Susceptibility of Avian Influenza virus subtype H5N1 to Stalosan® F

By

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Objective

To evaluate the virucidal efficacy of commercially available disinfectant manufactured by Stormøllen and distributed by A.I.P. Co., Ltd. against avian influenza virus subtype H5N1.

Materials and methods

Material

Material tested in this experiment was Stalosan®F,

Virus

The A/chicken/Thailand/1/04 (H5N1) highly pathogenic avian influenzavirus was propagating in 9-12 day old chick embryonated eggs. The virus was harvested from allantoic sac when the inoculated egg died. Allantoic fluid containing influenzavirus was measured by titration in embryonated eggs. The virus was re-passaging in embryonated eggs until the titer reached 10^9 mean embryo lethal dose (ELD_{50})/ml then aliquoted and stored at -80°C until use.

Test method

Toxicity test

Stalosan®F has been tested for its toxicity on 9-12 day embryonated egg before testing for the virucidal efficacy to avian influenzavirus (H5N1) to ensure that the products will not harm embryos or affect the virus titration method. The toxicity test was performed by egg inoculation using 0.125, 0.25 and 0.5 gm/ml of Stalosan®F. The product was diluted with minimum essential medium (MEM) then inoculated 100 µl of each dilution to 5 embryonated eggs. The eggs were candled every day for 7 days to detect dead embryos.

Virucidal test

The stock virus ($10^{8.3}ELD_{50}$/ml) 0.2 ml was placed and spread on sterile petridishes and allowed drying in biosafety cabinet at room temperature (25°C) for 30 minutes. Stalosan®F was added to the plate 0.5 g/cm^2 (equivalent to 50 g/m^2 as recommended by the manufacturer). The product was allowed to remain in contact in dry condition of the virus for 8 minutes, 60 minutes, and 24 hours respectively. After those exposure times, the Stalosan®F powder and virus was resuspended using 2.0 ml of MEM tissue culture medium then serially diluted with MEM to make dilution from 10^0 to 10^{-10}. Each dilution of the mixture was inoculated into the allantoic cavity of 9-12 day embryonated egg. The eggs were candled daily 7 days for detect dead embryo. After 7 day incubation period, the allantoic fluid was collected and hemagglutination test (OIE, 1996) was performed to detect the remaining infectious virus. Virus titers after neutralization by Stalosan®F were calculated by Reed and Muench calculation method and compared to the control virus at each contact time.

Note

All test procedures were performed in Biosafety Level 3 Laboratory at the Veterinary Diagnostic Laboratory, Chulalongkorn University.

Result

Table 1 Numbers of dead embryos during 7 days observation in the toxicity test of Stalosan®F

Concentration (g/ml)	1 dpi	2 dpi	3 dpi	4 dpi	5 dpi	6 dpi	7 dpi
0.125	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.25	0/5	0/5	0/5	0/5	0/5	0/5	0/5
0.5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

Table 2 Numbers of dead embryos during 7 days of observation in the virus control group at 0 minutes

dilution	1 dpi	2 dpi	3 dpi	4 dpi	5 dpi	6 dpi	7 dpi
10^-5	0/5	5/5	-	-	-	-	-
10^-6	0/5	5/5	-	-	-	-	-
10^-7	0/5	2/5	1/5	0/5	0/5	0/5	0/5
10^-8	0/5	1/5	1/5	0/5	0/5	0/5	0/5
10^-9	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10^-10	0/5	0/5	0/5	0/5	0/5	0/5	0/5

Table 3 Number of dead embryos during 7 days of observation in the virucidal test of Stalosan®F at 8 minutes contact time

dilution	1 dpi	2 dpi	3 dpi	4 dpi	5 dpi	6 dpi	7 dpi
10^0	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10^-1	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10^-2	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10^-3	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10^-4	0/5	0/5	0/5	0/5	0/5	0/5	0/5

Table 4 Numbers of dead embryos during 7 days of observation in the virus control group at 8 minutes contact time

dilution	1 dpi	2 dpi	3 dpi	4 dpi	5 dpi	6 dpi	7 dpi
10^-5	0/5	5/5	-	-	-	-	-
10^-6	0/5	5/5	-	-	-	-	-
10^-7	0/5	2/5	1/5	0/5	0/5	0/5	0/5
10^-8	0/5	1/5	1/5	0/5	0/5	0/5	0/5
10^-9	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10^-10	0/5	0/5	0/5	0/5	0/5	0/5	0/5

Table 5 Numbers of dead embryos during 7 days of observation in the virucidal test of Stalosan®F at 60 minutes contact time

dilution	1 dpi	2 dpi	3 dpi	4 dpi	5 dpi	6 dpi	7 dpi
10^0	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10^-1	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10^-2	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10^-3	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10^-4	0/5	0/5	0/5	0/5	0/5	0/5	0/5

Table 6 Numbers of dead embryos during 7 days of observation in the virus control group at 60 minutes contact time

dilution	1 dpi	2 dpi	3 dpi	4 dpi	5 dpi	6 dpi	7 dpi
10^-5	0/5	5/5	-	-	-	-	-
10^-6	0/5	5/5	-	-	-	-	-
10^-7	0/5	1/5	1/5	0/5	0/5	0/5	0/5
10^-8	0/5	1/5	0/5	0/5	0/5	0/5	0/5
10^-9	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10^-10	0/5	0/5	0/5	0/5	0/5	0/5	0/5

Table 7 Numbers of dead embryos during 7 days of observation in the virucidal test of Stalosan®F at contact time 24 hours contact time

dilution	1 dpi	2 dpi	3 dpi	4 dpi	5 dpi	6 dpi	7 dpi
10^0	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10^-1	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10^-2	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10^-3	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10^-4	0/5	0/5	0/5	0/5	0/5	0/5	0/5

Table 8 Numbers of dead embryos during 7 days of observation in the virus control group at 24 hours contact time

dilution	1 dpi	2 dpi	3 dpi	4 dpi	5 dpi	6 dpi	7 dpi
10^-2	0/5	5/5	-	-	-	-	-
10^-3	0/5	3/5	1/5	0/5	0/5	0/5	0/5
10^-4	0/5	2/5	0/5	0/5	0/5	0/5	0/5
10^-5	0/5	1/5	1/5	0/5	0/5	0/5	0/5
10^-6	0/5	0/5	0/5	0/5	0/5	0/5	0/5

Table 9 Percent Virus Reduction by Stalosan®F

Contact times	Virus Titer (log ELD_{50}/ml)		% Reduction
	Treated groups	Virus control groups	
0 minutes	-	8.31	-
8 minutes	0	8.31	100
60 minutes	0	7.57	100
24 hours	0	4.58	100

According to the result, Stalosan®F did not showed lethal effect to the chick embryos. The reduction of avian influenzavirus (H5N1) titers by Stalosan®F showed in table 9 indicated a very high virucidal efficiency of the product. The virus was completely inactivated by Stalosan®F at the recommend dose 50 g/m^2 within 8 minutes.

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