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Inhibition of infectious bursal disease virus transmission using bioceramic derived from chicken feces

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ABSTRACT

Bioceramic powder (BCX), at pH 13.0, derived from chicken feces, was evaluated for its efficacy to inactivate virus and inhibit virus horizontal transmission by fecal–oral route, using infectious bursal disease virus (IBDV) vaccine strain D78 as a challenge virus. Three 1-week-old SPF chicks were vaccinated per os and used as seeder birds. Six hours later, 3 sentinel 1-week-old SPF chicks were introduced into the same cage. Results revealed that BCX had excellent efficacy to inactivate IBDV within 3 min. Treating IBDV contaminated litter in the cage with BCX could prevent transmission of IBDV to new sensitive chicks completely. Further, transmission of IBDV to the sentinel chicks was significantly inhibited by adding BCX to litter and chicken feed. These data suggest that BCX at pH 13, derived from chicken feces, has excellent efficacy to inactivate IBDV, which can be applied in bedding materials for preventing viral transmission during production round. It is a good material that can effectively be used for enhancing biosecurity system in poultry farms.

1. Introduction

Poultry production systems are important for food supplying to humans. There are many production system types depended on propose and demand, such as backyard system for family consumption or farming and industries for mass production supplies to food chain at large. Success in optimal production yield is affected by breed, feed and management, within which disease controlling is included. Some diseases are highly contagious and harmful, often causing outbreaks in many areas across the world, such as avian influenza (AI), Newcastle disease (ND), infectious bursal disease (IBD) *etc.* The infectious diseases can be transmitted through various routes. However, the main routes attributed to many impacting pathogens usually take shape by ingestion and airborne transmission (Guan et al., 2013; Hugh-Jones and Allan, 1973; Sharma et al., 2000; Zhao et al., 2013).

Intensive biosecurity system is the topic of interest in terms of disease prevention and control. Nowadays, vaccination and disinfectants are the core measures for disease prevention. Disinfectants play the major role for farm cleaning and disinfection. But most of the disinfectants usually lost their efficacy under farm conditions (Lombardi et al., 2008), especially due to presence of organic materials (De Benedictis et al., 2007; Gehan et al., 2009). Their efficacy may not last long enough after being applied (Wanaratana et al., 2010). Thus, the restriction of disinfectant applications brings about vulnerability of biosecurity systems. Therefore, searching for new candidate materials that can be applied in livestock farming is essential key for enhancement of biosecurity systems serving for farming.

Bioceramics are the materials prepared by sintering process. Since a bioceramic powder (pH 10.6) was invented in Japan, its efficacy was first illustrated by the ceramic prepared from chicken feces to inactivate AI virus (Takehara et al., 2009). The present experiment investigates efficacy of a bioceramic powder (BCX)

Abbreviations: ADG, average daily gain; AI, avian influenza; BCX, bioceramic powder; BF, bursa of Fabricius; CEF, chicken embryo fibroblasts; dpi, days post-inoculation; FBS, fetal bovine serum; IBD, infectious bursal disease; IBDV, infectious bursal disease virus; MM, maintenance medium; MAFF, Ministry of Agriculture. Forestry and Fisheries; ND, Newcastle disease; PFU, plaque forming units; RF, reduction factor; RT-PCR, reverse transcription polymerase chain reaction; SPF, specific pathogens free; VN, virus neutralization.

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at pH 13, derived from chicken feces after sintering process, for applying during chick raising, aimed at stopping fecal–oral transmission, while using infectious bursal disease virus (IBDV) within the employed evaluation system.

IBDV is a non-enveloped virus that has a bi-segmented double-stranded RNA genome, and belongs to *Birnaviridae* (Kibenge et al., 1988; Müller et al., 2003; Van den Berg et al., 2000). This virus resists heat inactivation (Alexander and Chettle, 1998) as well as chemical treatments (Petek et al., 1973), and can endure in various environments, even during compost procedure (Guan et al., 2010). Although IBDV vaccine can be administered by intramuscular injection (Carballeda et al., 2011), aerosol (Banda et al., 2008; Zhao et al., 2012) or eye drop (Hedayati et al., 2005), under natural conditions, the most common route of IBDV infection appears to be *via* ingestion (Sharma et al., 2000). Naturally, IBDV is liable to be shed from infected chicks and transmits only through feces (Zhao et al., 2013) and contaminated litter, as viral depot. It seems to be a good candidate for establishing an evaluation system of BCX at pH 13 for *in vivo* test.

2. Materials and methods

2.1. Animal

Animal handling was performed in strict accordance with Animal Care guidelines of Tokyo University of Agriculture and Technology (Tokyo, Japan), with permit numbers 25–37 and 26–45. Two types of chicks were employed for different purposes, including specific pathogens free (SPF) and conventional male white leghorn chicks. The SPF chicks were purchased from Nisseiken Co., Ltd. (Tokyo, Japan). Alongside, twenty four conventional chicks were purchased from Kanto Co., Ltd. (Gunma, Japan) for safety test of BCX.

Raising chicks was done in experimental rat cages, sized 236 mm × 420 mm × 199 mm, while 30 g per cage of wood chip was spent as litter. All chicks were kept in isolators which were ventilated under negative pressure with high efficiency particular air (HEPA) filter, fed *ad libitum* by non-antibiotics commercial chick feed (Kumiai Shiryo Co., Ltd., Tokyo, Japan), and had tap water freely access.

2.2. Cell culture

Chicken embryo fibroblasts (CEF) were prepared from 9 to 11-day-old embryonated eggs as described (Takehara et al., 1987). CEF were subjected to titration of IBDV during *in vitro* evaluation of virucidal effect of BCX, and virus neutralization (VN) test. For CEF culturing, Eagle's minimum essential medium (EMEM; Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), supplemented with 5% fetal bovine serum (FBS), L-glutamine 0.3 mg/ml, $NaHCO_3$ 1.4 mg/ml, and antibiotic–fungicide cocktail (penicillin 100 IU/ml, Streptomycin 0.1 mg/ml and amphotericin B 0.5 μg/ml) were employed.

2.3. Virus

IBDV vaccine strain D78 (Intervet Co., Ltd., Tokyo, Japan) was used for *in vitro* evaluation for virucidal effect, virus challenging of chicks, as well as VN test.

2.4. Ceramic powder

The bioceramic powder (BCX) of pH 13, derived from chicken feces and prepared by sintering process, was provided by NMG Environmental Development Co., Ltd. (Tokyo, Japan).

2.5. Plaque assay

Plaque assay was used for virus titration and VN test. Briefly, the virus was diluted with maintenance medium (MM; EMEM without FBS and supplemented with L-glutamine, $NaHCO_3$ and antibiotic-fungicide cocktail as mentioned above) in serial 10 fold, then inoculated onto CEF, 2 dishes per dilution. The first overlay agar medium (MM containing 8 mg/ml of Bacto agar, supplemented with 1% FBS) was laid after virus absorption for 1 h. The second overlay medium (MM without FBS, containing 8 mg/ml of Bacto agar supplemented with 2.1 mg/ml of $NaHCO_3$, 0.005% neutral red) was laid at 5 days post-inoculation (dpi), and then the plaques were counted at 6 dpi. The titer was calculated as plaque forming units (PFU)/ml.

2.6. Virus neutralization test

Sera separated from blood samples were subjected to VN test. Briefly, the serum was diluted in serially 4 fold, mixed with equal volumes of IBDV D78 of 50 PFU/100 μl, and incubated at 37 °C for 1 h. Then, the mixture of 200 μl was inoculated onto CEF to titrate the remaining virus by plaque assay. The VN titer was calculated by 50% plaque reduction according Behrens-Kärber's method (Matumoto, 1949).

2.7. Reverse transcription polymerase chain reaction (RT-PCR)

Total nucleic acid was extracted using Agilent RNA Isolation Mini Kit (Agilent Technologies, Inc., DE, USA), according to the manufacturer's instructions. Briefly, 20 mg of bursa of Fabricius (BF) tissue was homogenized with lysis buffer. Excess lysate was filtrated by column in the kit. Recovered solution was subjected to sedimentation with 70% ethanol. After that, RNA was trapped by filtrate column, eluted by distilled water. One step RT-PCR (Takara Bio Inc., Shiga, Japan) was carried out as indicated in the leaflet's protocol. Forward and Reverse primer obtained according to recommendation by OIE (OIE, 2008), composed of primer IBDV upper U3VP2F: GGT ATG TGA GGC TTG GTG AC and IBDV lower L3VP2R: GAT CCT GTT GCC ACT CTT TC. These primers were aligned compared with VP2 sequence of IBDV obtained from GenBank, accession number E12060, revealed flanking between 526 and 1082 nt of the reference sequence, while 556 bp of PCR product was expected. The primers were mixed with RNA template, cocktail of enzymes, buffer and distilled water, for making master mixture, then subjected to RT-PCR. Temperatures during reverse transcription step were 50 °C for 30 min, followed by 94 °C for 2 min. The PCR was performed as follows: 94 °C 30 s, 65 °C 30 s and 72 °C 1 min. The PCR was done for 30 cycles. Finally, 1% agarose gel was applied for gel electrophoresis.

2.8. Experimental designs

2.8.1. Evaluation for virucidal effect of BCX

Previously, we demonstrated that if the virus liquid was not adsorbed completely in BCX, BCX could not inactivate the virus completely (Takehara et al., 2009). So the ratio of the amount of the powder and the volume of the virus liquid is important for inactivating the virus. To evaluate the virucidal effect of BCX, a quantity of 200 mg was mixed with IBDV 100 μl, then incubated at room temperature for 3 min or 1 h. After each incubation period, the virus was recovered by adding 900 μl of MM, followed by vigorous vortex, and centrifuged at 18,000 × *g* for 3 min. The recovered virus was titrated on CEF by plaque assay shown above. The virus titer was calculated and compared between treated and intact control virus. Triplication was carried out for both incubation periods. Viral inactivation efficacy was determined using reduction factor (RF),