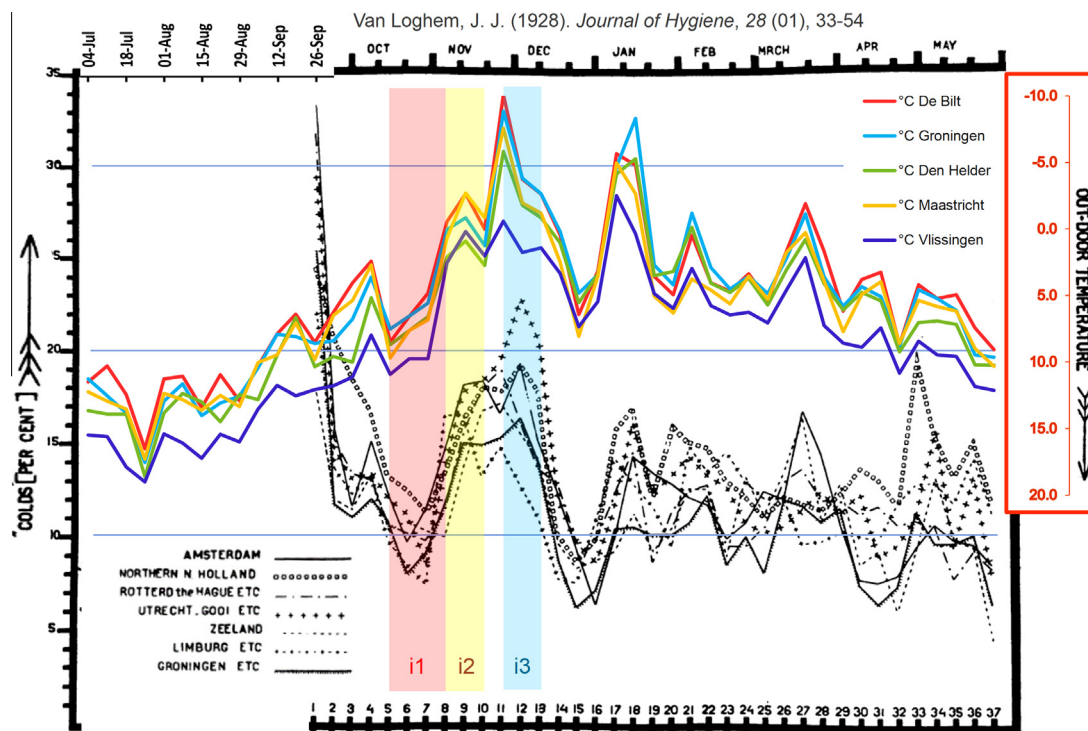
Fig. 1. Graph II from van Loghem's report [5] on the epidemiology of vARIs in the Netherlands in the winter of 1925/26, with ambient temperature superimposed. The graph shows the percentages of persons with colds in seven regions of the Netherlands for 37 weeks. The data was compiled from the reports 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 (also 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 vARIs was at the beginning of the study (September 1925), and that vARIs 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. Correspondents reported coryza, angina, laryngitis, bronchitis and "influenza". It is likely that a variety of viral "species" were present. See the main text for discussion of the events occurring during the intervals labeled i1, i2 and i3. ©1928, 2015. This figure was originally published in the Journal of Hygiene, 28(01), 33–54.

suggested that these variations might reflect fundamental differences in the mechanisms of replication or transmission of the viruses involved [4,9–11]. For example, rhinovirus is particularly prevalent in the autumn [12], while RSV and influenza usually cause outbreaks around the turn of the year [12]. Several comments can be made here: (1) very few vARIs have been found that consistently show the *opposite* seasonality, with more outbreaks in summer than in winter. For example, a study at a children's hospital in Mainz, Germany, that used modern diagnostic tests found that 7 out of 10 vARIs displayed normal seasonality, since these 7 showed significant inverse correlations with ambient temperature (p-value < 0.001) [4]. The remaining 3 were weakly inversely correlated with temperature. (A counter-example was reported by Hope-Simpson [13], although the tendency was weak: of the type 3 parainfluenza viruses isolated by him over 14 years, 66% were collected in the warm semester.) (2) Studies show that the reported variations in the timing of particular vARIs are in fact rather inconsistent. For example, RSV in children in Mainz peaked in spring in 2002 and 2006, but in the winters of the intervening years [4]. (3) It is possible that certain vARIs are more prevalent in, say, spring and autumn as a result of "interference" by other viral strains during winter. For example, the immune systems of hosts may frequently be activated by certain dominant viruses during the winter months, reducing the likelihood of subsequent infection by less active strains. The patterns of vARIs in the Mainz study suggest the existence of such interference (compare the relatively smooth curve of all respiratory illnesses in Fig. 1 in [4] with the irregular occurrences of the individual vARIs shown in Figs. 1 and 2 of that report).

These trends imply the existence of important mechanisms concerned with viral replication or transmission that are common to the majority of respiratory viruses, in spite of their widely-differing physical structures and biochemistry. It seems likely that an explanation of seasonality would have far-reaching practical and economic implications for treating and protecting humans and animals from vARIs.

Microbiologists have put forward many explanations of the seasonality of vARIs. Proposed explanations of influenza seasonality, for example, include factors that change host 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, as well as deficiency of selenium, vitamin C, vitamin D and vitamin E) [1]. (Factors that may change the behavior of viruses at the biochemical level are seldom considered.) The same or similar explanations have been put forward for other respiratory viruses [4,9–12,14–18]. However, these well-known explanations are very difficult to reconcile with a straight-forward observation: the vARIs in question are present in many tropical regions at intermediate levels throughout the year – often at much higher levels than in the summer in temperate locations [1,2]. Moreover, surveys show that the viral "species" that commonly cause vARIs in the tropics are similar to those in other climates. For example, the four most frequently identified viruses in two large hospitals in a tropical location (Singapore, 1990–1994) were, in order of prevalence, RSV, parainfluenza, influenza A and adenovirus [10]. (Most samples came from hospitalized children.)
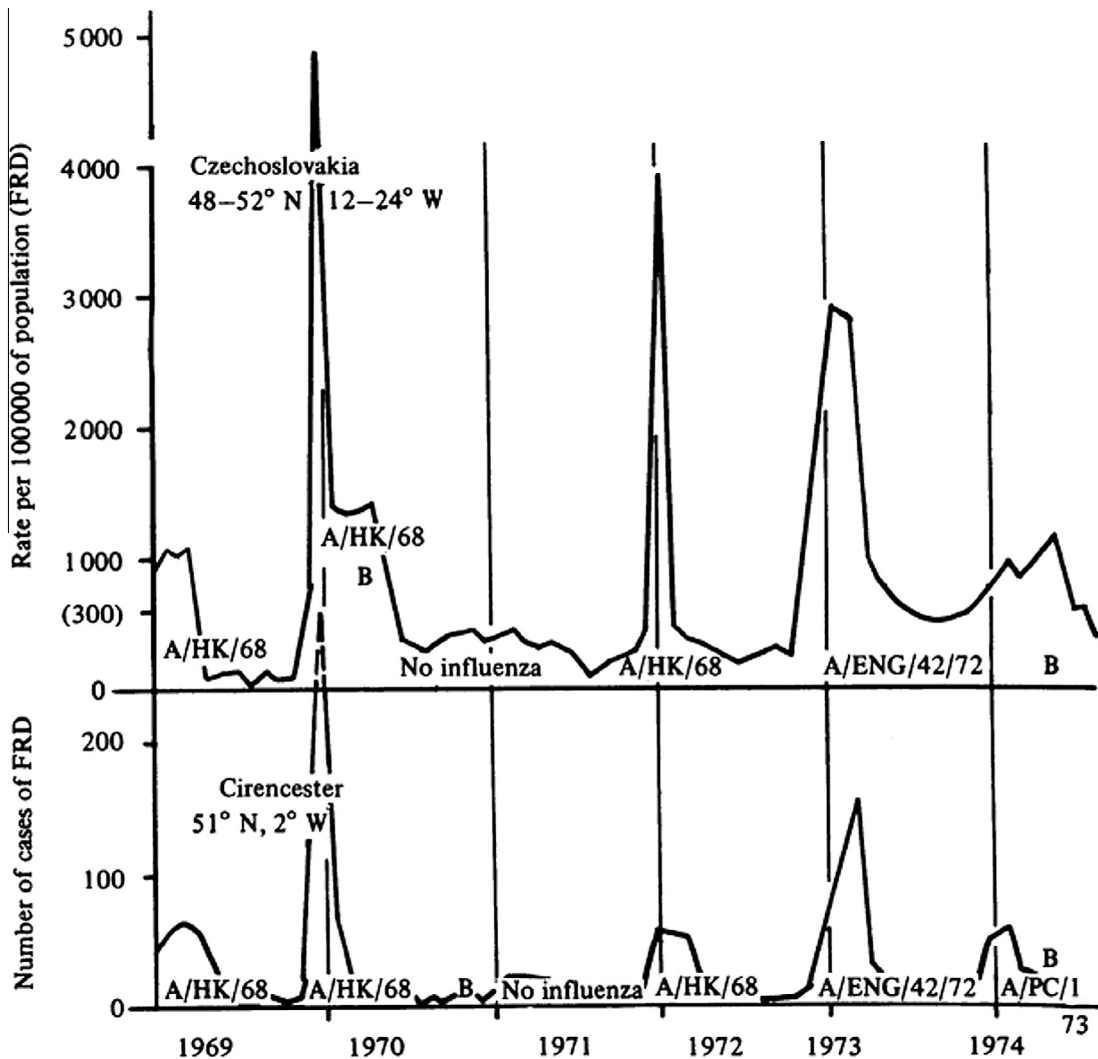
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Fig. 2. The Cirencester (UK) acute febrile respiratory diseases at 51.430 N, 1.590 W, compared with notifications of such diseases in Czechoslovakia (Prague, 50.050 N, 14-250 E), 1969–74, taken from Hope-Simpson's investigation into the role of season in the epidemiology of influenza [7]. This remarkable figure requires scientific explanation. The antigenic changes in influenza A virus (occurring at both sites) clearly show that novel influenza strains repeatedly moved across Europe during the period shown. There is, however, no evidence of moving "waves" of influenza because epidemics at the two sites are almost perfectly closely synchronized. Note that the shortest route between the two sites covers 1400 km by sea and road, crosses four national boundaries, and passes through some of the most densely-populated regions of Europe. This suggests that the virus moved to both sites prior to its manifestation, and a stimulus that was present at both sites triggered the concurrent epidemics. Bear in mind, however, that some influenza infections do not cause fevers. Influenza may have spread across Europe in the form of colds, before being strongly activated by low temperatures to yield febrile illness. These data are most readily explained by the fourth mechanism discussed below (M4), that virions can become dormant at some unknown location in the respiratory tract, and can subsequently be activated by host chilling. M3, which suggests that colder conditions weaken the immune defenses of hosts is also a possible explanation, but the very sudden onset of influenza in both locations is difficult to explain in this way. ©1981. This figure was originally published in the Journal of Hygiene, 86(01), 35–47.

A similar study in an oceanic climate (Mainz, Germany, 1998–2002) found that the four viruses that were most frequently identified among hospitalized children were, in order, rhinovirus, RSV, adenovirus and influenza A [4]. In Buenos Aires (2003–2006), a city with a humid subtropical climate, they were RSV, influenza A, Adenovirus, and parainfluenza [9]. Note that RSV, influenza A and adenovirus are present on all three lists.

Almost all of the explanations of influenza and other vARI seasonality mentioned above are associated with parameters that have more extreme values in the tropics throughout the year than in temperate summers. Since similar viral species are present in all these locations, it is likely that some strains will migrate from tropical to temperate regions, where (according to the explanations put forward) they should be replicated and transmitted without difficulty during the summer months.

The migration of influenza has been investigated in great detail recently using time-stamped viral sequences. These studies show that influenza does indeed frequently migrate from hotter to colder geographical regions. For example, the data of Bedford et al. shows that 50% of European A/H3N2 influenza strains were descended from strains that were in tropical or subtropical regions one year earlier [19]. For A/H1N1, B/Victoria-like and B/Yamagata-like influenza strains, the proportions were 62%, 17% and 32% respectively [19]. A/H3N2 influenza, in particular, circulates continuously in East and Southeast Asia, and spreads to temperate regions from this network [19,20], so we would certainly expect it to have properties that allow it to be active during temperate summers. The predominant direction of migration of influenza in China is also of interest. On a one-year timescale, H3N2, H1N1, and B/Yamagata/16/1988-like influenza were more likely to migrate from South to North China than in the opposite direction [19]. (The trend is not universal, however: B/Victoria/2/1987-like influenza was more likely to migrate from North to South China [19].) Note also that influenza and other vARIs often show clear
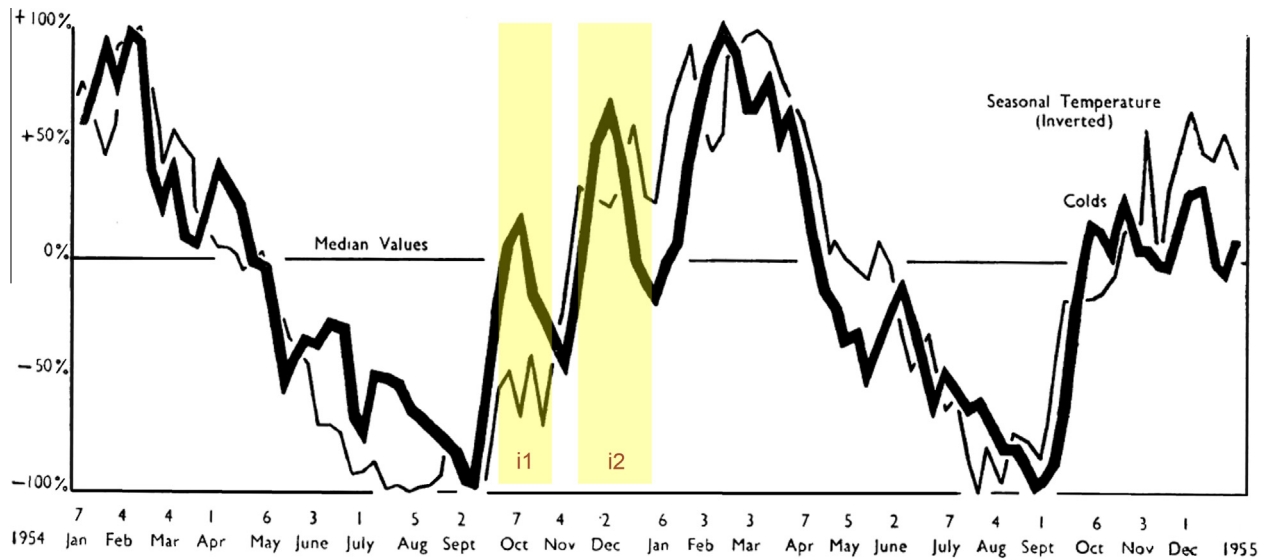
Fig. 3. Morbidity from colds in Cirencester, UK, 1954 and 1955, plotted alongside temperature [3]. Thick line – percentage of volunteers showing symptoms. Thin line – earth temperature (inverted). See the main text for discussion of the events occurring during the intervals labeled i1 and i2. ©1958. This figure was originally published in the Proceedings of the Royal Society of Medicine, 51(4), 267–271.
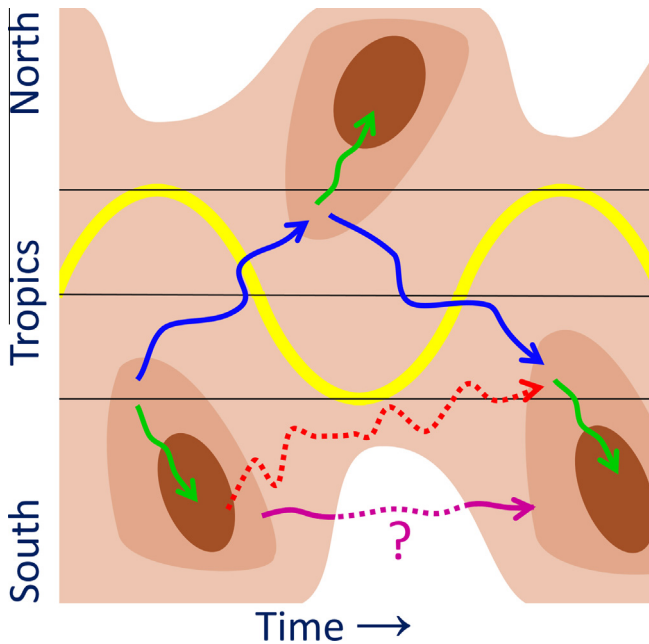

Fig. 4. The global distribution and seasonality of influenza and other vARIs, shown schematically. Time is indicated *across* the figure, while latitude is indicated from top to bottom. Levels of vARIs are indicated by brown shading, with dark brown showing the highest rates of infection. The figure shows general trends rather than specific data, but it is compatible with e.g. the Weekly Epidemiological Record of influenza A of the World Health Organization [7] and other studies [1]. The yellow curve shows the path of vertical solar radiation. The strange distribution of vARIs is shown, with more vARIs in the tropics throughout the year than in temperate regions during the summer months [1,2,20]. It is known that seed strains of influenza A (H3N2) circulate continuously in a network in East and Southeast Asia (blue arrows) and spread to temperate regions from this network (green arrows) [20]. Many studies show that personal chilling increases the prevalence of vARIs [5,14–16,21–25], and, since travel away from the tropical regions is associated with a decrease in temperature, it is likely that vARIs spread more quickly from the tropics to temperate regions (green arrows) than in the opposite direction (dotted red arrow). This is indeed the case for H3N2 influenza [20]. The degree to which viruses remain dormant during the summer in temperate regions (dotted purple arrow) is unknown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

seasonality in the tropics that is *not* correlated with temperature, humidity or solar radiation, but instead coincides with the rainy season [1,10]. The strange global and seasonal distribution of influenza and other vARIs is shown schematically in Fig. 4. These issues were discussed in two recent reviews of influenza seasonality, both of which also noted the lack of a satisfactory explanation [1,2].

A note on how the shared seasonality of most vARIs has been dealt with in this paper

It is a remarkable fact that almost all vARIs share the same seasonality in temperate regions. PCR-based studies, discussed below, confirm this common seasonality [9,4]. It is highly improbable that this common trend is coincidental, and this review will assume that an unrecognized common thread runs through the transmission or replication of respiratory viruses. If it were possible, more consistent conclusions might be arrived at by focusing instead on particular vARIs, but unfortunately the relevant data are not yet available. I will therefore consider influenza, the best-studied vARI, as well as colds and other vARIs, always bearing in mind that important differences may exist between the various viral species considered.

The effect of weather and host chilling

Ambient temperature often has a dramatic effect on respiratory disease. In the UK, Hajat et al. found that general practitioner consultations by elderly patients for lower respiratory tract infections in one UK City (Norwich) increased by 19% for every degree that average temperature dropped below 5 °C, observed 0–20 days before the consultation [14]. Absolute temperature, however, is not correlated with vARIs in a simple way. For example, we do not see a vARI that is limited to all global regions or seasons where temperatures never rise above, say, 15 °C (Fig. 4). Instead, evidence from many different sources shows that vARI incidence is related to temperature *fluctuations*. For example, van Loghem conducted a very extensive survey of vARIs in the winter of 1925/26 with 6933 participants from all regions of the Netherlands [5]. His data is shown in Fig. 1, together with the temperatures recorded by five Dutch weather stations. Epidemics of vARIs in all seven regions were very closely synchronized with each other, and closely
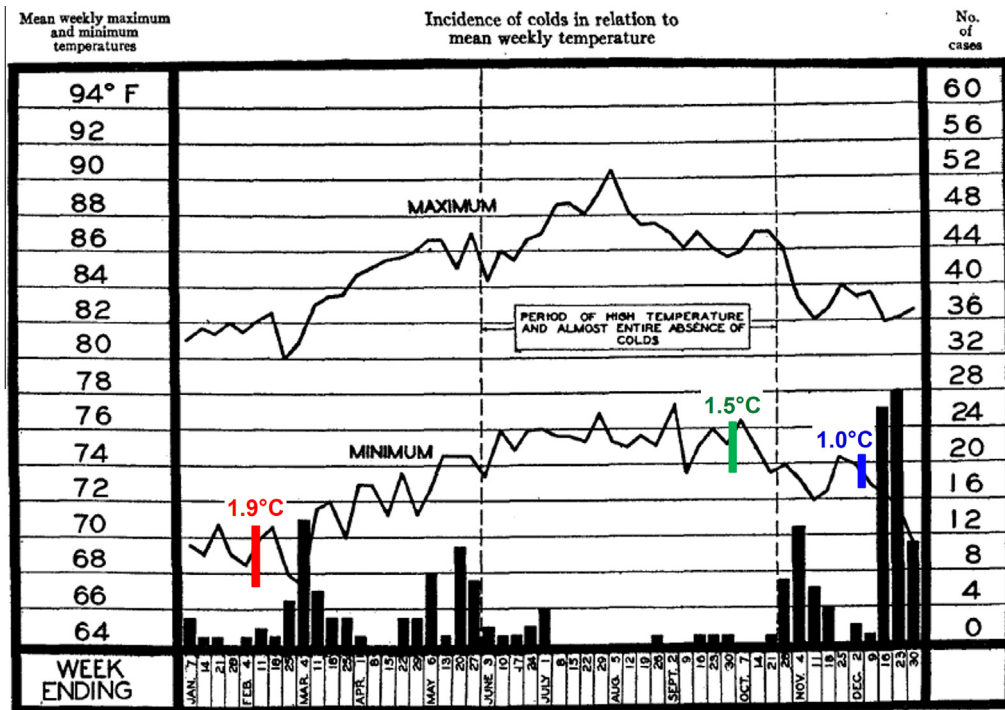
Fig. 5. Chart 1 from Milam and Smillie's 1929 study of colds on an isolated tropical island [21]. The authors noted that outbreaks of colds often followed temperature drops, and were almost absent in the summer months. The red, green and blue bars indicate temperature fluctuations of 1.9, 1.5 and 1.0 °C respectively. (The large outbreak in December seems to have been introduced to the island by a sailor on the mail boat.). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) ©1931. This figure was originally published in the Journal of Experimental Medicine. 53:733–752. doi: 10.1084/jem.53.5.733.

correlated with inverted temperature with roughly a one week lag (i.e. lower temperatures were associated with increased vARIs). These correlations were strongest during the period when temperatures were generally falling, i.e. the first half of the cold season. Note also that the highest infection rate, which occurred at the start of the study, coincided with almost the *highest* temperature in the study. Just prior to the start of the study, however, the temperature declined after remaining nearly steady during the summer months – implying that the temperature *dip* triggered the epidemic. This emphasizes that we need to consider temperature *fluctuations* rather than absolute temperature levels.

Milam and Smillie found similar patterns on the tropical island of St. John in the Virgin Islands in 1929, shown in Fig. 5 [21]. Between mid-afternoon and midnight each day the temperature on the island fell sharply by 5–7 °C. When the temperature dipped in the autumn by 1.5 °C (green bar) below the summer range, an epidemic of colds was triggered. More recently, Jaakkola et al. found that "sudden declines" in both air temperature and absolute humidity (in the three days that preceded the reporting of the sickness) increased the incidence of influenza A and B in military conscripts in Northern Finland [22]. Paradoxically, the incidence of influenza was lower at very low temperatures, and it was the sudden *decline* of temperature rather than low absolute temperature (and, the authors suggested, the decline of humidity) that increased the risk of influenza.

Historical studies have some advantages over modern investigations. The study by van Loghem was on a scale that would be difficult today, and he was able to collect data from multiple geographical locations within the Netherlands (Fig. 1). The study by Milam and Smillie has the advantage that the island was very isolated, so that a limited set of viruses was studied (Fig. 5). Modern studies, however, have the great advantage that they can

identify the viral species involved. Two recent studies in Argentina and Germany compared weather parameters with hospital admissions of children suffering from known vARIs [4,9]. Both studies found that the combined number of cases for all pathogens was strongly inversely correlated with outside temperature. Viegas et al. plotted the frequencies of RSV, adenovirus, influenza A virus and parainfluenza virus alongside mean temperature in Buenos Aires [9]. On all plots clear seasonality is apparent, but adenovirus and parainfluenza virus clearly *lagged* behind inverted temperature, peaking in early spring. Du Prel et al. presented similar plots and reported that rhinovirus, RSV, influenza A, adenovirus, metapneumovirus, influenza B and coronavirus were all strongly inversely correlated with temperature in Mainz, Germany ($p < 0.001$ in all cases). Rhinovirus was also correlated with relative humidity ($p < 0.001$) [4]. This study (and the Buenos Aires study) used Spearman's ranked correlation coefficients to find associations between meteorological parameters and hospitalizations for viral (and bacterial) infections [4,9]. This tabular information is difficult to interpret, however, because correlation coefficients dramatically *underestimate* the closeness of a relationship when one of the parameters lags behind the other (e.g. if two data sets are perfectly correlated except that one lags by a quarter of a cycle, the correlation coefficient will be 0). It is therefore possible that associations with temperature were present in Mainz for enterovirus, parainfluenza types 1 and 3, *Mycoplasma* and *Chlamydophila,* but not made evident by the correlation coefficients that the authors calculated. For example, rhinovirus in Mainz had a Spearman Rank Correlation Coefficient with inverted temperature of only 0.42, but inspection of the plots shows a clear relationship, with the first rhinovirus epidemic of each season closely following the first major drop in temperature at the end of summer [4]. We can conclude that both papers show important correlations with weather,

although other important relationships may not have been fully revealed by the analysis. It is also apparent that vARIs caused by completely unrelated viruses share the same seasonality both in a city with a humid subtropical climate, and in a city with an oceanic climate. It is very unlikely that these relationships are purely coincidental, which emphasizes that we should look for common causes of seasonality in these and other vARIs.

Studies at the individual level show that physical host chilling can increase the severity of vARIs. (The possible mechanisms involved will be discussed below.) The Eurowinter Group showed that shivering outside, being stationary outside, and wearing inadequate winter clothing increased respiratory disease-related mortality, while outdoor exertion sufficient to cause sweating was protective [15]. Yanagawa et al. found that 11 of 13 patients recovering from cardiopulmonary arrest who were treated with mild hypothermia developed pneumonia, as compared to 6 of 15 control patients who were maintained at normal body temperature (p-value < 0.02) [23].

Costilla-Esquivel et al. found a relationship between weather and acute respiratory illnesses in Monterrey (Mexico), 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 [16]. Rainfall and relative humidity were positively correlated with respiratory illnesses, while temperature was negatively correlated. Both rainfall and low temperature can obviously cause personal chilling, while high relative humidity is a consequence of the other two.

In summary, the available evidence shows that both sudden weather changes and factors that cause individual chilling frequently bring on vARIs, or increase their severity. This suggests that temperature sensitivity plays a role in seasonality, but the global patterns of vARIs rule out the possibility that viral activity is controlled solely by absolute temperature. Rather, respiratory viruses seems to adapt over a few weeks or months to the ambient temperature, such that temperature *fluctuations* outside the previous range trigger vARIs.

Relative and absolute humidity

Shaman et al. proposed absolute humidity as a "physically sound" driver of influenza seasonality in temperate regions [26]. However, unlike say concentrated sulfuric acid or anhydrous salts, influenza virions cannot "scavenge" low concentrations of water from dry air since virions are produced at moderate temperatures and pressures, and are in equilibrium with physiological fluids during their production. The absolute humidity that virions may be subjected to therefore has little or no significance. Instead, (as with most biological phenomena) temperature and *relative* humidity are the meaningful parameters that should be considered, either singly or in combination.

Absolute humidity does however have significance in the context of *indoor* viral survival and transmission. Since indoor temperatures in wealthy societies are maintained, by heating systems, at roughly constant levels throughout the year, indoor relative humidity is roughly determined by outdoor absolute humidity. Variations in absolute humidity may therefore contribute to influenza seasonality in some instances. Several points need to be considered, however: (1) reports of the effect of relative humidity on the survival of influenza virions at room temperature are inconsistent. Whereas Harper found that roughly twice as many viable PR8 virions were recovered after storage at 50% relative humidity in comparison to 64% [27], Schaffer et al. saw the opposite trend with their WSN virions from cell cultures, with roughly an order of magnitude *lower* recovery of virions at 50% relative humidity in comparison to 65% [28]. (2) Influenza and most other vARI epidemics are more frequent in the tropics during the rainy season, when both relative and absolute humidity levels are high [1,10]. For example, Fortaleza in Brazil has a single large peak of influenza [1] (and, from personal experience, other vARIs) that coincides with the rainy season (January to July). (3) Animal experiments with guinea pigs show that, while transmission of influenza generally decreased with increasing relative humidity, it actually *increased* at 20 °C when relative humidity moved from 50% to 65% [29]. (Animal experiments with influenza are further discussed below.)

Turning to another common vARI, Du Prel et al. explain the observed correlation of rhinovirus with relative humidity by pointing out that "rhinoviruses cannot survive in a dry environment", i.e. they suggest that the seasonality of rhinovirus is driven by changes in the survival rate of the virions outside the body [4]. However other interpretations are possible: increased relative humidity is associated with rainfall, which can wet individuals' clothing and cause chilling. Moreover, as noted above, *indoor* relative humidity in temperate climates is much *lower* in winter than in summer due to the effects of artificial heating in winter [3]. Changes in the survival of rhinoviruses due to indoor relative humidity should therefore generate the opposite seasonality if transmission mainly occurs indoors, which seems likely.

Observations of the incidence of colds in the UK found no correlation with relative humidity or water–vapor pressure [17]. Analysis of over 5000 person-years of data in two UK cities demonstrated that "it is the low outdoor temperature, independent of the humidity, which is associated with the increased number of winter colds" [17].

Clearly, correlations of vARIs with both relative and absolute humidity are at best inconsistent.

Animal experiments with human influenza virus

Experiments with guinea pigs suggest that the transmission of influenza A is more efficient at lower temperatures and lower relative humidity [29]. Lowen et 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 [29] (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 are in agreement with measurements of the stability of influenza A virions generated in cell cultures in air of different temperatures and humidities [27,28]. This might suggest that variations in transmission due to weather changes might determine or influence the seasonality of influenza (and other vARIs) in temperate regions. The results were, however, inconsistent; for example, transmission of influenza at 20 °C was higher at 65% relative humidity that at 50% [29]. Moreover, a more recent study 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 [30]. 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 are, however, seeded each year from a network of temporarily overlapping epidemics in East and Southeast Asia [20]. 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 unknown "additional factors, other than warm temperature and high relative humidity, which suppress influenza transmission by all routes during the

summer months" in temperate regions [30]. Although the authors may have identified the important routes of influenza transmission at different latitudes, they have therefore not provided a robust explanation of its seasonality.

Dormancy in vARIs

Viruses such as adenovirus [31], RSV [32], foot-and-mouth virus [33] and chickenpox virus - all of which can spread via the respiratory tract – are known to become dormant within their hosts. Other respiratory viruses show similar behavior, sometimes on shorter timescales. Morikawa et al. found human parechovirus, adenovirus, enterovirus, coronavirus 229E and HKU1, and rhinovirus in the gargle specimens of eight *asymptomatic* children using polymerase chain reaction (PCR) tests [34]. The tests required at least 100 copies of the genetic material, suggesting that the viruses in question had begun to replicate [34]. However, the authors noted that it is difficult to interpret PCR results because it is a very sensitive method, and the positive results may reflect an asymptomatic past or concurrent infection, or an imminent infection. Their data suggest, however, that at least some were imminent infections: four of the children had positive results when they were asymptomatic that were followed four weeks later by a vARI that was associated with the same virus (or a strain that was indistinguishable using the PCR test); rhinovirus was detected in a fifth child who was at first asymptomatic, but experienced the symptoms of a cold caused by rhinovirus one week later. Similarly, viral dormancy may explain the sudden arrival of autumn epidemics of rhinovirus in temperate regions; Granados et al. studied rhinovirus activity in university students, and showed that asymptomatic rhinovirus activity preceded peak symptomatic activity in September and October and was associated with lower viral load [18].

Influenza viruses have been detected several times in the absence of symptoms or an immune response in the host, which seems to indicate that dormant influenza virus is present. Foy et al. identified 10 asymptomatic individuals who were shedding influenza B virus but did not respond with antibody by any of the five test methods employed [35]. During the 2009 influenza A (H1N1) pandemic, Tandale et al. found that, of 65 asymptomatic individuals with PCR-confirmed H1N1, 12 had not seroconverted [36]. During the same pandemic, Papenburg et al. found two asymptomatic individuals with PCR-confirmed infections who had not seroconverted [37]. In Vietnam, Thai et al. found that, of 11 individuals shown by PCR to have been infected with pandemic H1N1 by other members of their household, one remained asymptomatic and had not seroconverted [38]. The authors commented that this "may indicate that viral RNA remained in the respiratory tract without being internalized and eliciting an immune response". These observations show the reality of influenza persistence in the respiratory tracts of asymptomatic individuals, and are compatible with the suggestion that virions can become dormant, and, later, be reactivated, for example by chilling.

Observations of vARIs in Antarctic stations after many months of complete isolation can provide evidence that is easy to interpret because vARIs are rare after the first month of isolation [24], and only one or a few viral species are active at a time. For example, after 12 months of complete isolation in 1965/6, a geologist ("J.E. H.") at the Mawson station picked up a respiratory virus from a visiting field party [24]. 17 days later he and three colleagues were exposed to cold and damp conditions, which brought on vARI symptoms including muscle aches and a sore throat in J.E.H. and two of his colleagues. 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 [25]. Both studies suggest that chilling caused by particular activities or weather changes can activate dormant virions, giving rise to vARIs. Similarly, Muchmore et al. reported parainfluenza shedding by healthy young adults throughout the 8½-month winter isolation period at Amundsen–Scott South Pole Station during 1978 [39]. The study recorded two episodes of respiratory illness caused by parainfluenza at the Station that year after 10 and 29 weeks of complete social isolation.

In principle, any failure or blockage of a biochemical or physical step in the replication or transmission of respiratory viruses might give rise to dormancy. Moreover, many respiratory viruses, including coronavirus, influenza, measles and mumps virus, require activation by proteolytic cleavage of viral proteins [40]. This (largely unexplained) phenomenon may provide opportunities for the establishment of dormancy. Nevertheless, visualization of the physical whereabouts of the virus, during the weeks or months that dormancy can last, is difficult. The virus might be located on the surface of the cells that line the respiratory tract (for example attached to the cell membrane or the cilia), or within the cells, possibly in the cytoplasm or the nuclei (although it seems unlikely that respiratory viruses are usually incorporated into the host genome as is HIV). There are problems with each of these explanations, but PCR and Antarctic studies show the reality of viral dormancy.

Taken together, the above observations strongly suggest that a variety of respiratory viruses can become dormant in human hosts for much longer periods than those reported in the literature [41] and that they can subsequently be activated by cold, giving rise to vARIs.

Mechanisms that would allow vARIs to respond to temperature changes

If we accept that host chilling (with various causes) triggers vARI epidemics and gives rise to vARI seasonality in both temperate and tropical regions, four possible mechanisms, M1–4 can be put forward as follows. M1: low temperatures and seasonal events increase crowding of human hosts, increasing transmission; M2: colder conditions allow the virus to survive outside the body for longer, increasing transmission; M3: the susceptibility of hosts increases as a result of chilling; M4: chilling increases the activity of viruses in the respiratory tract. I will now consider the evidence for and against these four possibilities.

M1: seasonal events increase the crowding of human hosts during the winter

A popular explanation of vARI seasonality is that contact rates are lower in summer when children are out of school, and when people spend more time outdoors. However, in the USA seasonal differences in "crowding" are minimal since the amount of time spent *indoors* varies by less than 10% between summer and winter [1]. In the UK, the number of school-days in the coldest six months of the year is less than 10% higher than in the warmest six months, but, like all temperate countries, the UK has marked vARI seasonality. Moreover, one of the two peaks of influenza activity in Singapore [1] actually coincides with the school holidays in June. Another negative observation is that festivals and sporting events are not associated with increased vARIs. For example, during the FIFA World Cup people spend more time indoors and often crowd together in bars etc. to watch matches on television, but no significant increase is apparent in Google Flu Search Activity in any country in the Northern Hemisphere during the 2014 FIFA World Cup [42]. Lofgren et al. agreed that theoretical and empirical studies do not adequately explain influenza A seasonality, noting in

particular that no published studies show directly that variations in crowding give rise to influenza seasonality [1].

Another problem for this explanation of seasonality is the simultaneous arrival of influenza [6,7] and other vARIs [5] throughout wide geographical areas, often following or coinciding with cold snaps (Figs. 1, 3 and 5). If cold weather acted on vARIs only by increasing viral transmission, then as the temperature falls e.g. in autumn, waves of infection should appear and move through populations. We would not expect to see epidemics that arise simultaneously in both neighboring (Fig. 1) and distant (Fig. 2) populations. This point is discussed in more detail in the next section.

In summary, temperature-dependent changes in human crowding may well play a role in the progress of vARI epidemics, but there is no evidence that they provide a general or the main driver of vARI seasonality.

M2: colder conditions allow virions to survive outside the body for longer

This is currently the most popular explanation of seasonality. The mechanism is, however, almost certainly not the main cause of seasonality, for several clear reasons.

Firstly, this explanation cannot explain why vARIs are present in many tropical regions all year round, but virtually absent from temperate regions during the summer months. If respiratory virions can adequately survive outside the body in the tropics, they should have no difficulty in surviving (according to this explanation) in the milder conditions of temperate summers. As noted above, a similar set of viruses give rise to vARIs that hospitalized children in temperate, subtropical and tropical cities [4,9,10], and they presumably migrate between these locations. Seed strains of influenza A (H3N2) spread from East and Southeast Asia to temperate regions every year [20], and other respiratory viruses may follow similar routes. (The suggestion [22,28,29] that low absolute or relative humidity may increase viral survival does not help, because vARI epidemics occur during the rainy season in many tropical locations [1].)

Secondly, consider van Loghem's data [5] (Fig. 1). While temperatures were generally decreasing (i.e. up to the end of January) vARIs were very well-correlated with inverted temperature, and extraordinarily well-synchronized across the country, with no evidence of "waves" of infection moving between different locations, which would be expected if cold temperatures increased *transmission* during the study. We don't know which viruses were most prevalent in the study, but if any had seasonality that was driven by M2 it is clear that they were not common. Hope-Simpson made similar observations when he compared influenza epidemics (1969–74) in the UK and in Prague, Czechoslovakia, showing the temporal correspondence of epidemics at widely separated localities at a similar latitude [7] (see Fig. 2). In another example, Magrassi was impressed by cases of influenza in 1948 among shepherds living in complete social isolation in open country in Sardinia, who developed the disease contemporaneously with the inhabitants of towns on the same island [6].

A third consideration is that records of vARI epidemics show that they frequently respond to temperature *fluctuations* rather than absolute temperature levels. For example, the rapid drop in temperature during weeks 6–8 of van Loghem's study (pink band, labeled i1 in Fig. 1) was followed, with a lag of roughly one week, by rapid increases in vARIs. When the temperature subsequently hovered around 0 °C in weeks 9 and 10 (yellow band, labeled i2), however, the number of vARIs stabilized. If the rapid 70% increase in vARIs that occurred at temperatures between 10 and 0 °C (pink band) was caused by increased transmission we would then expect vARIs to continue to increase when temperatures stabilized around

0 °C. A little later (weeks 12 and 13, blue band, labeled i3) the temperature was well below 0 °C, but now it was rising, and the vARIs fell from their highs, again emphasizing that vARIs tend to be sensitive to temperature fluctuations rather than absolute temperature.

A similar argument applies to the data of Hope-Simpson (Fig. 3), with the number of colds increasing very rapidly after temperature drops, but *falling* during two periods of constant low temperatures (yellow bands, labeled i1 and i2) in October and December 1954. Milam and Smillie's data (Fig. 5) also showed fast-acting sensitivity to very small temperature drops outside the recent range, with these events occurring at a range of temperatures throughout the year [21]. Jaakkola et al. reported that "sudden declines" of around 5 °C preceded the onset of influenza in Northern Finland [22], occurring at temperatures above 15 °C and also below −15 °C. Other vARIs can be considered. An interesting and clear-cut example comes from Singapore, where Hii et al. studied the strong association of hand, foot and mouth disease (HFMD) with the weather [11]. 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" [11]. Again, it is difficult to imagine that weather anomalies of the order of 1 °C can significantly change the survival of respiratory viruses outside the body (or, indeed, the susceptibility of human hosts to VRTIs, discussed in the next section).

If the transmission of vARIs including influenza were driven purely by low temperatures, we would not expect to see the rapid cessation of influenza epidemics in mid-winter [7], or the low attack rates that have been reported within families for influenza [43–45] and other vARIs [5].

In summary, we can see that temperature-dependent changes in viral transmission may well influence the progress of many vARI epidemics, but, unless new evidence comes to light, we need to look elsewhere for a general explanation of the seasonality of vARIs.

M3: chilling increases the susceptibility of hosts

An interesting and ingenious recent review looked directly at the seasonality of immune responses in humans by investigating antibody responses following vaccination [46]. Although the authors found seasonal variation in immunity, it could not explain vARI seasonality: seven of the studies of vaccines reviewed reported a stronger immune response in winter than in summer, with only 1 showing the opposite trend. There was no clear trend with regard to the dry and rainy seasons in tropical regions and several other studies showed no trend at all. These data therefore suggest that variations in general host susceptibility do not explain the seasonality of vARIs.

However, this leaves open the possibility that there are variations in host immune defenses that specifically affect the respiratory tract. Eccles suggested that host chilling may cause reflex vasoconstriction of the blood vessels of the upper airways, thereby reducing host defenses against viral infection during the winter [47–49]. Mudd and Grant [50] and van Loghem [5] made similar proposals. Two lines of evidence support the idea that chilling reduces host respiratory tract defenses by this or other mechanisms.

Foxman et al. found that mouse airway cells infected with mouse-adapted rhinovirus 1B exhibited significantly lower expression levels of type I and type III interferon genes and interferon-stimulated genes at 33 °C relative to 37 °C [51]. This is a very interesting but puzzling result, and the authors offer no explanation of the possible benefits to the mouse. If airway cells possess mechanisms that can reduce infections, it seems strange that they

should be down-regulated at lower temperatures. (One explanation is that the interferons severely damage airway cells, and the mouse prefers to deal with the virus with milder defenses unless it reaches sites where it may cause serious illness. A related proposal is that mice permit the growth of viruses in their respiratory tracts in order to raise antibodies against them before the virus can reach the internal organs.) We need to wait for equivalent results in the cells of other species, both *in vitro* and *in vivo,* before we can fully interpret these data.

Other support for M3 comes from a study by the Eurowinter Group, which found that exposure to cold outdoor air can affect vARIs in opposite directions [50]: shivering outside greatly increased respiratory disease-related mortality, in agreement with Eccles' suggestions. However, outdoor exertion sufficient to cause sweating *reduced* mortality (*p*-value = 0.02), although breathing cold air rapidly is known to *reduce* the temperature of the respiratory tract [52]. This is more difficult to explain, but it seems clear that variations in host defenses are involved. This is discussed in the next section.

Other evidence, however, does not support M3 (unless it is combined with M4 below). Since human immune defenses act well in a wide range of temperatures and climates (the climates of Northern Scandinavia and Southern India for example), we would not expect temperature changes of 1–2 °C to have a noticeable impact on host defenses against vARIs. Great sensitivity to temperature fluctuations is apparent, however. For example, consider again Chart 1 of Milam and Smillie's paper [21] (Fig. 5). Every night in the summer the temperature dropped by 5–7 °C. In the autumn the temperature fell by an extra 1.5 °C, which triggered an epidemic of colds. Can we believe that the islanders' immune systems could cope well with a regular 6 °C drop but succumbed after a 7.5 °C drop? Note that the absolute temperature after the dip – about 23 °C at night – was still very comfortable, and would certainly not cause a vARI epidemic in, say, Helsinki.

Note also that there is often a peak of vARIs in the early autumn, often associated with a rhinovirus epidemic [4,12,17]. This peak can be seen, for example in the very high level of colds at the beginning of the study by van Loghem, on 19 September, 1925 [5] (Fig. 1). The number of vARIs was then much higher than during the rest of the cold season. If we are going to say that the increase in vARIs in the cold season is due to the increased susceptibility of hosts in cold weather (M3), it is difficult to explain why the human immune system appears to be less efficient in early autumn than in, say, February.

Another problem for M3 is the abrupt cessation of influenza epidemics. Hope-Simpson noted that all the major influenza epidemics that he recorded in Cirencester, UK, (1951, 1957, 1959, 1969 and 1973) rose rapidly to a single peak within four weeks, then abruptly ceased in the following 4–5 weeks (Fig. 1 in Ref. [7]). In at least one case it was clear that this was 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 town's population at that time because this was the first H2N2 epidemic, and there was another H2N2 epidemic 16 months later, which was almost as large as the first [7]. The abrupt cessation of the first epidemic is therefore unexplained. The other four major epidemics listed above were in midwinter, when (according to this view) the immune system should be at its weakest, suggesting that the virus should spread and the epidemic should continue for more than eight weeks.

Numerous studies of the common cold in the 1950s and 60s used recycled "pedigree" viral strains that were collected from volunteers and used to inoculate subsequent volunteers. These studies weigh against M3 because they found that chilling did not increase the likelihood of volunteers getting colds [53–56]. These studies are discussed below.

Taken together, the available evidence suggests that temperature-dependent changes in host susceptibility (M3) may play a role in the epidemiology of vARIs, and may contribute to seasonality. However, changes in susceptibility alone cannot explain many observations of vARIs and it appears that this mechanism is not main driver of seasonality.

M4: chilling increases the activity of respiratory viruses as a result of their natural temperature sensitivity

Lwoff proposed in 1959 that the degree of virulence of viruses is related to their level of temperature sensitivity, i.e. greater sensitivity to heat is correlated with reduced virulence [57]. In 1979, Richman and Murphy confirmed this association and reviewed its implications for the development of live virus vaccines [58]. They noted that the replication of temperature-sensitive (*ts*)[1] influenza, parainfluenza, RSV, and foot-and-mouth viruses was consistently more restricted in the lungs of a variety of animals than in their nasal cavities. They also found that both naturally-occurring and synthetic *ts* viruses were very frequently less virulent than their non-*ts* counterparts in humans and animals, noting several cases (including influenza and vaccinia virus) where the loss of the *ts* phenotype resulted in the restoration of the virulence or growth capacity of the virus, both *in vivo* and *in vitro* [58]. Chu et al. later tested seven H1N1 strains with varying degrees of temperature sensitivity in volunteers and found a correlation between temperature sensitivity and the severity of symptoms, with strains that were more *ts* being less virulent [59]. It is reasonable to conclude that the *ts* phenotype facilitates the transmission of the wild virus because it prevents or reduces multiplication of the virus in the lungs or internal organs, which is likely to result in the immobilization, or possibly the death, of the host. One possible explanation of vARI seasonality (M4) is therefore that the lower temperatures of winter increase the activity and virulence of respiratory viruses, as a side-effect of their tropism. This may explain why nearly all respiratory viruses share the same seasonality.

It is known that there is a temperature gradient in the human respiratory tract, from around 24 °C at the glottis to around 35.5 °C at the subsegmental bronchi [52]. The temperature in the respiratory tract drops rapidly when the air being breathed is cold, when the host breaths rapidly, or – significantly – when the host is chilled [50,52]. Putting these observations together, a simple explanation (M4) of the seasonality and temperature sensitivity of vARIs can be proposed:

(1) One or several steps in the life-cycle of most "wild" respiratory viruses are *ts*. These *ts* steps might include the release of virions from cells, their binding to cells, their entry into cells, or any subsequent step in their replication (see the biochemical evidence reviewed below).

(2) *Ts* virions that bind to cells lower down the respiratory tract (therefore at relatively high temperatures) may remain dormant at some unknown cellular location (which might be on extracellular material, on the surface of, or within, the cells that line the respiratory tract).

(3) Virions that bind to cells higher up the respiratory tract (therefore at relatively low temperatures) may become active, but will not normally cause a vARI because they are active in low numbers at any one time and can be removed

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[1] In this article temperature-sensitive or *ts* refers to viruses that are more active at lower temperatures, i.e. they are heat-sensitive.

by the host's immune system (or, if a proportion of virions are defective, simultaneous invasion of each cell by several virions may be required to initiate infection).

(4) Each day the temperature of the respiratory tract varies, and this variation clears certain populations of virions from certain regions of the respiratory tract, leaving other populations intact.

(5) If the temperature of the respiratory tract drops suddenly below its normal range, batches of virions that were previously dormant may be activated simultaneously, giving rise to a vARI.

The virions' binding sites in the respiratory tract might also reflect the correlation, noted by Richman and Murphy, that more virulent strains tend to be less *ts* [58]. For example, avian influenza virions that can infect human hosts might be expected to bind to cells lower down the respiratory tract than typical seasonal influenza.

An important point is that M4 is sensitive to temperature *fluctuations*, not absolute temperature, because the temperature sensitivity of viruses may adapt quite rapidly as a result of selective pressures, and they can bind in different regions of the respiratory tract. This can explain the data of van Loghem, Milam, Jaakkola and others, discussed above [5,14–16,21–25]. It can also explain the seasonality of vARIs, because chilling outside of the range of the previous few days or weeks becomes more likely when the seasonal temperature drops in the autumn. In the spring, by contrast, exceptional chilling becomes less frequent as the seasonal temperature steadily rises.

M4 can explain the association of vARIs with wind and rain in the tropics, since these weather events often result in the chilling of individuals, even when the ambient temperature remains constant. It also provides an explanation for the strange epidemiology of influenza, including the low attack rate of many epidemics within families [5,43–45] and the rapid cessation of epidemics when many susceptible individuals remain in the population [7]; bear in mind that each member of a family has a different history of exposure to viruses and of chilling. For example, some family members may take regular outdoor exercise in the winter, which (I suggest) clears virions from the respiratory tract in small batches (outdoor exercise reduces mortality from vARIs [15]). Other family members may be exposed to a virus for the first time during particularly cold weather (e.g. in midwinter), so that virions bind and become dormant relatively low down the respiratory tract, and are therefore not activated unless even colder weather follows. Still others, who remain indoors much of the time, but are chilled occasionally may be prime candidates for infection (e.g. waiting for a bus in the rain may put individuals at risk - standing still outside in cold weather, and shivering outside, have both been shown to be a dangerous activities [15]). These trends can therefore explain the low attack rates and lack of transmission within families, as well as the rapid cessation of epidemics.

Note that the natural temperature sensitivity of respiratory viruses contributes to M4 in two steps; in a first step, it allows virions to become dormant; in a second step, it provides viral activation as a result of chilling. Step 2, however, could also be the result of the depression of immunity in the host – M3 above. There is no reason to rule this out, and, in the absence of experimental data, the relative contributions of changes in host physiology and virus biochemistry remain unknown. I noted above that changes in host susceptibility alone probably cannot account for the extreme sensitivity of vARIs to small temperature drops, but they may contribute. M3 may *contribute* to the simultaneous arrival of vARIs across wide geographical areas, and may be at least partly responsible for the arrival vARIs in tropical regions during rainy

seasons. However, it seems unlikely that M3 is the main driver of seasonality.

As noted above, experiments carried out in the 1950s and 60s with the common cold failed to find an effect where host chilling increased the likelihood of volunteers getting colds [53–56]. However, it seems likely that the procedure of recycling viruses used in these studies may have eliminated some of the wild virus's natural temperature sensitivity. M4 therefore offers an explanation of the negative results of these studies, as discussed in detail in the next section.

I noted above that the Eurowinter Group found that exposure to cold outdoor air can have opposite effects according to the situation: shivering outside greatly *increased* respiratory disease-related mortality (*p*-value = 0.001), while outdoor exertion sufficient to cause sweating greatly *reduced* mortality (*p*-value = 0.020) [15]. (The absolute values of the regression coefficients of mortality due to respiratory disease on these two cold exposure factors were far higher than any others found by the Group.) The first point, that shivering increased mortality, could be explained by either M3 or M4 – host defenses could be diminished, or virions could be activated, by cold. The second point, that outdoor sweating reduced mortality, is more difficult to explain. Although heat stress may boost the immune system above its normal level, the result would not be noticeably protective if virions are dormant and therefore invisible to the immune system. If they *were* visible they should be destroyed anyway during the much greater time spent indoors. A roughly neutral effect of M3 would therefore be expected. M4 would suggest that mortality should *increase*, because outdoor exertion clearly causes cooling of the respiratory tract, due to rapid breathing. The best explanation may be a combination of M3 and M4: the cold air and rapid breathing cools of the respiratory tract, which activates some of the dormant virions that are present. However, the good blood flow to the respiratory tract that is associated with heat stress allows the activated virions to be destroyed by the immune system.

There is extensive biochemical evidence for the temperature sensitivity in respiratory viruses, particularly influenza, which is discussed below. This includes temperature sensitivity that is associated with the steps of the uptake of virions into cells [60], RNA transcription [61–65] (including the *ts* control of the balance between the synthesis of mRNA and replicative products [66,67]), and the transport to the cell surface of hemagglutinin-esterase-fusion protein, the protein that promotes the fusion of influenza C virions with cells [68].

In summary, many observations of vARIs cannot be satisfactorily explained without invoking the activation of respiratory viruses by temperature drops or host chilling (M4). The evidence is outlined in Table 1. M4 is probably the most important driver of seasonality in vARIs, although other mechanisms may also contribute to seasonality, especially the increased susceptibility of hosts as a result of chilling (M3). Moreover, when we consider different virus families, we can expect variations in both the timing and underlying mechanisms of seasonality.

Early studies where volunteers were chilled

It is widely believed by doctors and scientists that chilling does not affect vARIs, and that this idea is "an old wives' tale" [75]. This belief seems to come from numerous studies from the 1950s and 1960s where volunteers who had been inoculated with respiratory viruses were chilled, including three influential reports by Andrewes, Dowling and Douglas [53–55]. Unfortunately, these studies generally used "pedigree" strains of cold virus that were recycled by being collected from volunteers and used to inoculate subsequent batches of volunteers in later experiments. (Volunteers

Table 1
Four mechanisms, M1–4, that might explain the vARI seasonality in temperate regions.

(M1) Seasonal events may increase crowding during the winter, increasing transmission
- School holidays and sporting events are not well-correlated with vARI epidemics [1]
- The simultaneous arrival of vARI epidemics throughout wide geographical regions [5–7] is a problem for this explanation
- There is little evidence for this mechanism [1,2]

(M2) Colder conditions and low relative humidity may allow virions to survive outside the body for longer, increasing transmission
- The prevalence of vARIs year-round in the tropics [1,2,10,11] is evidence against with this explanation since viruses (including influenza virus [19,20]) frequently spread from tropical to temperate regions
- The simultaneous arrival of vARI epidemics during cold snaps throughout wide geographical regions [5–7] is also a problem
- M2 cannot explain why vARIs respond to temperature dips rather than sustained low temperature [3,5,21,22].
- The rapid cessation of influenza epidemics in mid-winter in the presence of many susceptible individuals [7], and the low attack rate of vARIs including influenza within families [5,43–45] are difficult to explain by M2

(M3) Chilling may increase the susceptibility of hosts
- Vaccination studies [46] show that the human immune system is not in general weaker during the winter months than in summer
- It has been proposed that host chilling specifically reduces immune defenses in the respiratory tract [5,47–50]
- This mechanism is supported by a study using cultured mouse airway cells [51] and by the observation that outdoor exertion sufficient to cause sweating reduces mortality from respiratory disease [15]
- The prevalence of vARIs year-round in the tropics [1,2,10,11] but not in summers in temperate regions suggests that other explanations of seasonality are also required
- It is hard to reconcile M3 with the extreme sensitivity of vARIs to temperature dips [3,5,21,22]
- The peak of colds in the early autumn, when temperatures have dropped only a few degrees from their summer highs [4,5,12,17,21,42] is difficult to explain by M3
- Numerous studies of the common cold in the 1950s and 60s that used "pedigree" strains weigh against M3 [53–56]

(M4) Chilling may increase the activity of viruses in the respiratory tract as a result of their natural temperature sensitivity
- M4 is compatible with all of the above observations, including the extreme sensitivity of vARIs to temperature dips [3,5,21,22] (since temperature varies with position in the respiratory tract [52])
- It provides an explanation of the low attack rate of influenza within families [5,43–45], and the rapid cessation of influenza epidemics [7]
- M4 is compatible with studies using "pedigree" strains [53–56] and with Antarctic studies [24,25,39]
- It provides a biological explanation of the benefits of temperature sensitivity [57,58,69] to the virus, and an explanation of viral dormancy [18,24,25,32–39]
- M4 is compatible with the recovery of ts viruses from persistent infections of tissue cultures and animals [58,69–73], and the generation of non-ts viruses in conditions that allow rapid viral replication [59,74]

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were often kept in quarantine to avoid using natural "wild" strains that they happened to be carrying [53].) In these studies it seems likely the researchers selected strains that very quickly caused mild vARIs in a significant proportion of the volunteers – but without causing dangerous infections. This may well have removed some aspects of their natural temperature sensitivity, since temperature sensitivity might give rise to dormancy and thereby delay infection. This suggests that the results may have differed from those that would have been obtained using wild viruses, which might have shown an effect of chilling. One study, however, by Jackson et al., did use wild viruses that the volunteers were carrying [56]. In some of their 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% of those who were not chilled developed colds. However, the authors do not tells us what proportion of volunteers were chilled by breathing cold air and what proportion by wearing scant clothing; breathing cold air while remaining warm can be protective [15], presumably because viruses are activated but they can be removed by the immune system.

More recently, Johnson and Eccles used wild strains that the participants were carrying by chance, and saw an effect of chilling by immersing the participants' feet in cold water [48]. Of the chilled subjects, 28% developed colds, whereas only 9% of the control subjects who were not chilled did.

Simple experiments along similar lines need to be carried out to resolve these apparent contradictions.

The recovery of *ts* viruses from persistent infections

In an interesting review of 1975 [69], Preble and Youngner noted that *ts* strains often appear spontaneously in persistent infections of cell cultures with a variety of unrelated insect-transmitted and respiratory viruses, including Newcastle disease
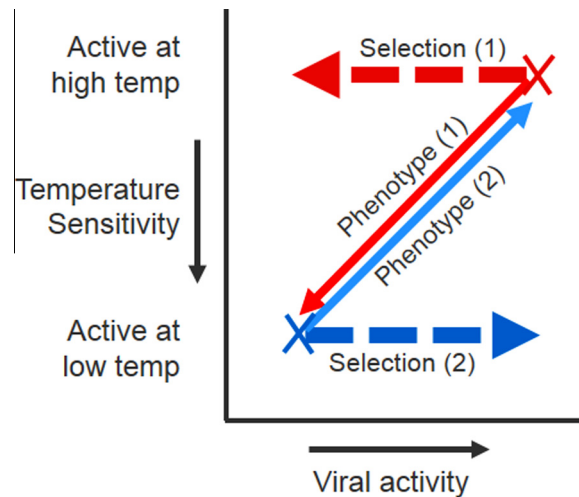

Fig. 6. The observed effect on temperature sensitivity of natural and (spontaneous) laboratory selection for increased and decreased viral activity. In this schematic 2-d plot the Xs indicate the starting levels of activity (virulence) and temperature sensitivity of two hypothetical viral strains. (The Xs could also indicate the properties of hypothetical viral proteins). 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 requires reduced viral activity so that viral and cell replication can be in balance [58,69]. The corresponding selective pressure is indicated by the dotted red arrow. Unexpectedly, reduced activity is often (though not always) accompanied by the spontaneous appearance of temperature (heat) sensitivity. This is indicated by the solid red arrow. See the main text for examples [70–72]. 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 has been lost (solid blue arrow) *even when selection takes place at low temperatures* (see main text [59,74]). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

virus, Western equine encephalitis virus, Sendai virus, measles virus, vesicular stomatitis virus, and Sindbis virus. (We can

speculate that their temperature sensitivity may allow insect-borne viruses to target the skin.) Similarly, Richman and Murphy found that persistent infections of cell-cultures with mumps virus and vesicular stomatitis virus frequently yielded *ts* virus, although they noted that persistent infections could also be established or maintained by non-*ts* mutations [58]. Three more recent reports described the recovery of spontaneously-generated *ts* strains of influenza A from persistent infections of cell cultures [70–72]. Similar tendencies are seen in persistent infections of animals; foot-and-mouth viruses recovered from carrier animals are frequently *ts*, and show evidence of high rates of mutation with frequent amino acid substitutions and rapid antigenic variation [73].

Preble and Youngner pointed out that since *ts* strains tend to be less virulent they may allow persistent infections to become established, because a balance between viral and cell replication is required [69]. They do not, however, explain why *ts* mutations in particular should be selected in persistent infections, as opposed to non-*ts* attenuating mutations. In most cases the cells were grown in the laboratory at 37 °C, so temperature sensitivity should be a *disadvantage.* If, however, the wild virus from which the laboratory strain was derived was *ts*, the probability of reverting to the *ts* phenotype may be relatively high. For example, a protein might lose its *ts* character by a point mutation caused by a single nucleotide change, so that the *ts* character could be restored by reinstating the original nucleotide. This may be more probable than other mutations that would attenuate the virus without conferring temperature sensitivity. Changes to RNA secondary structure (discussed below) may have similar effects. Bear in mind that many virus families include species that cause both systemic and respiratory tract infections, and strains may have jumped between these sites many times in their evolutionary history. For example influenza often infects the gut in birds, but it usually infects the respiratory tract in mammals. These considerations suggest that genetic pathways may exist that allow the rapid elimination and reintroduction of temperature sensitivity.

If this interpretation is correct, it may explain how respiratory viruses such as influenza, RSV and adenovirus are able to infect hosts throughout the world. A tropical virus might cause serious illness if it arrives in colder parts of the world, because it might infect sites lower down the respiratory tract. Since too much virulence may *reduce* viral transmission (as patients become bed-ridden), natural selection in the temperate site for reduced virulence may increase temperature sensitivity to a level that is appropriate to the virus's new location. A similar argument suggests that viral temperature sensitivity (and virulence) may be adjusted throughout the year by natural selection to a level that is appropriate to the season.

The converse trend: the loss of the *ts* phenotype in conditions that allow rapid replication

Since *ts* strains are generally less virulent *in vivo* [57,58,69], and are associated with persistent infections both *in vivo* and *in vitro* [58,69], it might be anticipated that the *ts* character would be lost in *in vitro* conditions that allow rapid replication of viruses, and this has indeed been observed. Chu et al. found a naturally-occurring *ts* influenza A strain that was a subclone of the H3N2 strain Ningxia/11/72 [59]. When they passaged the strain three times through chicken embryos at a *low temperature* (33 °C), they were surprised to find that a non-*ts* strain was produced. Similarly, Oxford et al. [74] 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 – which allow rapid replication of influenza virions. Again we can speculate that viru-

lence and greater viral activity in general is genetically linked to temperature sensitivity as a result of the evolutionary history of the virus.

The unexpected loss and gain of temperature sensitivity (in a wide variety of viruses) when increased or decreased viral activity is selected is shown schematically in Fig. 6.

Temperature sensitivity in wild and laboratory viruses

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 [76], and this is the temperature that is recommended today for their isolation by the Clinical and Laboratory Standards Institute [77]. Coronaviruses were first isolated at 33 °C [78] although laboratory strains are now frequently propagated at 37 °C. Naturally occurring influenza strains are also frequently *ts*. For example, in 1962 Stern and Tippett [79] propagated four viral specimens from patients with H2N2 "Asian" influenza, all of which were *ts*. All four gave cytopathic effects in monkey cells and the production of hemagglutinatin-positive fluids 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. The authors also found (in 1962) that the FM1 (H1N1, 1947) and PR8 (old-style H0N1, 1934) strains grew more slowly in monkey cells at 37 °C than at 33 °C. In 1977, Kung et al. found that nine of ten isolates of the newly emerged "Russian" H1N1 influenza were *ts* [80]. 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 after incubation at 34 °C compared to 38.5 °C [74].

Membrane fusion and *ts* entry into cells

Takashita et al. found that, in influenza C (C/Ann Arbor/1/50), roughly half the amount of the hemagglutinin-esterase-fusion protein (HEF) was found on the cell surface at 37 °C compared to 33 °C [68]. (HEF in influenza C carries out the functions of both hemagglutinin and neuraminidase 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 saw an "unexpected" result when he measured the uptake of the triple reassortant influenza virus A/Jap/Bel into cells [60]. 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 (Fig. 2 of Ref. [60]). 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*.

The temperature sensitivity of viral transcription

Most laboratory respiratory viruses are propagated at 37 °C, which may result in the rapid loss of *ts* characters, especially since viruses mutate very rapidly when they are introduced to new hosts. If, however, temperature sensitivity is a common feature of wild respiratory viruses, we might expect to see remnants of temperature sensitivity in the biochemistry of laboratory strains. It turns out that such remnants are quite common.

For several decades virologists have found that maximum RNA transcription in influenza viruses occurs below normal body temperature. In 1977, Plotch and Krug [61] reported that the greatest activity of the RNA polymerase of WSN virus was at 30–32 °C. This

is similar to the optimum temperature of the polymerase of influenza C, which is 33 °C [62,63]. Ulmanen et al. [64] found that the rate of transcription by detergent-treated WSN viruses (influenza A) was about 10 times greater at 33 °C than at 39.5 °C, and also that the binding of a cleaved primer cap (the "A13 fragment") to the viral cores was "unexpectedly" much weaker at 39.5 °C than at 33 °C. Scholtissek and Rott [65] 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). 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 [66]. For all strains, vRNA unexpectedly decreased when the temperature was increased from 37 °C to 42 °C. 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 [67]. 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. 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.

The secondary structure of RNA

Much recent attention has focused on the role of RNA secondary structure in influenza A, although discussion of its role in temperature sensitivity has been limited. "RNA thermometers" are RNA segments (found in both microorganisms and higher organisms) that respond to temperature changes with three-dimensional conformational changes that alter gene expression [81]. They are frequently (but not always) found in the 5'-untranslated regions of mRNA, and can act in both directions – translation can be augmented or suppressed at high temperatures [81]. Chursov et al. used bioinformatic techniques to search for pronounced differences between mRNA from cold-adapted *ts* influenza strains and the corresponding wild-type sequences [82]. Pronounced differences were found in the mRNAs of four viral proteins. The authors suggest that temperature-induced structural changes of mRNA may constitute an "unappreciated molecular mechanism of the cold adaptation/temperature sensitivity phenomena" [82].

Little secondary structure is predicted in influenza vRNA outside the untranslated 5′ and 3′ terminal ends of the vRNA strands that form the promoter necessary for the initiation of RNA synthesis [83]. However, the positive-sense RNA is predicted to have extensive secondary structure, which is conserved, in segments 1, 5, 7 and 8 [83]. Since ordered RNA is intrinsically *ts*, and since individual base changes may have a cumulative effect on the overall secondary structure of RNA, it is likely that viral temperature sensitivity can be fine-tuned by small sequence changes to untranslated regions of RNA. For example changes to the secondary structure of vRNA, cRNA and mRNA might all affect temperature sensitivity in influenza. (Changes to protein sequences may also be in involved of course.)

Temperature sensitivity and the evolution of viral tropism

We can speculate that temperature sensitivity might have profound effects on viral tropism. Many or most respiratory viruses possess temperature sensitivity, and we can imagine that a respiratory virus that loses its temperature sensitivity might infect the lungs, gut or internal organs. (Some method of limiting virulence other than temperature sensitivity might then be selected that can allow the virus to survive in the long-term.) Conversely, viruses originating in the internal organs that develop temperature sensitivity could safely cause severe local infections that would be limited to the upper respiratory tract (possibly in addition to other cold parts of the body such as the feet), without greatly incapacitating the host (incapacitation would limit opportunities for transmission of the virus). Obviously the resulting irritation of the respiratory tract might cause coughing, sneezing and runny noses, all of which might help to transmit the virus – in other words a respiratory virus has been generated. Influenza infects the gut of water fowl but the respiratory tract of mammals (and birds), and is able to move between these two ecological niches. Similarly, some adenovirus serotypes are mainly respiratory, others mainly cause gut-related disease. Viruses that are transmitted via skin rashes and blisters that burst, such as chickenpox, measles, smallpox, and hand, foot and mouth disease in humans, and foot-and-mouth disease in cloven-hoofed mammals, could also benefit from temperature sensitivity that might allow them to infect the skin preferentially and so to spread by direct contact. (In the diseases mentioned above contact transmission from blisters etc. coexists with aerosol transmission.) Several examples indicate that what we think of as respiratory viruses can occasionally cause systemic infections: virulent human influenza strains sometimes give rise to viremia [84,85], and three children who were infected with pandemic H1N1 influenza in 2009 ("swine flu") presented with petechial rashes [86]. Similarly, 33 patients presented with hemorrhagic cystitis caused by H3N2 influenza A in 1975 [87].

Conclusions and suggestions for experimental verification

Many suggestions have been put forward to explain the seasonality of vARIs, especially influenza. Changes in crowding (M1), and changes in the survival rate of viruses outside the body (M2) cannot be completely ruled out, but they fall short in at least one important respect: they cannot explain why vARIs including influenza are transmitted in the tropics, especially during wet weather, but are mostly absent from temperate regions in the summer months. An alternative suggestion (M3), that chilled hosts are more susceptible as a result of *ts* changes to their immune defenses, is a better candidate. A biochemical study using cultured mouse cells supports the idea [51], and M3 also seems to be part of the best explanation of the protective effect of winter outdoor exercise [15]. However, evidence from vaccination studies, epidemiological evidence that vARIs respond to small temperature changes throughout a wide range of absolute temperatures (Figs. 1 and 3), and evidence that vARIs frequently appear simultaneously throughout large geographical regions (Figs. 1 and 2), seem to rule out changes in host susceptibility as the main driver of vARI seasonality.

This leaves the explanation that might seem most intuitive to the lay-person – that viruses can become temporarily dormant, and are reactivated by chilling, which changes their behavior at the biochemical level (M4). This explanation is seldom considered by microbiologists, who seem to have ruled it out on the basis of historical reports from the 1950s and 1960s, which concluded that chilling does not bring on vARIs [53–56]. However, there is now so much clear evidence that chilling *does* increase vARIs that we need to look more closely at those historical experiments. I suggest above that they were flawed because they generally used "pedigree" viral strains which were passed by the investigators from volunteers to subsequent batches of volunteers in later

experiments. Obviously the investigators would have thought carefully about the choice of strains that they used; they certainly didn't want to put the volunteers at risk, so mild strains needed to be selected. However they naturally wanted their experiments to fit into the time available – for example Andrewes worked with volunteers who each stayed at his unit for 10 days, and his experiments did not start until the volunteers had been in quarantine for three days. The incubation period of the strain that he used was most frequently two to three days. By selecting such a strain he and his colleagues may have eliminated the virus's natural temperature sensitivity during the early stages of infection (temperature sensitivity that is predicted by M4) – a flaw in the design of his experiments with potentially far-reaching implications.

Note, however, that the negative results of the host-chilling experiments using pedigree strains nevertheless weigh against M3 – because the lack of temperature sensitivity of the virus (or possibly other characters of a virus that had undergone prolonged serial passage) should not influence host susceptibility.

Leaving aside these early experiments, what is the evidence for or against the idea (M4) that temperature fluctuations can activate respiratory viruses and give rise to vARI seasonality? Epidemiological evidence shows that (a) surges in vARIs often follow a few days after temperature dips (even minor dips) [2,3,5,14,21,22]; (b) in all climates, it is temperature *drops* that are generally associated with vARI epidemics rather than sustained low absolute temperature [3,5,21,22]; (c) the attack rate and the rate of transmission within families are often low, especially for influenza [5,43–45]; (d) vARI epidemics often appear simultaneously throughout wide geographical areas, with no evidence of waves of transmission that move between neighboring (Fig. 1) or widely separated (Fig. 2) localities [5–7]; and (e) influenza epidemics often occur in mid-winter but cease while many susceptible individuals remain in the population [7]. All of these observations are compatible with M4, as discussed above. Moreover, observations of the timing and location of vARI epidemics suggest that respiratory viruses can become dormant, a suggestion that is confirmed by the occasional presence of vARIs in polar communities after many weeks or months of complete isolation [24,25,39]. Biochemical tests of asymptomatic individuals who shed influenza A and B without seroconversion [35–38] are compatible with dormancy, and PCR tests showed directly that individuals harboring a variety of viral pathogens subsequently developed the corresponding vARIs [18,34]. If we accept this evidence of viral dormancy and reactivation, then the idea (M4) that chilling increases the activity of respiratory viruses that were present before the epidemic began can provide the rapid response, sensitivity to temperature dips, and occasional lack of transmission that we need to explain the epidemiological events (a–e) above. It is unclear how these events can be explained by the alternative mechanisms M1 – M3.

We also need to explain the clear trend in the laboratory for persistent infections of cells to yield *ts* strains of a variety of viruses in the absence of obvious selective pressures [58,69–72], and also the converse observation, that temperature sensitivity is lost when viruses are grown in cell cultures at low temperature in conditions that allow rapid replication [59,74] (Fig. 6). Moreover, biochemical studies show that many steps in the replication of laboratory strains of influenza and other viruses have residual natural temperature sensitivity [60–68,83], in spite of many cycles of replication of most laboratory viruses at 37 °C. We can explain these data by suggesting a link between loss of temperature sensitivity and the acquisition of increased virulence in respiratory viruses, which seems to be a legacy of their evolutionary history.

Lwoff (1959), and Richman and Murphy (1979) suggested that the temperature sensitivity of respiratory viruses allows them to target the respiratory tract, and to avoid the lungs [57,58]. This idea, and the related idea that less *ts* strains tend to be more viru-

lent, seems to be widely accepted by microbiologists. However, the corollary, that ambient temperature dips can activate viruses that were previously inactive, is less popular. It is, however compatible with the well-known "trade-off" model of virulence. This model suggests that the benefits of virulence (in particular the increased rate of virus production and shedding) are balanced against the reduction of the time during which shedding takes place (and also changes to the behavior of hosts that reduce transmission) if virulence is too great. The implication is that mechanisms are required to moderate virulence, and temperature sensitive mechanisms can clearly achieve this in the case of respiratory viruses. An analogy in the form of a joke may be helpful here. Two scientists are out hiking, when they meet an angry-looking bear. "It's no use," says one scientist to the other, "you can never outrun a bear". "I don't need to outrun the bear," replies his companion, "I only need to outrun you". It is often assumed that an "evolutionary arms race" exists between viruses and the host immune system, with each making a series of small improvements to gain an advantage over the other [88]. This assumption may be incorrect – it may be that most viruses could easily overcome the defenses of hosts that lack specific immunity, but that selective pressures nevertheless reduce the virulence of well-adapted viruses, as predicted by the trade-off model. Some of the evidence for this comes from viruses that have recently jumped from one host species to another, such as myxomatosis in European rabbits, and, in humans, HIV, SARS, Middle Eastern Respiratory Syndrome, avian influenza and Ebola. These viruses are unusually virulent, in spite of – or because of – having had little time to adapt to their hosts. The main arms race may not be between viruses and hosts, but between competing hosts, where each needs to reduce its susceptibility to outcompete other members of the same species.

Of course, respiratory viruses comprise many unrelated families, and there may be important differences in the mechanisms that give rise to their seasonality. Multiple mechanisms could contribute to seasonality, and the mix could vary between viruses. Nevertheless it seems likely that that the common seasonality of these diverse viruses is not coincidental, that common mechanisms give rise to this near-universal trend, and that some variant of M4 is the most important in many or most cases.

It is unlikely that either evidence gleaned from studies that were designed to investigate other aspects of viral biochemistry or epidemiological observations can determine the causes of vARI seasonality with certainty. Instead it will be necessary to investigate temperature sensitivity directly *in vivo* and *in vitro*, working with viruses that are as close as possible to wild viruses. As a start, wild and laboratory viral samples should be "deep sequenced" (i.e. the relative proportions of different sequences in a sample should be established at multiple genetic sites) to determine the mix of *ts* and non-*ts* sequences in wild samples, and to establish the impact on temperature sensitivity of propagating wild viruses in the laboratory. This analysis needs to include consideration of RNA secondary structure. The information gained can be applied at many levels, from observations and experiments with living organisms to experiments with cell cultures and in solution. It may be possible to image the distribution of virions in the respiratory tracts of animals, and to see e.g. differences in animals that were housed at high and low temperatures prior to the investigation. Virions might be released from tissues or cells by raising the temperature, or captured by lowering the temperature. (Note that the genetic information remains attached to the chemical probe, in a manner analogous to the phage display technique.) The entry of virions into cells can be investigated by measuring the escape rate of pre-adsorbed virus from neutralization by antibody in temperature shift experiments [60]. In tissue cultures, transcription and the production of genetic material can be followed during temperature shifts (for example the production of mRNA, cRNA and vRNA can

each be studied in influenza). Similarly, the production of viral proteins can be followed during temperature-shift experiments. Experiments in solution can also yield valuable information. For example, the thermal stability of mutants of influenza hemagglutinin and neuraminidase can be investigated in solution by thermal shift assays, and similar experiments can be undertaken with other respiratory viruses. The thermal stability of secondary structures of wild-type and laboratory viral RNA and RNA/protein complexes can be measured in solution. Bioinformatics can be applied to the problem. For example the sequences of hemagglutinin, neuraminidase and other viral proteins from wild and laboratory strains, and from the viruses obtained from different animal hosts and laboratory procedures can be analyzed. This analysis can consider the impact of mutations on the structures of viral proteins and complexes that have been determined by X-ray crystallography and other techniques. For example the effect of changing a particular residue can be anticipated or investigated experimentally. Secondary structure prediction techniques can be applied to viral RNA, DNA and protein/nucleic acid complexes. Yet another approach is to investigate and model viral epidemiology with these ideas in mind. Finally, experiments can be performed at the whole organism level. For example, chilblains and chapped lips can be examined for the presence of respiratory and other viruses. Other, very simple, experiments can be performed with human volunteers. For example, groups of volunteers can be subjected to chilling in the autumn or midwinter (which are the seasons when individuals are particularly susceptible to vARIs) and compared to control groups who are kept warm. The number of vARIs suffered by both groups can then be compared. This approach would make use of dormant viruses that the participants were already carrying by chance.

Competing interests

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 750 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 vARIs. I started to write a short note, but everything fell into place so neatly that it rapidly grew into the current document.

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