Survival Characteristics of Airborne Human Coronavirus 229E

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SUMMARY

The survival of airborne human coronavirus 229E (HCV/229E) was studied under different conditions of temperature (20 ± 1 °C and 6 ± 1 °C) and low (30 ± 5%), medium (50 ± 5%) or high (80 ± 5%) relative humidities (RH). At 20 ± 1 °C, aerosolized HCV/229E was found to survive best at 50% RH with a half-life of 67·33 ± 8·24 h while at 30% RH the virus half-life was 26·76 ± 6·21 h. At 50% RH nearly 20% infectious virus was still detectable at 6 days. High RH at 20 ± 1 °C, on the other hand, was found to be the least favourable to the survival of aerosolized virus and under these conditions the virus half-life was only about 3 h; no virus could be detected after 24 h in aerosol. At 6 ± 1 °C, in either 50% or 30% RH conditions, the survival of HCV/229E was significantly enhanced, with the decay pattern essentially similar to that seen at 20 ± 1 °C. At low temperature and high RH (80%), however, the survival pattern was completely reversed, with the HCV/229E half-life increasing to 86·01 ± 5·28 h, nearly 30 times that found at 20 ± 1 °C and high RH. Although optimal survival at 6 °C still occurred at 50% RH, the pronounced stabilizing effect of low temperature on the survival of HCV/229E at high RH indicates that the role of the environment on the survival of viruses in air may be more complex and significant than previously thought.

Coronaviruses are large, enveloped viruses containing a single-stranded RNA genome of positive polarity. In animals, they are known to cause many diseases of economic importance which involve mainly the respiratory, digestive and/or nervous systems (Macnaughton & Davies, 1981; Riski & Hovi, 1980; Wege *et al.*, 1982). In man, they have been reported to be responsible for 16 to 20% of common colds (Robb & Bond, 1979) and they have also been isolated from cases of gastroenteritis (Caul & Clarke, 1975; Caul & Egglestone, 1982). While the mechanisms of spread of these agents in nature are not fully understood, air has been suspected as a vehicle for their dissemination (Robb & Bond, 1979). The present study was therefore designed to investigate the capacity of these viruses to survive in the airborne state. There also appears to be a seasonal pattern to the occurrence of epidemics of colds caused by coronaviruses. Such outbreaks have been reported to occur in the late winter or early spring months in North America (McIntosh *et al.*, 1970; Cavallaro & Monto, 1970); however, no marked seasonal pattern was found in Great Britain (Macnaughton, 1982). It was therefore of interest to determine the effects of both relative humidity (RH) and air temperature on the survival of airborne coronaviruses.

Virus stocks for use in the aerosol experiments were prepared in L132 cells, a continuous line of human lung cells, as described previously (Kennedy & Johnson-Lussenburg, 1975/76). Human coronavirus strain 229E (HCV/229E) was harvested after incubation at 33 °C for 36 h, separated into aliquots and stored at −80 °C. The titres of our batches varied from 3·0 × 10⁷ to 4·2 × 10⁷ p.f.u./ml. Poliovirus type 1 (Sabin vaccine strain) has been used as the standard reference virus in all our aerosol studies (Ijaz *et al.*, 1984, 1985 *a*).

Titrations of each of these viruses were performed by a standard plaque assay in monolayers of L132 cells in 75 cm^2 disposable culture flasks (Lux Scientific Corp.) as previously described (Kennedy & Johnson-Lussenburg, 1975/76). Suspensions containing both HCV/229E and poliovirus type 1, which were obtained when sampling aerosols of virus mixtures, required a differential plaque assay procedure. For the plaque assay of HCV/229E, specific neutralizing antiserum against poliovirus type 1 was added to one-half of the sample and incubated for 1 h before use in the standard procedure described above. HCV/229E plaques developed in 6 days at 33 °C. The other half of the sample was plaque-assayed without additional treatment. Plaques developing within 2 days at 37 °C represented poliovirus recovered in the sample. Control tests demonstrated that there was no interference in the plaquing ability of HCV/229E in the presence of poliovirus antiserum and conversely that the presence of HCV/229E did not interfere with poliovirus plaquing.

The methods used for the generation, storage and collection of the virus aerosols have been described in detail previously (Sattar *et al.*, 1984; Ijaz *et al.*, 1985*a*). The air temperature of the cabinet containing the drum was maintained either at 20 ± 1 °C or 6 ± 1 °C and the relative humidity (RH) was kept at either high (80 ± 5%), medium (50 ± 5%) or low (30 ± 5%) RH depending on the experiment. The procedures for achieving the desired test temperature and humidity level have been reported previously (Ijaz *et al.*, 1985*a, b*). The high and low levels of RH selected in this study correspond to the two extremes in indoor atmospheric conditions generally encountered in the temperate regions in the summer and winter seasons. The medium RH level was chosen to represent the inside atmosphere of climatically controlled buildings. The temperatures reflect the range encountered in late winter and early spring seasons (6 to 20 °C) or in indoor environments (20 °C).

After a 15 min period of stabilization, the first air sample was collected into an impinger containing 10 ml of collection fluid consisting of tryptose phosphate broth (TPB) with 0·001% antifoam A (Sigma) added. The volume of drum air sampled by operating the impinger for a 2 min period was 11·2 l (5·6 l/min). Depending on the duration of the test run, additional aerosol samples were collected at regular intervals post-aerosolization. In order not to deplete the aerosol by dilution during sampling, no more than five samples were taken over the entire time course of an experiment.

After collection, the fluid from the impinger was divided into two portions. One of these was used for estimating the amount of uranine, the physical tracer, and the other was used for virus plaque assay (Ijaz *et al.*, 1985*a b*). The extent of biological decay of the virus in the aerosol was calculated using the following formula: % virus survival = $[(D_0/D_t) \times (V_t/V_0)] \times 100$, where D_0 and D_t are the dye concentrations at times 0 and t h respectively. It should be noted that time 0 is taken as the end of the 15 min equilibration period in the drum. The difference between the input virus concentration and the 15 min sample concentration expressed as a percentage represents the initial loss due to the process of generating the virus cloud.

The survival of the aerosolized virus under various experimental conditions was plotted against time using an exponential model, % survival = $A \times \exp(-B \times time)$ and the coefficients A and B were estimated by least squares curve fitting. At least three experiments at each RH level and temperature were carried out and the half-lives of the viruses, expressed as the mean ± standard error, were calculated by regression analysis (Lawless, 1982).

Preliminary control experiments were carried out as described previously (Ijaz *et al.*, 1985*a, b*). It was found that neither TPB used as the suspending medium nor the additives uranine and antifoam A caused any change in the viability of the viruses used in these tests. No significant loss in virus infectivity was found as a result of the strong shearing forces encountered during the nebulization process. Finally, although it has been suggested that the passage of relatively dry air through the spray fluid during aerosolization could result in accelerated evaporation with subsequent increase in the tracer dye concentration (Beard & Easterday, 1965), we could detect no change in the uranine concentration before and after nebulization for 10 min. These results thus confirm the reliability of the method of quantifying the amount of infectious virus in aerosol both initially and throughout the experiment.

During the 15 min equilibration period following aerosol loading of the drum, there is always

Table 1. *Recovery of coronavirus and poliovirus after aerosolization and equilibration of the aerosol cloud*

Relative Humidity (%)	Recovery			
	Coronavirus 229E		Poliovirus type 1 (Sabin)†	
	20 ± 1 °C	6 ± 1 °C	20 ± 1 °C	6 ± °C
30 ± 5	87·0 ± 2·5	91·0 ± 2·6	0	ND‡
50 ± 5	90·9 ± 1·6	96·5 ± 3·0	0	ND
80 ± 5	55·0 ± 3·5	104·8 ± 5·1	90·0 ± 1·3	ND

* Values are the average of all experiments, minimum of three at each RH.
† Standard reference virus included as an aerosol biological decay control.
‡ ND, Not done.

an initial loss of virus. This loss is an important inherent characteristic of different viruses and is dependent on a number of factors; i.e. suspending medium, viral concentration, temperature and RH in the aerosol storage container. This loss was calculated as described previously (Ijaz *et al.*, 1985*a*, *b*) and the results recorded as the percentage of virus recovered (Table 1). At 20 ± 1 °C the initial loss of both HCV/229E and the standard reference poliovirus type 1 (Sabin) varied according to the RH. As reported previously poliovirus served as a reliable control and no changes were found in its aerosolization characteristics, i.e. no virus was recovered at medium and low RH and 100% was recovered at high RH. At 20 ± 1 °C, HCV/229E recovery was better at 30 ± 5% RH and 50 ± 5% RH (87% and 91% respectively) but at high RH, only 55% of the original input HCV/229E was detected (initial loss = 45%). On the other hand, at 6 ± 1 °C there was an overall enhancement of HCV/229E recovery at all three RH levels. This enhancement was most remarkable at high RH where, contrary to the results at 20 °C, there was essentially no initial loss (100% recovery). The reasons for this dramatic reversal in the effects of aerosolization at high RH seem to be due solely to temperature and are not immediately clear. They are, however, consistent with the survival results which are reported below.

The airborne stability of poliovirus has been well characterized under different environmental conditions (deJong, 1970; deJong *et al.*, 1973; Harper, 1961; Hemmes *et al.*, 1960), so that this virus could be used as a control for the performance of the aerosol equipment. To be absolutely sure that the test conditions were reliable poliovirus was included as an internal control, and the rate of biological decay of mixtures of HCV/229E and poliovirus were determined under identical experimental conditions. The viruses recovered from the mixed aerosols were quantified using the differential plaque assay described above. All the survival data reported here are based on a minimum of three experiments performed under each of the test conditions.

The survival characteristics of both poliovirus type 1 and HCV/229E in mixed aerosols at 20 ± 1 °C over a 24 h period at the three different RH levels are shown in Fig. 1. HCV/229E was found to survive best at medium RH and nearly as well at low RH, 75% and 65% virus survival respectively. High RH was found to be very deleterious to the survival of aerosolized HCV/229E. More than 50% of the infectious virus was lost after 3 h in aerosol and only 3% of infectious virus was recovered in the 24 h cloud sample. As expected, no poliovirus was recovered at low and medium RH whereas after 24 h at high RH nearly 30% of the infectious virus could be recovered. This result is in agreement with our previous findings (Ijaz *et al.*, 1985*a*).

The effect of relative humidity at 20 ± 1 °C on the survival of HCV/229E over a period of 72 h was then tested and the results are shown in Fig. 2. Since more than 50% of the virus in the cloud was still infectious at both low and medium RH, in order to determine how long infectious HCV/229E remained detectable in the drum air at the optimum 50 ± 5% RH, two separate long-term experiments were carried out. After 6 days in aerosol, at 20 ± 1 °C, nearly 20% of the virus was still detectable and the virus half-life was approximately 70 h. The half-lives of the
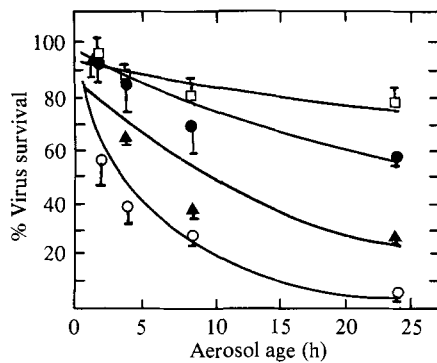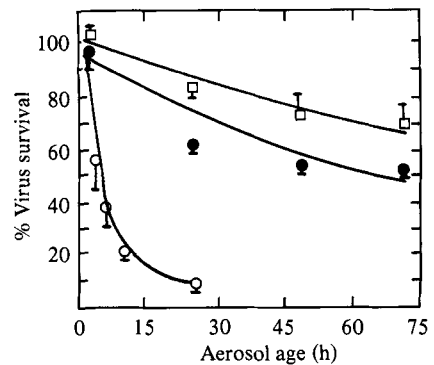
Fig. 1   Fig. 2

Fig. 1. Effect of relative humidity (RH) at 20 ± 1 °C on the survival of aerosols of mixtures of poliovirus type 1 (Sabin) [▲, high RH (80 ± 5%)] and HCV/229E [O, high RH (80 ± 5%); □, medium RH (50 ± 5%); ●, low RH (30 ± 5%)]. No poliovirus was recovered at medium and low RH. See text for calculation of regression curves. The standard deviation bars are depicted on one side only for clarity.

Fig. 2. Effect of relative humidity on the survival of aerosols of HCV/229E stored at 20 ± 1 °C for 72 h. O, High RH (80 ± 5%); □, medium RH (50 ± 5%), ●, low RH (30 ± 5%).

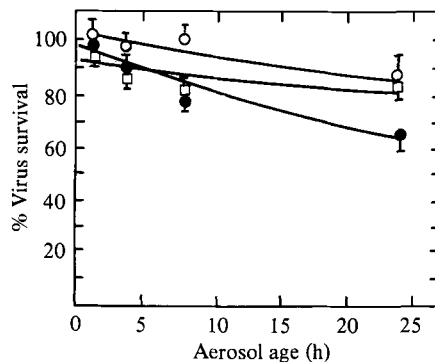Fig. 3. Effect of low temperature (6 ± 1 °C) on survival of airborne HCV/229E at different RH levels. O, High RH (80 ± 5%); □, medium RH (50 ± 5%); ●, low RH (30 ± 5%).

airborne viruses at each RH are summarized in Table 2. It can be seen that HCV/229E not only survived best at 50% RH, but its half-life was nearly 3 days longer than that of poliovirus type 1 at its optimal high RH. These results demonstrate clearly that, at 20 °C, coronavirus has great potential for airborne transmission.

The effect of low temperature on the survival of HCV/229E was tested at high, medium and low RH by holding the virus cloud at 6 ± 1 °C for 24 h. Virus half-lives were calculated by regression analysis of the survival data (Fig. 3) and the results are included in Table 2. Under experimental conditions of low temperature and medium or low RH, the pattern of biological decay was similar to that observed at 20 ± 1 °C; however, virus survival was significantly enhanced. There was an increase of around 53% in the virus half-life at medium and 30% at low RH. In marked contrast to the very poor survival of HCV/229E at 80 ± 5% RH and 20 ± 1 °C, the half-life of HCV/229E was calculated to have increased from 3 h to 86·01 ± 5·28 h at low temperature (6 ± 1 °C). Furthermore, instead of only 3% HCV/229E survival after 24 h in aerosol at 20 °C and high RH (Fig. 1), 86% of the virus survived after 24 h in aerosol, an increase

Table 2. *Half-life of aerosolized viruses under different conditions of relative humidity and temperature*

Virus	Temperature (°C)	Half-life (h)		
		High RH (80 ± 5%)	Mid RH (50 ± 5%)	Low RH (30 ± 5%)
Coronavirus 229E	20 ± 1	3·34 ± 0·16	67·33 ± 8·24	26·76 ± 6·21
	6 ± 1*	86·01 ± 5·28	102·53 ± 9·38	34·46 ± 3·21
Poliovirus type 1 (Sabin)	20 ± 1	9·07 ± 1·82	NR†	NR

* Half-life values were predicted by regression analysis of the 24 h results shown in Fig. 2.
† NR, No virus recovered.

of more than 80%. Although HCV/229E survival at 6 ± 1 °C was still best at medium RH, the long-term survival at high RH was predicted to differ by very little (Table 2).

Akers (1973) and deJong *et al.* (1973) both proposed a tentative rule that "lipid-containing viruses were generally more stable in aerosols than lipid-free viruses and that lipid-containing viruses are more labile in moist air (above 50% RH) than in dry air". Poliovirus, a lipid-free virus, which we have used as a standard reference clearly follows this rule. Disregarding temperature, this is also true of HCV/229E, a lipid-containing virus, which at its optimal RH was considerably more stable in aerosol than poliovirus; however, its behaviour at the different levels of RH and temperature did not follow this rule. At low temperature, virus survival at medium and low RH was markedly enhanced but, in addition, virus survival at high (80%) RH was the reverse of that seen at room temperature; under these conditions, HCV/229E was very stable. It would seem, therefore, that the survival of vertebrate viruses in the airborne state and under different environmental conditions cannot be predicted on the basis of viral structure and composition. Furthermore, the role of temperature on the airborne survival of HCV/229E appears to be more complex than anticipated. It is suggested that under conditions of high humidity, the fluidity of the lipid-containing envelope is stabilized at low temperature, thus protecting the virion; however, further studies are needed to explain these phenomena.

It is premature at this stage to draw any conclusions regarding the epidemiological relevance of these findings. However, it is tempting to speculate on an environmental role in the seasonal dissemination of this human respiratory virus. Questions relating to interepidemic reservoirs as well as the transmission of infection need also to be addressed.

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