Assessment of the Immunogenic Potential of the Infectious Bursal Disease Vaccine Virus (G-61) in Broiler Chicks

By
Basim, I.H. Al-Ibady
Vet. Med. College, University of Diyala, Iraq

Abstract

Using of numerous methods of vaccination of broiler chicks leading to variation in the average of immune response that elicited against Gumboro vaccine viruses. The study aimed to determine the effect of the immunogenicity of the live attenuated infectious bursal disease (IBD) vaccine virus (G-61 strain) in Broiler Chicks. A total of (160) chicks were divided into four equal groups namely A, B, C and D (40 chicks per group). The chicks of groups(A, B and C) were vaccinated with live attenuated IBD(G-61) vaccine at (14) days old, via the aerosol, intranasal and drinking water routes respectively while chicks of group D were left without vaccination as a control. An indirect enzyme linked immunosorbent assay (ELISA) was used to detect the antibodies titres against infectious bursal disease (IBD) vaccine virus in sera of chicks at (28) days of age. At (29) day of age, chicks of all groups were challenged by using of a virulent IBDV. At (40) day of age, the levels of antibodies against infectious bursal disease virus have been measured by indirect ELISA. The results obtained showed that higher levels of antibodies were noted when the vaccine administered via aerosol route as compared to the intranasal and drinking water. Following challenge of vaccinated chicks, the protection rates noted are correlated to the levels of antibodies elicited. It is concluded that the choice of approach to achieve higher and protective immune status against infectious bursal disease viruses in chicks is to apply IBDV (G-61) vaccine strain in broiler chicks via the aerosol route.

Key words: IBDV, antibody titre, aerosol, vaccination methods.
Introduction

Infectious bursal disease virus (IBDV) primarily affects the bursa of Fabricius in young birds resulting in impaired immunological capabilities\(^1\,^2\). The disease is responsible for high mortality in 3 to 4 week-old chicks, but adult birds remained clinically less affected\(^3\). The control of the disease mainly through proper immunization as well as maintaining a good
Assessment of the Immunogenic Potential of the Infectious Bursal Disease Vaccine Virus (G.61) in Broiler Chicks

Many virus strains had been used as vaccines and classified into mild, intermediate and hot vaccines\(^1\)\(^-\)\(^5\). Intermediate vaccines were proved to be immunogenic without residual pathogenic effects on the vaccinated chicks\(^6\)\(^-\)\(^8\). The enzyme linked immunosorbed assay (ELISA) is more sensitive, specific and reproducible in detecting antibodies against IBD virus\(^9\)\(^,\)\(^10\).

**Materials and Methods**

**Experiment Design**

One hundred and ninety chicks were used in this study. They were obtained as one day old from Iraqi Company for production and marketing of meats, Al-Khalis poultry fields. Thirty chicks were selected randomly and blood samples collected by sacrificing these chicks at one day of age for demonstration of indirect ELISA in order to measure the derived maternal antibody titer against infectious bursal disease virus in their sera.

The rest chicks were divided into four equal groups namely A, B, C and D (40 chicks per group). The chicks in the groups A, B and C were vaccinated with live attenuated IBD (G-61) vaccine at (14) days old, via the aerosol, intranasal and drinking water routes respectively while chicks in group D were left without vaccination as a control. At (28 days old), blood samples were collected. At (29 days old), chicks were challenged using a virulent IBDV. At (40) day of age, six blood samples were collected randomly from chicks of treated groups plus control group by puncture of heart for demonstration of indirect ELISA in order to measure the antibody titer against infectious bursal disease virus in their sera.

**Collection of bursae samples**

Infected bursae were collected from an outbreak of infectious bursal disease at a local poultry farms in Diyala governorate. Complete history of outbreak was taken. These samples were stored at \(-20\) °C till used.

**Field virus isolation and purification**

A 10% (w/v) suspension of infected bursae was made by chopping and grinding them in sterilized pestle and mortar with sterilized sand after the method of Reddy *et al.*\(^1\)\(^1\)\(^1\)\(^1\). The suspension was made in phosphate buffered saline (PBS) containing antibiotics (100 IU
penicillin-G/ml and 50 µg gentamicin sulfate/ml). This suspension was later centrifuged at 5000 r/min for 20 min and the supernatant was collected. The supernatant fluid was mixed with chloroform (1:1, v/v) in centrifuge tubes and centrifuged at 5000 r/min for 20 min. Three distinct layers were obtained: top layer containing virus, middle one containing bursal tissue debris and bottom layer was containing chloroform. The clear supernatant was collected in sterilized screw capped test tubes.

**Titration of virus**

The embryo lethal dose for (ELD\textsubscript{50}) for the virulent infectious bursal disease virus were determined by inoculation of the virus in the incubated chicks embryos at 9 day-of-age embryos after making of series of ten fold serial dilution. Fertile eggs with 9 day-of-age embryos were used and injected with virus through chorioallantoic membrane (CAM) route. Ten fold serial dilutions of the virus suspension were performed from 10–1 to 10–10 in sterile normal saline and each dilution was injected in a batch of five eggs. The eggs were inoculated with each dilution at rate of 0.1 ml per egg. Ten eggs were inoculated with sterile saline and were kept as control. After inoculation all the eggs were sealed with melted wax and were re-incubated at 37.5 °C. Inoculated eggs were candled daily. Mortality during first 24 hours was discounted as non-specific. After 96 hours the eggs were opened and embryos were checked for lesions.

**Methods of vaccine application**

For administration of the vaccine in drinking water (DW), the vaccine was dissolved in an amount of water which should be consumed by the birds within approximately two hours. When using the aerosol method of vaccination, the vaccine was dissolved in a quantity of water equal to 1000 doses per liter and spread as a coarse spray evenly over the birds at a distant of 30-40 cm. For the intranasal (I/N) route of vaccination, the vaccine was dissolved in physiological saline solution (usually 30ml per 1000 doses) and administrated by means of a standardized dropper by which drop should be applied intranasally.

**Statistical analysis**

The two-way analysis of variance (ANOVA) and Least Significant Differences were used to determine the differences among groups of data obtained.
Results

Results of values of antibodies titer against IBDV of indirect ELISA test to at (28) day of age.

The results of table (1) revealed that there were statistically significant differences (P<0.05) in the mean values of titer of antibodies against (IBDV) among the following treated groups (A, B and C) in compared with control group at (28) day of age. The results showed that there was significant variations (P<0.05) in the mean values of titer of antibodies against (IBDV) between groups (A and C) whereas no significant differences (P<0.05) in the mean values of titer of antibodies against (IBDV) between groups (A and B) at (28) day of age as shown in table (1).

Table (1): Values of Antibody titer of indirect ELISA test against (IBDV) at (28) day of age.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>S .E</th>
<th>L .S .D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>a 1774.1</td>
<td>± 1792</td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>a 1000</td>
<td>± 1889</td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>b 1370</td>
<td>± 498</td>
<td>2742.16</td>
</tr>
<tr>
<td>Group D</td>
<td>c 1735.1</td>
<td>± 525</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SE "Standard Error". L.S.D means Leatest Significant Differences. The letters that differ vertically indicate to statistical significantly variations (P<0.05).

Results of values of antibodies titer against IBDV of indirect ELISA test to at (40) day of age.

The results showed that there were an important statistically significant variations (P<0.05) in the mean values of titer of antibodies against (IBDV) among the treated groups (A, B and C) respectively in comparrison with control group at (40) day of age as shown in table (2). The recorded results in table (2) revealed that there was a significant increasing (P<0.05) in the mean values of titer of antibodies against (IBDV) of group "A" in compared with groups (B and C) respectively at (40) day of age. Control group gave the lowest mean values of titer of antibodies against (IBDV) in comparrison with all the treated groups at (28and40) day of age.
Assessment of the Immunogenic Potential of the Infectious Bursal Disease Vaccine Virus (G.61) in Broiler Chicks

Table (2): Values of Antibody titer of indirect ELISA test against (IBDV) at (40) day of age

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>S.E</th>
<th>L.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>2192.4</td>
<td>± 1770</td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>1993.3</td>
<td>± 1130</td>
<td>2192.4</td>
</tr>
<tr>
<td>Group C</td>
<td>2208.3</td>
<td>± 1133</td>
<td></td>
</tr>
<tr>
<td>Group D</td>
<td>0</td>
<td>± 0</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SE "Standard Error". Values followed by different letters on the table are significantly different (P<0.05).

L.S.D means Leatest Significant Differences.

Figure (1): Values of Antibody titer of indirect ELISA test against (IBDV) at (28) day of age.
Assessment of the Immunogenic Potential of the Infectious Bursal Disease Vaccine in Broiler Chicks

Discussion

The antibody responses against IBD vaccine (G-61 strain) as detected in chicks by indirect ELISA are shown in tables 1 and 2 respectively. Using indirect ELISA, a significantly (p <0.05) higher serologic response was found among chicks vaccinated via the aerosol followed by DW and the least response when chicks vaccinated via I/N. When indirect ELISA technique was employed to measure the antibody levels to the vaccine, it was observed that significantly (p<0.05) higher antibodies levels were obtained when aerosol route was used as compared to intranasal and drinking water (Table 1and 2). Following challenge of vaccinated birds with the virulent IBDV, the protection rates of the data obtained were in the groups of birds vaccinated via the aerosol, intranasal and drinking water respectively. The serologic response to the intermediate vaccine of IDB (G-61), administered via three commonly used routes of application, was assessed in the present study.

The protective potential of the immunogenicity of the IDB (G-61) vaccine and correlation of that protection to the antibody responses measured by indirect ELISA tests was also targeted in this study. It is interestingly that the highest response to the vaccine was observed
Assessment of the Immunogenic Potential of the Infectious Bursal Disease Vaccine Virus (G.61) in Broiler Chicks

when given via aerosol route. The ability of this route to elicit high levels of antibody responses to avian viruses was previously confirmed by \(^{[12]}\). The recorded results in table (2) revealed that there was a significant increasing (P<0.05) in the mean values of titer of antibodies against (IBDV) of chickens of group of aerosol in compared with chickens of groups intranasal and drinking water respectively at (40) day of age. This promotes the vaccine as a good immunizing agent as these sites are not major sites for the virus replication. The virus was proved specifically replicating in the lymphoid \(^{[13]}\) tissues especially those of the bursa of Fabricius. The protection rates obtained following challenge of chicks with the virulent virus strain were observed to correlate to the antibody responses induced by them. This confirmed the potential role of antibody in protection against the IBDV infection, the fact that recently confirmed by Hassan \(^{[14,15]}\).

Conclusions

In conclusion, the intermediate IBD vaccine (G-61 strain) proved highly immunogenic and protective response when administered in chickens via aerosol route and indirect ELISA is a better serologic technique to monitor that potential of the virus.

References

Assessment of the Immunogenic Potential of the Infectious Bursal Disease Vaccine Virus (G.61) in Broiler Chicks


