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Original Article

Evaluation of effects of Levamisole hydrochloride on the humoral immune response of broiler chickens vaccinated against Infectious Bursal Disease (IBD)

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ABSTRACT

Infectious bursal disease (IBD) is one of the factors that weakens the immune system, which causes severe economic damage to the poultry industry. Levamisole hydrochloride as an anti-parasitic drug also has immunogenic effects. This study was aimed to investigate the stimulating and strengthening effects of Levamisole hydrochloride on the immune system of broiler chickens vaccinated against infectious bursal disease. A total of 108 one-day-old chicks (Ross308) were divided into three groups considering 18 chicks in each group. In the first group, oral Levamisole hydrochloride from the first day until the end of the breeding period and in the second group, was administrated simultaneously with the infectious bursal disease vaccine from the 19th day until the end of the breeding period. The third group (control) did not take any medicine. Blood samples were taken from each group on the 29th and 40th days for ELISA test and the percentage of blood lymphocytes. The statistical results showed a significant difference in the effect of Levamisole hydrochloride in the first and second groups compared to the control group ($P<0.05$). The weight of the cloacal bursa and spleen increased in the study groups compared to the control group at the end of the period. According to the results, it can be concluded that Levamisole hydrochloride can strengthen the immune effect of IBD vaccine.

بررسی اثرات لوامیزول هیدروکلراید بر پاسخ ایمنی هومورال جوجه های گوشتی واکسینه شده علیه بیماری بورس عفونی

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چکیده

بیماری بورس عفونی (IBD) به عنوان یکی از عوامل تضعیف کننده سیستم ایمنی است که خسارت اقتصادی شدید به صنعت طیور وارد می کند. هدف از این مطالعه، بررسی اثرات تحریکی و تقویتی لوامیزول هیدروکلراید بر سیستم ایمنی جوجه های گوشتی واکسینه شده بر علیه بیماری بورس عفونی می باشد. تعداد ۱۰۸ قطعه جوجه یک روزه (Ross308) به سه گروه با در نظر گرفتن ۱۸ جوجه در هر گروه، تقسیم شدند. گروه اول، لوامیزول هیدروکلراید خوراکی از روز اول تا پایان دوره پرورش و گروه دوم، لوامیزول هیدروکلراید خوراکی همزمان با واکسن بیماری بورس عفونی از روز ۱۹ تا پایان دوره پرورش تجویز شد. گروه سوم (شاهد) هیچ دارویی مصرف نکردند. نمونه خون از هر گروه در روز ۲۹ و ۴۰ برای تست الایزا و درصد لنفوسیت‌های خون گرفته شد. نتایج آماری بیانگر تفاوت معنی داری تاثیر داروی لوامیزول هیدروکلراید در گروه اول و دوم در قیاس با گروه کنترل بود ($P<۰/۰۵$). وزن بورس کلواکی و طحال در گروه های مورد مطالعه نسبت به گروه کنترل در پایان دوره افزایش یافت. با توجه به نتایج، می توان نتیجه گرفت که لوامیزول هیدروکلراید می تواند اثر ایمنی واکسن IBD را تقویت کند.

واژه های کلیدی: جوجه گوشتی، الایزا، بیماری بورس عفونی، لوامیزول هیدروکلراید

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INTRODUCTION

Infectious bursal disease (IBD), in addition to having clinical signs and related mortalities, is also a suppressive immune system. The virus is a member of the Birnaviridae family and has two serotypes (serotypes 1 and 2). The Target of the virus is to destroy the B lymphocytes of cloacal bursa and cause depletion of this organ. Timely vaccination based on virulence and antigenic diversity is one of the important prevention methods. Commercially available vaccines include live attenuated (mild, mild intermediate, intermediate, intermediate plus, or "hot), viral vectored recombinant and inactivated vaccines [1, 2]. Levamisole HCl is a synthetic anthelmintic drug for the animals and poultry. The mechanism of action of the drug is through neuromuscular toxicity. In addition to the anti-parasitic properties of Levamisole, it also strengthens the immune system [3,4,5]. The first immunization of Levamisole on mouse against *Brucella abortus* was reported by Renoux on 1971[6]. Over the years, Levamisole has been widely used in human and animal medicine for its immune properties as well as the treatment of autoimmune, chronic and recurring diseases and its anti-cancer properties [4]. The results of numerous studies show that *Levamisole* is stimulant of immunity system due to its immunization with the activation of macrophages and secretion of IL_2 and cytokines. Levamisole can cause modification of cell immune responses on host immunity which functions of macrophages and T lymphocytes can be better. Levamisole also increases the number of T cells and the ratio of (CD4/CD8) cells. On the other hand, secretions of cytokines caused stimulation and differentiation of T cells activities and subsequently simulated the B cell [3, 7, 8]. Therefore, this study was carried out to specify the immunomodulatory and reinforcement effect of Levamisole on antibody titer against the infectious bursal disease vaccine.

MATERIALS AND METHODS

The 108 one-day-old chickens (*Ross308*) were divided to three equal groups (with three replications). From day one to the end of the period, the rearing conditions were the same for all chicks and the only difference was in the Levamisole medication schedule. The first group (G1), oral Levamisole hydrochloride at a dose of 10mg/kg were administered from day one to the end of the breeding period. The second group (G2), oral Levamisole hydrochloride at a dose of 10 mg/kg was used simultaneously with Infectious Bursal disease –vaccine to the end of the breeding period. The control group (G3) did not take any medicine. Oral IBD vaccine was used on the 19^{th} day. Blood samples (wing vein) were taken at days 29 and 40 and evaluated by ELISA testing (IDEXX standard kit) to evaluate the antibody titer against infection of IBD. The Differential Leukocyte Count (DLC) test was performed on blood samples and the percentage of blood lymphocyte concentration was calculated after preparing the blood sample and Giemsa staining. The weight of the Cloacal bursa and spleen organs in the studied groups was recorded at the end of the breeding period (day 40). For analyzing data, software SPSS15 and Tukey testing were used.

RESULTS

The results indicated that a significant difference of ($P<0.05$) was seen between the groups in terms of the antibody titer and percentage of blood lymphocytes concentration (Tables 1 and 2). On day 29 of the breeding period, the mean antibody titer against IBD virus in the first group (G1) was significantly different from the second and control (G2 and G3) studied groups ($P<0.05$). On day 40 of the breeding period, the mean antibody titer against IBD virus in the first and second groups (G1 and G2) was significantly different from the control (G3) groups ($P<0.05$). There was no significant difference among the first (G1) and second (G2) groups on day 40 in the view of antibody titer ($P>0.05$). The percentage of lymphocytes on days 29 and 40 in the first and second groups was significantly different from the control group ($P<0.05$). The weight of the Cloacal bursa and spleen increased in the first and second groups compared to the control group at the end of the breeding period (Table 3). No signs of disease or dangerous challenges were observed from the beginning to the end of the breeding period in the studied groups.

Table 1. Comparison of the mean and standard deviation (Mean ± SD) of antibody titer on days 29 and 40 among the studied groups.

Group	Day	
	29	40
G1	708.78 ± 168.9 [a]	2104.22 ± 134.85 [a]
G2	448.39 ± 177.5 [b]	2031.11 ± 194.52 [a]
G3	401.78 ± 70.39 [b]	1613 ± 96.53 [b]

Note: Different letters and the numbers in each column above indicate the existence of a significant difference at the 95% level among the groups ($P<0.05$)

Table 2. Comparison of the mean and standard deviation (Mean ± SD) of percentage of lymphocytes on day 29 and 40 among the studied groups.

Group	Day	
	29	40
G1	75.67 ± 1.75 [a]	75.89 ± 1.32 [a]
G2	71.28 ± 2.54 [b]	74.22 ± 2.32 [b]
G3	63.78 ± 3.35 [c]	60.83 ± 2.09 [c]

Note: Different letters and the numbers in each column above indicate the existence of a significant difference at the 95% level among the groups ($P<0.05$).

Table 3. The weight (gr) of Cloacal bursa and spleen at the end of the period (day 40) among the studied groups

Group	Organ	
	Cloacal bursa	spleen
G1	11.3	7.0
G2	10.9	7.2
G3	9.9	6.5

Note: Different letters and the numbers in each column above indicate the existence of a significant difference at the 95% level among the groups ($P<0.05$)

DISCUSSION

IBD is a viral disease that suppresses the immune system. Although the immunosuppression caused by IBDV is principally directed towards B lymphocytes, an effect on cell-mediated immunity has also been demonstrated [1, 2]. Levamisole hydrochloride, an imidazole-thiazide group derivate, is an effective and safe broad spectrum anthelmintic commonly used in veterinary and human medicine. In addition, the drug has immunogenic effects [9]. The use of the factors that stimulate and strengthen the immune system can be considered as a strategy to adjust and increase the response of the immune system after vaccination. This study indicated the effect of stimulating and reinforcing of Levamisole hydrochloride on the immune system of broilers vaccinated against infectious bursal disease. Several studies show the effects of stimulating and modulating the immune system of birds by Levamisole during the suppression of the immune system and the use of vaccines [10, 11, 12, 13]. Basically, Levamisole does its immunization by activating macrophages and also increases the production and secretion of cytokines. In this process, activated T lymphocytes can stimulate humoral immunity [3, 7, 9, 14]. Abraham et. al showed that a low dose of Levamisole has stimulatory effects upon secretion of thymus and thymopoietin in the chickens. These hormones stimulate cellular immunity and indirectly stimulate humoral immunity [7]. Soppi et al. showed that Levamisole is able to enhance both humoral and cellular immune responses in normal chickens [15]. Singh et. al indicated the moderating effects of immunity by Levamisole in *Leghorn* chickens that were infected with infectious bursal virus [16]. Shomali et al. showed that administration of Levamisole in a repeated regimen increased cell-mediated immunity (CMI) response against AI (H9N2) virus and decreased virus shedding in quails [17]. Analysis of the data from this study, shows the effect of stimulating and strengthening of Levamisole on immune system bird. On the 29th day after vaccination, the significant difference in the antibody titer of the first group compared to the second group and the control indicated a quick response and better and stronger effects of Levamisole in the first group, which was taking the medicine from the first day ($P<0.05$). On the 41th day after vaccination, there was a significant difference in the antibody titer in both the first and second groups compared to the control group, indicating the immunomodulation effects of Levamisole ($P<0.05$). The increase in the weight of the Cloacal bursa and spleen in the first and second groups compared to the control group at the end of the period (day 40) and the significant and permanent difference in the percentage of blood lymphocytes ($P<0.05$) in the first and second groups in comparison with the control group indicate the stimulation of immune organs. According to the results of this study and a review of other researches, it can be concluded that the use of Levamisole hydrochloride drug is effective in strengthening and stimulating the immune system. Also, the consumption of the drug from the first day can cause a rapid response and production of antibodies against the *bursal* vaccine virus.

CONCLUSION

Due to its reasonable price, convenient use, low side effects, and according to the results of this study and other studies, Levamisole hydrochloride drug can be a suitable option to strengthen the immune system in birds.

ETHICS

All ethical standards have been respected in this study.

CONFLICT OF INTEREST

None declared.

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