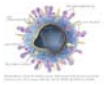
PERSISTENCE OF THE INFLUENZA A(H1N1) PANDEMIC VIRUS IN WATER AND ON NON-POROUS SURFACE

Virion structure



Enveloped virus
Negative, single-stranded
and segmented RNA genome

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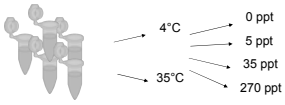
Introduction

The threat of pandemic Highly Pathogenic Avian Influenza Virus (HPAIV) H5N1 and the recent outbreak caused by the novel A(H1N1) influenza virus generated a renewed interest in the study of influenza virus transmission. Aerosols, large droplets and direct contact of the nasal mucosa by contaminated hands probably all contribute to a certain extent to the transmission of influenza viruses. Virus survival in different environmental settings is a key element for control measures and decisions introduced by national health authorities and policy makers. We studied the survival of the H1N1 pandemic (H1N1pdm) virus which emerged in 2009 and the seasonal A/New Caledonia/20/99 virus strain, in water and on non porous surface.

Methods to study virus survival

▪ in liquid medium



Virus preparation diluted 1:10 in distilled water. Salinity was adjusted using sodium chloride. Saline concentrations were selected to represent natural saline environments. The values of 0, 5, 35 and 270 ppt respectively correspond to the average levels of salinity encountered in river freshwaters, the Baltic Sea, oceans and the Dead Sea or a number of food processing operations.

▪ on smooth surface

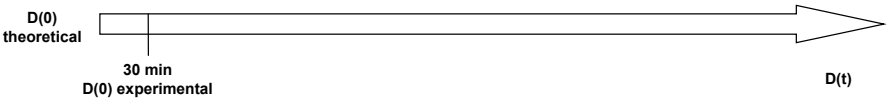
50 µL of initial viral suspension were put on dry-cleaned sterile watch glasses, which were placed into a sealed box containing silica gel with moisture indicator.

Infectivity assays

Endpoint titration
Tissue Culture Infectious Dose 50
(TCID50)

Genomic RNA quantification

Quantitative real-time RT-PCR
targeting the M gene
(amplicon from position 32 to 186).



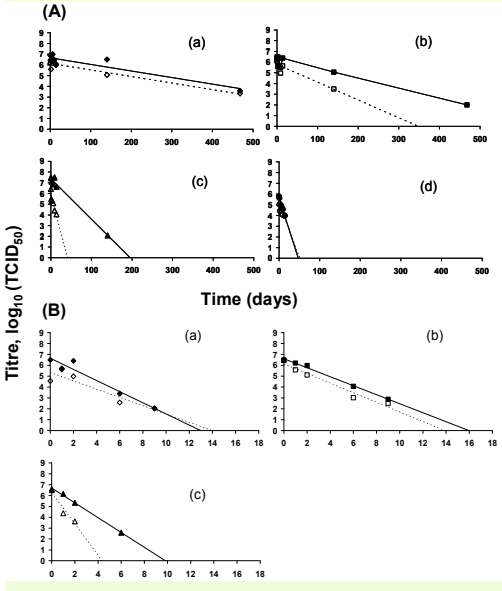
"D0 theoretical" corresponded to the tenth of the titre of the viral stock. For trials in water, "*D0 experimental*" was the titre obtained after the virus suspension was diluted and left under different conditions during 30 minutes. For trials on smooth surfaces, two experimental D0s were calculated: "*D0 wet*" calculated after viral suspension was inoculated onto the surface and left 30 minutes under different temperatures and "*D0 dry*" obtained when the viral suspension was dried.

Results and discussion

***** From experimental data (Figure 1A & B), times of persistence were calculated by linear regression (Table 1).
- At low temperature, the pandemic and the seasonal H1N1 strains were very stable even with a high level of salinity. At 4°C, viruses remained infectious at least 986-1081 days and 50 days at 0 and 270 parts per thousand (ppt) of NaCl respectively. A marked difference was observed between the A/NewCaledonia/20/99 virus and the H1N1pdm virus in the following conditions: at 5 ppt of salt (348 versus 698 days respectively) and at 35 ppt (42 versus 197 days respectively). This suggests that the H1N1pdm strain was more stable than the seasonal H1N1 virus in this liquid environment (Table 1).
- Increasing environmental temperature to 35°C had a negative effect on the survival of both strains (Figure 1B). Infectious H1N1pdm and seasonal H1N1 virions were present at 14 days at 0 ppt. In contrast, at 270 ppt, the time required for a total loss of infectivity was reached in about two days (Table 1). The pH of water was checked over time and found to be very stable at 6.9 for each condition.

***** On smooth surface, virus survival was evaluated after the virus spotted on the surface was dried. However, desiccation had a strong negative effect on viruses survival at 25 and 35°C (the loss of titre ranged from about 3 to 4 $\log_{10}$) (Figure 2A). Relative humidity values measured at 4, 25 and 35°C were 28, 16 and 12% respectively. The duration of the persistence of both viruses was very similar whatever the temperature (Figure 2B). After 70, 20 and 7 days no infectious particle was detectable anymore at 4, 25 and 35°C respectively (Table 2).

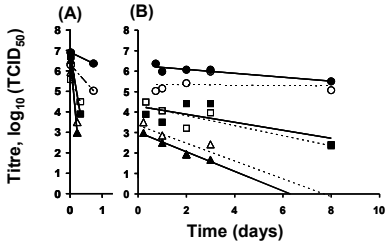
***** In water and on smooth surface, over time, there was no decrease in genomic RNA concentration as estimated by quantitative real-time RT-PCR (data not shown). The whole HA1 region of the HA gene was amplified by endpoint RT-PCR to check whether RNA was degraded after 30 minutes. Taken together the drop in $TCID_{50}$, the stable quantity of viral RNA and the presence of long fragments of RNA (after first 30 min) suggest that external viral structures in direct contact with the environment were involved in virus loss of infectivity.



Strain	Temperature	Salinity (ppt)	Persistence time (days)	Confidence interval (days)
A/Paris/2590/2009 (H1N1)pdm	4°C	0	1081	[637 ; 1125]
		5	698	[425 ; 643]
		35	197	[135 ; 174]
		270	47	[24 ; 64]
	35°C	0	14	[8 ; 15]
		5	16	[10 ; 15]
		35	10	[6,7 ; 8,2]
		270	< 1,8	
A/NewCaledonia/20/1999 (H1N1)	4°C	0	986	[585 ; 935]
		5	348	[190 ; 362]
		35	42	[23 ; 45]
		270	52	[23 ; 102]
	35°C	0	14	[7,3 ; 15]
		5	14	[8,7 ; 12]
		35	4.4	[2,5 ; 4,7]
		270	< 2,0	

▲ **Table 1:** Persistence time estimated by linear regression and calculated from data generated in liquid medium.

◄ **Figure 1:** Viral persistence of A/Paris/2590/2009 (H1N1)pdm (—) and A/New Caledonia/20/99 (····) in water at 4°C **(A)** and 35°C **(B)**. Linear regression for persistence at 0 ppt (a), 5 ppt (b), 35 ppt (c) and 270 ppt (d) are represented.



▲ **Figure 2:** Viral persistence of A/Paris/2590/2009 (H1N1)pdm (—) and A/New Caledonia/20/99 (····) on smooth surface at 4 (●;○), 25 (■;□) and 35°C (▲;Δ). Linear regression for persistence during drying **(A)** and after drying **(B)** are represented.

Strain	Temperature	Persistence time (days)	Confidence interval (days)
A/Paris/2590/2009 (H1N1)pdm	4°C	66	[33 ; 504]
	25°C	22	[13; 145]
	35°C	7	[5 ; 16]
A/NewCaledonia/20/1999 (H1N1)	4°C	63	[25 ; 80]
	25°C	19	[12 ; 91]
	35°C	6	[4 ; 17]

▲ **Table 2:** Persistence time estimated by linear regression and calculated from data generated on smooth surface.

Conclusion

Our results showed that the H1N1 viruses had the ability to persist in water and on glass surface for extended periods of time, even at 35°C. As described previously, low temperature increased virus survival. At low salinity levels (0 and 5 ppt), maximum survival times varied between 698-1081 and 348-986 days for pandemic and seasonal H1N1 viruses respectively. In some experimental conditions, H1N1pdm virus remained infectious for longer period of time than its seasonal counterpart. Increasing environmental temperature and salinity levels had a strong negative effect on the survival of both viruses which retained their infectivity no more than 2 days at 35°C and 270 ppt of salt. Influenza viruses are thought to be transmitted from individual to individual through direct or indirect contact with respiratory secretions and when touching surfaces contaminated with influenza viruses. Although the initial drying of the viral suspension had a strong negative effect on viral infectivity, once dried both strains unexpectedly remained infectious for long periods of time: at least 6 days at 35°C and up to 66 days at 4°C. Persistence observed in some experimental conditions has to be considered for Influenza viruses ecology. The environment could be a source of transmission and a reservoir. Fortunately, data obtained at 270 ppt confirmed that food processing operations are able to alter the viability of these Influenza viruses.

RIVERS & FONDATION BNP PARIBAS / BNP PARIBAS

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