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Original Article

PRIMING OF CHIKENS WITH LIVE AND INACTIVATED IBC VACCINE

Babak V.A.[1,*], Gusev A.A.[2]

[1] Branch of National Center for Biotechnology LLP (4-88/17, Stepnogorsk, Akmola region, 021500, Republic of Kazakhstan).
[2] JSC "Pokrovsky Plant of Biopreparations", 601125, Vladimir region, Petushinsky district, village Volginsky, Russian Federation
[*]vgavm2003@mail.ru

ABSTRACT

The research gives the results of the experiments, which studied the effect of priming the chickens with a live and inactivated vaccine against the IBC virus, and subsequent immunization with a live vaccine. The research demonstrates the results of the study of optimal schemes of birds' immunization with live and inactivated vaccines, when the greatest specific effect is achieved. It has been established that priming chickens that do not contain antibodies against IBC with both live and inactivated vaccines causes the formation of intense immunity in them, which indicates a mature immune system capable of actively responding to a foreign antigen in the first days of life. Priming chickens with a live vaccine based on the background of maternal antibodies is not accompanied by the synthesis of antibodies, unlike priming with an inactivated vaccine, which leads to the formation of insignificant humoral immunity. Vaccination chickens containing maternal antibodies with live vaccine, primed with both live and inactivated vaccine depends on the content of specific antibodies in their body and is more effective when the level of antibodies in their body is low.

Key words: infectious diseases, poultry farming, vaccination, chicken priming, transovarial antibodies, humoral immunity, infectious bronchitis of chickens (IBC).

INTRODUCTION

Viral diseases of birds remain one of the most difficult problems of the infectious pathology of birds in most countries of the world, including Russia. They have a massive coverage of the livestock, are accompanied by high morbidity in birds, mortality, loss of productivity, and cause significant damage to poultry farming [1, 2, 3].

Among the infectious diseases of birds of viral etiology, infectious bronchitis of chickens (IBC, Bronchitis infectiosa avium) occupies a special place, causing great economic damage to the poultry industry. In chickens, the infection manifests itself as a respiratory and uremic syndrome, in hens - it damages the germinal organs, which leads to a long-term decrease in egg production. The causative agent is an RNA-containing virus of the *Coronaviridae* family, virions have a lipoprotein envelope; they are polymorphic and range in size from 80 to 200 nm, and multiply on chicken embryos. The low stability of the virus in the external environment is compensated by its extremely high horizontal contagiousness through the airborne and alimentary route. The main source of infection are sick and recovered chickens and hens that shed the virus into the environment or remain virus carriers up to 100 days after the illness [4].

Isolation of the virus from the body of a sick bird occurs with saliva; discharge from the nose, eyes, and with feces. The bird gets infected mainly by aerogenic route, as well as by ingestion of infected feed and water. Sick roosters shed the virus with semen within 20 days after the infection, so sexual transmission is possible. In addition, the virus is transmitted transovarially at acute, chronic and asymptomatic course of the disease [4].

In modern poultry farms, the protection of young birds from IBC remains relevant despite the availability of highly effective vaccines. Vaccine manufacturers offer not only their own preparations, but also recommend the use of various schemes for their use in poultry farms of various directions [5, 6].

Live and inactivated vaccines are used for the specific prevention of infectious bronchitis in chickens (IBC). Both field isolates of the IB virus and attenuated strains are used when manufacturing inactivated vaccines. The vaccines made from "H-120" and "4/91" strains of the virus are most widely used among live vaccines. The vaccine is applied by various methods: ocularly, intranasally, by drinking with drinking water and with a spray method [5, 7, 8].

Currently, to create early active immunity in chickens, immunization of chickens of the first days of life with live and inactivated vaccines (priming) is applied in poultry farming. The spray method has received the greatest application. The spray method of introducing the vaccine involves spraying it, followed by keeping the birds in an aerosol cloud for

15-20 minutes. The respiratory tract is the gateway of infection for many pathogenic microorganisms for poultry; therefore, good local immunity to the mucous membranes of the respiratory organs is the most important condition for protection against these pathogens. This method ensures effective penetration of vaccine strains against respiratory infections into immunocompetent tissues of the upper respiratory tract [9, 10, 11, 12].

The spray method is an efficient and cost-effective way to vaccinate large numbers of birds. Spray vaccination provides a number of advantages in the mass processing of poultry: it requires less time and labor, minimizes the stress that the bird experiences, and induces good local and systemic immune responses of the body [1, 2].

Priming chickens with an inactivated IBC vaccine has also gained recognition and is being introduced into the poultry industry. Both monovalent and bivalent vaccines against IB and NB are used as preparations for priming [8, 13, 14].

Chicks from vaccinated parent flocks are known to contain maternal antibodies that suppress the immune response to «priming», especially when live vaccines are used. Against the background of passive antibodies, the replication of the vaccine virus can be significantly suppressed or will not occur at all. Therefore, immunization with a live vaccine against the background of maternal antibodies will not give the desired effect. The negative effect of maternal antibodies is less expressed when immunized with live vaccines on the mucous membranes compared with parenteral administration. There is some information that oral vaccination with live vaccines can induce systemic and local immunity despite the presence of maternal antibodies to the virus. When emulsion inactivated vaccines are used, maternal antibodies are not such a critical factor in the development of active immunity, because when they are introduced, there is the synthesis of a small amount of antibodies and the immunological memory is formed. [5, 9, 15].

At the same time, priming with live and inactivated vaccines according to the background of maternal antibodies does not contribute to the formation of intense and long-term humoral immunity, therefore primed chickens are vaccinated again. In this case, the most common method to prime chickens is to make them drink a live vaccine and sometimes to use an inactivated vaccine parenterally. At the same time, before vaccination of primed chickens, it is necessary to conduct preliminary studies to determine the amount of antibodies in the blood serum of chickens, the content of which may adversely affect the formation of humoral immunity [9, 13, 16, 17]. Therefore, in order to form full-fledged active immunity in chickens primed with both live and inactivated vaccines, re-immunization should be carried out after the disappearance or significant weakening of maternal immunity. However, waiting can lead to the susceptibility to infection, which is especially dangerous when the environment is highly infected with pathogenic pathogens [9, 14, 15, 16].

The main purpose of this study was to assess the intensity of immunity in chickens primed with live and inactivated vaccine and subsequently immunized with live IBC vaccine as well as to study the most effective schemes to immunize primed chickens with a live vaccine.

MATERIALS AND METHODS

In the studies, a live IBC vaccine from the H-120 strain and an inactivated emulsified IBC vaccine with an ISA-70 adjuvant from “Seppik” company were used. The spray method was used to prime chickens with a live vaccine. Immunization with the inactivated vaccine was carried out parenterally at a dose of 0.2 ml. The experiments were carried out on the chickens Free from Pathogenic Flora (FPF) from the “Loman” company and commercial chickens of the “Cobb” cross which were 2 days old, obtained from a poultry farm.

During the experiment, the level of antibodies to the IBC virus was monitored in the blood serum of birds. ELISA kit from “Synbiotics” company was used to assess the intensity of immunity. The level of production of antibodies to the IBC virus gave the idea about the severity of the immune response in chickens.

At all stages of the experiments, chickens were primed at the age of two days.

In the first experiment, we studied the formation of humoral immunity in chickens free of antibodies against the IBC virus and chickens containing maternal antibodies, in response to priming with live and inactivated vaccines. Five groups of chickens, 5 heads in each (n=5) were involved in the experiment.

The first and second groups of FPF chickens

did not contain antibodies against IBC. The third, fourth and fifth groups of chickens contained maternal antibodies.

Chickens of the first and third groups at the age of two days were primed with a live vaccine, and the chickens of the second and fourth groups - with an inactivated vaccine. The fifth group served as a control one for the dynamics of the decrease in maternal immunity.

In the second experiment, we studied the formation of humoral immunity in the chickens primed with a live vaccine containing maternal antibodies in response to subsequent immunization with a live vaccine. Three groups of chickens, 10 heads in each (n=10), were involved in the experiment. The first group of chickens was vaccinated with a live vaccine on the 10th day, the second on the 17th day. The third group served as a control one for the dynamics of the decrease in maternal antibodies, the chickens of this group were primed, but not vaccinated.

In the third experiment, we studied the formation of humoral immunity in the chickens primed with an inactivated vaccine containing maternal antibodies in response to immunization with a live vaccine. Three groups of chickens, 10 heads in each (n=10), were involved in the experiment. The first group of chickens was immunized with a live vaccine on the 10th day and the second group on the 17th day. The third group served as a control for the dynamics of the decrease in maternal antibodies, the chickens of this group were primed, but not vaccinated.

Statistical processing of the research results was carried out in Microsoft Office Excel 2016 using a statistical data analysis package, and using the "STATISTICA 8.0" program. The significance of all published values was not lower than the first criterion threshold of reliability ($p<0.05$). We used generally accepted methods of statistical processing of experimentally obtained samples of varying variables for biotechnological research 18.

RESULTS

The results of the **first** experiment on the effect of priming chickens with live and inactivated IBC vaccine are presented in Table 1.

From the data of Table 1, it can be seen that in the chickens of the first group, free from maternal antibodies, priming with a live vaccine was accompanied by the synthesis of antibodies from the 10th day, reaching a maximum amount by the 17th day (2770 units). In the following days, up to the 35th day, their slight decrease was noted.

In the second group of chickens free of maternal antibodies, primed with an inactivated vaccine, antibody synthesis was also observed from the 10th day and gradually increased up to the 3rd day. On the 35th day, the antibody titer in this group was significantly higher than in the first group of chickens primed with the live vaccine (1.50 times, at $p \leq 0.05$).

Priming of chickens of the third group containing maternal antibodies with a live vaccine did not lead to a decrease in maternal antibody titers, but in the period from 10th up to 17th day, it was accompanied by an insignificant increase of the amount of antibodies, followed by their decrease and complete disappearance by day 35th.

In the chickens of the fourth group, containing maternal antibodies and primed with an inactivated vaccine, the synthesis of antibodies began from the 10th day, their number gradually increased and by the 17th day their level increased up to 2550 units, but by the 35th day it insignificantly decreased up to 2190. The dynamics of the decrease of maternal immunity was monitored using the fifth group.

Similar experimental data in response to priming were obtained by the authors of studies using live vaccines against Newcastle disease and IBD [9, 13, 17].

Table 1. Antibody titers in chickens primed with live and inactivated vaccines

№№ Chicken groups	Vaccine/Group	Antibody titer in ELISA (mdl mean, M)				
		2 day	10 day	14 day	17 day	35 day
1	Live IBC	negative	1053	1980	2770	2540
2	Inactivated IBC	negative	920	1840	2980	3820
3	Live IBC (**on antibodies**)	**3740**	2190	2550	1550	350
4	Inactivated IBC (**on antibodies**)	**3620**	2040	2360	2550	2190
5	Control of maternal antibodies	3730	2400	1960	1170	220
(n=5, $p \leq 0,05$)						

Thus, it has been established that priming chickens that do not contain antibodies against IBC with both live and inactivated vaccines causes a significant formation of intense immunity in them. This indicates a mature immune system capable of actively responding to a foreign antigen in the first days of life. At the same time, priming chickens with a live vaccine using the background of maternal antibodies is not accompanied by the synthesis of antibodies, in contrast to priming with an inactivated vaccine, which leads to the formation of insignificant humoral immunity.

The results of the **second** experiment on the study of the formation of immunity in chickens primed with a live vaccine containing maternal antibodies, and subsequently vaccinated with a live vaccine on days 10th and 17th, are presented in Table 2.

Table 2 shows that in the chickens of the first group, primed with a live vaccine against the background of maternal antibodies, subsequent immunization with a live vaccine on day 10th did not lead to any increase in antibody titers. Their gradual decrease to almost complete disappearance by day 25th and day 35th was noted. The dynamics of their decrease practically did not differ from the decrease in maternal antibodies in the control third group of chickens. The results obtained correlate with Simakova's N.M. researches on the effect of vaccination against IBC on immunity indicators in chickens with high titers of transovarial antibodies [16].

In the second group of chickens primed with a live vaccine against the background of maternal antibodies, followed by immunization with a live vaccine on day 17th, the amount of antibodies increased by day 25th and decreased by day 35th. The level of antibodies in the control, primed group of chickens began to decrease from the 17th day and was practically not recorded by the 25th and 35th days.

It has been established that the effectiveness of chicken vaccination with a live vaccine containing maternal antibodies and primed with a live vaccine depends on the content of antibodies in their body and is more successful when the level of antibodies in their body is low (for example, as in the second experiment on day 17th).

The results of the **third** experiment on the study of the formation of immunity in chickens primed with an inactivated vaccine containing maternal antibodies and subsequently vaccinated with a live vaccine on days 10th and 17th are presented in Table 3.

Table 3 shows that in primed chickens of the first group, immunization with a live vaccine on the 10th day was accompanied by a slight increase in antibody titers on day 17th, followed by a slight decrease by days 25th and 35th.

In the second group of primed chickens, immunization with a live vaccine, which was carried out at the 17 days of age, was accompanied by a high rise in antibody titers by day 35th - up to 4560 units. This was an extremely significant increase, both in relation to the control group and experimental group No. 1, vaccinated on the 10th day, (which confirms the two-sample t-test statistical data analysis tool (t-statistic, t-critical two-tailed, and P(T<=t) two-tailed < specified significance level P≤0.05)).

In control group chickens containing maternal antibodies and primed with an inactivated vaccine, antibody synthesis began on the 10th day, and was maintained at the level of 2280 units on the 14th day, and remained at the level of 2200 units by 35th day.

The data in Table 3 show that in chickens primed with the inactivated vaccine and vaccinated with the live vaccine, the formation of antibodies was noted both in the first and second groups. The most significant amount of antibodies up to 4560 units was noted in the second group of chickens vaccinated on 17th day with a lower content of specific antibodies.

Table 2. Antibody titers in chickens primed and vaccinated with a live vaccine on days 10th and 17th.

№№ Chicken group	Vaccine/group	Antibody titer in ELISA (mdl mean, M)					
		2 day	10 day	14 day	17 day	25 day	35 day
1	Live IBC vaccinated on the 10th day	3490	2640	2280	1570	680	250
2	Live IBC vaccinated on the 17th day	3570	2250	2560	1480	2120	1590
3	Control group (primed with live vaccine)	3770	2520	2510	1650	150	100
(n=10, p ≤ 0,05)							

Table 3. Antibody titers in chickens primed with inactivated vaccine and vaccinated live on days 10th and 17th.

№№ Chicken group	Vaccine/group	Antibody titer in ELISA (mdl mean, M)					
		2 day	10 day	14 day	17 day	25 day	35 day
1	Live IBC vaccinated on the 10th day	3950	2250	2160	2470	2360	2270
2	Live IBC vaccinated on the 17th day	3760	2270	2140	1880	3120	4560
3	Control group (primed with inactivated vaccine)	3740	2190	2280	1900	2150	2200
(n=10, p ≤ 0,05)							

DISCUSSION

In our work, we investigated the humoral response of chickens that do not contain maternal antibodies and those ones that contain them to priming with live and inactivated vaccine against IBC. We also studied the effect of priming chickens on the formation of humoral immunity in response to vaccination with a live vaccine.

It has been established in the experiments that priming of chickens that do not contain maternal antibodies against IBC is accompanied by the formation of humoral immunity, both to the live IBC vaccine and to the inactivated vaccine.

The study of humoral immunity in chickens containing maternal antibodies, when primed with a live vaccine, was accompanied by a slight increase in antibody titer in the period from 10th to 14th day, followed by their decrease and complete disappearance to 350 by the 35th day of observation.

In chickens primed with an inactivated vaccine against the background of maternal antibodies, there was a slight decrease in antibody titers by day 10th (2040 units). On following days, the antibody titer slightly increased up to 2550 units by the 17th day and gradually decreased up to 2190 on the 35th day. Therefore, priming chickens containing maternal antibodies with a live vaccine does not cause the formation of an active immunity. This can be explained by the fact that the antibodies contained in the body of chickens block the reproduction of the vaccine virus and it, (the virus), without accumulating in the body, does not cause the activation of the immune system.

When vaccinated with an inactivated vaccine, antibodies also partially neutralize the viral antigen, which is contained in the vaccine, but its remaining amount activates the formation of specific immunity. These results indicate the dependence of the formation of humoral immunity on the content of maternal antibodies that suppress the reproduction of the vaccine virus and partially block the immune response to the inactivated vaccine.

Priming chickens containing maternal antibodies with a live vaccine (second experiment), followed by their vaccination with a live vaccine on day 10th, was not accompanied by an increase in the content of antibodies. In this case, the reproduction and accumulation of the vaccine virus did not occur, since it was blocked by a high concentration of maternal antibodies. In the group of primed chickens immunized with a live vaccine on the 17th day, the formation of antibodies was noted, which was recorded until the 35th day. This is explained by the fact that by the 17th day the amount of maternal antibodies in the body of chickens has decreased, and priming with a live vaccine against the background of maternal antibodies was not accompanied by the formation of humoral immunity and by the synthesis of antibodies. A small amount of maternal antibodies present in chickens did not prevent the reproduction of the vaccine virus, which contributed to the formation of active immunity and the synthesis of specific antibodies.

Priming chickens containing maternal antibodies with an inactivated vaccine (third experiment), followed by their vaccination on day 10th with a live vaccine, was accompanied by a slight increase in the content of antibodies from days 10th up to 17th, and further the intensity of immunity practically did not differ from the control group of chickens primed but not vaccinated.

An insignificant increase of humoral antibodies from day 10 to day 17 is explained by the fact that when chickens are primed with an inactivated vaccine, by day 10 an immunological memory is formed for the viral antigen present in the inactivated vaccine. Immunological memory responds positively to immunization with a live vaccine by the synthesis of antibodies.

In the group of primed chickens immunized with a live vaccine, active synthesis of antibodies was observed on day 17th, which reached 4560 units by 35th day. This is due to the fact that vaccination coincided, on the one hand, with the period of a decrease in maternal antibodies. On the other hand, it coincided with the period of completion of the productive phase of immunogenesis induced by the primary administration (priming) of an inactivated vaccine, i.e. with the time of insignificant content of synthesized antibodies in response to priming.

Thus, an increase in antibody titers can be explained by a positive reaction of immunological memory cells to a multiplying vaccine virus based on a low antibody background.

Based on the results obtained, we can concluded that when priming chickens containing maternal antibodies, the period of activity of immune plasma cells producing antibodies caused by live and inactivated vaccines is limited to two weeks, during which an insignificant amount of antibodies is formed. Therefore, revaccination of chickens with a live vaccine after 10 days was not accompanied by an increase in antibody titer, as it coincided, on the one hand, with the period of persistence of maternal antibodies, and on the other hand, with a period of insignificant accumulation of synthesized antibodies.

Vaccination of primed chickens with a live vaccine 17 days after the initial administration of the vaccine, that is, during the completion of the productive phase of immunogenesis induced by the primary, administration (priming) of live and inactivated vaccines and a decrease in maternal antibodies was effective. This is explained by the fact that a small amount of antibodies was unable to prevent the reproduction of the vaccine virus and neutralize the viral antigen contained in the inactivated vaccine.

At the same time, it was established that chickens primed with an inactivated emulsion vaccine, when immunized with a live vaccine, formed more intense immunity than those primed with a live vaccine.

CONCLUSION

1. Priming chickens that do not contain antibodies against IBC with both live and inactivated vaccines causes the formation of intense immunity in them, which indicates a mature immune system capable of actively responding to a foreign antigen in the first days of life.

2. Priming chickens with a live vaccine against the background of maternal antibodies is not accompanied by the synthesis of humoral antibodies, in contrast to priming with an inactivated vaccine, which leads to the formation of insignificant humoral immunity.

3. Vaccination of primed chickens with live vaccine de-

pends on their specific antibody content and is more effective when their antibody level is low.

4. It was stated that in chickens primed with inactivated vaccine, immunization with a live vaccine carried out when they are 17 days old, was accompanied by a high rise in antibody titers by the 35th day - up to 4560 units. It was considered an extremely significant increase, both in relation to the control group and to the experimental group vaccinated on day 10th.

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ПРАЙМИРОВАНИЕ ЦЫПЛЯТ ЖИВОЙ И ИНАКТИВИРОВАННОЙ ВАКЦИНОЙ ПРОТИВ ИБК

Бабак В.А.[1,*], Гусев А.А.[2]

[1] Филиал ТОО «Национальный центр биотехнологии» (4-88/17, г. Степногорск, Акмолинская область, Республика Казахстан, 021500)
[2] АО «Покровский завод биопрепаратов», Владимирская область, Петушинский район, пгт. Вольгинский, 601125, Россия.
**vgavm2003@mail.ru*

АБСТРАКТ

В исследовании приведены результаты опытов по исследованию влияния праймирования цыплят живой и инактивированной вакциной против вируса ИБК, и последующей иммунизации живой вакциной. Приведены результаты по изучению оптимальных схем иммунизации птиц живой и инактивированной вакцинами, при которых достигается наибольший специфический эффект. Установлено, что праймирование цыплят, не содержащих антител против ИБК, как живой, так и инактивированной вакциной вызывает у них формирование напряжённого иммунитета, что свидетельствует о сформировавшейся иммунной системе, способной в первые дни жизни активно реагировать на чужеродный антиген. Праймирование цыплят живой вакциной по фону материнских антител не сопровождается синтезом антител, в отличии от праймирования инактивированной вакциной, которое приводит к формированию незначительного гуморального иммунитета. Вакцинация живой вакциной цыплят, содержащих материнские антитела, праймированных как живой, так и инактивированной вакциной, зависит от содержания в их организме специфических антител и более эффективна, когда уровень антител в их организме низкий.

Ключевые слова: инфекционные заболевания, птицеводство, вакцинопрофилактика, праймирование цыплят, трансовариальные антитела, гуморальный иммунитет, инфекционный бронхит кур (ИБК).

БАЛАПАНДАРДЫ ТЖБ ҚАРСЫ ТІРІ ЖӘНЕ ИНАКТИВИРЛЕНГЕН ВАКЦИНАМЕН ПРАЙМЕРЛЕУ

Бабак В.А.[1,*], Гусев А.А.[2]

[1] «Ұлттық биотехнология орталығы» ЖШС Филиалы (4-88/17, Степногорск қ-сы, Ақмола облысы, Қазақстан Республикасы, 021500).
[2] РҒА корреспондент-мүшесі, профессор, «Покровский завод биопрепаратов» АҚ, Владимир облысы, Петушин ауданы, пгт. Вольгинский, 601125, Ресей.
**vgavm2003@mail.ru*

ТҮЙІН

Зерттеуде балапандарды тірі және инактивирленген ТЖБ вирусына қарсы вакцинамен праймерлеудің және кейіннен тірі вакцинамен иммундаудың әсерін зерттеу бойынша тәжірибелердің нәтижелері келтірілген. Құстарды тірі және инактивирленген вакциналармен иммундау кезінде ерекше әсерге қол жеткізілетін оңтайлы схемаларды зерттеу нәтижелері келтірілген. ТЖБ қарсы антиденелері жоқ балапандарды тірі және инактивирленген вакцинамен праймерлеу оларда қарқынды иммунитеттің қалыптасуына әкелетіні анықталды, бұл өмірдің алғашқы күндерінде бөгде антигенге белсенді жауап бере алатын қалыптасқан иммундық жүйені куәландырады. Аналық антиденелердің фонында балапандарды тірі вакцинамен праймерлеу инактивирленген вакцинамен праймерлеуден айырмашылығы шамалы гуморальдық иммунитеттің қалыптасуына әкелетін антиденелер синтезімен қатар жүрмейді. Тірі және белсенді емес вакцинамен праймерленген аналық антиденелері бар балапандарды тірі вакцинамен вакцинациялау олардың денесіндегі арнайы антиденелердің құрамына байланысты және олардың денесіндегі антиденелердің деңгейі төмен болған кезде тиімдірек болады.

Негізгі сөздер: жұқпалы аурулар, құс шаруашылығы, вакцина профилактикасы, балапандарды праймерлеу, трансвариальды антидене, гуморальды иммунитет, тауықтардың инфекциялы бронхиті (ТИБ).