A survey of selective trends and seasonality in viral respiratory tract infections

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Changes to this version:

1. The word “Holland” has been replaced with “the Netherlands”
2. A section has been added on RNA thermometers
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Abstract

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

Key index phrases
Respiratory tract infections, viral infections, temperature changes, temperature sensitivity, seasonality of the common cold, influenza seasonality, epidemiology of viruses.

The seasonality of colds and ‘flu

The viruses that cause viral respiratory tract infections (VRTIs) include many unrelated groups of viruses including 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 (e.g. hand foot and mouth virus, rhinovirus, rubella virus). It is striking that completely unrelated strains have very similar lifecycles and produce indistinguishable symptoms in the early stages of infection. Respiratory viruses clearly occupy a very popular and well-defined ecological niche. Most respiratory viruses show clear seasonality, with far more VRTIs occurring in the autumn and winter than in the summer in temperate regions. This implies that selective pressures associated with the mechanisms of replication or transmission during the colder months outweigh the selective disadvantages of inactivity during the summer, and also that surviving the summer months is not a major difficulty. Moreover, it suggests that similar mechanisms of transmission and replication are used in a wide variety of respiratory viruses.

Virologists have put forward many explanations of the seasonality of VRTIs. For example, proposed explanations of influenza seasonality 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]. However, none of these explanations is satisfactory, because they cannot explain simultaneously the virtual absence of VRTIs in temperate regions during the summer and their occurrence throughout the year at intermediate levels in the tropics. The strange global and seasonal pattern of VRTIs is shown schematically in Figure 1. Any factor that can prevent VRTIs in temperate summers has more extreme values in the tropics throughout the year, so, according to these explanations, VRTIs should not be present in the tropics at all. Moreover, H3N2 influenza A (and presumably other influenza strains) circulates continuously in East and Southeast Asia, and spreads to temperate regions from this network [2], so we would expect it to have properties that allow it to be active during temperate summers. Note also that influenza and other VRTIs show clear seasonality in the tropics that does not coincide with fluctuations in temperature, humidity or solar radiation [1]. A popular explanation of VRTI seasonality is that contact rates are lower in the 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 VRTI seasonality. Moreover, one of the two peaks of influenza activity in Singapore [1] coincides with the school holidays in June. Lofgren et al. agreed that theoretical and empirical studies do not adequately explain influenza A seasonality, noting that no published studies directly show that variations in crowding cause influenza seasonality, and that a linkage between viral evolution and the wide assortment of other proposed factors in influenza seasonality is lacking [3]. Current explanations of seasonality clearly lack credibility.

Although temperature per se does not determine the incidence of VRTIs, evidence from many different sources shows that VRTIs are correlated with temperature fluctuations. For example, van Loghem conducted a very extensive survey of VRTIs in the winter of 1925/26 with 6,933 participants from all regions of the Netherlands [4]. His data is shown in Figure 2, together with the temperatures recorded by five Dutch weather stations. Epidemics of VRTIs in all seven regions were very closely correlated with each other, and with inverted temperature (i.e. lower temperatures were associated with increased VRTIs). Note that these correlations were strongest in the first half of the cold season. Milam & Smillie found similar patterns on the tropical island of St. John in the Virgin Islands in 1929, shown in Figure 3 [5]. Between mid-afternoon and midnight each day the temperature on the island dropped sharply by 5 - 7°C. When the temperature dipped in the autumn by 1 - 2°C below the summer range, cold epidemics were triggered. More recently, Jaakkola et al. found that a sudden decline 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 [6]. 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 humidity that increased the risk of influenza. In the UK, Hajat et al. found that general practitioner consultations 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 [7]. Studies have shown that personal chilling increases the incidence of VRTIs. The Eurowinter Group showed that shivering outside, and wearing inadequate winter clothing increased respiratory disease-related mortality, while outdoor exertion sufficient to cause sweating was protective [8]. 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 controls who were maintained at normal body temperature (p<0.02) [9].
Costilla-Esquibel et al. found a relationship between weather and acute respiratory illnesses in Monterrey, which they were able to model very accurately using only three weather parameters: weekly accumulated rainfall, minimum temperature in the week, and weekly median relative humidity [10]. Of the three parameters rainfall had the highest impact, humidity the lowest. High relative humidity is expected as a consequence of increased rainfall and low temperature.

Studies of VRTIs in Antarctic stations after many months of complete isolation allow observations of (almost certainly) one viral strain at a time. For example, a geologist (“J.E.H.”) at the Mawson station in 1966 picked up a respiratory virus from a visiting field party [11]. 17 days later he and three colleagues were exposed to cold and damp conditions, which brought on VRTI 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 [12]. These studies suggest that chilling caused by particular activities or by weather changes can activate dormant viruses, giving rise to VRTIs.

Absolute and relative humidity have recently been suggested as factors that influence VRTI levels [6, 13, 14]. Absolute humidity is a convenient single parameter that can be used for statistical analysis, but it has no physico-chemical meaning in the context of viral transmission and replication because viruses are produced and are active at biological (i.e. moderate) temperatures and pressures. They are therefore subject to the normal drying capacity of air, which is defined by relative humidity. Observed correlations of VRTI epidemics with absolute humidity are therefore almost certainly due to separate correlations with temperature and relative humidity. Relative humidity can undoubtedly influence viral transmission [13 – 15] but it is not universally correlated (either positively or negatively) with VRTI epidemics. For example, while high relative humidity is associated with winter influenza epidemics in Bismarck, ND (USA), this parameter is almost constant in Singapore, and varies little in Fortaleza (Brazil), and Sydney (Australia). All four cities have clear influenza seasonality [1]. Note also that animal and tissue culture experiments found the opposite relationship – that high relative humidity reduces transmission, as described below. In all four cities, however, influenza is correlated with parameters that cause personal chilling. In Bismarck and Sydney, influenza arrives during the cold months of the year, while in Fortaleza it coincides with the rainy season. In Singapore there are two peaks of influenza activity that coincide with the two monsoon seasons, which are associated with strong winds that can clearly chill human hosts.

In summary, available evidence shows that both sudden weather changes and factors that cause individual chilling frequently bring on VRTIs. This suggests that temperature sensitivity plays a role in seasonality, but the global patterns of VRTIs rule out the possibility that viral activity is controlled solely by absolute temperature. (For example, we do not see a VRTI that is limited to all global regions or seasons where temperatures remain below say 10°C.) 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 VRTIs.

**Mechanisms that would allow VRTIs to respond to temperature changes**

If we accept that exposure to cold triggers VRTI epidemics and gives rise to VRTI seasonality in both temperate and tropical regions, three possible mechanisms can be put forward: (1) colder conditions may allow the virus to survive outside the body for longer, increasing transmission. (2) The susceptibility of hosts may increase as a result of chilling. (3) Chilling may increase the activity of viruses in the body. I will now consider the evidence for these three possibilities.
(1) Colder conditions may allow respiratory viruses to survive outside the body for longer

This is currently the most popular explanation of seasonality. It is, however, almost certainly not the correct explanation, for several clear reasons. Firstly, this explanation cannot explain why VRTIs are present in many tropical regions all year round, but virtually absent from the temperate regions during the summer months. If

they can adequately survive outside the body in the tropics, respiratory viruses should certainly survive (according to this explanation) during temperate summers. (The suggestion that low absolute or relative humidity may increase viral survival does not help, because VRTI epidemics occur during the rainy season in many tropical locations.) Secondly, consider van Loghem’s data [4] (Figure 2). While seasonal temperatures were falling or at constant low levels (i.e. up to the end of January), changes in VRTIs were very well-synchronized with changes in temperature, with a lag of less than a week. Since the average incubation period of seven common VRTIs (excluding measles) reported in a recent review was 3.9 days [16], there is time for only one or two cycles of infection per week. The response of VRTIs appears to be too fast to be the result of changes in viral transmission. It was also extraordinarily well-synchronized across the country, with no evidence of “waves” of infection moving between different locations. Thirdly, it seems unlikely that virus transmission is acutely sensitive to small temperature fluctuations (about 4°C) over such a large range (about 20°C). A similar argument applies to the data of Milam & Smillie (Figure 3), which also showed fast-acting sensitivity to small temperature drops that occur at a range of temperatures throughout the year [5]. Jaakkola et al. reported that “sudden declines” of around 5°C preceded the onset of influenza in Northern Finland [6], and these events were observed at temperatures above 15°C and also below -15°C, which implies that transmission needs to vary over roughly 30°C. Fourthly, we should consider the effects of chilling on individuals; if changes in viral transmission were responsible for seasonality, why would wearing an anorak and outdoor physical exertion reduce mortality from VRTIs, while shivering outside increases it [8]? It seems that we need to look elsewhere for an explanation of VRTI seasonality.

(2) Chilling may increase the susceptibility of hosts

Eccles suggested that physical chilling may cause reflex vasoconstriction of the blood vessels of the upper airways, thereby reducing host defenses against infection during the winter [17-19]. This hypothesis can explain the results of Eccles’ own study where the chilling of volunteers’ feet increased the number of VRTIs in the following 4 to 5 days [18], and it can also explain the simultaneous appearance of VRTIs across wide geographical regions. For example, Magrassi was impressed by cases of influenza in 1948 among shepherds living in complete social isolation in open country in Sardinia, who developed influenza contemporaneously with the inhabitants of towns on the same island [20]. Eccles’ suggestion, however, has great difficulty in other areas.

The difficulty with scales that was noted above for explanation (1) above applies even more strongly to this explanation. Since the mechanism needs to apply in different climates and at different times of year (from early autumn to mid-winter) it must act over a wide range of temperatures. It is very difficult to reconcile this with the observed sensitivity to small temperature drops. Consider, for example, Chart 1 of Milam & Smillie’s paper [5], reproduced in Figure 3. Every night in the summer the temperature dropped by about 5 - 7°C. In the autumn the temperature fell by an extra 1.7°C, which triggered an epidemic of colds. Can we believe that the inhabitants’ immune systems could cope well with a regular 6°C drop but succumbed after a 7.7°C drop? Note that the absolute temperature after the dip - about 23°C at night - was still very comfortable.

Note also that there is often a peak of VRTIs in the early autumn [4, 5, 21]. This can be seen, for example, in Google Flu Trends for Germany [29], especially away from coastal regions. In Germany, Flu Trends models “acute respiratory illness” (ARI) which includes all VRTIs whether or not they cause fever. Another example is
the very high level of colds at the beginning of the study by van Loghem, which began on 19 September, 1925 [4]. (He found that 33% of the population of Amsterdam suffered from colds at that time.) VRTI levels were much higher at the start of the study than in the rest of the study, and it is difficult to explain why the human immune system should be weaker in early autumn than in midwinter.

Another problem for this idea is the abrupt cessation of influenza epidemics. Hope-Simpson noted that all of the major influenza epidemics that he recorded in Cirencester, UK, (1951, 1957, 1959, 1969 and 1973) rose rapidly to a single peak within four weeks, then abruptly ceased within 4-5 weeks [Figure 1 in ref. 22]. 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 population because there was a second major H2N2 epidemic 16 months later [22]. The abrupt cessation of the first epidemic is therefore unexplained. Each of the other 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 nine weeks.

An interesting and ingenious recent review looked directly at the seasonality of immune responses in humans by investigating antibody responses following vaccination [24]. Although the authors found that seasonal variations in immunity, it could not explain VRTI seasonality: seven of the studies of vaccines reported a stronger immune response in winter than in summer, with only 1 showing the opposite seasonality. There was no clear trend with regard to the dry and rainy seasons in tropical regions and several studies showed no trend at all. These data therefore strongly suggest that variations in host susceptibility do explain the seasonality of VRTIs.

(3) Chilling may increase 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 resulted in reduced virulence [25]. In 1979, Richman & Murphy confirmed this association and reviewed its implications for the development of live virus vaccines [26]. These authors noted that the replication of temperature-sensitive (ts) influenza, parainfluenza, RSV, and foot-and-mouth viruses was consistently more restricted in the lungs of a variety of animals than in their nasal tubinates. 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 virulence or growth capacity of the virus, both in vivo and in vitro [26]. It is reasonable to conclude that the ts phenotype facilitates the transmission of the virus because it prevents or reduces multiplication of the virus in the lungs or internal organs, which might result in the death or immobilization of the host.

The evidence discussed in previous sections, including the simultaneous appearance of VRTIs across wide geographic areas, their sudden appearance in the autumn in temperate regions, and their appearance in polar communities after weeks or months of complete isolation, suggests that respiratory viruses can become dormant in human hosts. There is also biochemical evidence of dormancy in influenza, which will be discussed below.
Putting these observations together, a simple proposal can be put forward that may explain the seasonality of VRTIs and the anomalous behavior of respiratory viruses described above. 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 [27]. The temperature in the respiratory tract drops rapidly when the host is chilled, when the air being breathed is cold, or when the host breaths rapidly [27, 28]. The proposal therefore takes the following form:

1. One or several steps in the life-cycle of most respiratory viruses are ts. These ts steps might include the binding of viruses to cells, their entry into cells or any subsequent step in their replication.
2. Viruses that are immobilized in the respiratory tract at relatively high temperatures will remain dormant.
3. Viruses that are immobilized high in the respiratory tract (therefore at low temperatures) will become active, but will not normally cause a VRTI because they are active in low numbers at any one time and are removed by the host’s immune system.
4. Each day the temperature of the respiratory tract varies, and this variation clears certain populations of viruses from certain regions of the respiratory tract, leaving other populations intact.
5. If the temperature of the respiratory tract drops below its normal range, batches of viruses that were previously dormant will be activated simultaneously.

It seems likely that the binding of many respiratory viruses to their target cells is ts, because it would be wasteful for viruses to become bound in e.g. the lungs where they would remain inactive indefinitely. For the virus to become dormant it is necessary for the “restrictive temperature” for binding (the temperature at which binding becomes impossible) to be higher than the restrictive temperature for activation. Richman & Murphy noted that more virulent strains tend to be less ts, which may be reflected their binding sites in the respiratory tract [26]. (For example, virulent pandemic influenza strains would be expected to be less ts than seasonal influenza and to bind lower down the respiratory tract.) Chu et al. tested seven H1N1 strains with varying degrees of temperature sensitivity in volunteers and found a correlation between temperature sensitivity and the severity of VRTI symptoms, with more ts strains being less virulent [50].

An important point is that this mechanism can be sensitive to temperature changes, not absolute temperature. This can explain the data of van Loghem, Milam, Jaakkola and others [4 – 6, 10 - 12]. It can also explain the seasonality of VRTIs, since chilling outside of the range of the previous few days or weeks becomes more likely as the seasonal temperature drops in the autumn. Similarly, in the spring chilling becomes less frequent as the seasonal temperature rises.

The proposed mechanism also explains the association of VRTIs with wind and rain in the tropics, since these weather events often result in the chilling of individuals, even when the temperature remains constant. It also provides an explanation for the strange epidemiology of influenza, including the rapid cessation of epidemics when many susceptible individuals remain in the population [22]; bear in mind that each member of a family will have a different history of chilling and of exposure to viruses. For example, some members of the family may take regular exercise outdoors, which can clear viruses from the respiratory tract in small batches (outdoor exercise is known to reduce mortality from VRTIs [8]). Other individuals 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 in the respiratory tract, and are not then activated unless even colder weather follows. These trends can therefore explain the low attack rates [23] and lack of transmission within families [23], as well as the rapid cessation of epidemics [22].
Animal experiments with human influenza virus

Experiments with guinea pigs show that the transmission of influenza A is more efficient at lower temperatures and lower relative humidity. Lowen at al. found a 3.5-fold increase in the transmission of human influenza A between guinea pigs at 5°C compared to at 20°C [13] (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 [14]. Variations in transmission due to weather changes could therefore contribute to the seasonality of influenza (and other VRTIs) in temperate regions. 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 [15]. The authors postulate that the normal mode of influenza transmission varies depending on climate: in temperate regions aerosol transmission may predominate, while in the tropics short-range and contact transmission may be more important. Epidemics of H3N2 influenza in the temperate regions, however, are seeded each year from a network of temporarily overlapping epidemics in East and Southeast Asia [2]. There is therefore no reason why influenza cannot be transmitted by the contact route in the summer months into and within the temperate regions. Lowen et al. recognize this difficulty, and they postulate the existence of “additional factors, other than warm temperature and high relative humidity, which suppress influenza transmission by all routes during the summer months” in temperate regions [15]. Although the authors may have identified the routes of influenza transmission at different latitudes, they have therefore not provided a robust explanation of its seasonality.

Dormancy in VRTIs

Viruses such as adenovirus [30], RSV [31], foot-and-mouth virus [32] and chickenpox virus - all of which can spread via the respiratory tract - are known to become dormant within their hosts. Other respiratory viruses may show similar behavior if they possess or develop appropriate patterns of temperature sensitivity.

Influenza viruses have been detected several times in the absence of symptoms or an immune response in the host, which indicates that dormant influenza virus is present. Foy et al. identified 37 individuals who were shedding influenza B virus, of whom 10 were asymptomatic and did not respond with antibody by any of the five test methods employed [33]. During the 2009 influenza A (H1N1) pandemic, Tandale et al. found that, of 65 asymptomatic individuals with PCR-confirmed H1N1, 12 had not seroconverted [34]. During the same pandemic, Papenburg et al. found two asymptomatic individuals with PCR-confirmed infections who had not seroconverted [35]. 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 [36]. The authors commented that this “may indicate that viral RNA remained in the respiratory tract without being internalized and eliciting an immune response”. The two studies mentioned above of VRTIs in Antarctica also demonstrated dormancy of respiratory viruses [11, 12]. In addition, 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 [37]. Moreover, they recorded two episodes of respiratory illness caused by parainfluenza at the Station that year after 10 and 29 weeks of complete social isolation. Dormancy clearly exists in a variety of respiratory viruses.

Early studies where volunteers were chilled

It is widely believed by medics and scientists that chilling does not affect VRTIs, and that this idea is “an old wives’ tale” [38]. 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 [39–41]. Unfortunately, these studies used “recycled” viruses that rapidly caused VRTIs in a significant proportion of the volunteers (as opposed to natural strains that the volunteers happened to be carrying). It is known that “serial passage” experiments, where parasites are deliberately introduced to many hosts in succession, cause rapid changes to the parasite, since the need for transmission is eliminated [42]. In these studies it seems likely that strains that caused mild infections very quickly were consciously or unconsciously selected by the researchers. This probably removed some aspects of their natural temperature sensitivity. However, one study, by Jackson et al., did use “wild” viruses that the volunteers were carrying at the time of the experiment [43]. In some experiments, volunteers in scant dress were exposed to 15.5°C air for four hours. In others, warmly-dressed volunteers breathed air at -12°C for two hours. Of those who were chilled, only 10% developed colds in the next 7 days, whereas 12% who were not chilled developed colds. However, the authors do not tell us what proportion of volunteers were chilled by breathing cold air and what proportion by wearing scant clothing; in some cases breathing cold air while remaining warm can be protective [8], presumably because viruses are activated but they can be removed by the immune system.

More recently, Johnson & 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 [18]. 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.

Membrane fusion and ts entry into cells

Takashita et al. found that, in influenza C (C/Ann Arbor/1/50), roughly half the amount the hemagglutinin-esterase-fusion protein (HEF) was found on the cell surface at 37°C compared to 33°C [65]. (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 [66]. 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 [Figure 2 of ref. 66]. 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 recovery of ts viruses from persistent infections

In an interesting review of 1975 [44], Preble & Youngner noted that ts strains often appear spontaneously in persistent infections of cell cultures with a variety of unrelated viruses (including Newcastle disease virus, Western equine encephalitis virus, Sendai virus, measles virus, stomatitis virus, and Sindbis virus). Similarly, Richman & Murphy found that persistent infections of cell-cultures with mumps virus and vesicular stomatitis virus consistently yielded ts virus, although they noted that persistent infections could also be established or maintained by non-ts mutations [26]. Three more recent reports described the recovery of spontaneously-generated ts strains of influenza A from persistent infections of cell cultures [45–47]. Similar tendencies are seen in persistent infections of animals; foot-and-mouth viruses recovered from carrier animals are frequently ts [49], and show evidence of high rates of mutation with frequent amino acid substitutions and rapid antigenic variation [49].
Preble & 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 [44]. They do not, however, explain why ts mutations in particular should be selected in persistent infections, as opposed to non-ts attenuating mutations.

The loss of the ts phenotype in conditions that allow the rapid replication of viruses

Since ts strains are generally less virulent in vivo and are associated with persistent infections in vitro and in vitro [26, 44], 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 [50]. When they passaged the strain three times through chicken embryos at 33°C, a non-ts strain was unexpectedly produced. Similarly, Oxford et al. [51] 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.

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 Figure 4.

Temperature sensitivity in wild and laboratory viruses

Numerous studies have found that it is easier to propagate respiratory viruses when they 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 [52], and this is the temperature that is recommended today for their isolation by the Clinical and Laboratory Standards Institute [53]. Coronaviruses were first isolated at 33°C [54] although laboratory strains are now frequently propagated at 37°C. Naturally occurring influenza strains are also frequently ts. For example, in 1962 Stern & Tippett [55] propagated four viral specimens from patients with H2N2 “Asian” influenza, all of which were ts. All four gave cytopathic effects in monkey cells and agglutination in eggs at 33°C but not at 37°C. Subcultures were able to adapt to culture at 37°C but grew more slowly than at 33°C. The authors also found (in 1962) that the FM1 (H1N1, 1947) and PR8 (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 [56]. Oxford et al. found that 17 of 26 recent H1N1 isolates, and 2 of 11 recent H3N2 isolates were ts, producing cultures that gave at least 10 times more viral plaques at 34°C than at 38.5°C [51].

Biochemical studies of the temperature sensitivity of respiratory viruses

For several decades virologists have found that maximum RNA transcription in influenza viruses occurs below normal body temperature. In 1977, Plotch & Krug [57] 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 [58, 59]. Ulmanen et al. [60] 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 & Rott [61] 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
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 [71]. They are frequently (but not always) found in the 5’-untranslated regions of mRNA, and can act in both directions – translation can be enabled at either high or low temperature [71]. Chursov et al. used bioinformatic techniques to identify pronounced differences between mRNA from cold-adapted TS influenza strains and the corresponding wild-type sequences [72]. 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 cold adaptation and temperature sensitivity [72]. Little secondary structure is predicted in influenza vRNA outside the untranslated terminal ends of the vRNA strands that form the “panhandle” structure [64]. However, the positive-sense RNA is predicted to have extensive secondary structure, which is conserved, in segments 1, 2, 5, 7 and 8 [64]. Since ordered RNA is intrinsically TS, and since single base changes usually have a small impact on the overall secondary structure of an RNA molecule, it is likely that temperature sensitivity can be fine-tuned by changes to untranslated regions of viral RNA. Changes to protein sequences may also be involved.

**Temperature sensitivity and the evolution of viral tropism**

We can speculate that temperature sensitivity might have profound effects on viral tropism. Most or many respiratory viruses possess temperature sensitivity, and we can suggest that a respiratory virus that loses its temperature sensitivity it might infect the gut or other internal organs. (Some method of limiting virulence other than temperature sensitivity might then be necessary to ensure the long-term survival of the virus.) Conversely, viruses from internal organs that develop temperature sensitivity could safely cause severe infections that would be limited to the upper respiratory tract, 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 would 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 presumably able to move between these two ecological niches. 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 all those diseases, and others, contract transmission from blisters coexists with aerosol transmission. More virulent human influenza strains can cause viremia [67, 68], and three children who were infected by pandemic H1N1 influenza in 2009 (“swine flu”) presented with
petechial rashes [69]. H3N2 influenza A caused hemorrhagic cystitis in 33 patients who were infected by the strain [70].

Conclusions and suggestions for experimental confirmation

There are many problems with conventional explanations of VRTI seasonality. The suggestion that VRTI seasonality arises from changes in the survival rate of viruses outside the body cannot explain why VRTIs including influenza can be transmitted in the tropics, especially during wet weather, but are almost absent in the summer months in temperate regions. Moreover, VRTIs are too sensitive to small temperature changes (and respond to them too quickly) for changes in virus survival to be the correct explanation. This problem is even greater for explanations based on changes in host susceptibility, and studies of antibody responses following vaccination also militate against this mechanism [22]. There is, however, ample evidence that many or most naturally-occurring respiratory viruses are ts, and that this explains VRTI seasonality. The view is compatible with observations that temperature changes (rather than absolute temperature levels) are correlated with VRTI epidemics, and with the virtual absence VRTIs in temperate regions during the summer in spite of their presence in the tropics throughout the year. Observations of the timing of VRTIs suggest that respiratory viruses can become dormant, a suggestion that is confirmed by the presence of VRTIs in polar communities after weeks or months of complete isolation, and by biochemical tests of asymptomatic individuals who shed influenza A and B. The view that temperature dips trigger VRTIs is compatible with data showing that chilling by wind, rain, standing still in cold weather and wearing inadequate clothing can cause VRTIs, and that outdoor exercise is protective. Biochemical data shows that many steps of replication are naturally ts in wild and laboratory viruses.

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 VRTI 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 viruses 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. Viruses 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 viruses into cells can be investigated by measuring the escape rate of pre-adsorbed virus from neutralization by antibody in temperature shift experiments. 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 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 hemagglutinin and neuraminidase from influenza virus 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 in 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 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 and RNA/protein 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 VRTIs) and compared to control groups who are kept warm. The number of VRTIs suffered by both groups can then be compared. This approach would make use of dormant viruses that the participants were already carrying by chance. These experiments can be extended by subjecting the volunteers to cyclical chilling, which may activate viruses that require more than a single temperature shift.

(For referees: an extended version of this article is available at http://vixra.org/abs/1310.0166)

**List of abbreviations used**

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

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

**Competing interests**

I declare that I have no competing interests.

**Author’s information**

I am one of the two founders and Directors of Douglas Instruments Ltd, a small UK company that manufactures automatic systems for protein crystallization. I worked with Professor David Blow in the 1990s, and have published 15 papers about protein crystallization that have together been cited over 700 times. A few years ago I began to think about respiratory viruses when a friend bet me that I couldn’t find biochemical evidence that chilling could trigger VRTIs. I started to write a short note, but everything fell into place so neatly that it grew until it became the current document. A longer version is available at viXra.org.

**References**


Figure 1. The global distribution and seasonality of VRTIs. Levels of VRTIs are indicated by brown shading, with dark brown indicating the highest rates of infection, while the yellow curve shows the path of vertical solar radiation. The strange distribution of VRTIs is shown, with more VRTIs in the tropics throughout the year than in temperate regions during the summer months [2, 3]. 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) [2]. Several lines of evidence suggest that personal chilling will increase the prevalence of VRTIs [4 - 12], and, since travel away from the tropical regions is associated with a decrease in temperature, it is likely that VRTIs spread more quickly from the tropics to the temperate regions (green arrows) than in the opposite direction (dotted red arrow). The degree to which viruses remain dormant during the summer in temperate regions (dotted purple arrow) is unknown.
Figure 2. Graph II from van Loghem’s report [4] on the epidemiology of VRTIs 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 (averaged over 7 days at weekly intervals) from five Dutch weather stations, with the temperature scale inverted (lowest temperatures at the top). Note that by far the highest rate of VRTIs was at the beginning of the study (September 1925), and that VRTIs in different regions are closely correlated with each other and with inverted temperature. These correlations are strongest in the first half of the cold season.
Figure 3. Chart 1 from Milam and Smillie’s 1929 study of colds on an isolated tropical island. The authors noted that outbreaks of colds often followed temperature drops, and were almost absent in the summer months. The red, green and blue bars indicate temperature fluctuations of 1.9, 1.7 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.) ©1931. This figure was originally published in the Journal of Experimental Medicine. 53:733-752. doi: 10.1084/jem.53.5.733.
Figure 4. The observed effect on temperature sensitivity of selection for increased and decreased viral activity. Selective pressures are indicated by dotted arrows, while the resulting changes to viral phenotype are indicated by solid arrows. The establishment of persistent viral infections of cell cultures generally requires reduced viral activity so that viral and cell replication can be in balance [26, 44]. The corresponding selective pressure is indicated by the dotted red arrow. Unexpectedly, reduced activity is often accompanied by the spontaneous appearance of temperature (heat) sensitivity. This is indicated by the solid red arrow. See the main text for examples [45 - 47]. The converse trend is equally surprising: when ts viruses are propagated in conditions that allow rapid growth (thereby selecting the most active mutants, dotted blue arrow), heat sensitivity is often lost (solid blue arrow) even when selection takes place at low temperatures (see main text [50, 51]).