鳥取大学
Tottori University

Test Report

This is a report concerning tests performed by The Department of Veterinary Public Health, Faculty of Agriculture, Tottori University.

National University Corporation Tottori University

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Faculty of Agriculture, Tottori University
Department of Veterinary Public Health

Test 1: Antiviral effect of processed dolomite

This test was performed to determine whether or not processed dolomite can inactivate representative respiratory viruses.

Materials

Test suspension:

Test suspension was prepared by dispersing 6% of processed dolomite powder and 2.5% urethane into deionized water.

Viruses:

The viruses used in this test were as follows:

Avian infectious bronchitis virus strain Beaudette 42 *(family Coronaviridae)*

Newcastle disease virus strain La Sota *(family Paramyxovoridae)*

Human Influenza virus strain A/Aichi/2/68 (H3N2) *(family Orthomyxoviridae)*

Avian Influenza virus strain A/whistling swan/Shimane/499/83 (H5N3) *(family Orthomyxoviridae)*

These strains had been grown in the allantoic cavity of 10-day-old embryonated hen's eggs for 3 days at 37℃ prior to being tested.

Hen's eggs:

Ten-day-old embryonated SPF hen's eggs were used for virus titration.

Methods

1. The antiviral activity of the processed dolomite was tested as follows: 0.9 ml of each viral suspension was poured into two small tubes. Then 0.1 ml of the dolomite suspension being tested was put into one of these small tubes and the same amount of phosphate buffered saline solution (PBS) (pH7.2) was put into the other and shaken well. The final concentration of the processed dolomite in the mixture was 0.6%. These tubes were put into chilled water at 4℃ and shaken carefully for 10 minutes.

   Then, the effect of the processed dolomite was determined by diluting 10-fold with PBS and the virus titration was then performed. That is, 0.09ml of the mixture of viral medium and the test suspension or PBS was poured into a small tube containing 0.81ml of PBS to dilute the mixture 10-fold. This procedure was repeated nine times to give a series of ten 10-fold ditutions. Then 0.2ml of each dilution was inoculated into the allantoic cavity of three 10-day-old embryonated SPF hen's eggs. These eggs were incubated for 3 days (6 days for infectious bronchitis virus) at 37℃. The incubated eggs were then chilled in a refrigerator overnight. On the following day, the allantoic fluid was collected from each egg except

the egg inoculated with infectious bronchitis virus and was tested for hemagglutination activity by mixing with a 0.5% chicken erythrocyte suspension (1). In the case of infectious bronchitis virus, characteristic morphology of chicken embryo curling was recorded in order to determine the virus titer.

The virus titer was determined by the Reed and Muench method (2). The antiviral effect was evaluated based on the virus titer.

2. The antiviral effect of processed dolomite sprayed onto gauze was tested as follows: The testing suspension was sprayed 15 times on gauze (a stack of ten sheets, 15 mm x 15 mm). Then the gauze was placed in a plastic bag (100 mm x 100 mm) and 0.6 ml of the virus suspension was dropped onto the gauze. After 15 minutes at 4°C, 0.09ml of this virus suspension was removed and added to 0.81ml of PBS. Afterwards, the virus titer of each solution was determined by the methods described above.

Results

1. As shown in Table 1, the infectivity titer of all strains of infectious bronchitis, influenza and Newcastle disease viruses fell by at least 10,000 fold following contact with 0.6% processed dolomite within 10 minutes.

Table 1. Antiviral effect of processed dolomite

Virus	Virus titer (EID_{50}/0.2ml)		
	Pre-testing	Control (PBS)	Treated with Dolomite
Avian infectious bronchitis virus	$10^{8.5}$	$10^{8.3}$	$<10^{1.5}$
Human Influenza virus (H3N2)	$10^{7.8}$	$10^{8.8}$	$10^{2.8}$
Avian Influenza virus (H5N3)	$10^{8.3}$	$10^{7.8}$	$10^{3.0}$
Newcastle disease virus	$10^{7.5}$	$10^{7.8}$	$10^{2.5}$

2. As is shown in Table 2, when placed in gauze sprayed with testing suspension for 15 minutes, the infectivity titer of avian infectious bronchitis virus and human influenza virus fell by at least 10,000 fold.

Table 2. Antiviral effect of processed dolomite sprayed on gauze

Virus	Virus titer (EID_{50}/0.2ml)		
	Pre-testing	Control (PBS)	Treated with Dolomite
Avian infectious bronchitis virus	$10^{6.5}$	$10^{6.3}$	$<10^{1.5}$
Human Influenza virus (H3N2)	$>10^{8.5}$	$10^{8.3}$	$10^{3.5}$

References

(1) Sever, J. L. (1962) Application of microtechnique to viral serological investigations. J. Immunol. 88: 320-329.

(2) Reed, L. J. and Muench, H. 1938. A simple method for estimating fifty percent endpoints. *Am. J. Hyg.* **27**: 493-497.

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Department of Veterinary Public Health

Test 2: Antiviral effect of non-woven textile containing processed dolomite

This test was performed to determine whether or not non-woven textile containing processed dolomite can inactivate influenza viruses.

Materials

Test samples: Non-woven textile containing processed dolomite

Viruses: The viruses used in this test were as follows: Avian Influenza virus strain A/tufted duck/shimane/510/02 (H1N1), A/duck/Hong kong/278/76 (H2N2), and A/whistling swan/Shimane/277/01 (H3N9). These strains had been grown as described above.

Hen's eggs: Ten-day-old embryonated SPF hen's eggs were used for virus titration.

Methods

In a polyethylene bag, 15 pieces of non-woven textiles that had been cut into 1.5 cm squares were stacked. Then 0.6 ml of the virus suspension was added and the fluid allowed to soak into the textile. After 10 minutes incubation at 4°C, 0.09ml of this viral suspension was removed and mixed with 0.81ml of PBS in a small tube, to dilute the viral medium 1:10. This operation was repeated nine times to give a series of ten 10-fold dilutions. Then 0.2ml of each dilution was inoculated into allantoic cavity of three 10-day-old embryonated hen's eggs. The eggs were incubated for 3 days at 37℃. The inoculated eggs were chilled in a refrigerator overnight. On the following day, allantoic fluid was collected from each egg and mixed with a 0.5% chicken erythrocyte suspension to observe hemagglutinating activity (1).

The antiviral effect was evaluated by determining the virus titer, according to the Reed and Muench method (2).

Results

As is shown in Table 3, non-woven textile containing processed dolomite can reduce the infectivity titer of avian influenza viruses more than 100,000 fold.

Table 3. Antiviral effect of non-woven textile containing processed dolomite

Virus	Virus titer (EID_{50}/0.2ml)		
	Pre-exam	Control (Non-woven textile without processed dolomite)	Non-woven textile with processed dolomite
A/tufted duck/Shimane/510/02(H1N1)	$10^{6.25}$	$10^{5.50}$	$<10^{0.50}$
A/duck/Hong Kong/278/76(H2N2)	$10^{6.50}$	$10^{5.25}$	$<10^{0.50}$
A/whistling swan/Shimane/277/01(H3N9)	$10^{6.50}$	$10^{6.25}$	$<10^{0.50}$

References

(1) Sever, J. L. (1962) Application of microtechnique to viral serological investigations. J. Immunol. 88: 320-329.

(2) Reed, L. J. and Muench, H. 1938. A simple method for estimating fifty percent endpoints. Am. J. Hyg. 27: 493-497.

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These tests were performed by the Department of Veterinary Public Health, Faculty of Agriculture, Tottori University using materials received from Mochigase Electric Co., Ltd. (now Mochigase Co., Ltd.).

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