# STUDIES ON THE ATTENUATION OF INFECTIOUS BRONCHITIS VIRUS

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Infectious bronchitis is an acute, highly contagious, respiratory disease of chickens. The disease may occur in all ages of poultry. The outbreak of infectious bronchitis in Bangkok in 1954–1955 was first reported by Chaiyasittiyuthaparn (1957). Virus isolations were carried out during the spread of the disease, and confirmed later by virus neutralization tests, using the known sera supplied by Dr. Salsbury's Laboratories and the United States Department of Agriculture. These findings were positive (Chaiyasittiyuthaparn, 1958).

The control measure of the disease is, however, possible, by immunization of chickens with the modified live virus vaccines (Luginbuhl and Jungherr, 1952: Crawley, 1953, 1955: Hofstad, 1954, 1956). Therefore, attempts have been made in order to attenuate the isolated local virus for the production of vaccine, since infectious bronchitis has become a problem to poultry industry in Thailand.

## MATERIALS AND METHODS

The S–strain of infectious bronchitis virus (IBV–S) isolated from a diseased bird of an infected poultry farm in Bangkok was serial passaged into the allantoic sac of 9 or 10–day chick embryos by Chaiyasittiyuthaparn (1957.) The mortality rate of inoculated embryos was gradually increased in higher serial transfers. On 138 th passage, mortality was nearly a hundred percent afer 36–48 hours of incubation, and the remainders died either on the 3 rd or 4 th day with lesions of dwarfing, stunting and curling on the dead embryos as shown in Fig. 1. The titrations of the virus of 143 rd and 144 th passages, titers were $10^{-7.3}$ and $10^{-7.2}$ to $10^{-7.8}$ respectively and remained constant, which indicated that the virus has been fixed for chick embryos.

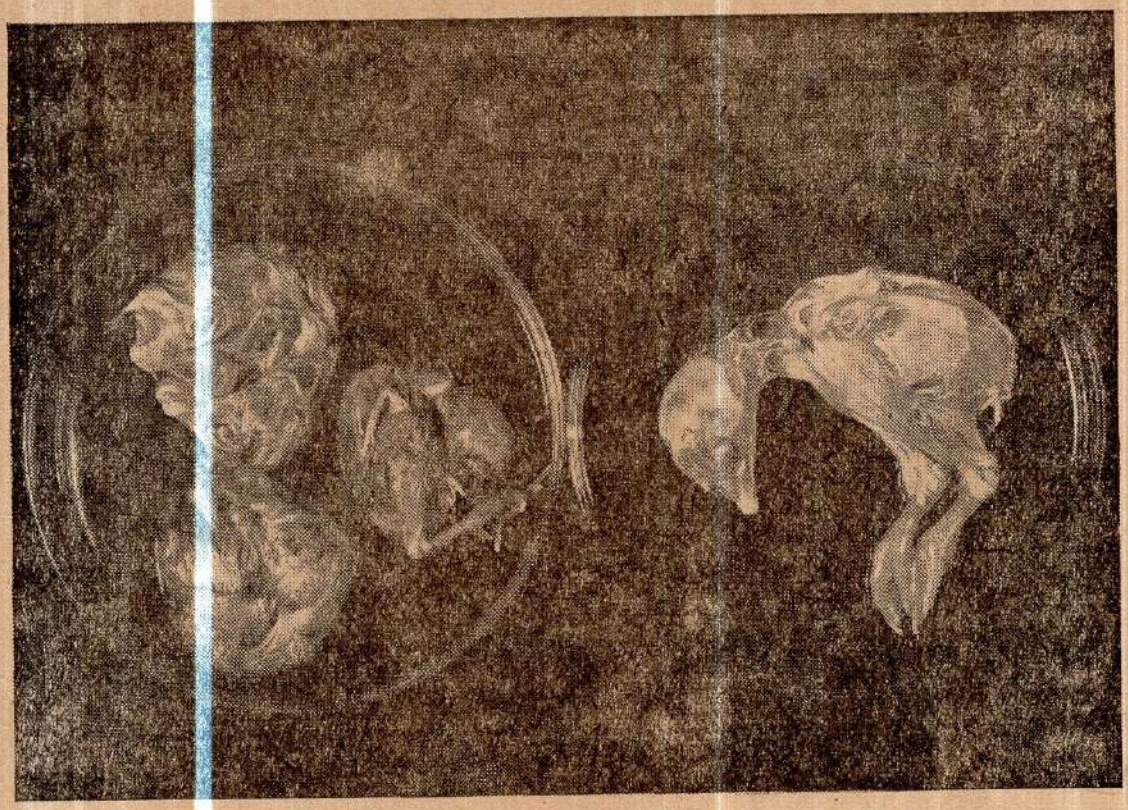

*Fig. 1 Comparision of normal 14 day old embryo (right) and infected embryos of the same age (left).*

The virus of 143 rd passage (IBV–S143) was used for the study on vaccination. Fourtyseven of 10 day-old white leghorn chickens were divided into 4 groups. In order to find out the optimal effective dose of the vaccine, a few drops of different dilutions of the virus were given by intranasal routeas follows:

Group I: 14 birds, vaccinated with undiluted virus (allantoic fluid of infected embryos);

Group II: 14 birds, vaccinated with 1:10 of virus suspension;

Group III: 14 bird, vaccinated with 1:20 of virus suspension; and

Group IV: 5 birds, non–vaccinated for controls.

The chickens in group IV were placed in an isolated room to prevent the virus contamination. All vaccinated birds were closely observed for the clinical reactions and symptoms for a period of fourteen days. (Table 1).

Blood samples were periodically collected from the heart of every chick in the four groups at 3 weeks, 3 months and 6 months postvaccination for the determinations of antibody and duration of immunity by serum neutralization (SN) test. The virus used in SN tests were IBV–S 143 and IBV–S 144.

SN tests were performed on pooled sera of each group after their inactivation at 56° C for 30 minutes. Undiluted serum samples were combined with equal volumes of decimal virus, and the mixtures were then incubated at room temperature for 30 minutes prior to injection into the allantoic sac of 9 day-old embryos. Each embryo received 0.2 ml of serum-virus mixture. Five embryos were used per dilution, and 50 percent end points were determined by the method of Reed and Muench (1938) on the basis of mortality (Table 2, 3, and 4). $LD_{50}$ Neutralization Index ($LD_{50}$ NI) of $10^2$ or greater were considered positive (Cunningham, 1952).

## RESULTS

No signs of illness were observed in all vaccinated birds during 14 days after the vaccination. This indicated that, the virus was attenuated for 10 day-old chicken (Table 1). The results of SN test at 3 weeks postvaccination were highly positive in sera of group I and II, whereas group IV, (normal serum) was negative (Table 2). The serum titer of group III could not be calculated, because of its irregular results on the mortality rates of embryos, but in the data, it showed partial neutralization.

### Table 1

Intranasal vaccination of 10 day-old chickens with IBV-$S_{143}$ (April 17, 1961).

| Group | Number of chicks | A few drops of virus suspension | Symptoms and reactions | Remarks |
|---|---|---|---|---|
| I | 14 | undiluted virus | -no- | General conditions of |
| II | 14 | 1 : 10 | -no- | birds in group I were |
| III | 14 | 1 : 20 | -no- | poor during 7th-10th |
| IV | 5 | non-vaccinated | -- | weeks of age, which caused by round worms infestation. They were treated with Safersan and Phenovis. |

**Table 2**

Serum neutralization tests
3 weeks postvaccination

| | Virus dilutions | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ | $10^{-9}$ |
| IBV–S143 | *5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 3/5 | 1/5 | 0/5 |
| Virus + pooled serum of group I | 4/5 | 3/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| Virus + pooled serum of group II | 5/5 | 4/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| Virus + pooled serum of group III | 5/5 | 5/5 | 2/5 | 1/5 | 1/5 | 2/5 | 1/5 | 0/5 | 0/5 |
| Virus + pooled serum of group IV (normal serum) | 5/5 | 5/5 | 5/5 | 5/5 | 4/5 | 3/5 | 3/5 | 0/5 | 0/5 |

* numerator = number of embryos died ;
denominator = number of embryos inoculated.

**Table 3**

serum neutralization tests
3 months postvaccination

| | Virus dilutions | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ | $10^{-9}$ |
| IBV–S144 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 3/5 | 0/5 | 0/5 |
| Virus + pooled serum of group I | 5/5 | 5/5 | 3/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| Virus + pooled serum of group II | 5/5 | 4(5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| Virus + pooled serum of group IV (normal serum) | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 4/5 | 1/5 | 0/5 | 0/5 |

The results of SN test at 3 months postvaccination in Table 3 were similar to those in Table 2, except the neutralization index of serum of group I which dropped from 5.3 to 4.0 (Table 5). SN test on serum of group III was absent because of the limited number of chick embryos.

**Table 4**

Serum neutralization tests
6 months postvaccination

| | Virus dilutions | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{5}$– | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ | $10^{-9}$ |
| $IBV–S_{144}$ | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 2/5 | 0/5 |
| Virus + pooled serum of group I | 5/5 | 5/5 | 5/5 | 5/5 | 4/5 | 4/5 | 2/5 | 1/5 | 0/5 |
| Virus + pooled serum of group II | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 4/5 | 3/5 | 1/5 | 0/5 |
| Virus + pooled serum of group IV (normal serum) | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 4/5 | 2/5 | 0/5 |

The determinations of antibody in the sera of chickens of group I and II at 6 months postvaccination in Table 4 were negative. These findings demonstrated that the duration of immunity last only three months or a little longer. The virus used for SN tests in Table 4 was freshly prepared, its titer was thus greater than the virus previously used in Table 3.

The figures of $LD_{50}$ Neutralization Index ($LD_{50}$ NI) of those serum neutralization tests from Table 2, 3, and 4 were summarized in Table 5.

**Table 5**

Summaries of $LD_{50}$ Neutralization Indices

| Serum of birds in group | $LD_{50}$ NI Postvaccination | | |
|---|---|---|---|
| | 3 weeks | 3 months | 6 months |
| I | 5.3** | 4.0 | 1.1 |
| II | 4.9 | 4.8 | 0.8 |
| III | no calculation | — | — |
| IV | 0.6 | 0.7 | 0.1 |

**A neutralization index of 5.3 indicates that 0.1 ml of serum neutralizes 200,000 $EID_{50}$ of virus while 4.0 indicates neutralization of 10,000 $EID_{50}$.

## DISCUSSION

Although the same strain of virus was used in the studies of vaccination and SN test, the figures of NI in Table 5 clearly showed that the immunogenic property of the virus did not loss. In addition, the results of vaccination indicated the loss of its pathogenicity and spreading ability. A significant drop of antibody occurred in chicks of group I at 3 months postvaccination, this caused by round worm infestation. SN tests of serum of group III at 3 months and 6 months postvaccination were absent in Table 3 and 4, because the first test in Table 2 could not be calculated, and the number of embryonating eggs were also limited on the days of test. According to the results of these studies, a minimal effective dose of the virus vaccine for intranasal application of 10 day-old chick was a few drops of 1 : 10 of the virus suspension. The immunity was completely developed by 21 days after the vaccination and last for at least 3 months or a little longer.

Antigenic property of the virus on further passage as well as field trials vaccination should be investigated. Its antigenic may or may not decrease. However, IBV—S143 may be used for the production of a modified live virus vaccine in the near future.

At present, there are several strains of IBV which have been used as the modified vaccines with different methods of administration e.g. the spray or aerosol (Crawley, 1953 ; Hofstad, 1954), the dust (Markham, et al., 1955), and drinking water (Luginbuhl et al., 1955) procedures. Moreover, the IB vaccines have also been combined with ND (B 1 strain) vaccines as convenience and apparently without interference in the immune response from each vaccine (Markham, et al., 1956). These methods of application of IB vaccines have been a topic of interest that should be carried out.

## SUMMARY

A local strain of infectious bronchitis virus isolated from a diseased bird of an infected poultry farm in Bangkok, has become attenuated for 10 day–old chickens after 142 serial passages into allantoic sac of 9 or 10 day–chick embryos. The immunogenic property of the virus does not loss. Studies on intranasal application of chickens with a few drops of undiluted virus or 1 : 10 of the virus suspension, the immunity was completely developed by three weeks postvaccination and last for three months or longer. This attenuated infectious bronchitis virus may be used for the production of a modified live virus vaccine.

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