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The effect of two dogs' multivalent vaccines on selective immune indices

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Abstract

Vaccination is the most important preventive measure to protect companion animals against infectious diseases. The aim of the present study was evaluation and comparison the effects of vaccination with two dogs' multivalent vaccines on selective immune indices. twenty healthy 4-month-old dogs of mixed breed and both sexes were randomly divided into two groups. The first and second group of the dogs were vaccinated with polyvalent Biocan (Group A) and Hipradog (Group B) vaccines on days 0 and 28, respectively. Blood was taken from the dogs of both groups on days 0, 28 and 35; The collected sera were assessed for lysozyme, complement, bactericidal activities, and total serum immunoglobulin. The type of vaccine and time had a significant effect on the amount of complement, but their interaction had no significant effect. Also, time had a significant effect on the amount of lysozyme, but the type of vaccine and the interaction between vaccine and time did not have a significant effect on this index. Regarding the bactericidal activity, the type of vaccine, time, and their interaction had no significant effect on the anti-*Salmonella* and anti-*Staphylococcal* activity. Vaccine type, time and the interaction between vaccine and time had a significant effect on the immunoglobulin trend changes based on the ELISA method. The results obtained in the present study indicated that there is no significant difference between these two polyvalent vaccines in terms of antimicrobial properties; however, the Hipradog vaccine in comparison with Biocan vaccine, stimulates the production of more complement, lysozyme, and immunoglobulin, so it is recommended to use the Hipradog vaccine for dog's vaccination.

Key words: Immune, Multivalent Vaccine, Dog

Introduction

The animal's survival depends on its successful defense against the invasion of microbes. Different defense mechanisms in the body are collectively called the immune system (Day, 2016).

The immune system consists of several parts. Innate or non-specific immunity, which is the body's first defense barrier, consists of macrophages, neutrophils, dendritic cells, and natural killer cells (NK cells), and the products of these cells (including lysozyme, complement, and innate immune cytokines such as tumor necrosis factor, some interleukins, etc.) (Fagiolo et al, 1993). Lysozyme is one of the components of innate immunity that has antibacterial activity; This enzyme is lytic and in addition to its antibacterial activity,

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it can destroy parasitic organisms with the participation of the complement system and phagocytes (Ganz and Lehrer, 1994; Zanetti et al, 1995). Another component of the innate immune system is the complement system. Activation of this system eventually leads to opsonization and lysis of many invading pathogens (Gadjeva et al, 2008). When bacteria enter the host body, the bactericidal activity of serum acts as the first line of defense as an immediate innate response that is mainly controlled by polymorphonuclear cells (Barquero-Calvo et al, 2013).

The acquired immune system consists of two main parts. One part deals directly with extracellular invaders. Soluble proteins called antibodies are responsible for destroying these invaders. This type of immune response is usually called the humoral immune response in which B lymphocytes play a role. The second major type of the acquired immune system is called cellular immunity, which deals directly with intracellular invaders (Chan and Carter, 2010; Weiner et al, 2010).

Passive immunity transmitted through the placenta (which is less significant in dogs and cats) as well as receiving colostrum can protect neonates in the first few weeks after birth, but this ability to protect against diseases, begins to decline, as soon as the maternal antibody levels decrease; Here, vaccines, which are probably one of the greatest veterinarian tools to prevent diseases and maintain the health of the individual and society, become important (Davis-Wurzler, 2014). Vaccines play a vital role in controlling and preventing infectious diseases in small animals. Although adverse reactions such as vaccine ineffectiveness may occur randomly, the evaluation of the benefits and risks of vaccination strongly recommends the use of the vaccine. It is the duty of the veterinarian to draw the attention of the animal owner to the dangers of incorrect vaccination (Davis-Wurzler, 2014; Gaskell et al, 2002). In addition to the lack of proper storage of the vaccine and incorrect vaccination schedule, which are considered as human causes of failure in vaccination, the condition of the immune system should also be considered in post-vaccination immunization (Hall and German, 2010). It is important to vaccinate the animal at an early age (before being exposed to the pathogen) and use a product with long-term immunity and minimal side effects. Currently, there are a number of products with such features. One of these categories of products is attenuated vaccines that are made by weakening the pathogen in such a way that they no longer cause serious disease in the target species. On the other hand, the demand of veterinarians for the simplicity of vaccine administration and saving time has led to the widespread use of polyvalent vaccines, and today, products that contain 8 or more pathogens per dose are commonly prescribed for dogs (Greene CE, 2006; I, 2013). Dogs are vaccinated with multivalent vaccines containing canine distemper virus, adenovirus type-2, parainfluenza virus, parvovirus and selective pathogenic *leptospira* species bacteria to save time (Ettinger, 2016). However, choosing between vaccine brands has always been challenging because they have different immunization levels. Thus, comparing immunization responses with the two most used vaccines will help have a good choice (Truyen and Streck, 2019). The importance of vaccination in the prevention and control of the mentioned diseases and the non-specific immune response as the first defense barrier of the body, raises the need for more studies on the immunogenicity of multivalent vaccines in dogs.

In this study, the effect of vaccination with two common multivalent vaccines in Iran, on some non-specific immune parameters (serum lysozyme and complement levels, as well as its bactericidal activity) and total serum Immunoglobulin has been evaluated.

Materials and Methods

After preparing 20 doses of Biocan® vaccine (Ivanovice na Hané, Czech Republic) and 20 doses of Hipradog® vaccine (Girona, Spain) and keeping them at temperatures between 2 °C and 8 °C, 20 × 4-month-old healthy dogs of mixed breeds and both sexes were randomly selected and divided into two groups (Group A and B).

The dogs of group A were vaccinated through subcutaneous injection with multivalent Biocan vaccine. The dogs of the group B received multivalent Hipradog vaccine through subcutaneous injection. Vaccinations were repeated 4 weeks (day 28) later and the blood samples were taken from all dogs on days 0, 28 and 35 post vaccination through the cephalic vein and the collected blood samples were kept at 37°C for one hour and after centrifugation at 4000 rpm/minute for 5 minutes, the blood serum was separated and stored at -70 °C until the immunological tests.

To collect data, the spectrophotometer was used according to the following methods:

Measurement of serum lysozyme level

The serum lysozyme level was measured by the method of Dall'Ara et al. (2015) with some modifications. This method is based on the ability of lysozyme to lyse *Micrococcus lysodeikticus*. and each 0.001 decreases in absorption per minute was considered equivalent to one unit of lysozyme (Dall'Ara et al, 2015). An average of two repeated measures of each serum sample was recorded for statistical analysis.

Evaluation of serum complement levels

Serum complement was measured according to the method of Lie et al. (1986) with some modifications. The technique is based on the complement-dependent lysis of heterologous (rabbit) erythrocytes. The difference between the average optical absorbance of the active serum sample and the average optical absorbance of the inactivated serum control was considered as a criterion to evaluate the hemolytic activity of the alternative complement pathway (Lie et al, 1986).

Assessment of serum bactericidal activity

Serum bactericidal activity was estimated by Clausse et al. (2017) method with some changes. The percentage of bactericidal activity was calculated according to the following formula (Clausse et al, 2017): Bactericidal activity = Mean absorption of each sample / Mean absorption of control - 1

Measurement of total serum immunoglobulin

Total serum immunoglobulin concentration was measured by zinc sulfate turbidity test (ZST) according to the method of McEvan et al. (1970) and also by an ELISA test by the method of Mila et al. (2015).

Statistical analysis

The collected data were analyzed descriptively and analytically with SPSS software. Data analysis was done with two-way repeated measures analysis of variance and LSD supplementary test. $P \leq 0.05$ was considered significant.

Results

The effect of the vaccine type on the complement level

The mean and standard deviation of serum complement level according to the vaccine type and time after vaccination are presented in Table 1.

Table 1: Mean and standard deviation of dog's serum complement level after vaccination with two multivalent vaccines

35	28	0	Days after vaccination / Vaccine type
0.158 ± 0.068^{Ba}	0.202 ± 0.035^{Aa}	0.121 ± 0.024^{Bb}	Biocan (A)
0.238 ± 0.052^{Aa}	0.215 ± 0.072^{Aab}	0.143 ± 0.033^{Ab}	Hipradog (B)

Different lowercase letters indicate differences in rows and different uppercase letters indicate differences in columns ($P<0.05$).

The repeated measures analysis of variance showed that the vaccine type (P≤0.05) and time (P<0.01) have a significant effect on the complement level, but their interaction has no significant effect (P>0.05). The overall mean of the complement level and its standard deviation for groups A and B were 0.15±0.01 and 0.2±0.01, respectively, and this difference was significant (P≤0.05). Examining the complement trend changes showed that the average complement in group A, on the day 0 compared with days 28 and 35 and in group B, on day 0 compared with the day 35 has a significant difference (P≤0.05). The comparison of the average complement on different days showed that there is a significant difference between Biocan and Hipradog vaccine on day 35, unlike days 0 and 28 (P<0.05).

The effect of the vaccine type on the lysozyme level

The mean and standard deviation of lysozyme content, according to the vaccine type and time, are presented in Table 2.

Table 2: Mean and standard deviation of dog's serum lysozyme level after vaccination with two multivalent vaccines

35	28	0	Days after vaccination / Vaccine type
130.22 ± 63.13^{Ab}	200.24 ± 64.25^{Aa}	219.18 ± 48.27^{Bab}	Biocan (A)
195.23 ± 75.66^{Ab}	188.27 ± 88.33^{Ab}	259.21 ± 38.79^{Aa}	Hipradog (B)

Different lowercase letters indicate differences in rows and different uppercase letters indicate differences in columns (P<0.05).

The repeated measures analysis of variance showed that time has a significant effect on the lysozyme levels (P<0.05), but the type of vaccine and the interaction between vaccine and time have no significant effect (P>0.05).

The overall mean of lysozyme and its standard deviation for groups A and B were 172.63±15.98 and 214.67±15.98, respectively, and this difference was not significant (P>0.05). Examining the lysozyme trend changes showed that the mean of lysozyme in group A, on day 28 compared with day 35 and in group B, on day 0 compared with days 28 and 35 has a significant difference (P<0.05). The comparison of average lysozyme on different days showed that there is no significant difference between Biocan and Hipradog vaccine on days 0, 28 and 35 (P>0.05).

Effect of vaccine type on serum anti-salmonella activity

The mean and standard deviation of salmonella killing percentage in the blood serum of dogs, according to the vaccine type and time, are presented in Table 3.

Table 3: The mean and standard deviation of the dogs' serum anti – *Salmonella* activity after vaccination with two multivalent vaccines

35	28	0	Days after vaccination / Vaccine type
^{Aa} 15.84±1.76	^{Aa} 19.16±1.41	^{Aa} 12.5±0.5	Biocan (A)
^{Aa} 18.39±0.72	^{Aa} 26.75±2.02	^{Aa}20.65±0.4	Hipradog (B)

Different lowercase letters indicate differences in rows and different uppercase letters indicate differences in columns (P<0.05).

The repeated measures analysis of variance showed that vaccine type, time and their interaction had no significant effect on the serum salmonella-killing activity levels (P<0.05). The overall mean of serum salmonella killing activity and its standard deviation for groups A and B, were 15.69±1.23 and 21.58±1.04, respectively, and this difference was not significant (P<0.05). Examining the Salmonella killing

activity trend changes showed that the trend of changes in this ability in these two groups is not significant (P<0.05). Comparison of the average salmonella-killing ability on different days showed that there is no significant difference between Biocan and Hipradog vaccine on days 0, 28 and 35 (P<0.05).

Effect of vaccine type on serum anti-staphylococcal activity

The mean and standard deviation of anti-staphylococcal percentage in the blood serum of dogs according to the vaccine type and time are presented in Table 4.

Table 4: The mean and standard deviation of the dogs' serum anti-*staphylococcal* activity after vaccination with two multivalent vaccines

35	28	0	Days after vaccination / Vaccine type
^{Aa} 24.8±2.51	^{Aa} 34.74±1.61	^{Aa} 28.76±0.67	Biocan (A)
^{Aa} 23.94±0.91	^{Aa} 29.13±2.34	^{Aa} 29.35±0.53	Hipradog (B)

Different lowercase letters indicate differences in rows and different uppercase letters indicate differences in columns (P<0.05).

The repeated measures analysis of variance showed that vaccine type, time and their interaction did not have a significant effect on the serum anti-staphylococcal activity levels (P>0.05). The overall mean of serum anti-staphylococcal activity and its standard deviation for groups A and B, were 29.38±1.59 and 27.46±1.3, respectively, and this difference was not significant (P>0.05). Examining the serum anti-staphylococcal activity trend changes showed that the trend of these changes in dogs receiving Biocan or Hipradog vaccine is not significant (P>0.05). Comparison of the average anti-staphylococcal activity of serum on different days after vaccination showed that there is no significant difference between Biocan vaccine and Hipradog vaccine on days 0 and 35 (P>0.05).

Effect of vaccine type on the total serum immunoglobulin level by ELISA method

The mean and standard deviation of immunoglobulin concentration in milligrams per milliliter by ELISA method according to the vaccine type and time are presented in Table 5.

Table 5: The mean and standard deviation of total serum immunoglobulin concentration (mg/ml) measured by ELISA method after vaccination with two multivalent vaccines

35	28	0	Days after vaccination / Vaccine type
^{Ab} 14.0±0.82	^{Ba} 19.0±2.31	^{Aa} 17.5±3.01	Biocan (A)
^{Ac} 14.7±0.5	^{Aa} 28±5.76	^{Ab} 18.6±3.81	Hipradog (B)

Different lowercase letters indicate differences in rows and different uppercase letters indicate differences in columns (P<0.05).

The repeated measures analysis of variance showed that vaccine type (P<0.05), time (P<0.001) and the interaction between vaccine and time (P<0.001) have a significant effect on the immunoglobulin trend changes. The overall mean and standard deviation of immunoglobulin for groups A and B were 16.83±2.04 and 20.43±3.35, respectively, and this difference was significant (P<0.05). Examining the immunoglobulin trend changes showed that the mean of immunoglobulin in group A, on days 0 and 28 compared with day 35 and in group B,

on day 0 compared with days 28, 35 and also day 28 compared with day 35 has a significant difference (P<0.05). The comparison of average immunoglobulin evaluated by ELISA on different days showed that there is a significant difference between Biocan and Hipradog vaccine on day 28, unlike days 0 and 35 (P<0.001).

Effect of vaccine type on the total serum immunoglobulin level by zinc sulfate turbidity test (ZST)

The mean and standard deviation of immunoglobulin concentration in milligrams per milliliter by zinc sulfate method according to the vaccine type and time are presented in Table 6.

Table 6: The mean and standard deviation of the immunoglobulin concentration (mg/ml) by zinc sulfate method after vaccination with of two multivalent vaccines

35	28	0	Days after vaccination / Vaccine type
^{Ab} 20.6±4.63	^{Aa} 26.3±3.13	^{Bb} 19.2±6.33	Biocan (A)
^{Aa} 23.8±6.92	^{Aa} 24.4±7.83	^{Aa} 26.7±3.33	Hipradog (B)

Different lowercase letters indicate differences in rows and different uppercase letters indicate differences in columns (P<0.05).

The repeated measures analysis of variance showed that vaccine type, time and the interaction between vaccine and time did not have a significant effect on the immunoglobulin trend changes (P>0.05). The overall mean and standard deviation of immunoglobulin for groups A and B were 22.03±4.69 and 24.9±6.02, respectively, and this difference was not significant (P>0.05). Examining the immunoglobulin trend changes showed that the immunoglobulin amount after the use of the Biocan vaccine, unlike the Hipradog vaccine, has significant changes during the time of the study (P<0.05); mean of immunoglobulin in group A, on day 28 in comparison with days 0 and 35 has a significant difference. The comparison of average immunoglobulin on different days showed that there is a significant difference between Biocan and Hipradog vaccines on day 0, unlike days 28 and 35 (P<0.05).

Discussion

Immediately after birth, neonates are susceptible to infectious diseases due to the immature immune system. While complete immunological ability is usually achieved a few weeks after birth, the newborn must cope with the new unhealthy extra uterine environment, where multiple factors are often responsible for morbidity and mortality. Meanwhile, the innate immune system, which consists of the function of several non-specific factors such as lysozyme, becomes important (Barrios et al, 1996; Schlievert et al, 1977).

Dog vaccination is the most important part of keeping them. The vaccine prevents some diseases of bacterial and viral origin. The most likely time to catch fatal diseases is at the age of puppies; therefore, it is necessary to inject the vaccine in puppies at the age of 6 weeks (approximately 40 days old) and repeat it one month later, and basically, this repetition of vaccination should continue until the age of 16 weeks (Dall'Ara et al, 2021; Davis-Wurzler, 2006). The animal's immune system response to vaccination includes both specific and non-specific immune aspects (Dhein and Gorham, 1986).

Components of innate immunity constitute a set of defense mechanisms against disease that are not pathogen-specific. Cells associated with innate immunity, such as dendritic cells and macrophages, directly kill pathogens through phagocytosis or produce cytokines that facilitate the elimination of pathogens. The innate immune response is essential for pathogen-specific long-term adaptive immune responses (Kumar et al, 2009).

A wide variety of vaccine adverse effects are currently recognized in domestic animal species. A variety of physiological and environmental factors influence the animal's immune response and thus may influence the response to vaccination (Dhein & Gorham, 1986; Myer, 2001; Roth, 1999).

As determined in the present study, no significant difference was observed between Biocan and Hipradog multivalent vaccines in terms of antibacterial properties against two examined bacteria, Salmonella and Staphylococcus. Thus, these two vaccines have the same and similar effects in terms of bactericidal activity induction. Shams et al. (2022) by comparison of the two vaccine brands, Biocan and Duramune Max, stated that these two vaccines had a similar effect on the studied dogs.

In a study, Jacobs et al. (2007) investigated the effect of two types of commercial vaccines on dogs; 8 puppies (group 1) were vaccinated once with a modified bivalent live vaccine against infectious tracheobronchitis intranasally and at the same time with an injectable trivalent vaccine against canine parvovirus, canine distemper virus and canine adenovirus. The results indicated that these two types of vaccines had the same antimicrobial effect, which is almost consistent with the findings obtained in the present study (Jacobs et al, 2007).

In the current study, the amount of total immunoglobulin in the serum of dogs after receiving two doses of Hipradog and Biocan vaccine was evaluated by ELISA and ZST. On day zero, as expected, the amount of total serum immunoglobulin was not statistically different with both measurement methods and in both groups of studied dogs; While in both groups A and B, 28 days after receiving the first dose of the vaccine, there was a significant increase in total serum immunoglobulin, based on which it can be concluded that both vaccines were immunogenic; however, on the 35th day, the amount of serum total immunoglobulin decreased in both groups, which could be due to the elimination of antibodies in the serum after receiving the second dose of the vaccine.

In other words, according to the references, if the third time blood sampling was done at least two weeks after receiving the second dose of the vaccine, there was an increase in total serum immunoglobulin expected (Dodds, 2021; Welch, 2021). In the comparison of two types of vaccines based on ELISA method which is a more accurate method for measuring the amount of immunoglobulin, the Hipradog vaccine has been able to stimulate humoral immunity better than the Biocan vaccine; but it is necessary to reevaluate this importance in more accurate evaluations which included specific antibodies measurement, that also require a big budget which made it impossible to do in the current research.

The results obtained in the present study indicated that there is no significant difference between the two groups that recieved Biocan and Hipradog multivalent vaccines in terms of antimicrobial properties; however, the Hipradog vaccine (group B) caused more stimulation of the immune system, so that the amount of complement and lysozyme in this vaccine was higher than the Biocan vaccine (group A), on the other hand, since serum total immunoglobulin level in the Hipradog vaccine group was significantly higher than the Biocan vaccine group on day 28 after vaccination, it is recommended to use the Hipradog vaccine for dog vaccination. Considering the low accommodation of the two evaluated methods and more accuracy of ELISA, the mentioned method is recommended in similar future research.

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Conflict of interest

The authors declare that there are no conflicts of interest in this article.

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تأثیر دو نوع واکسن چندگانه سگ بر برخی از شاخص‌های ایمنی ذاتی

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چکیده

واکسیناسیون مهم‌ترین ابزار پیش‌گیری در جهت محافظت حیوانات خانگی در برابر بیماری‌های عفونی است. مطالعه حاضر به منظور ارزیابی و مقایسه تأثیرات واکسیناسیون با دو نوع واکسن چندگانه سگ بر برخی از شاخص‌های ایمنی ذاتی انجام شد. ۲۰ قلاده سگ سالم ۴ ماهه از نژاد مخلوط و از هر دو جنس به طور تصادفی به دو گروه تقسیم شدند. گروه اول و دوم سگ‌ها به ترتیب با واکسن‌های چندگانه بیوکن (گروه الف) و هیپراداگ (گروه ب) در روزهای ۰ و ۲۸ واکسینه شدند. خون‌گیری از هر دو گروه از سگ‌ها در روزهای ۰، ۲۸ و ۳۵ صورت گرفت؛ سرم‌های جمع‌آوری شده برای مقادیر لیزوزیم، کمپلمان، فعالیت باکتری‌کشی و میزان آنتی‌بادی تام سرم ارزیابی شدند. نوع واکسن و زمان تأثیر معنی‌داری بر مقدار کمپلمان داشتند اما اثر متقابل آن‌ها تأثیر معنی‌داری نداشت. همچنین زمان تأثیر معنی‌داری بر مقدار لیزوزیم داشت، اما نوع واکسن و اثر متقابل بین واکسن و زمان تأثیر معنی‌داری بر این شاخص نداشت. در خصوص فعالیت باکتری‌کشی، نوع واکسن، زمان و اثر متقابل آن‌ها تأثیر معنی‌داری بر فعالیت ضد سالمونلایی و ضد استافیلوکوکی نداشت. نوع واکسن، زمان و اثر متقابل آن‌ها، بر روند تغییرات ایمونوگلوبولین بر اساس روش الایزا، تأثیر معنی‌داری داشتند. نتایج به دست آمده در این مطالعه نشان داد که اختلاف معنی‌داری بین این دو واکسن چندگانه در خصوص خواص ضد میکروبی وجود ندارد؛ با این حال واکسن هیپراداگ در مقایسه با واکسن بیوکن باعث تحریک تولید مقادیر بیش‌تری لیزوزیم، کمپلمان و آنتی‌بادی تام سرم شده و به همین خاطر استفاده از آن در واکسیناسیون سگ‌ها پیشنهاد می‌شود.

کلمات کلیدی: ایمنی، واکسن چندگانه، سگ

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