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Efficacy and Stability of Ceramic Powder to Inactivate Avian Influenza Virus

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***Abstract*—**This experiment aims to demonstrate the efficacy of ceramic powder derived from various sources to inactivate avian influenza virus and its possibility to use in the environment. The ceramics used in the present experiment were derived from chicken feces (CF), scallop shell (SS), polyvinyl chloride (PVC) and soybean (SB). All ceramics were mixed with low pathogenic AIV (LPAIV) H7N1, and then kept at room temperature. The recovered virus was titrated onto Madin-Darby canine kidney (MDCK) cells. All ceramics were assessed the inactivation stability in the environment by keeping under sunlight and under wet-dry condition until reached 7 week or 7 resuspension times respectively. The results indicate that all ceramics have excellent efficacy to inactivate LPAIV. This efficacy can be maintained under the simulated condition. The ceramics are expected to be the good materials for application in the biosecurity system at farms.

***Keywords*—**Avian Influenza, Ceramics, Efficacy, Stability.

I. Introduction

THE outbreaks of highly pathogenic avian influenza (HPAI) by AIV subtype H5N1 in Asia is causing the serious problems to the poultry production and concerning to human health. This disease spread widely in Asia, Europe, and Africa [1], [2]. It effects to the global health and economic losing with poultry production system.

The transmission of this disease can be passed through the live birds, then distribute to the live bird markets, which plays a crucial role in the maintenance, amplification and dissemination of avian influenza viruses and hence are considered high risk locations for potential zoonotic transmission of influenza viruses to humans [3], [4].

For HPAI controlling, the minimization of vaccine using or unvaccination is preferred in general concept of controlling this disease. Hence the biosecurity measure is become the topic of interest and it is very important to search to good materials that can inactivate this virus and are stable in the environment.

Ceramic powder is the product obtained from sintering or burning process. Its efficacy to inactivate avian influenza virus is first report from ceramic derived from chicken feces by Takehara et al. [5]. In this experiment, we had demonstrated the efficacy of ceramic powder derived from various sources to inactivate avian influenza virus. We also measure the possibility of application if these products will be used in the environment.

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II. Materials and Methods

A. Ceramic Powder

The ceramics used in the present experiment were derived from various sources, including from chicken feces (CF), scallop shell (SS), polyvinyl chloride (PVC) and soybean (SB).

B. Inactivation Efficacy Testing

The ceramics 200 mg were mixed with 100µl of low pathogenic AIV (LPAIV) H7N1 (A/duck/Aomori/395/04), and then kept at room temperature until reached 20h, except SS which 3min was used. After that, 900µl of maintenance medium was added for virus recovering. Finally, 10 fold dilutions were carried out serially and titrated onto Madin-Darby canine kidney (MDCK) cells in a 96 wells plate. The cytopathic effect (CPE) was observed and determined at 3 days post-inoculation (dpi) and confirmed by hemagglutination assay (HA).

C. Stability in Environment Testing

All ceramics were assessed the stability efficacy to inactivate AIV after applied into the environment. All ceramics with amount 3g were poured into 90mm petri dishes. Then all dishes were kept under sunlight from morning to evening until reached 7 week.

One other dish of each ceramic were kept separately, aim to measure the efficacy after kept under wet and dry condition. These dishes were suspended by chlorine free tap water 10ml per dish. After that, all dishes were leaved until dried under the sunlight. Every dish was resuspended by the same volume of tap water following by dried again until reach 7 times.

The samples were collected from every dish including tested dishes under sunlight and wet-dried condition when the experiment period was finished. All samples were kept at room temperature for further inactivation efficacy testing.

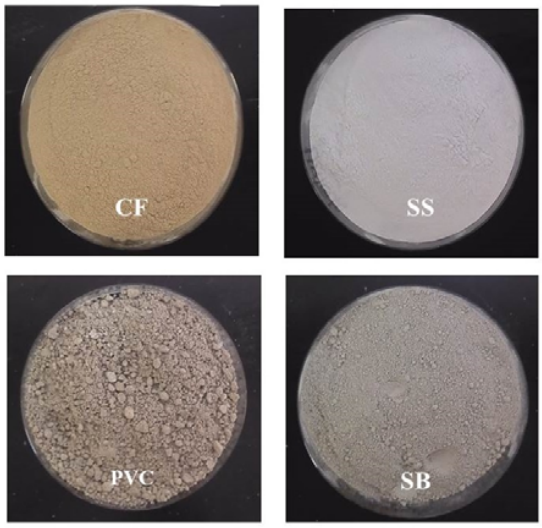

Fig. 1 Ceramic powder derived from chicken feces (CF), scallop shell (SS), polyvinyl chloride (PVC) and soybean (SB), pouring in 90mm dishes. The color of each ceramic is different according to the sources. The CF illustrates brown color, while grey were found from others

III. Result

After virus recovery was carried out and titrated, the viral titer was calculated into Log10 $TCID_{50}$/ml. The obtained viral titer of treated virus with the various ceramics before and after testing under sunlight for 7 weeks and after wash with the water and dry under sunlight until 7 times were shown in Tables I and II.

TABLE I
Efficacy of Ceramics after Kept under Sunlight

Sources of ceramic	Viral titer (Log10 $TCID_{50}$/ml)	
	At 0 weeks	At 7 weeks
Chicken feces	< 2.5	< 2.5
Scallop shell	< 2.5*	< 2.5*
Poly vinyl Chloride	< 2.5	< 2.5
Soybean	< 2.5	< 2.5
Virus control 20 h	7.75	7.5
Virus control 3 min	7.75	7.75

* Incubation period = 3 minutes

In Table I, all ceramics were incubated with LPAIV for 20h except SS, which 3min was decided to use. The results demonstrated the excellent efficacy including SS which tested under short incubation period. All treated samples shows the viral reduction titer lower than detectable level (< 2.5 Log10 $TCID_{50}$/ml) since the beginning until 7 weeks under sunlight.

In Table II, the viral titer recovered from all ceramics after resuspension with the water and dry under sunlight up to 7 times illustrated the some difference titer among samples. The recovery virus obtained from CF and SB reveals the titer 2.5 Log10 $TCID_{50}$/ml. The titer 3 Log10 $TCID_{50}$/ml was illustrated by SS whilst the viral titer recovered from PVC reveals under detectable level.

TABLE II
Efficacy of Ceramics after Kept under Wet and Dry

Sources of ceramic	Viral titer (Log10 TCID50/ml)	
	At 0 times	At 7 times
Chicken feces	< 2.5	2.5
Scallop shell	< 2.5	3
Poly vinyl Chloride	< 2.5	< 2.5
Soybean	< 2.5	2.5
Virus control	7.75	6.75

IV. Discussion

In the present experiment, first we demonstrated the inactivating efficacy of CF, SS, PVC and SB to LPAIV H7N1. All ceramics disclosed the good efficacy to inactivate LPAIV by 20h of incubation period, which the titer can be reduced more than 10,000 times when compared with positive controls, the untreated viruses which were kept at the same incubation period.

The measurement whether possible to use these ceramics in the environment or not was designed by using the simulated condition under sunlight and wet-dry. The results indicated that all ceramics still had satisfactory efficacy even kept under sunlight up to 7 weeks, when the samples were incubated with LPAI H7N1 for 20 h, especially the SS, we decide to test with a short incubation period as 3min due to its excellent efficacy was described by previous report [6], which using short incubation period.

Under wet and dry condition, all ceramics were examined by 20h of incubation period, all still illustrate the good efficacy after resuspension up to 7 times. There are some differences among obtained titer. However, all are satisfying due to the high level of viral reduction was demonstrated, more than 1,000 times.

Generally, Influenza virus is the enveloped RNA viruses belonging to the family of Orthomyxoviridae [7]. The AIV has lipid envelope, can be inactivated by organic solvent and detergents [8]. This virus can survive long period in the environment. Both simulation methods can be referred to the harsh condition in the field application especially the high temperature, long exposure time with sunlight, and wet condition after expose by rain. The results indicate that all ceramics can be used in the field practice due to their efficacy to inactivate LPAIV rather stable under harsh condition. We expect that these ceramics can be applied in the biosecurity and HACCP at farms for controlling AIV due to their long lasting efficacy to inactivate AIV.

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