

Avian Influenza Virus inactivation with lime

N. DEBOOSERE, V. ALEXANDRE and M. VIALETTE*

* Institut Pasteur de LILLE – Microbiological Safety Unit – 1 rue du Pr Calmette – BP245-59000 LILLE – FRANCE

The recent outbreaks of Avian Influenza (AI) worldwide have highlighted the difficulties in controlling this disease¹. These difficulties can be linked to the easy transmission of the disease, as well as to the resistance in the environment of the AIViruses (AIV).

The efficiency of liming in inactivating in a short term different nude and enveloped² viruses has been demonstrated and the lime has been successfully used to prevent and control outbreaks of diseases to be notified, for instance, the U.K and Irish Foot and Mouth disease epidemic in 2001. Besides, since 1997, Germany includes lime treatment in its guideline for disinfection in case of epizootic. Up to now no information on the AIVs inactivation by lime was available in the literature and even its inactivation kinetic at the laboratory scale wasn't established. Nevertheless, lime has been successfully used to avoid the extension of the H5N1 *Influenza* virus disease in Turkey and more recently in Germany.

The objective of this investigation was to evaluate chemical inactivation of H5N1 by lime treatment.

The experiments were carried out on a 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.

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. This disinfectant concentration, significantly below the ones of the lime suspension commonly used in veterinary hygiene (higher than 2 % Ca(OH)₂ (w/w)), has been chosen to permit an immediate inactivation of the disinfectant, by the neutralisation procedure described, hereafter, in the experimental protocol.

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.

Controls have shown that neither the lime-neutralisation procedure nor the duration of the experiment sensitively decrease the viral titre. This validates that a 0.5% Ca(OH)₂ suspension was effective to inactivate more than 4 log₁₀-units H5N1 within 5 minutes at 4°C.

The definition of practical guidelines on the way to disinfect with lime the animal house walls, soil, litter, manure and water that could have been or be contaminated, will be of great help to prevent and control H5N1 *Influenza* A virus' outbreaks. So these results are very useful and would have to allow recommending the use of lime to control outbreaks of H5N1 *Influenza* virus in case of epizootic. Nevertheless, a study of contaminated solid and liquid manures would be interesting in order to investigate further potential protective effects on chemical inactivation and to obtain knowledge of virus behaviour in this kind of matrix. Virus inactivation in pig slurry by Ca(OH)₂ was effectively shown as not a pH effect alone, but was accelerated by increasing temperature and was dependent on the characteristics of the infected matrix².

¹ De Benedictis P., Beato M.S., and Capua I. *Inactivation of Avian Influenza Viruses by Chemical Agents and Physical Conditions: A Review*, Journal compilation © 2007 Blackwell Verlag, Berlin Zoonoses Public Health. 54 (2007) 51-68.

² Turner C. and S.M. Williams, *Laboratory-scale inactivation of African swine fever virus and swine vesicular disease virus in pig slurry*, Journal of Applied Microbiology 1999, 87, 148-157