Original Article

An Inactivated Vaccine for Prevention and Control of Inclusion Body Hepatitis in Broiler Breeders

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Abstract

Inclusion bodies hepatitis (IBH) in Thailand is caused by Fowl Adenovirus (FAdv) serotype 2. Vertical transmission is the important route for disease outbreak. Biosecurity and vaccination are the major keys for disease control and prevention. Vaccination of broiler breeders results in maternal antibody production that can be transferred to their progenies. Therefore, the aim of present study was to develop an inactivated FAdv serotype 2 vaccine. The FAdv was isolated from liver of infected chickens by inoculation onto chicken embryo liver cells (CEL). Virus purification was performed and then inactivated by Binary Ethylene Imine (BEI). The 10^7 $TCID_{50}$ of virus concentration was inactivated for chicken immunization. The 0.01M BEI at 37°C for 72 h was the optimal condition for FAdv inactivation. Infectivity test, virus isolation and PCR technique were used for complete virus inactivation. In addition, sterility test was performed before and after the inactivated FAdv was mixed with Montanide™ ISA 70 VG, an adjuvant. Specific pathogen free chickens were vaccinated once during rearing period by 0.5 ml/bird intramuscular injection. Results showed a significant antibody response (p<0.05). Moreover, the embryonic eggs and chicks from the vaccinated breeders showed disease protection at 90% and 100%, respectively, when challenged with 10^7 $TCID_{50}$ of FAdv serotype 2. These results suggested that the inactivated FAdv serotype 2 vaccine could be prepared locally for broiler breeder farms in Thailand.

Keywords: antibody response, broiler breeder, Fowl adenovirus, inactivated vaccine

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Introduction

Inclusion body hepatitis (IBH) was initially described in the 1960s in the United State (Helmbelt and Frazier, 1963) and was distributed widely throughout the world (McFerran and Adair, 2003). IBH is caused by group 1 Fowl adenovirus (FAdv) consisting of 12 serotypes (Benko et al., 2005). At present, in Thailand, IBH is caused by FAdv serotype 2 and has caused economic loss to the poultry industry (Songserm, 2007; Witoonsatian et al., 2008). Horizontal transmission is mainly oral-fecal route (McFerran and Smyth, 2000). In addition, vertical transmission is the important route and establishes latent infection (Helena et al., 2006). Infected breeder flocks spread the virus vertically during laying period resulting in low hatchability, poor chick quality, and high mortality in young broilers (Songserm, 2007). Therefore, an effective control should be initiated at broiler breeder farms to provide maternal antibody transferring via egg yolk, in order to prevent the disease in offspring (McFerran and Adair, 2003; Grimes, 1977; Grimes, 2007). Several studies have reported the control of FAdv infection and revealed successful protection by immunization with either attenuated vaccines or inactivated vaccines. Complete protection of progenies by injecting broiler breeders twice with an autogenous killed vaccine was earlier reported (Alvarado et al., 2007). Dual or polyvalent vaccines applied in breeders were also described earlier (Toro et al., 2002; Fadly and Winterfield, 1975). Furthermore, many groups have succeeded in controlling the disease by applying inactivated homogenates that were prepared from the livers of infected birds clinically showing hydro-pericardium syndrome in Pakistan (Afzal and Ahmad 1990; Anjum, 1990; Roy et al., 1999). At present, most commercial vaccines are prepared by combinations of the FAdv-4 and other viruses which are the Newcastle virus (NDV) and the Chicken anemia virus (CAV) (Toro et al., 2002). Apart from the virus serotype in the commercial vaccines, FAdv serotype 2 was identified as the cause of IBH in Thailand. The purpose of the present study was to develop an inactivated FAdv vaccine to be experimentally injected into broiler breeders and evaluate the vaccine efficacy monitored by an indirect enzyme linked immuno-sorbent assay (ELISA) test which was developed based on recombinant Hexon protein as described earlier (Junnu et al., 2014).

Materials and Methods

Virus preparation

Virus propagation: The FAdv serotype 2 virus isolated in Thailand in 2007 (Songserm, 2007) was propagated in chick embryo liver (CEL) cell culture which was prepared as described earlier (Adair et al., 1979). Briefly, the CEL cells were prepared from 12- to 14-day-old chicken embryos. The cells were dispersed in 0.25% Trypsin (HyClone®, Thermo scientific, UT, USA), grown in growth media (GM): 1XMinimum Essential Medium (MEM) (Gibco™, Invitrogen Corporation, CA, USA) with L-glutamine, 10% Fetal Bovine Serum (FBS) (HyClone®, Thermo scientific, UT, USA), 10% Tryptose Phosphate Broth (TPB) and Penicillin-Streptomycin (PS). Master seed of the virus at a titer of 10^7 mean Tissue Culture Infectious Dose ($TCID_{50}$)/0.1 ml reconstituted from freeze-dried condition in phosphate buffered saline (PBS) was inoculated onto the CEL cells, incubated at 37°C for 1 hour, and then maintenance media (MM): (1XMEM with L-glutamine, 1% FBS, 10% TPB and PS) was added and further incubated at 37°C with 5% CO_2. Cytopathic effect (CPE) was daily observed. CPE containing CEL cells were harvested by freeze-thaw method and centrifuged at 10,000 rpm for 30 min, at 4°C. The supernatant was filtered through syringe filters with porosity of 0.20 µm and stored at -80°C until used.

Plaque purification: The virus was prepared by 10-fold serial dilutions in GM and then 0.5 ml of each dilution was overlaid onto the CEL cells in duplicate dishes. One hour after overlaying on the CEL cells incubated at 37°C with 5% CO_2, the remaining virus suspension was discarded. Four percent agarose gel was prepared in growth media and was melted in microwave (about 3 min for 100 ml). Twenty-five ml of 4% agarose gel was mixed with 225 ml GM and placed in 37°C water bath for 1 hour. Then, the combination of agarose and GM was gently poured onto the infected CEL cells. Later, the dishes were incubated at 37°C with 5% CO_2. The plaques were found to be visible within 3 to 5 d. The plaques of suitable dilutions were harvested by using sterile pasture pipette and were collected in MM. The plaque purification was performed three times. For the second and third times, the purification was performed with 10-fold dilution of these plaques from 1:10 to 1:100. The plaques were picked up and mixed with the MM, and vortexed for dispersing the virus particle and for preparing the master virus seed.

Titration of FAd Virus: The FAdv infected CEL cells were titrated by means of $TCID_{50}$ (Reed and Muench, 1938). Briefly, a 10-fold serial dilution of the virus was prepared in MM from 1:10 to 10^{10}. Monolayer of CEL cells was prepared in 96-well tissue culture micro-titration plate (SPL®, SPL Life Sciences, Korea). One hundred microliters of each dilution of the virus were consecutively inoculated in 10 wells of the first row. The last 2 wells of the same row were served as the positive and negative control, respectively. The plate was incubated at 37°C with 5% CO_2 for 5 d. The CPE was daily observed under an inverted microscope.

Vaccine preparation

Inactivation of FAd Virus: The FAdv infected CEL cells were inactivated using binary ethylene imine (BEI) following the method described earlier (Bahnemann, 1990). In short, 0.1 M BEI was prepared by dissolving 0.041 g of 2-bromo-ethylamine HBr (BEA) (Sigma, USA) in 2 ml of 0.175 N NaOH (Merck, Germany) and was incubated at 37°C for 60 min. The BEI was diluted 1:10 and 1:100 in FAdv supernatant to a final concentration of 0.01 and 0.001M, respectively. BEI treated FAdv supernatant solution of both concentrations was incubated at 37°C and were collected at 0, 6, 12, 24, 48 and 72 h. Then, the treated supernatants were tested for pH and virus infectivity. The BEI- FAdv supernatant treatment was stopped by using sterilized 1M sodium thiosulfate (Merck,

Germany) at the concentration of 10 times of the BEI final concentration (Sarachai et al., 2010). The inactivated virus was inoculated onto the fresh and confluent monolayer of CEL cells. Ten-serial passage was performed for the residual infectivity test of virus *in vitro*. Fifteen ml of the inactivated virus suspension were tested for total bacterial count contamination following conventional plate count method in Bacteriological Analysis Manual (Maturin and Peeler, 2001). Briefly, the sterility of vaccines was checked on bacteriological and fungal media which were synthetic media including standard plate count agar (SPA) and yeast extract glucose chloramphenicol agar (YGC), respectively.

Virus isolation test: The inactivated FAdv were tested for virus infectivity by the inoculation onto the monolayer CEL cells. One hundred microliters of ten-fold dilution from 0.01 or 0.001M BEI-FAdv supernatant solution at each time point were inoculated onto the monolayer CEL cells. The CPE of the inoculated CEL cells were daily observed for 5-7 d.

Immuno-peroxidase monolayer assay: The FAdv inactivation was tested for virus infectivity by the inoculation of the inactivated FAdv onto monolayer CEL cells. Two hundred microliters of inactivated FAdv suspension from each time point were inoculated onto the monolayer CEL cells. The inoculated CEL cells were observed for a few or more CPE affected cells within 24 h. The infected cells were washed with 1xPBS for 3 times and air-dried completely in a safety cabinet. The infected CEL cells were then fixed with 4% paraformaldehyde at room temperature (RT) for 10 min. After the 4% paraformaldehyde was rinsed, the cells were washed with 1xPBS for 3 times and incubated with 0.5% saponin at RT for 10 min. Then, saponin was rinsed and the CEL cells were washed 3 times again. Mouse anti Hexon protein serum (Junnu et al., 2014) was diluted with 1xPBS at a ratio of 1:500 and then were added onto monolayer CEL cells and incubated at RT for 1 hour. The CEL cells were washed with 1xPBS for 3 times and incubated with goat anti-mouse IgG (H+L) (KPL, USA) which was diluted with 1xPBS at 1:500 for 1 hour. Color was developed using 3, 3′-Diaminobenzidine (DAB) as a chromogen.

The sterile inactivated FAdv suspension was mixed with an adjuvant (Montanide® ISA 70 VG, SEPPIC Inc, France) at a ratio of 1:1 (v/v) and was slowly homogenized about 100 rounds per minute (rpm) at RT for 1 hour.

Animals

Chickens and housing: Specific pathogen free (SPF) white leghorn chickens and commercial broiler chickens (Arbor Acres) provided by Better Foods Company Ltd., Thailand were tested for antibody response to vaccine evaluation. They were raised in positive-pressure high-efficiency particulate air-filtered stainless steel isolation cabinets at a biosafety level 3 laboratory, Faculty of Veterinary Science, Mahidol University. All animal procedures performed in this study were reviewed, approved, and supervised by the Faculty of Veterinary Science-Animal Care and Use Committee of Kasetsart University and Mahidol University.

Study plans

Antibody response of vaccinated chickens: Twenty-four one-day-old SPF chickens which were free from avian viruses including AI, ND and FAdv, and of which antibody were negative to FAdv were divided into 4 groups (A, B, C and D), 6 birds each. Group A served as the control group and was intramuscularly injected with PBS or the culture media (maintenance media mixed with adjuvant). Group B, C and D were intramuscularly immunized with the inactivated FAdv vaccine initially prepared from three different virus concentrations including 10^8, 10^7, 10^6 $TCID_{50}$, 0.5 ml per bird. Twelve days post vaccination, serum samples of the vaccinated chickens were collected once and antibody response was tested by an indirect ELISA, as previously described (Junnu et al., 2014). This ELISA kit has been carried out and routinely used to detect antibodies against Hexon protein of FAdv serotype 2 in our laboratory.

Protection study in progeny of vaccinated broiler breeders: In this study, forty one-day-old commercial chicks (group A) and forty embryonic eggs of seven days (group B) were obtained from vaccinated broiler breeders immunized at ten weeks of ages. Those chicks were randomly divided into 2 groups; negative control (NC) and challenged (C). In addition, chicks of a positive control (PC) group were obtained from free FAdv flock. Group NC served as the control group and was not inoculated with the virus, but was orally inoculated with PBS. Group C and PC, were orally inoculated with 10^7 $TCID_{50}$ of FAdv serotype 2. For the embryonic eggs, they were divided and were virus inoculated similar to the chicks. However, those eggs were virus inoculated via allantoic route and were incubated until hatching. Both chicks from one-day-old and chicks from embryonic eggs were reared in different isolation units in a biosecurity animal building. Feed and water were provided *ad libitum*. All birds were daily observed for clinical signs and mortality for three weeks after FAdV challenge.

Serological Tests: Serum samples of the vaccinated chickens were collected and stored in aliquot at -20°C until used.

Enzyme-linked immune-sorbent assay (ELISA): Detection of antibody against FAdv was performed by using an indirect ELISA as reported earlier (Junnu et al., 2014). Briefly, the recombinant Hexon protein diluted 1:200 was used. Chicken sera were 10-fold diluted. Horseradish peroxidase (HRP)-labeled goat *anti*-chicken IgG (KPL, Galthersburg MD, USA) was diluted 1:10,000. Results were determined by ELISA reader at a wave length of 650 nm.

Serum Neutralization (SN) test: The CEL cells were prepared for SN test. Chicken serum samples were performed using 2-fold dilution with medium from the first to twelfth wells. The 50 µl 100 $TCID_{50}$ of FAdv was loaded in each well and mixed well. The plates were incubated at 37°C with 5% CO_2 for 1 hour. Then, 100 µl

(2.5×10^5 cells/ml) of CEL cells were added to each well. The plates were further incubated for 5-7 d and observed every 24 h. Results were determined by inhibition of CPE in the wells.

Statistical Analysis: Values of the SN titer were transformed to $\log_2$ values. Mean comparison of parameters (S/P ratio and $\log_2$ SN titer) between the vaccinated and control groups was analyzed by one way ANOVA. Significance level (p-value) for all parameters was set at 0.05.

Results

Vaccine preparation: There was no total bacterial mold growth by the sterility test. Moreover, there was no CPE of FAdv infection in the CEL cells after the 5- to 7-day incubation by the infectivity test. These revealed that the virus suspension was completely inactivated and had no bacterial or mold contamination. .

Antibody response of vaccinated chickens: The ELISA Hexon test kit and SN test were performed in duplicate on total serum samples. The mean group S/P ratio and mean $\log_2$ SN titers between the control and vaccinated groups had difference in viral concentration and are summarized in Table 1. Prior to vaccination, all pre-serum samples of the vaccinated SPF chickens were negative to antibody against FAdv by the SN test and ELISA. Twelve days post vaccination, all serum samples of the vaccinated chickens showed antibody titer in the SN test and ELISA. The chickens vaccinated with a 10^7 and 10^6 $TCID_{50}$ had better anti-Hexon IgG response compared to those vaccinated with 10^5 $TCID_{50}$ and the control chickens. The mean S/P ratio in the chickens of 10^7 and 10^6 $TCID_{50}$ groups (0.396±0.08 and 0.216±0.06) were significantly higher than that of the control chickens (0.009±0.02) (p<0.05). However, the chickens vaccinated with 10^6 $TCID_{50}$ (mean S/P ratios: 0.216±0.06) were not significantly different from those vaccinated with 10^5 $TCID_{50}$ (mean S/P ratios: 0.088±0.04) (p>0.05). The $\log_2$ SN titers of the chickens of 10^7 and 10^6 $TCID_{50}$ groups (log2 SN titers: 8.00±1.04 and 6.17±1.42) revealed significant difference from those of the chickens of 10^5 $TCID_{50}$ and the control groups (p<0.05).

In the SPF chickens, the mean S/P ratios and mean $\log_2$ SN titers between the vaccinated and control groups of different sampling weeks are shown in Figs. 1 and 2, respectively. The chickens vaccinated with FAdv inactivated vaccine had better anti-Hexon IgG and neutralizing antibody response than the control chickens. The ELISA results showed the mean S/P ratios of the vaccinated chickens at 2-8 weeks post vaccination being significantly higher than those of the control chickens. However, the mean S/P ratios of the vaccinated chickens at 9-13 weeks post vaccination were not significantly different from those of the control chickens. The SN titers of the vaccinated chickens were higher than those of the control chickens at 3-13 weeks post vaccination (p<0.05).

Although the S/P ratio of the ELISA test decreased at six weeks post vaccination, the SN titer of all tested serum samples still remained at high levels.

Protection study in progeny of vaccinated broiler breeder: The mean body weights of the chickens in the trial groups after challenged are presented in Table 2. The chickens derived from challenged chicks and embryonic eggs had lower mean body weight than those of the control chickens. Together with reduced weight gain, the chickens of the challenged groups showed mild depression with reduced feed intake throughout the study.

To evaluate the protective efficacy of immunization against FAdv serotype 2 challenge, all vaccinated and positive control chickens were challenged with 10^7 $TCID_{50}$/0.1 ml of FAdv, at one day old for the chicks and 7 d old for the embryonic eggs. For the results of protection efficiency as shown in Table 3, the challenged chicks did not find mortality, showing 100% protection, comparable to the challenged embryonic eggs, which revealed 90% protection. In addition, the death of the challenged embryos showed hemorrhagic, swollen and friable livers including pancreas hemorrhage.

Discussion

Fowl adenovirus has been identified as a causative agent of IBH and hydropericardium syndrome (HPS) in chickens and has become a major economic impact to poultry industry worldwide (Adair and Fitzgerald, 2008). At present, epidemiological studies indicate that FAdvs cause economic loss in the global poultry population (Cheema et al., 1989; Singh et al., 1996; Ojkic et al., 2008; Nakamura et al., 2011; Steer and Noormohammadi, 2011; Zadravec et al., 2011; Choi et al., 2012). In Thailand, IBH is caused by FAdv serotype 2 (Songserm, 2007). Although a vaccine prepared from FAdv serotype 4 and combined with Newcastle disease virus clone 30 and Lasota strain is now available on the market, it is still questionable whether antibody raised by this vaccine can provide protection to the disease caused by the FAdv serotype 2 in Thailand.

Table 1 Mean group of antibody titers measured by ELISA Hexon test kit and SN test on different trial dosage (Group A: Control, Group B: 10^7 $TCID_{50}$, Group C: 10^6 $TCID_{50}$ and Group D: 10^5 $TCID_{50}$)

Group	ELISA Hexon test kit (Mean S/P ratio)	Mean antibody titer ($\log_2$ SN titer)
A	0.009 ± 0.02^{a}	0^{a}
B	0.396 ± 0.08^{b}	8.00 ± 1.04^{b}
C	0.216 ± 0.06^{bc}	6.17 ± 1.42^{b}
D	0.088 ± 0.04^{ac}	0^{a}

[a, b, c] Mean antibody titers with different superscripts indicate statistical significance (p<0.05) between different vaccinated groups.

Table 2 Body weight in grams (Mean±SE) at weekly intervals in trial groups after challenged

Group		One day old	1 week	2 weeks	3 weeks
A	NC	46.25±0.67[a]	150±5.77[c]	292.9±8.60[c]	641.5±17.53[b]
	PC	45.50±0.89[a]	208.4±8.96[b]	406.22±7.47[b]	630.33±14.34[b]
	C	44.05±0.79[a]	125±3.56[a]	224.87±4.23[a]	562.57±11.06[a]
B	NC	49.27±1.81[a]	226.11±11.89[c]	422.44±30.82[b]	742.85±41.56[b]
	PC	46.48±1.60[a]	124.25±4.49[b]	345.00±53.00[ab]	616±127.03[ab]
	C	47.87±0.68[a]	154.03±5.39[a]	331.93±14.38[a]	583.89±15.57[a]

*Broiler chickens and embryonic eggs were challenged at one day old of age and 7 days, respectively.
[a, b, c] values with different superscripts within column vary significantly ($p<0.05$).
A: one-day-old chicks, B: embryonic eggs, NC: negative control, PC: positive control, C: challenged

Table 3 Protection level of inactivated FAdv vaccine after challenged

Group	Total number	Total mortality	% mortality	% protection
Chick of vaccinated broiler breeder	30	0/30	0	100
Embryonic egg of vaccinated broiler breeder	30	3/30	10	90

*Broiler chickens and embryonic eggs were challenged at one day old of age and 7 days, respectively.

Our inactivated FAdv serotype 2 vaccine could induce antibody raised post vaccination, presented by the high level of both ELISA S/P ratio and SN titers. Additionally, it was efficient to protect the disease after challenge in the experimental chickens. Other groups conducting the studies by inactivating FAdv serotype 4 and vaccinating experimental chickens were successful in controlling the disease earlier (Sahidullah et al., 2008; Aslam et al., 2012). Our successful study is in accord with those previous studies in that the inactivated FAdv vaccine could be an efficient tool to control the FAdv infection.

The mean of ELISA antibody from SPF chickens could be detected twelve days post vaccination and declined at 5 weeks post vaccination. The results support the finding of some previous studies (Akhtar et al., 2000; Sahidullah et al., 2008; Kim et al., 2014) which revealed the increasing antibody titer at one week and decreasing at four weeks post vaccination. The increasing antibody at 11-13 weeks post vaccination was detected by the ELISA test kit although the S/P ratios were less than the cut-off value (S/P ratio cut-off = 0.106). However, the SN titers were not changed at this period. This might indicate the high sensitivity and specificity of the test kit. Since all experimental chickens were raised in the biosecurity level 3 isolator, it was unlikely to be caused by the FAdv infection. On the other hand, there might be an error in serum collection, especially at week 10 post vaccination.

In this study, the maternal antibody of vaccinated broiler breeders transferred to the embryonic eggs could protect the embryos and one-day-old chicks after FAdv serotype 2 challenged, with 90% and 100%, respectively. The result of 90% protection in the challenged embryos may be questioned whether the protective level of maternal antibody transferred to the embryos was not uniformed at challenge. This may depend on the different rate of transferring of maternal antibody to the embryos. One hundred percent of the survived chickens after challenged indicated that the antibody-disease intervention was successful. However, the mean body weights of the challenged chickens were significantly lower than those of the control chickens ($p<0.05$) (Table 2). This feature may be caused by the intervention resulting in decreased feed intake. It is worthwhile to induce the specific antibody against FAdv infection in the breeders with inactivated vaccine because the inactivated vaccine does not lead to the viral shedding from the breeders to the environment. Apart from the inactivated vaccine, virus in live vaccines or homogeneous infected tissues do replicate in the GI tract and liver resulting in viral shedding to the environment although clinical signs are not present. Shedding of attenuate or live vaccine virus is commonly found in many vaccines (Meeusen et al., 2007). Furthermore, virus shedding from chickens fed infected homogenate or orally inoculated with live virus is at high risk of the problem. The virus can be mechanically transmitted by several vectors including flies, cockroaches, beetles and others.

To our best field experience, single or twice vaccination with inactivated vaccine has been successful in controlling the disease. The first vaccination should be done in the breeders at least 2 weeks before laying period. The protective maternal antibody will last longer than 45 weeks of egg production period (unpublished data). However, farm biosecurity is the most important control measure of this problem. Vaccination can be helpful to decrease the economic loss.

Even though infected parent stocks did not show any clinical signs, the virus could be vertically transmitted to their offspring, resulting in economic loss of chick production (Adair and Fitzgerald, 2008).

Several previous studies of vaccine development for IBH and HPS disease control and prevention either attenuated adaptation vaccine (14-16 passages) or inactivated vaccine which was treated with formalin and BEI. However, formalin affects virus protein membrane and reduces the antigenicity of an antigen. These effects can also cause disease in vaccinated chickens (Bahnemann, 1990). We have used

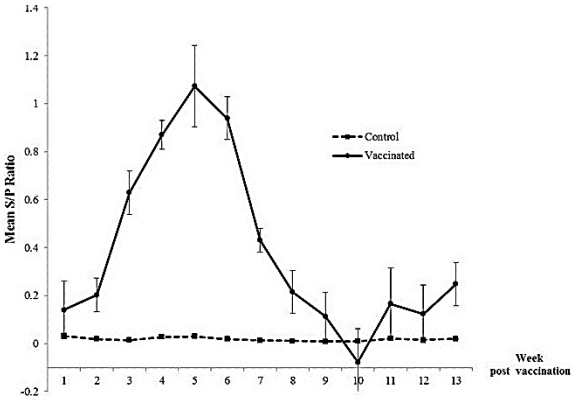

Figure 1 Mean group of sample-to-positive (S/P) ratios in serum from vaccinated group (n = 5) and control group (n = 5) on different weeks (at 0 to 13 weeks post vaccination). Error bars represent standard errors.

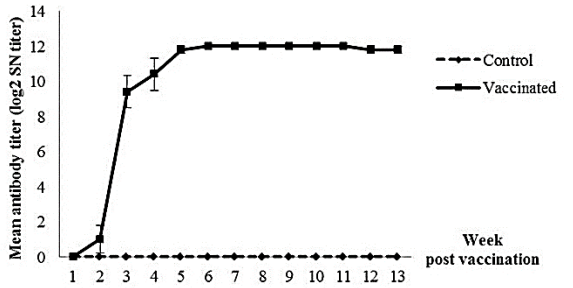

Figure 2 Mean group of log2 serum antibody titers measured by SN test between vaccinated group (n = 5) and control group (n = 5) on different weeks (at 0 to 13 weeks post vaccination). Error bars represent standard errors.

BEI for inactivating the FAdv because BEI is an alkylating substance which reacts very little with proteins. Therefore, the antigenic components of virus are not altered (Akhtar et al., 2000).

In conclusion, the inactivated FAdv serotype 2 vaccines could stimulate specific immunity against the FAdv serotype 2 in this study. Hence, humoral immunity induced by the inactivated FAdv serotype 2 vaccines could be a tool of IBH control in both breeders and their progenies.

Acknowledgements

This work was partially supported by the Center for Advanced Studies for Agriculture and Food, Institute for Advanced Studies, Kasetsart University, Under the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, Ministry of Education, Thailand, Center for Agricultural Biotechnology, Kamphaeng Saen Campus, and Center of Excellence on Agricultural Biotechnology, Science and Technology Postgraduate Education and Research Development Office, Commission on Higher Education, Ministry of Education (AG-BIO/PERDO-CHE). The authors would also like to thank Center of Veterinary Research and Service, Faculty of Veterinary Medicine, Kasetsart University, and Betagro Science Center Laboratory.

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บทคัดย่อ

การพัฒนาวัคซีนเชื้อตายสำหรับป้องกันและควบคุมโรคอินคลูชั่น บอดี เฮปาไตติสในประเทศไทย

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เชื้อฟาว์ล อะดีโนไวรัส ซีโรไทป์ 2 ก่อให้เกิดโรคอินคลูชัน บอดี เฮปาไตติส (ไอ บี เอช) ที่มีการแพร่ระบาดอยู่ในประเทศไทย การถ่ายทอดเชื้อนี้จากแม่สู่ลูกถือว่าเป็นการแพร่กระจายเชื้อที่สำคัญ ในส่วนของการควบคุมและป้องกันโรคนี้คือ การมีระบบความปลอดภัยทางชีวภาพที่เข้มงวดของฟาร์มและการให้วัคซีนในพ่อแม่พันธุ์ไก่เนื้อเพื่อถ่ายทอดภูมิคุ้มกันไปสู่ลูกไก่ได้ ดังนั้นวัตถุประสงค์ในการศึกษาครั้งนี้ คือ การพัฒนาวัคซีนเชื้อตายจากเชื้อฟาว์ล อะดีโนไวรัส ซีโรไทป์ 2 ซึ่งผลิตจากไวรัสที่ได้จากตับไก่ที่ติดเชื้อและนำมาเพิ่มจำนวนไวรัสในเซลล์ chicken embryo liver (CEL) จากนั้นนำไวรัสที่มีความเข้มข้น 10^7 $TCID_{50}$ มาทำให้หมดฤทธิ์ด้วย binary ethylene imine (BEI) ที่ความเข้มข้น 0.01 โมลาร์ ที่อุณหภูมิ 37 องศาเซลเซียส เป็นเวลา 72 ชั่วโมง เมื่อนำมาทดสอบความสามารถในการติดเชื้อ ทดสอบหาสารพันธุกรรม และทดสอบหาโปรตีนเฮกซอนด้วยวิธีการทางซีรั่มวิทยา พบว่า ไวรัสหมดฤทธิ์อย่างสมบูรณ์ รวมทั้งทดสอบการปลอดเชื้อแบคทีเรียและเชื้อราของไวรัสที่หมดฤทธิ์แล้วเมื่อผสมกับสื่อวัคซีน Montanide™ ISA 70 VG ในการทดสอบให้วัคซีนในไก่ปลอดเชื้อปริมาณ 0.5 มิลลิลิตรต่อตัว เข้ากล้ามเนื้อ จำนวน 1 ครั้ง ผลปรากฏว่าไก่ที่ได้รับวัคซีนสามารถสร้างภูมิคุ้มกันได้สูงกว่าไก่ที่ไม่ได้รับวัคซีนอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) สำหรับประสิทธิภาพในการป้องกันโรคของวัคซีน พบว่า ตัวอ่อนในไข่ไก่ฟักและลูกไก่จากพ่อแม่พันธุ์ที่ให้วัคซีนแล้วให้เชื้อทับนั้นสามารถป้องกันโรคได้ร้อยละ 90 และร้อยละ 100 ตามลำดับ ดังนั้นการพัฒนาและทดสอบวัคซีนเชื้อตายสำหรับป้องกันโรคไอ บี เอช นี้ สามารถทำการเตรียมและผลิตสำหรับใช้ในฟาร์มไก่พ่อแม่พันธุ์ภายในประเทศไทยต่อไป

คำสำคัญ: การตอบสนองทางภูมิคุ้มกัน พ่อแม่พันธุ์ไก่เนื้อ ฟาว์ล อะดีโนไวรัส วัคซีนเชื้อตาย

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