

دوره کاغذی      بیماری      واکسن

## Efficacy of Experimental Inactivated Vaccine for Infectious Bronchitis Disease

واکنش

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### Summary

Experimental oil-emulsion vaccine was formulated with a ratio of one part of 0.05%  $\beta$ -propiolactone inactivated antigen and four parts oil adjuvant ISA-70. Four hundred white Hy-Line (w-36) chicks were reared in four groups. Groups 1 and 2 were vaccinated by live attenuated HI20 at day 10 and reared separately from groups 3 and 4. At week 16 groups 1 and 2 were received experimental oil-emulsion and commercial oil-emulsion vaccines respectively. At this time, group 3 was received single dose of experimental oil-emulsion vaccine and group 4 was unvaccinated control. At week 29 all of groups were challenged by  $0.25 \times 10^7$  EID<sub>50</sub>/bird of infected allantoic fluid when they were on peak of production. All groups were bled frequently and the sera were assayed by ELISA and AGID tests. Clinical signs and high percentage drop in egg production in groups 3 and 4 were noted. Moreover, no virus and/or viral antigen in the trachea of groups 1 and 2 were detected at days 5 and 10 postchallenge. The results of clinical observation egg production, virus isolation and detection in the trachea and levels of antibody suggested that layers vaccinated with a combination of live attenuated and the experimental inactivated vaccines had the highest protection.

**Key word:** infectious bronchitis, oil-emulsion vaccine,  $\beta$ -propiolactone, AGID, ELISA

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### **Introduction**

Infectious bronchitis virus (IBV) is a positive sense, single-stranded RNA and the prototype member the family *Coronaviridae* and the cause of major economic losses in the poultry industry. It can be involved in respiratory disease, nephritis, and poor production and quality. Mortality may occur in young chickens due to respiratory or kidney manifestations of the infection (Cavanagh & Naqi 1997). The signs are not specific to IBV (De Wit 2000), however IBV is highly specific to chicken (Dhinakar Raj & Jones 1996).

The pathogenicity and tissue tropism vary among IBV strains (Ignjatovic *et al* 2002). The highly transmissibility nature of the disease, and the occurrence more than 20 IBV serotypes (Johnson *et al* 2003), have complicated and increased the cost of attempts to prevent the disease by immunization. Both live and inactivated virus vaccines are used for immunization against IB (Cavanagh & Naqi 1997). Live vaccines are used in broiler and the initial vaccination of breeders and layers (Cavanagh & Naqi 1997, Cook 2001). However, live IBV vaccination may protective on basis of virus isolation from the trachea after challenge with homologous strain only, but not protective against drop in egg production and kidney damages (Ladman *et al* 2002). Inactivated vaccines are usually given after priming with live virus and are administered a few weeks before production commences. Commercial inactivated vaccines may be given in combination with other inactivated vaccines (Cavanagh & Naqi 1997).

Vaccine strains are selected to represent the antigenic spectrum of isolates in a particular country or region. The Massachusetts (M41) strain is used widely because initial isolates from many countries are of that serotype (Cavanagh & Naqi 1997). Whilst the Massachusetts serotype continues to be of worldwide importance, the dominant type in any area changes with time. In addition to the continuing problems caused by the number of different serotypes that are circulating, recently the apparent emergence of different pathotypes of the virus is seen (Cook 2001).

$\beta$ -propiolactone ( $\beta$ -PL) inactivated IBV initially described by Christian and Mack (1957). They point out that the live virus vaccine had several disadvantages such as spread infection to neighbouring susceptible stock, predispose vaccinated bird to chronic respiratory disease, depress egg production and be contaminated with other pathogens.  $\beta$ -PL treatment had no adverse effect on IBV antigenicity properties (Cavanagh & Naqi 1997) so, to prepare an inactivated vaccine, 0.05-0.1% of the agent recommended for elimination IBV infectivity (King 1984, Cavanagh & Naqi 1997). Potency of a formalin-inactivated IBV vaccine by precipitin antibody response in vaccinated and control chicks after challenge was discussed (Witter 1962). Many trials carried out to demonstrate the efficacy of inactivated IB vaccine (Box 1980, Ladman *et al* 2002).

This paper describes an inactivated IB vaccine produced by a local isolated of the virus on laboratory scale and trials carried out to demonstrate its efficacy.

### ***Materials and Methods***

**Antigen.** Of several IBV isolates from outbreaks in Iran (Razi Institute, Karaj) one isolate, which was recognized as Massachusetts type was selected for propagation as killed vaccine antigen and challenge virus. It identified as pathogen by Veterinary Laboratories Agency-Weybridge, UK. According to De Wit *et al* (1997) pathogenicity of the virus for chicken was identified. Initially ten 9-week-old SPF chickens in controlled condition were inoculated with 0.2ml/bird of infected allantoic fluid (AF). Then the clinical signs and gross lesions of IB disease, and antibody rise in sera were observed. The virus was also inoculated in 9-11-day-embryonated SPF eggs. Infected embryos were dwarfed and curled. Chorioallantoid membranes of these eggs were used as antigen in AGID for detection of antibody in sera. Harvested AF was possessed approximately  $10^7$ EID<sub>50</sub>/ml in the third passage and was concentrated to one-fourth the original volume by centrifugation and inactivated with 0.05%  $\beta$ -PL according to Cavanagh & Naqi (1997). The mixture

was incubated 90min at 37°C. To examine for complete virus inactivation, the mixture was passaged three times into allantoic cavity of ten 9-11-day-old fertilized SPF eggs. Also, to confirmation that there was not any cross infection with NDV and AIV, harvested AF was tested for hemagglutination activity (HA) before inactivation and without any treatment.

**Vaccine preparation.** Water-in oil-emulsion vaccine was prepared with one part of inactivated AF as antigen and four parts of ISA-70 (SEPPIC, Cosmetics-Pharmacy Division, Paris, France) as adjuvant. The final product was tested for possible bacterial and fungal contamination.

**Challenge virus.** It was the same origin as the vaccine virus. All chickens in four groups were challenged by spray with infectious allantoic fluid containing approximately  $10^7$ EID<sub>50</sub> /ml that was diluted with equal volume of sterile distilled water ( $0.25 \times 10^7$ EID<sub>50</sub>/bird). The ventilation fans were switched off and diluted suspension was dispersed. The fans were restarted after 30min. Following challenge, the chickens were observed daily for clinical signs and dropping in egg production and eggshell quality.

**Serotype antiserum.** Ten 9-week-old SPF chickens that were tracheally infected with the virus were inoculated by intravenous injection at two weeks postinfection. Serum of the birds was harvested, pooled and used as antiserum in AGID for antigen detection and positive control serum.

**Statistics analysis.** Statistical analysis was performed on egg production among four groups by *t*-test and, on isolation and detection of virus in trachea at days 5 and 10 postchallenge by chi-square test.

**Experimental design.** Four hundred white Hy-Line (w-36) chicks were reared in 4 groups and grown in controlled conditions. Groups 1 and 2 were placed in one site and groups 3 and 4 in other site with adequate distance. The birds in groups 1, 2 and 3 were vaccinated and kept in cages since 16-week-old as described follow. Also, rearing and growth condition of group 4 was similar to other groups. Groups 1 and 2

were inoculated with live attenuated H120 by eye drop at day 10 then 0.5ml of experimental and commercial oil-emulsion vaccines were subcutaneously injected at the back of the neck at week 16, respectively. In group 3 only single dose experimental oil-emulsion vaccine was delivered subcutaneously, at the back of the neck at week 16. Group 4, any vaccine was delivered and considered as control till the time of challenge. Before challenge 10 chickens from this group were kept separately (without any expose to IBV) as blank control. Ten percent of each group were tagged and bled at days 24, 34, 44 (days 10, 20 and 30 after live vaccination) and at days 112, 142 and 152 (after killed vaccination). All groups were challenged when they were on peak of production at week 29. Then, clinical signs, virus isolation and virus (or antigen) detection in trachea, antibodies rise in sera and egg production before and after of challenge were recorded in all groups. After challenge 30 percent of each group at days 4, 8, 15 and 25 were bled for detection of precipitin reaction.

**Virus isolation.** Attempt to isolation the challenge virus in all groups at days 5 and 10 postchallenge was done as a single criterion of immunity. Virus isolation was done on SPF embryonated eggs. Tracheal samples were kept in equal volume of sterile TPB and glycerol in -70°C for virus isolation and, in a classical way were passaged three times into allantoic cavity of ten 9-11-day-old embryonated SPF eggs. The eggs were incubated 5-6 days for dwarfing, curling or embryo mortality if virus was present in the samples.

**Antibody detection.** Blood were collected from 10 percent of chickens representing in each group and sera stored at -20°C. A commercial ELISA (IDEXX kit) was used to determine the Antibody titers at 24, 34 and 44 and at days 112, 142 and 152 days old. The titer of the Antibody was calculated based on the formula of IDEXX kit. Also, precipitin reaction was tested on fresh sera at days 0, 4, 8, 15 and 25 after challenge.

**Clinical signs and egg production.** Clinical signs and egg productions of each group before and after challenge were recorded daily. Total numbers of egg laid by

each group during weeks of 26-38 were recorded, and the means weekly egg productions for each group over this period were calculated.

**AGID test.** For antibody detection AGID assay was performed in 0.7-1.0% agar containing 8.0% NaCl as described by Gazdzinski (1977). To perform the test, fresh tracheal mucosa was collected by scraping and suspended in 0.2ml of PBS and used as antigen with appropriate precipitating antiserum (Lohr1981).

## **Results**

*Virus isolation.* The challenge virus was isolated from all samples of groups 4 at day 5 and 80% of samples at day 10 postchallenge. Also, the virus was isolated of all samples of group 3 at day 5 and 70% at day 10. However, no virus was isolate from samples collected from birds in groups 1 and 2.

*Clinical signs and egg production.* Egg production in all groups was showed a similar pattern (92%) until challenge. The clinical signs and mean weekly egg production of each group were compared to group 1 as follow. Egg production and eggshell quality were normal at postchallenge and no clinical signs of disease were observed in this group. In group 2 eggshell quality was approximately normal but egg production was slightly (5%) dropped and after 10 day postchallenge it rapidly repaired ( $P<0.05$ ). No respiratory signs of disease were detected. Group 3 was shown mild respiratory signs and drop in egg production (15%) and eggshell quality was seen and did not return to normal through out the trial ( $P<0.05$ ). In group 4 the primary clinical signs including watery eye and conjunctivitis, coughing, sneezing and sever head shaking were observed. Coughing, sneezing and head shaking were present in all of the birds 4 days postchallenge and persisted for approximately 8 days. Drops in egg production and eggshell quality were started at day 4 postchallenge. 31% drop in egg production and high percentage in eggshell quality was observed at week 2 postchallenge and did not return to normal through out the trial ( $P<0.05$ ). Data are shown in figure 1. Mortality was not observed in any of the

treatment groups after IBV challenge and as all birds were white egg layers loss of shell color could not be assessed.

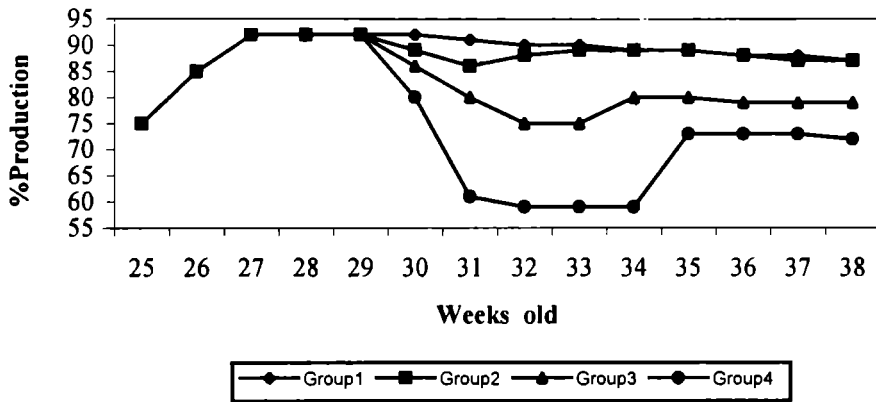


Figure 1. Egg production rate for all experimental groups at 25-38 weeks of age

*AGID test.* In antibody detection trial, the AGID positive reaction was demonstrated in sera of unvaccinated group from 8th day postchallenge and the higher number of positive reaction was detected from 8 to 25 days of postchallenge in all groups. In antigen detection trial, the AGID reaction demonstrated in 70% and 40% of the unvaccinated group (group 4) that collected at days 5 and 10 postchallenge, respectively. In groups 1 and 2 precipitin reaction was not detected at days 5 and 10 postchallenge. The AGID test in group 3 was demonstrated in 60% at day 5 postchallenge, but at day 10 precipitin reaction was not detected.

*ELISA test.* The result of ELISA in each group was shown in figure 2. The results in groups 1 and 2 were similar and a rise in antibody titer was seen at day 142. In group 3 that was not primed but exposed to IBV, the titer of antibody was negative through the rearing and at day 142 the mean titer was increased. In group 4 that was not primed but exposed to virus challenge at 29th week old, both ELISA and AGID tests were negative through out the rearing. After challenge, antibody titer rose to 10200-14000 in ELISA.

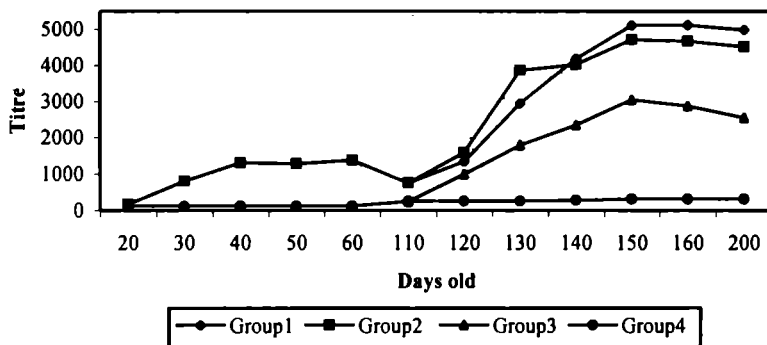


Figure 2. *IBV* specific antibody titers measured by ELISA

### Discussion

The aim of the present study was to prepare an inactivated vaccine for efficient immunization chicken against IBV. The antigenic spectrum and dominant strain of isolates in Iran is Massachusetts (Seify abad Shapouri *et al* 2002, Momayes *et al* 2002, Vasfi Marandi & Bozorgmehri Fard 2001). Respiratory signs and sharp decline in egg production in exposed chicken indicate that the virus was virulent for the respiratory and reproductive system but non-nephropathogen because renal gross lesion was not observed.

Respiratory signs and virus isolation in control chickens (group 4) indicate that no exposure and no local immunity were developed and virus could replicate in respiratory tract. Also, failure in virus isolation in groups 1 and 2 may be due to develop a mucosa immunity produced by the combination of live IBV priming and experimental or commercial inactivated vaccine. In group 3, the chicken did not protected against challenge virus. Although, virus isolation and AGID results were similar to group 4 at day 5 after challenge, but AGID reaction was not detected in this group at day 10. It could be due to reduction in virus particle reacts in this test. Despite use of killed vaccination in group 3 and presence humoral antibody, clinical signs were observed. It suggests that the single dose of experimental oil vaccine may



offer only partial protection against challenge with the same virus. As respiratory immunity is critical in controlling IB by reducing the potential spread of the virus to no infected flocks (Ladman *et al* 2002), so vaccination with single dose of killed vaccine cannot effective. The same result using a killed vaccine follow by booster either H120 with aerosol or H52 in drinking water also reported (Box and Sizer 1973). Commercially reared chickens which had received live IB vaccine initially to a recommended schedule were found at point of lay to have only modest levels of antibody to IB virus and relative inadequacy of the immunity conferred by challenge (Box 1980, Ladman *et al* 2002). Lack of local immunity in trachea and oviduct epithelia resulted in fewer drop egg production and eggshell quality in this group compared to group 1 ( $P<0.05$ ). But lower drop in egg production and quality, may be due to humoral immunity developed that neutralised virus challenge in circulation before complete effect on epithelium of oviduct. This results confirm the finding that antibody level alone may not be a good indication of immunity for the respiratory and reproductive tracts and contact with live IB vaccine is necessary in the late growing period in order to obtain adequate protection in the laying hen (Box & Sizer 1973). A sharp drop in egg production in unvaccinated laying chickens showed after challenge (Box 1980). In contrast, in our study birds that had been further immunized at point of lay with live and experimental inactivated IB vaccines showed no detectable fall in egg production after the same challenge. Sharp drop in egg production was also observed in group 4, which confirm the finding of Box (1980) but egg production did not return to normal by the end of experiment.

Chicken injected with live and experimental inactivated vaccine (group 1) raised statistically higher ( $P<0.05$ ) antibody titer than the other groups. In groups 1 and 2, vaccinations with live and at point of lay with a killed IB oil-emulsion vaccines increased antibody titer ELISA and offered good protection against challenge. No clinical signs of disease were observed. While egg production and eggshell quality were lower in group 2 ( $P<0.05$ ) antibody titers were similar in both

groups. These results may be due to the presence of slight differences between antigenicity and immunogenicity of commercial vaccine and challenge viruses. The same result was achieved when the virus challenge was the same virus as killed vaccine (Ladman *et al* 2002).

Comparison between AGID and ELISA tests indicate that precipitin antibodies against IBV appeared rapidly by day 8 after infection when antibody titer was still low by ELISA. AGID test, to detect virus antigen in trachea, was positive in group 4 at days 5 and 10 postchallenge and group 3 at day 5. This result suggests that AGID test is applicable to detection of acute phase IB disease using tracheal mucosa. Additionally, the test is rapid, inexpensive, available and more sensitive and specific (De Wit *et al* 1997). Due to the same result for group 3, precipitin reaction on trachea of group 4 at day 10 was lower than at day 5 postchallenge ( $P<0.05$ ). Inexistence of precipitin reaction and no virus isolation in groups 1 and 2 was due to local immunity in trachea. The AGID reaction for virus detection in tracheal mucosa decreased rapidly after day 5, so this test can be used only in early stage of disease in susceptible chicks ( $P<0.05$ ).

Equivalent protection achieved by revaccination of pullets with the less attenuated H52 live vaccine or oil vaccine (Box 1980), but live H52 vaccine has risk of spread of IBV to contiguous birds or premises. According to recommendation of Cook (2001) where oil-emulsion IB vaccine was used to immunize point of lay pullets, H52 live vaccine should not be used at least 8 weeks before. As seen in this study and the others (Song *et al* 1998, Ladman *et al* 2002), inactivated IBV vaccination stimulates increased local respiratory as well as systemic immunity upon challenge with a homologous strain. By studying parameter such as postvaccination challenge with infectious virus and serological follow up of the vaccination program, and the application of AGID and ELISA tests it concluded that the prepared experimental oil-emulsion IB vaccine could be as efficient as the commercial one and stimulate protective concentration antibodies for at least 8

weeks in period of observation. Although, the environmental exposure in field condition seems very important to protection and cause significantly increased protection.

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