Research Regarding some Live Attenuated Vaccines Used in Immunoprophylaxis of the Avian Infectious Bursitis

Emil Tirziu1, Tatiana Rugea2, Ileana Nichita1, Ciceronis Cumpanasoiu1, Daniela Mot3, Monica Seres1, Radu Valentin Gros1

1Faculty of Veterinary Medicine, 300645, Timisoara, Calea Aradului, 119, Romania
2DSVSA Timis, Timisoara, Romania,
3Faculty of Animal Sciences and Biotechnologies, 300645, Timisoara, Calea Aradului, 119, Romania

Abstract
In our research four live attenuated vaccines against avian infectious bursitis (two inland produced and two imported) were tested: Biavac, Biaromvac-Pa, Gumboro Vaccine Nobilis 228e and Live Virus Vaccine Tablets Gumboro, M.B. Strain. The research was made in production conditions on 44,400 broiler chickens maintained in industrial system and raised on bedding and in batteries. The broilers were kept in four poultry houses, each of them representing an experimental group. We mention that vaccines were administered only one time. Vaccines efficiency was assessed by immunoenzymatic test. In that purpose, for each poultry house, 20 broilers were isolated and identified by a tibial ring, their immune response being followed between 5 and 42 days of age. Analyzing the results about individual antibodies titer during the experiment, the significant differences were observed both in poultries and phases. The best results were obtained using Live Virus Vaccine Tablets Gumboro, M.B. strain.

Keywords: antibodies, avian infectious bursitis, ELISA, vaccination.

1. Introduction
Avian infectious bursitis is one of the most important infectious diseases which affect the immune system of the young birds causing morphological and functional alteration of the lymphoid organs and a significant diminution of the immune response by immunosuppression [1,2]. Also, the disease causes depression, general and digestive disorders, reduced feed conversion index, weight loss, morbidity and mortality. Because of infectious bursitis disease virus (IBDV) dissemination and resistance, the general prophylaxis is insufficient. This fact imposes a specific prophylaxis, by vaccination [3,4]. In that purpose there have been used different types of vaccines: inactivated vaccines, vaccines which contain live microorganisms in various degree of attenuation, monovalent or polyvalent vaccines, each of them being used in circumstances specified by producer [5]. In research conducted in the study we tested four live vaccines with different degrees of attenuation.

2. Materials and methods
To determine the immune response after vaccination in broiler chickens four vaccines against avian infectious bursitis (two inland produced and two imported) were tested: 

BIAVAC Vaccine – lyophilized live vaccine obtained from an intermediary strain of IBDV. The vaccine was administered in drinking water - 1000 doses were dissolved in 10l of clean and cold water added with 50g milk powder for the vaccine protection.

BIAROMVAC-PA Vaccine – lyophilized live vaccine obtained from an attenuated strain of IBDV (BIA-PA). The vaccine was administered in drinking water - 1000 doses were dissolved in 20l
of clean, cold, and without disinfectants water added with 0.5% defatted milk.

**GUMBORO VACCINE NOBILIS 228E**

Vaccine – lyophilized live vaccine obtained from an intermediary strain of IBDV (228E). The vaccine was administered in drinking water, 1000 doses/10l water for 1000 broilers.

**LIVE VIRUS VACCINE TABLETS GUMBORO, M.B. STRAIN**

Vaccine – lyophilized live vaccine that contains intermediary and attenuated MB strain. The vaccine was administered diluted in drinking water, 1000 doses in 20l water added with 0.5% defatted milk for the vaccine protection against chlorine and disinfectant residues.

The research was made in production conditions on 44,400 broiler chickens maintained in industrial system and raised on bedding and in batteries. The broilers were kept in four poultry houses, each of them representing an experimental group. We mention that vaccines were administered only one time, according with the following scheme (Table 1).

Vaccines efficiency was assessed by immunoenzymatic test. In that purpose, for each poultry house, 20 broilers were isolated and identified by a tibial ring, their immune response being followed between 5 and 42 days of age.

To establish the antibodies titer, blood samples were gathered at the age of 5, 8, and 11 days (before vaccination), 14 days (in the day of vaccination), and after that at the age of 21, 28, 35, and 42 days. The level of antibodies was measured using Infectious Bursal Disease Virus Antibody Test Kit (IDEXX Laboratories, USA).

### 3. Results and discussion

#### Evolution of the anti-IBDV antibodies titer in broilers vaccinated with BIAVAC

To evaluate the efficacy of the vaccine was considered necessary to establish the level of maternal antibodies in the moment of his administration. The obtained data showed that maternal antibodies had a uniform decrease during the four checking (Table 2, Figure 1).

Diminution of maternal antibodies from a determination to another was significant ($p<0.0001$), from a 2607 value recorded at the age of 5 days, to 2287 at 8 days, 1841 at 11 days, as the time of vaccination the titer reached the value of 1331. Immune status of the poultry was uniform at the age of 5 days, coefficient of variation being under 10% (7.46%). This characteristic was maintained until age of 11 days, as the time of vaccination (the age of 14 days) the individual decrease of the maternal antibodies being slightly uneven (C.V. = 11.77%).

Analyzing the results about individual antibodies titer during research, the significant differences were observed both in poultries and phases. Thus, if the maternal antibodies titer was 1331 in the beginning of the experiment, at seven days post-vaccination presented a significant decrease ($p<0.0001$) reaching the minimal value of 185 units, with a distribution of values between 1005 and 254, issue highlighted by the high coefficient of variation (19.43%).

We consider this significant decrease ($p<0.0001$) being determined both by the action of administered antigen to the maternal antibodies and by the latency period specific for initiation of the immune response [6].

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>BIAVAC</th>
<th>BIAROMVAC-PA</th>
<th>GUMBORO VACCINE NOBILIS 228E</th>
<th>GUMBORO VACCINE LIVE VIRUS “MB”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of poultry</td>
<td>7000</td>
<td>7000</td>
<td>6400</td>
<td>24000</td>
</tr>
<tr>
<td>Keeping mode</td>
<td>industrial system, on bedding</td>
<td>industrial system, on bedding</td>
<td>industrial system, on bedding</td>
<td>industrial system, in batteries</td>
</tr>
<tr>
<td>Age of the poultries (days)</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Vaccine administration</td>
<td>in drinking water</td>
<td>in drinking water</td>
<td>in drinking water</td>
<td>in drinking water</td>
</tr>
<tr>
<td>Drinking diet (hours)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Diluted vaccine administered/poultry (ml)</td>
<td>10</td>
<td>20</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>

### Table 1. Vaccines administration protocol
Table 2. Antibodies titer’s values determined by experimental vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Maternal antibodies titer at the age of:</th>
<th>Vaccination at the age of:</th>
<th>Post-vaccination antibodies titer after administration at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 days</td>
<td>8 days</td>
<td>11 days</td>
</tr>
<tr>
<td>BIAVAC</td>
<td>Average</td>
<td>2607</td>
<td>2287</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>194.44</td>
<td>195.49</td>
</tr>
<tr>
<td></td>
<td>C.V.</td>
<td>7.46</td>
<td>8.55</td>
</tr>
<tr>
<td>BARIOMVAC - PA</td>
<td>Average</td>
<td>2526</td>
<td>2179</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>171.18</td>
<td>183.27</td>
</tr>
<tr>
<td></td>
<td>C.V.</td>
<td>6.77</td>
<td>8.41</td>
</tr>
<tr>
<td>GUMBORO VACCINE NOBILIS 228 E</td>
<td>Average</td>
<td>2187</td>
<td>1892</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>168.7</td>
<td>136.5</td>
</tr>
<tr>
<td></td>
<td>C.V.</td>
<td>7.71</td>
<td>7.21</td>
</tr>
<tr>
<td>LIVE VIRUS VACCINE TABLETS GUMBORO, M.B. STRAIN</td>
<td>Average</td>
<td>2091</td>
<td>1783</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>146.99</td>
<td>123.90</td>
</tr>
<tr>
<td></td>
<td>C.V.</td>
<td>7.03</td>
<td>6.95</td>
</tr>
</tbody>
</table>

Figure 1. Antibodies titer dynamics

Moreover, many speciality data show this trend in antibodies titer. Thus, Hair-Bejo and Ng [7] have tested the effectiveness of a live attenuated vaccine with intermediary pathogenity to induce antibodies anti-IBDV in broilers vaccinated at the age of one and 14 days. Based on antibodies titer determined by ELISA at the age of 1, 7, 14, 21, 28, 35, and 42 days, they have found that the vaccine induced high antibodies titer in the poultries vaccinate at the age of 14 days, while in the birds vaccinated in the first day of life the antibodies anti-IBDV level was significantly reduced due to the presence of maternal antibodies in high titer.

The obtained results show that while maternal antibodies level decreases, antibodies anti-IBDV induced by the vaccine ensure adequate immunological protection of the broilers. Thus, at day 14 after vaccination, there was an antibodies titer of 1157, which is considered to provide adequate protection. Also, it was registered a uniformity of the immune response after vaccination, the coefficient of variation being 9.67%.
At 21st day post-vaccination there was an increase in antibodies level with an average of 2374, which is 12.83 time higher than the mean antibodies titer in the 7th day after vaccination [8], with a uniformity of the immune response.

At 28th day post-vaccination the antibodies titer maintains his ascensional trend, although this increase is not so dramatic and uniform (C.V. = 14.05 %). At the end of the experimental period, the antibodies level reached a peak of 3859, and the coefficient of variation decreased to 7.22%.

Although titer of the antibodies anti-IBDV is characterized initially by low values because of maternal antibodies, at the end of the experiment reach peak of 3859. We mention that values obtained after immunization with Biavac vaccine during experimental period are lower than those reported by the producer [9].

Evolution of the anti-IBDV antibodies titer in broilers vaccinated with BIAROMVAC

The broilers group vaccinated with Biaromvac showed an antibodies titer of 2526 at the age of 5 days, with a decrease to the 1361 value in the moment of vaccination (Table 2).

Maternal antibodies level was uniform during the first 11 days of life, the coefficient of variation being under 10%. At the age of 14 days, in the moment of vaccine administration, the level showed a dispersion of individual values between 976 and 1948, resulting in a coefficient of variation of 18.98%.

Analyzing the results of the antibodies titer evolution is found that at 7 days post-vaccination antibody levels have a strong reduction (217) comparing to the titer recorded at the moment of vaccination (1361) and a coefficient of variation of 20.85%.

At 14th day after vaccination there was a significant increase (p<0.0001) in antibody titer which is maintained until the end of the experimental period. At the end of experimental the level of anti-IBDV antibodies reaches a peak of 3799.

Although the action of viral antigen was stronger on maternal antibodies, after 7 days of vaccination there was an intense immune response, the antibodies titer reaching the value of 2613, and subsequently the dynamic of immune response growth was less intense.

Scutaru et al. [10] researching the dynamics of antibodies induced by the PA vaccine strain used for obtaining Biaromvac vaccine, determined that antibodies level have increased since the 15th day after vaccination, with a peak value at 25-30 days after immunization.

Evolution of the anti-IBDV antibodies titer in broilers vaccinated with GUMBORO VACCINE NOBILIS 228 E

Maternal antibodies level recorded at the moment of vaccination was 1007, compared to a value of 2187 in the 5 days old broiler. Note that the immune status of poultries in the first two weeks of life was uniform, but with a decreasing dynamic (Table 2, Figure 1). Coefficients of variation recorded in each stage were 7.43% in average.

Analyzing the results is found that antibodies titer after vaccination with Gumboro vaccine nobilis 228 E have the same chart as Biaromvac vaccine, but with different levels.

The results about the dynamics of antibodies induced by Gumboro vaccine nobilis 228 E are similar to those reported by Ceica and Vasiu in 2008 [11]. Note that starting with 21st day after vaccination, the level of post-vaccination antibodies was significant higher than maternal antibodies titer recorded in 5 days old broilers (p<0.0001).

Evolution of the anti-IBDV antibodies titer in broilers vaccinated with LIVE VIRUS VACCINE TABLETS GUMBORO, M.B. STRAIN

The group of broilers isolated from the flock which was to be vaccinated with Live virus vaccine Gumboro, M.B. strain had a uniform immune status at the age of 5 days, coefficient of variation being under 10%. This characteristic was maintained until age of 14 days, but with a downward dynamics (Table 2, Figure 1).

At 7th day after vaccination an uneven decreasing of maternal antibodies was recorded, this trend determining a different immune response after vaccination. Thus, at this moment, the broilers were found without immunological protection, the average of maternal antibodies titer being 49, with a coefficient of variation of 47.81%.

At 14 days post-vaccination, although antibodies level experienced a significant increase (p<0.0001), the immune response of broilers was different (C.V. = 22.50%). We mention that the immune response has remained uneven at 21 days after vaccination (14.67 %), although it presented values significantly higher than those recorded at 14 days post-vaccination.
At the end of the experimental period there was a uniformity of the broilers' immune response and antibodies level reaches the value of 4029.

Analyzing the dynamics of the post-vaccination immune response in the 14 days age broilers, a decrease in maternal antibodies titer was observed in the first week. Then, the titer begin to rise, the values registered at the end of the experiment being significantly higher than in the moment of vaccination (p<0.0001).

Diminution of the antibodies level at 14 days post-vaccination was 20.14 times higher than maternal antibodies titer, then postvaccinal antibodies titer had a substantial increase of 82.22 times at 28 days.

In conclusion we can say that the dynamics of post-vaccination antibodies titer determined by the four vaccines considered for the study differ from one vaccine to another, although the maternal immune status of the broilers was without significant differences, values recorded providing better protection of poultries until vaccination.

Data obtained on the evolution of maternal antibodies titer was similar to those observed by other authors [12,13]. Moreover, Lucio and Hitchner [14], Baxendale and Lutticken [15] indicates that oil emulsion vaccines can induce an adequate maternal immunity to protect poultries for 4-5 weeks, while chickens coming from parents vaccinated with live vaccines are protected only for 1-3 weeks.

It is noted that in the moment of vaccination, the differences registered between antibody levels of the groups vaccinated with Biavac vaccine and with Biaromvac aren’t significant, but from the 14th day after the administration of vaccines we recorded significant differences. Also, the vaccine Biaromvac neutralizes less maternal antibodies than Biavac vaccine, the average antibodies titer recorded being 217 comparing to 185.

The maternal antibodies level in groups vaccinated with Gumboro vaccine nobilis 228 E and Live virus vaccine tablets Gumboro, M.B. strain was similar, but lower comparing to the titer registered in Biavac and Biaromvac groups.

At 7 days after vaccination, a marked decrease of maternal antibodies was registered, more pronounced for Live virus vaccine. It was also found that differences between antibodies titer registered at 14 days post-vaccination are maintained (Gumboro vaccine nobilis 228E – 1813; Live virus vaccine tablets Gumboro, M.B. strain – 833). We mention that in the end of the experimental period the antibodies level induced by Live virus vaccine tablets Gumboro, M.B. strain reaches a value significantly higher than Gumboro vaccine nobilis 228 E.

Note that antibodies titer after administration of the four tested vaccines showed a dynamic upward by the end of the experimental period, but with different values from one vaccine to another (Figure 1).

Overall analysis shows that at 7th day after vaccination, maternal antibodies titer decreases below the limit considered to protect against possible infection.

Thus, Lucio and Hitchner [14] showed that if antibody levels fall below 1:100, poultries are 100% susceptible to infection, and titers between 1:100-1:600 gives an approximately 40% protection against exposure to virus.

Skeeles [16] argues that the titers must be under 1:64 before the vaccination with an attenuated strain of avian infectious bursitis virus to have maximum efficiency.

The antibodies level recorded at 7th day post-vaccination is not consistent with the results obtained by other authors. Thus, in a study conducted during 1990-1992 in a poultry unit on broilers obtained both from parents vaccinated against avian infectious bursitis virus and parents unvaccinated, Pop et al. [17] observed that the immune response after vaccination with two live attenuated vaccines was different. For broilers coming from parents vaccinated against avian infectious bursitis virus and parents unvaccinated, antibody titers after administration of vaccines were maintained until 14th day post-vaccination.

At 21st day post-vaccination the highest values of antibody titer occurred in group vaccinated with Biaromvac and in the end of the experimental in group vaccinated with Live virus vaccine tablets Gumboro, MB strain.

4. Conclusions

Maternal antibody titers showed a downward trend, but with elevated values in the moment of vaccines administration, between 1007 and 1361. Between antibodies titers induced by Biavac, Biaromvac vaccines and Gumboro vaccine nobilis 228E during experiment differences were observed, these becoming insignificant at 28 days post-vaccination.
Live virus vaccine, M.B. strain works most prominent on maternal antibodies comparing to the other vaccines, resulting the highest antibodies titer at the end of the experiment. Antibody titers recorded at 7th day after vaccination does not protect broilers against possible infections.

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