Avian *Influenza* Virus inactivation with lime

Nathalie DEBOOSERE, Virginie ALEXANDRE and Michèle VIALETTE

INSTITUT PASTEUR DE LILLE - Microbiological Safety Unit, 1 rue du Pr Calmette, BP 245, 59019 LILLE Cedex, FRANCE

michele.vialette@pasteur-lille.fr

INTRODUCTION

The recent outbreaks of Avian *Influenza* (AI) worldwide have highlighted the difficulties in controlling this disease. These difficulties can be linked to the easy transmission of the disease, as well as to the resistance in the environment of the AI Viruses (AIVs).

There are many ways for inactivating viruses. These include physical and chemical methods. In this last case, the efficiency of liming in inactivating different nude or enveloped virus has been demonstrated. But no information on the AIVs inactivation by lime was available in literature yet, and even its inactivation kinetic at the laboratory scale wasn't established.

In this way, the objective of the present work was to **evaluate chemical inactivation of H5N1 virus by lime treatment.**

MATERIAL AND METHODS

Virus and cells

The experiments were carried out on an H5N1 strain (A/Cambodia/408008/2005). The MDCK cells (Madin Darby Canine Kidney) were used for the viral propagation to prepare inocula and to measure virus infectivity.

Endpoint titration procedure

Infectivity of influenza A virus subtype H5N1 was quantified using a microtiter endpoint titration, expressed in units of tissue-culture infective dose per millilitre ($TCID_{50}$/ml), Endpoints were recorded as 100% monolayer destruction with $TCID_{50}$ calculated as described in the European standard NF EN 14476. The dilution producing more than 50% monolayer destruction was used to determine the virus titre of the sample assayed. Examination for cytopathic effect was performed with light microscopy (Figure 1).

Experimental procedures

Chemical inactivation experiments were performed at a pH value of about 12.5 using a 0.5% (w/v) suspension of calcium hydroxide in sterile distilled water. The 0.5% $Ca(OH)_2$ suspension, pre-cooled to 4°C, was contaminated with virus and samples were removed at required time intervals over 30 minutes. In order to stop the $Ca(OH)_2$ disinfecting activity, the samples were immediately neutralized by a 1:5 dilution in serum-free culture medium (MEM) containing 5% of citric acid 0.17 M /sodium citrate 0.83 M.

The assay was conducted in duplicate.

RESULTS

The virus inactivation kinetics obtained in the study are presented in Figure 2. The temperature monitored throughout chemical treatments is also indicated.

The control values (assay without lime) have shown that neither the lime-neutralization procedure nor the duration of the experiment significantly decreased the viral titre (Table 1).

Results of infectivity assays (Table 1) showed that a 0.5% $Ca(OH)_2$ suspension was effective in inactivating H5N1 virus by more than 4 log_{10}-units within 5 minutes at 4°C.

Figure 2: Temperatures and Inactivation curves of H5N1 virus

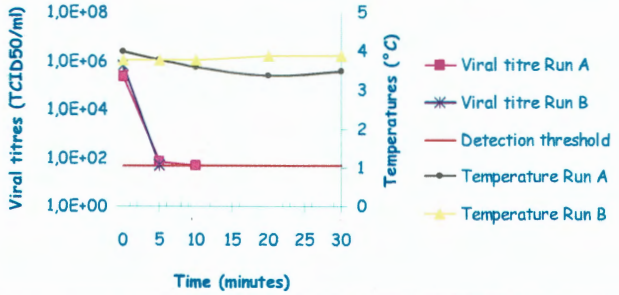
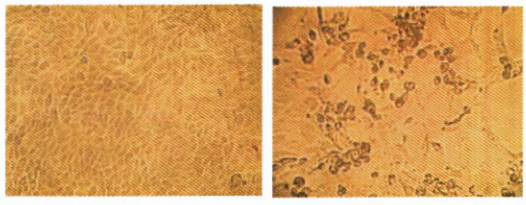


Figure 1: Cytopathic effect of H5N1 influenza A virus on MDCK cells



a/ Mock control b/ Cytopathic effect

Table 1: Inactivation effect of lime suspension on H5N1 virus infectivity

Sample		Infectious titre ($TCID_{50}$/ml)	Reduction Factor Evaluation
Positive controls			
Virus stock spiked in serum-free culture medium (MEM) kept at 4°C and sampled:			Virus stock control
- immediately after the spiking	Run A	$3.53\ 10^6$ (a_1)	
	Run B	$4.32\ 10^6$ (a_2)	
- after 30 minutes			Effect on virus in culture medium of the duration of the experiment
	Run A	$5.27\ 10^6$ (b_1)	$\log(a_1) - \log(b_1) \approx 0$
	Run B	$2.37\ 10^6$ (b_2)	$\log(a_2) - \log(b_2) = 0.26$
Virus stock spiked in lime suspension, neutralized and 5-fold diluted in MEM, kept at ambient room and sampled :			Effect on virus of conditions used to neutralize and dilute lime solution
- immediately after the spiking	Run A	$3.52\ 10^6$ (c_1)	$\log(a_1) - \log(c_1) \approx 0$
	Run B	$5.81\ 10^6$ (c_2)	$\log(a_2) - \log(c_2) \approx 0$
			Effect on virus in neutralized and diluted lime solution of the duration of the experiment
- after 1 hour	Run A	$4.32\ 10^6$ (d_1)	$\log(c_1) - \log(d_1) \approx 0$
	Run B	$4.32\ 10^6$ (d_2)	$\log(c_2) - \log(d_2) = 0.13$
Experiments			
Virus stock spiked in lime suspension kept at 4°C and sampled:			Effect on virus of lime treatment
- immediately after the spiking	Run A	$2.59\ 10^5$ (e_1)	$\log(c_1) - \log(e_1) = 1.13$
	Run B	$7.06\ 10^5$ (e_2)	$\log(c_2) - \log(e_2) = 0.92$
- after 5 minutes	Run A	$8.18\ 10^1$ (f_1)	$\log(c_1) - \log(f_1) = 4.63$
	Run B	$< 5.46\ 10^1$ (f_2)	$\log(c_2) - \log(f_2) > 5.03$
- after 10 minutes	Run A	$< 5.46\ 10^1$ (g_1)	$\log(c_1) - \log(g_1) > 4.81$
	Run B	$< 5.46\ 10^1$ (g_2)	$\log(c_2) - \log(g_2) > 5.03$
- after 20 minutes	Run A	$< 5.46\ 10^1$ (h_1)	$\log(c_1) - \log(h_1) > 4.81$
	Run B	$< 5.46\ 10^1$ (h_2)	$\log(c_2) - \log(h_2) > 5.03$
- after 30 minutes	Run A	$< 5.46\ 10^1$ (i_1)	$\log(c_1) - \log(i_1) > 4.81$
	Run B	$< 5.46\ 10^1$ (i_2)	$\log(c_2) - \log(i_2) > 5.03$

CONCLUSION

The present study shows that the **H5N1 virus is effectively and rapidly inactivated by lime.** Lime may thus be recommended as a way of controlling AIVs in environment in case of epizootic outbreaks. Practical guidelines have been defined in Germany since 1997. This could be useful to prevent and control H5N1 *Influenza* A virus' outbreaks.

In environment, it is well known that interactions exists between environmental factors. For example, temperature, matrix characteristics, pH should have an effect on the chemical inactivation of virus. In this way, a study of contaminated solid and liquid manures would be interesting in order to investigate further potential protective effects on chemical inactivation by lime and to obtain knowledge on virus behaviour in this kind of matrix.